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Physiological responses to brain tissue hypoxia and blood flow after acute brain injury

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2018

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Preface

This dissertation is submitted for the degree of Doctor of Philosophy at the University of Edinburgh. The research described herein was conducted under the supervision of Professor Peter Andrews in the Centre for Clinical Brain Sciences, University of Edinburgh, between September 2013 and April 2017.

This work is to the best of my knowledge original, except where acknowledgements and references are made to previous work. Neither this, nor any substantially similar dissertation, has been or is being submitted for any other degree, diploma or other qualification at any other university.

Part of this work has been presented in existing publications (Appendix 2):

Flynn L, Andrews P. Advances in the understanding of delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. F1000Research. 2015;4:F1000 Faculty Rev-200.

Flynn LM, Rhodes J, Andrews PJ. Therapeutic Hypothermia Reduces Intracranial Pressure and Partial Brain Oxygen Tension in Patients with Severe Traumatic Brain Injury: Preliminary Data from the Eurotherm3235 Trial. Ther Hypothermia Temp Manag. 2015.

Flynn LMC, Macleod MR, Begg CJ, Andrews PJ. Efficacy of alpha-calcitonin gene-related peptide in dilating cerebral arteries: protocol for a systematic review and meta-analysis of in vivo animal studies. Evid Based Preclin Med. 2016;3(1):1-3.

Acknowledgements

I wish to thank my supervisor, Professor Peter Andrews, for his kind support and guidance throughout my degree and for encouraging me to continue with an academic career. I must acknowledge Professor Andrews, along with Dr Caroline Begg and Professor Malcolm Macleod, for their involvement in the meta-analysis in Chapter 3. They have not directly contributed to Chapter 3 and the writing here is solely my own, but they have all contributed to the work which has led to the entry in this thesis as follows: Dr Begg enabled us to perform an independent search and data entry; Professor Macleod assisted with design and data analysis and Professor Andrews assisted with design of the study and editing of the submitted manuscript. I would also like to take this opportunity to thank Dr Jonathan Rhodes, who has given me a great deal of advice with regards to statistical analysis and interpreting data and has been collecting data on patients with traumatic brain injury for many years. My thanks go to Cat Graham for her help with statistical analysis and study design. Finally, I would like to thank Bridget Harris for her advice throughout and for kindly proof reading this thesis.

Abstract

This thesis explores physiological changes occurring after acute brain injury. The first two chapters focus on traumatic brain injury (TBI), a significant cause of disability and death worldwide. I discuss the evidence behind current management of secondary brain injury with emphasis on partial brain oxygen tension ($P_{bt}O_2$) and intracranial pressure (ICP). The second chapter describes a subgroup analysis of the effect of hypothermia on ICP and $P_{bt}O_2$ in 17 patients enrolled to the Eurotherm3235 trial. There was a mean decrease in ICP of 4.1 mmHg (n=9, p<0.02) and a mean decrease in $P_{bt}O_2$ (7.8 ± 3.1 mmHg ($p < 0.05$) in the hypothermia group that was not present in controls. The findings support previous studies in demonstrating a decrease in ICP with hypothermia. Decreased $P_{bt}O_2$ could partially explain worse outcomes seen in the hypothermia group in the Eurotherm3235 trial. Further analysis of $P_{bt}O_2$ and ICP guided treatment is needed.

The third chapter focuses on delayed cerebral ischaemia (DCI) after aneurysmal subarachnoid haemorrhage (aSAH), another form of acute brain injury that causes significant morbidity and mortality. I include a background of alpha-calcitonin gene-related peptide (αCGRP), a potential treatment of DCI, along with results from a systematic review and meta-analysis of nine experimental models investigating αCGRP. The meta-analysis demonstrates a 40.8 ± 8.2% increase in cerebral vessel diameter in those animals treated with αCGRP compared with controls ($p < 0.0005$, 95% CI 23.7 to 57.9). Neurobehavioural scores were reported in four publications and showed a

standardised mean difference of 1.31 in favour of αCGRP (CI -0.49 to 3.12). I conclude that αCGRP reduces cerebral vessel narrowing seen after SAH in animal studies but note that there is insufficient evidence to determine its effect on functional outcomes. A review of previous trials of αCGRP administration in humans is included, in addition to an original retrospective analysis of CSF concentrations of αCGRP in humans. Enzyme-linked immunosorbent assay of CSF (n = 22) was unable to detect αCGRP in any sample, which contrasts with previous studies and was likely secondary to study methodology. Finally, I summarise by discussing a protocol I designed for a dose-toxicity study involving the intraventricular administration of αCGRP to patients with aSAH and provide some recommendations for future research. This protocol was based upon the systematic review and was submitted to the Medical Research Council's DPFS funding stream during the PhD.

Lay Summary

This thesis explores the physiological changes that occur after acute brain injury. The first two chapters focus on traumatic brain injury (TBI), a significant cause of disability and death worldwide. TBI involves primary and secondary brain injuries. Primary brain injury is the initial trauma to the brain and is best managed with prevention strategies. Secondary brain injury is that which occurs in the minutes to days following the initial trauma and is due to several injury processes, with a final common pathway of brain ischaemia. Secondary brain injury can be attenuated by maintaining basic physiological variables within an equilibrium. Two such variables are intracranial pressure (ICP, the pressure within the skull) and partial brain oxygen tension ($P_{bt}O_2$, a measure of brain oxygenation). Increased ICP and decreased $P_{bt}O_2$ are associated with worse outcomes in patients with TBI. Therapeutic hypothermia (TH), the controlled reduction in core body temperature below 36°C, has been trialled as a treatment for TBI and does not appear to be beneficial. The second chapter of this dissertation documents the effect of TH on ICP and $P_{bt}O_2$ in 17 patients enrolled to the Eurotherm3235 trial (a trial of hypothermia as a treatment for patients with raised ICP after TBI). The analysis demonstrated a decrease in ICP and $P_{bt}O_2$ in patients randomised to hypothermia. These findings support other studies by indicating a reduction in ICP with cooling. Decreased $P_{bt}O_2$ could partially explain worse outcomes seen in the hypothermia group in the Eurotherm3235 trial. However, I conclude by stating the importance of further analysis of $P_{bt}O_2$ data.

Physiological responses to brain tissue hypoxia and blood flow after acute brain injury

Whilst researching changes in brain blood flow and $P_{bt}O_2$ in TBI I became interested in other pathologies that can alter brain tissue perfusion and conducted a review of delayed cerebral ischaemia (DCI) after subarachnoid haemorrhage (SAH, a type of stroke). The third chapter of this thesis concerns DCI, a life-threatening complication of SAH that may respond to early treatment because its onset is delayed. There is a strong association between brain vessel narrowing and DCI. Alpha calcitonin gene-related peptide (αCGRP) is a potent vessel dilator that may be effective at reducing brain vessel narrowing after SAH. I report analysis of data from nine animal SAH models in which αCGRP was administered. There was a 40% increase in brain vessel diameter in those animals treated with αCGRP compared with controls. There was evidence to suggest that the treated animals had a better functional outcome (less neurological disability). I conclude that αCGRP reduces brain vessel narrowing after SAH in animal studies but note that there is insufficient evidence to determine its effect on functional outcomes. The results of previous studies examining the concentration of αCGRP in cerebrospinal fluid (CSF, fluid around the brain and spinal cord) in humans with SAH is summarised. I describe my study of αCGRP concentrations within the CSF of patients with and without SAH. In contrast to previous studies, I did not detect any αCGRP in 22 CSF samples. I argue that this was due to methodological problems. Finally, I summarise by discussing a protocol for a study involving the administration of αCGRP into the CSF of patients with aSAH and provide recommendations for future research.

Abbreviations

ARDS	Acute respiratory distress syndrome	HSS	Hypertonic saline solution
ACA	Anterior cerebral artery	ICA	Internal cerebral artery
ANOVA	Analysis of variance	ICP	Intracranial pressure
aSAH	Aneurysmal subarachnoid haemorrhage	ICU	Intensive care unit
ATP	Adenosine triphosphate	LP	Lumbar puncture
BBB	Blood brain barrier	MAP	Mean arterial pressure
BP	Blood pressure	MCA	Middle cerebral artery
BTF	Brain Trauma Foundation	MRI	Magnetic resonance imaging
CALC	Calcitonin	n	Number (often used for number in trial)
CaO2	Arterial content of oxygen	NGF	Nerve growth factor
CBF	Cerebral blood flow	NICE	National Institute for Health and Clinical Excellence
CGRP	Calcitonin gene-related peptide	NO	Nitric oxide
CI	Confidence interval	OR	Odds Ratio
$CMRO_2$	Cerebral metabolic rate for oxygen	$PaCO_2$	Arterial partial pressure of carbon dioxide
CNS	Central nervous system	PaO_2	Arterial partial pressure of oxygen
CPP	Cerebral perfusion pressure	$P_{bt}O_2$	Partial brain oxygen tension
CSD	Cortical spreading depolarisation	PCTA	Percutaneous transluminal angioplasty
CSF	Cerebrospinal fluid	PCWP	Pulmonary capillary wedge pressure
CT	Computed tomography	PKC	Protein kinase C
CTP	Computed tomography perfusion	RCT	Randomised controlled trial
CVP	Central venous pressure	RR	Relative risk
CXR	Chest x-ray	RTC	Road traffic collision
DCI	Delayed cerebral ischaemia	SAH	Subarachnoid haemorrhage
DSA	Digital subtraction angiography	SaO_2	Oxygen saturation
EBI	Early brain injury	SBP	Systolic blood pressure
ED	Emergency department	SD	Standard deviation
EDH	Extra dural haematoma	SDH	Subdural haematoma
EEG	Electroencephalogram	SE	Standard error
EIA	Enzyme-linked immunosorbent assay	SEM	Standard error of the mean
ET	Endothelin	SIGN	Scottish Intercollegiate Guidelines Network
ERA	Endothelin receptor antagonist	TBI	Traumatic brain injury
EVD	External ventricular drain	TCD	Transcranial Doppler
FiO_2	Fraction of inspired oxygen	TCDB	Traumatic coma data bank
GCS	Glasgow come scale	TH	Therapeutic hypothermia
GOS-E	Extended Glasgow outcome scale	TRPV	Transient receptor potential vanilloid
Hb	Haemaglobin	VAD	Ventricular access device
HIE	Hypoxic ischaemic encephalopathy	WFNS	World Federation of Neurosurgeons Scale

Chapter 1: Traumatic Brain Injury

The first two chapters of this thesis concern traumatic brain injury (TBI). Here I explain the epidemiology of TBI and the evidence base for current management with a focus on intracranial pressure (ICP) and partial brain oxygen tension ($P_{bt}O_2$). I go on to provide a brief outline of therapeutic hypothermia (TH). I conclude the chapter by summarising the difficulties with TBI research and suggest recommendations for future research.

Traumatic Brain Injury

Traumatic brain injury, defined as a blunt or penetrating trauma to the brain, can be broadly divided into mild, moderate or severe, depending upon the patient's Glasgow Coma Scale (GCS) following the injury (1). The Glasgow Coma Scale is a basic clinical indicator of neurological function. The three parameters that make up the GCS are based on best responses from the patient's eye, voice and motor function (E, V, M, Table 1). A GCS of 3-8 following the injury represents a severe TBI and usually indicates a need for neuro-intensive care (2). The GCS was never intended to be used as a sum score and the greater importance of its three individual components was highlighted by Teasdale and Jennett as early as 1983 (3). A common criticism of the GCS is that it is not linear for outcome prediction (4). Indeed, a GCS of four predicts a mortality rate of both 48% and 19% depending on the components that make up the score (E1 V1 M2 and E2 V1 M1 respectively) (5). GCS is also poorly reproducible. In a study of emergency physicians

independently assessing GCS, scores were the same in only 38% and were two or more points apart in 38% (6). In another study involving a written clinical scenario, the GCS was correctly identified by less than half of all physicians and only 56% of neurosurgeons (4). Despite these limitations, and evidence suggesting that simpler scales perform just as well, the GCS is still ubiquitous in clinical practice and is referenced in National Institute for Health and Clinical Excellence (NICE), Scottish Intercollegiate Guidelines Network (SIGN) and Brain Trauma Foundation (BTF) guidelines (4, 7).

Table 1: Glasgow Coma Scale

Behaviour	Response	Score
Eye Opening Response	Spontaneously	4
	To speech	3
	To pain	2
	No response	1
Verbal Response	Orientated to time, place and person	5
	Confused	4
	Inappropriate words	3
	Incomprehensible sounds	2
	No response	1
Motor Response	Obeys commands	6
	Localises painful stimulus	5
	Withdraws from painful stimulus	4
	Abnormal flexion	3
	Abnormal extension	2
	No response	1
Total Score	*Mild brain injury*	≥13
	Moderate brain injury	9 - 12
	Severe brain injury	<9

Table 1: Glasgow Coma Scale

Epidemiology

Traumatic brain injury is a significant cause of disability and death and a huge economic burden on society. In the UK, TBI is the commonest cause of death and disability in those aged 1-40 (8). Annually, there are approximately 200,000 hospital admissions attributable to head injuries, of which 11,000 are severe and require treatment in an intensive care unit (ICU). Most of these admissions are young adult males (8, 9) but there is a trimodal distribution with peaks in young children, young adults and the elderly. Most head trauma is secondary to falls, assaults and road traffic collisions (RTC), with RTCs accounting for the greatest proportion of severe TBI and death. In the US, approximately 50,000 people die following TBI each year and of those who survive to discharge, approximately 43% have an ongoing disability at one year after the initial injury (10).

In addition to the morbidity and mortality associated with TBI there is a significant economic burden. In Europe the annual monetary cost exceeds €33 billion (11, 12). In the US, it was estimated to be $60 billion in the year 2000 (13).

It is difficult to provide an accurate estimate of the effect of TBI in third-world countries due to incomplete records, but it is thought to be at least as significant due to the larger incidence of RTCs. An estimated ten million people suffer severe TBI worldwide each year (14). Because of the enormous incidence of TBI occurring in third-world countries, any intervention for TBI should be affordable and practical to low income areas.

Primary and Secondary Brain Injuries

Primary injury after TBI reflects the initial trauma to the head causing direct physical damage to the brain parenchyma, tissue damage, shearing or vascular damage in addition to destabilising cell membranes. Public health measures to prevent TBI may be the best management strategy for these injuries.

Much of the morbidity associated with TBI can be attributed to secondary insults. Secondary brain injury occurs hours to days after the initial event and can have an intracranial or systemic aetiology. Intracranial causes include haematomas, cerebral oedema, hydrocephalus, raised ICP, excitotoxicity, infection and imbalances in free radicals, and oxygen and substrate delivery. Systemic brain insults are often related to brain tissue hypoxia and are ischaemic in nature (15-17). It is these secondary injuries that we aim to influence and which this thesis focuses on. Surrogate indicators of brain perfusion and oxygenation measured on the ICU in an aim to reduce secondary brain injury include intracranial pressure (ICP), cerebral perfusion pressure (CPP), and partial brain oxygen tension ($P_{bt}O_2$)(18).

In severe TBI, additional changes occur to accommodate fluctuations in cerebral blood flow (CBF) and ICP. A basic understanding of these changes is necessary for the management of TBI. Cerebral blood flow must be maintained to ensure adequate delivery of oxygen and glucose to the brain to prevent secondary brain injury. Maintaining this blood flow is dependent upon the mean arterial pressure (MAP) of blood entering the brain, and the ICP exerted

on vessels within the skull. When the MAP decreases, there are physiological changes which take place to maintain CBF. Similarly, if the MAP increases, putting the brain at risk of cerebral oedema and increased ICP, there are changes which take place to reduce the MAP. This is pressure autoregulation and is key to understanding the correct management of patients with severe TBI (19, 20).

Intracranial Pressure

The skull can be thought of as a fixed box, containing three compartments of approximately 80% nearly incompressible brain parenchyma, 12% blood and 8% cerebrospinal fluid (CSF). The total volume within this box is approximately 1600mls. If the volume of any one of these compartments increases, the ICP will increase unless the volume of another compartment is reduced to compensate. There is limited volumetric compensation from CSF and blood before increased ICP causes cerebral herniation. When intracranial volume increases after TBI, secondary to oedema, hydrocephalus or increased blood volume, several changes occur. Firstly, CSF leaves the skull into the spinal canal, then venous blood and more CSF is forced out of the skull. Eventually, this compensatory mechanism is fatigued as the venous sinuses are emptied and there is minimal CSF remaining that can be displaced. This describes the Monro-Kellie hypothesis, stating that the cranium is incompressible with a fixed volume that exists in a state of equilibrium (21-23). Figure 1 demonstrates a CSF-pressure-volume curve indicating that small increases in volume lead to minimal changes in ICP until a further small change in volume leads to a

dramatic increase in ICP. Areas 3-4 on the curve reflect the rapid decompensation that occurs when the brain has exhausted its ability to compensate for increases in volume. An increase in volume greater than 100-120mls is sufficient to cause a dramatic increase in ICP (20).

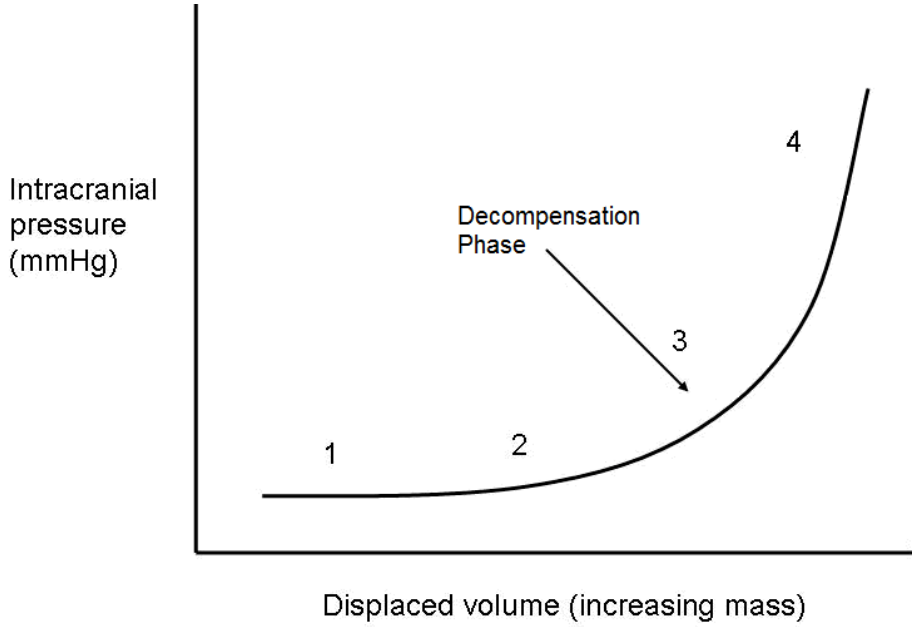


Figure 1: Intracranial Pressure-Volume Curve

A normal ICP value in a supine adult has been reported as between 7-15 mmHg, although it varies by 1-2 mmHg as we change position, cough or sneeze (24). The standard reference for ICP is derived from ventricular-sited ICP monitors. Less accurate sites include the subarachnoid, epidural and subdural spaces, but intraparenchymal monitors are now standard of care (Table 3).

In healthy adults ICP is maintained through changes in CSF volume and venous blood volume. Patients admitted to the ICU with severe TBI may have an intracranial bolt inserted into the skull with a probe placed in the brain

parenchyma, ventricles, epidural or subarachnoid spaces, providing a continuous measure of ICP (Fig.2).

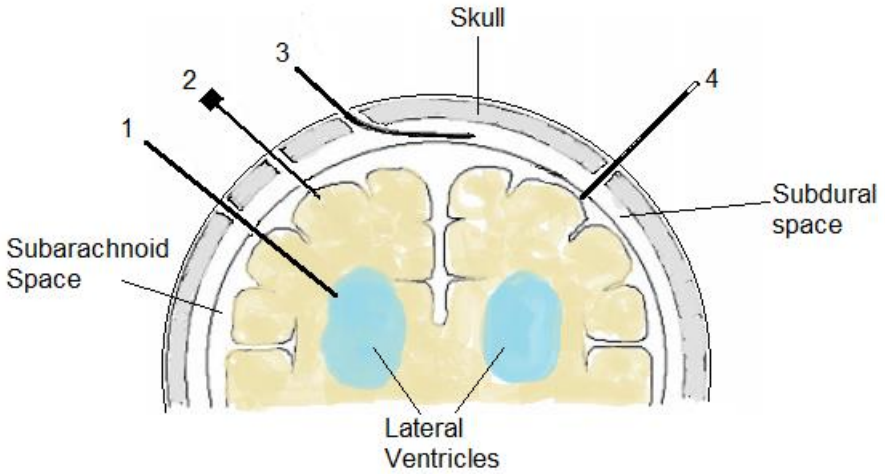


Figure 2: Potential Sites of ICP Monitoring. 1, Intraventricular drain; 2, Intraparenchymal probe; 3, Extradural probe; 4, Subarachnoid probe.

Despite trials of non-invasive methods of measuring ICP, an intraventricular drain placed in one of the lateral ventricles or brain parenchyma connected to an external pressure transducer remains the gold standard (25, 26). Intraventricular drains are the preferred choice in the presence of hydrocephalus because of their ability to drain CSF (27). This route also offers the ability to administer thrombolytics in patients with intraventricular haemorrhage and antibiotics in patients with ventriculitis (28, 29). In patients with intracranial hypertension and small ventricles, intraventricular monitors can be technically difficult to site. The risk of infection from intraventricular monitoring is also higher than alternative methods and is estimated to be between 5-11% (30-32). Intraparenchymal pressure monitors have a lower

rate of infection (approximately 1%) but give a localised estimated of ICP in certain cases (33). If there is a disturbance in CSF circulation, as may occur after TBI, the measured intraparenchymal pressure may reflect a compartmentalised pressure and not the true ventricular ICP. In addition to this, intraparenchymal monitors can suffer from a small drift from their baseline measurement and recalibration cannot be performed once the sensor is in place (26). Due to fluctuations in ICP a mean value should be taken over at least 30 minutes (31). The transducer should be zeroed at the external auditory meatus and may require repeat zeroing if possible. Traditionally, the monitor was placed on the right because this is the non-dominant side in approximately 80% of the population, but may also be placed on the pathological side (34-36). Note that patients who have $P_{bt}O_2$ measured will have an intraparenchymal probe measuring ICP, $P_{bt}O_2$ and brain temperature. It is unclear whether probes should be placed at the site of injured tissue to better reflect the $P_{bt}O_2$ and ICP in the affected areas or in healthy tissue.

Advantages and Disadvantages of ICP Monitoring Sites		
Location of Sensor	Advantages	Disadvantages
Intraventricular	Is the current gold standard and allows drainage of CSF to decrease ICP	Highly invasive with associated infection rate. Requires more technical ability from physician.
Intraparenchymal	Low infection rate, easier to place than intraventricular	Measure focal pressures rather than CNS pressure
Subarachnoid	Low infection rate, no brain tissue damage	High failure rate and limited accuracy
Extradural	Low infection rate, no brain tissue damage and simplest to place	Not as accurate as those above

Table 2: Intracranial Pressure Probe Sites: advantages and disadvantages. CSF, cerebrospinal fluid; ICP, intracranial pressure; CNS, central nervous system

Cerebral Perfusion Pressure

Cerebral perfusion pressure in the healthy adult is the pressure gradient between arterial and venous blood. It is the difference between MAP and central venous pressure (CVP), both calibrated at the level of the right atrium. It is commonly estimated as the difference between the MAP and the ICP due to the increase in pressure surrounding the cerebral vessels. The MAP is the mean average arterial pressure of a single cardiac cycle and can be calculated as the cardiac output (heart rate x stroke volume) x the systemic vascular resistance (the resistance to blood flow throughout the circulation). The MAP can be estimated by adding the diastolic pressure to one third of the pulse pressure (systolic minus diastolic pressure). It is preferred to the systolic pressure because it reflects the perfusion pressure at which organs, such as the brain and kidneys, receive their blood supply (20).

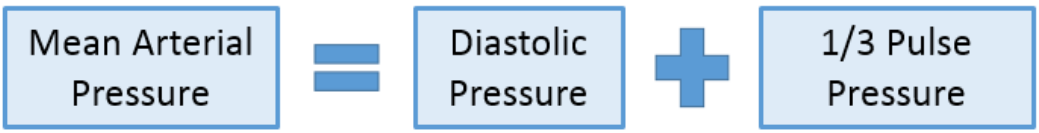


Figure 3: Mean Arterial Pressure

I have stated that the MAP and CVP are calibrated at the level of the right atrium in healthy subjects, but in TBI patients managed with head elevation the level of calibration may be different. Some authors advocate that MAP and ICP should be set at the level of the foramen of Monro/the tragus of the ear to avoid CPP over-estimation (37, 38). Rao et al. highlight the importance of standardised measurement points. Measuring blood pressure (BP) at the level

of the heart in a patient with 30 degree head elevation results in a CPP 11 mmHg higher than that measured at the tragus of the ear (37, 38). Kirkman et al. cite two papers referenced by the BTF that describe worse outcomes in patients with a CPP below 60 mmHg (37). However, these two studies used the level of the heart to zero their BP measurements and as such represented a 'true' CPP below 50 mmHg (39, 40). Rao et al. found that of 58 European centres, 62% calibrated MAP at the heart, while 36% calibrated at the head (the remaining centre calibrated at the heart unless ICP was above 20 mmHg, when they would then recalibrate at the head) (38).

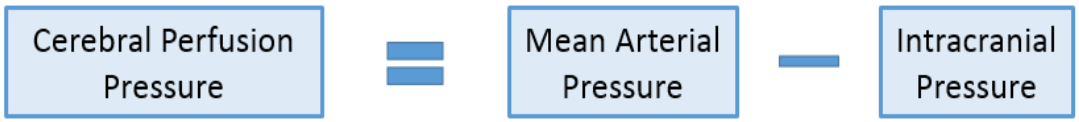


Figure 4: Cerebral Perfusion Pressure

In a healthy adult, with a MAP of 80-100 mmHg and an ICP of 5-10 mmHg, CPP is maintained at about 70-85 mmHg. If a patient's MAP is low and their ICP is high, the effect can be a catastrophic decrease in CPP leading to brain tissue ischaemia (41). Figure 5 shows the traditional Lassen curve demonstrating a rapid increase and decrease in cerebral blood flow when the body decompensates for changes in cerebral perfusion pressure (42).

There is no class I evidence (defined as good-quality randomised controlled trials (RCT)) that CPP-guided therapy improves outcomes, and some evidence suggests it makes no difference, whilst others suggest it may worsen

outcomes. In the early nineties the lower threshold for CPP was 70 mmHg based on prospective data demonstrating improved outcomes associated with higher CPP (43, 44). The benefit of a higher CPP may come from the higher MAP and avoidance of the adverse effects of hypotension. Decreased CPP below 50 mmHg has been associated with brain tissue hypoxia and ischaemia from surrogate markers (one RCT n=189, and two small cohort studies) (45-47). However, a CPP above 70 mmHg has been shown to increase the risk of acute respiratory distress syndrome (ARDS) and is not associated with improved outcomes (46, 48, 49). The Selfotel trial (a multi-centre RCT of 427 patients) found no correlation between CPP and neurological outcomes as a secondary measure as long as CPP was ≥60 mmHg. The authors concluded that a CPP >60 mmHg has little influence on the outcomes of patients with TBI (50). The Lund concept introduced still different targets of CPP based on a 'volume-targeted' approach over 20 years ago. Despite not being adopted and remaining controversial, it is still described and reviewed in contemporary literature. The Lund concept is based on blood-brain barrier (BBB) and autoregulation impairment and advocated a CPP of 50 mmHg to minimise intra-capillary hydrostatic pressure and water content and avoid rises in ICP (51). A 2013 Cochrane review noted that there was no evidence that the Lund concept is a preferable treatment option (no studies met the inclusion criteria). Another review highlights that there is evidence against the use of some individual components of this strategy (52, 53).

It is still unclear what the optimal target CPP is but the current 2016 BTF guidelines state the following:

- *There is insufficient evidence to support a level I or II A recommendation for CPP targets.*
- *Aim for a target CPP of 60 to 70 mmHg.*
- *It is unclear whether 60 or 70 mmHg is the minimum optimal threshold.*
- *Aggressive attempts to maintain CPP above 70 mmHg should be considered due to the risk of ARDS* (54).

The BTF also recommend using guideline-based CPP monitoring to reduce two-week mortality. This is based upon one retrospective cohort study of 2,300 patients that demonstrated a decrease in two-week mortality from 22 to 13% (OR 0.52, 95% CI 0.39-0.70; p < 0.0001) which appears to be associated with an increased adherence to guidelines. However, it should be noted that both ICP and CPP monitoring guideline adherence increased in this study, not solely adherence to CPP guidance (55).

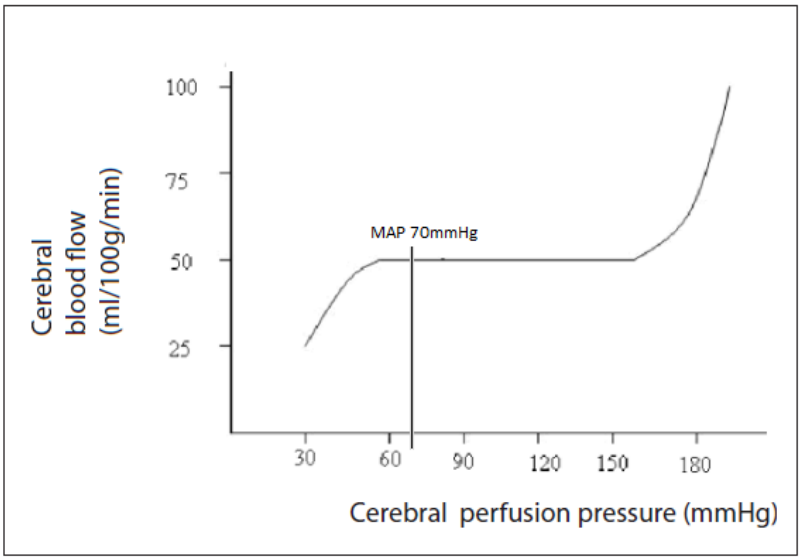


Figure 5: Cerebral Blood Flow Change with Cerebral Perfusion Pressure

Cerebral Blood Flow

There is roughly 150mls of blood in the skull, 100mls of which is in the venous system, but CBF is relatively high. Cerebral blood flow is the blood supply to the brain and is typically 45-50ml $100g^{-1}$ min^{-1}, ranging from 20ml $100g^{-1}$ min^{-1} in white matter to 80ml $100g^{-1}$ min^{-1} in grey matter. This equates to approximately 15% of the cardiac output, or $700ml.min^{-1}$ (56). The electrochemical function of cells in the brain begin to fail when CBF falls below 20ml $100g^{-1}$ min^{-1}. In addition to this, changes in CBF lead to changes in the cerebral arterial blood volume and can subsequently lead to increases and decreases in ICP as the artery dilates or constricts to accommodate the different blood volumes. The blood flow is equal to cerebral perfusion pressure (MAP - ICP) divided by the cerebrovascular resistance. One of the limitations of these figures is that they are derived from data based on the Kety and Schmidt technique (a method of assessing blood flow based on the change in a substance delivered by the arterial system and returned to the vascular system) which can be limited to global, rather than regional data. They may also be more applicable to healthy tissue than pathological tissue (57, 58).

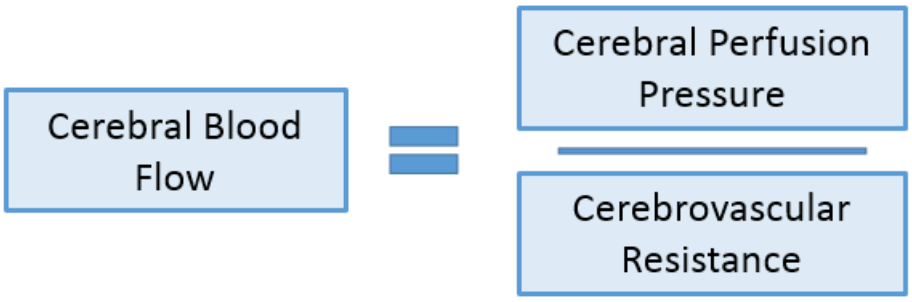


Figure 6: Cerebral Blood Flow

CBF is broadly dependent on factors which can modulate cerebrovascular resistance (the radius of the cerebral blood vessel):

- CPP;
- Cerebral metabolic rate for oxygen ($CMRO_2$);
- Arterial partial pressure of carbon dioxide ($PaCO_2$);
- Arterial oxygen content (CaO_2).

More specifically, CBF can also be altered by hypoxia, hypercapnia, metabolic demand, pain, low CPP and raised MAP as well as drugs which induce arterial dilatation, such as high-dose volatile agents (all except nitrous oxide) (59). Because neurons produce adenosine triphosphate (ATP) almost entirely by the oxidative metabolism of glucose and ketones and have a very limited capacity for anaerobic metabolism, they can only survive extremely short periods of ischaemia compared with other tissues. This is typically three to eight minutes (41).

Increased metabolic demand, as occurs during TBI, is directly proportional to CBF (and therefore oxygen and glucose delivery) and is often referred to as flow-metabolism coupling. It is thought that vasoactive substances such as nitric oxide, adenosine, carbon dioxide, potassium and hydrogen ions control this response (41).

In healthy individuals, CBF is said to be maintained with a CPP of 50-150 mmHg (26, 60). In patients following TBI or with ischaemic brain tissue, CBF

is more reliant on BP. When MAP is increased, CBF increases, as does cerebral volume and hence ICP. When CPP decreases, CBF decreases, reducing the ICP, but perhaps reducing CBF to an insufficient level. Normal autoregulation has been shown to be present in up to approximately 70% of patients with TBI (61). When CPP is lower than 60 mmHg, there may be insufficient cerebral perfusion leading to cerebral vasodilation in an attempt to provide more oxygen and glucose, which actually increases the cerebral blood volume and further increases ICP. The rise in ICP further decreases the CPP and a vicious cycle is born (Fig.7). The solution may be to increase MAP, thereby increasing CPP, causing vasoconstriction and decreased cerebral volume, lowering ICP. As discussed above, this is an oversimplification of a complex disease and increased CPP >70 mmHg is not associated with improved outcomes (46, 50).

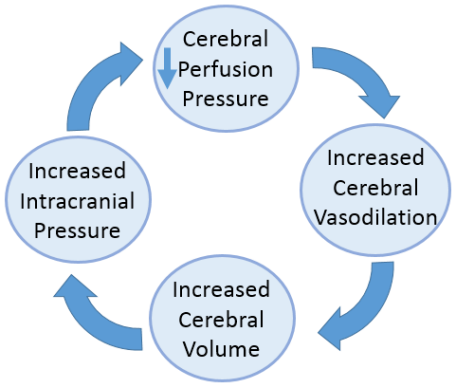


Figure 7: The ROSNER Cascade

Optimal Cerebral Perfusion Pressure (CPPopt)

I have discussed cerebral autoregulation and acknowledged that there is interpatient variability in autoregulatory function after TBI (61, 62). Therefore, targeting non-individualised CPP may be an oversimplified management of a complex physiological process (63). Autoregulation relies, in part, upon cerebrovascular pressure reactivity, the contraction and relaxation of cerebral arterial walls in response to increases and decreases in transmural pressure respectively (64, 65). With this response intact, increases in MAP are said to cause cerebral vasoconstriction within 5-15 seconds, with associated decreases in cerebral volume and ICP. The opposite, with a decrease in MAP, is also said to be true (66, 67). It has been proposed that individual patients have different pressure reactivity thresholds (63). Patients with chronic hypertension may have a rightward shift of the Lassen curve (i.e. a higher MAP requirement, Fig.5) (68). Research has been concentrated on identifying an individualised optimal CPP (CPPopt), based upon cerebrovascular pressure reactivity (63, 69-71). Ventilated patients with severe TBI demonstrate slow arterial blood pressure (ABP) variations of 20 seconds to three minutes which are transmitted to ICP (67, 72). This transmission between ABP variations and ICP has been used by Czosnyka et al. to determine a cerebrovascular pressure reactivity index (PRx) (67, 72). The PRx can be defined as *the moving correlation coefficient between mean intracranial pressure and mean arterial pressure* (73). It is calculated by 40 consecutive five second averages of MAP and ICP being captured at 60 Hz. The value generated is between -1 and +1, with normal reactivity seen with a coefficient of -1 and abolished reactivity seen

at +1 (i.e. passive behaviour of a non-reactive vascular bed). The relationship between CPP and PRx appears U-shaped when plotted on a graph (Fig. 8) (63, 67, 69-71, 74, 75). Steiner et al., demonstrated from a retrospective analysis of 114 consecutive TBI patients, that PRx can be used as a means of identifying CPPopt for individual patients using long-term ICP and MAP monitoring (75). Aries et al., have gone on to use a similar algorithm to find that CPPopt can be calculated for 55% of patients' monitoring period. The same authors have shown that CPP too high in comparison to CPPopt is associated with increased incidence of severe disability, and CPP too low in comparison to CPPopt is associated with increased incidence of mortality (70, 76). Interestingly, the CPPopt graphs published by Aries et al., show a mean CPPopt of approximately 75 mmHg, with certain patients' CPPopt as high as 107.5 mmHg, much higher than the BTF recommended 60-70 mmHg (54, 70). PRx calculation requires high frequency data sampling (>60Hz), which requires additional software to extract and process the waveform data. This is not commonplace in many ICUs (77, 78). Because of this limitation, Depreitere et al., have developed a low-resolution autoregulatory index (LAx), a moving index using minute-by-minute data sampling of ICP/MAP correlation coefficients over 3-120 minutes (78). Different LAx values and time windows can be combined and weighted to provide CPPopt recommendations which appear to be more easily obtainable than PRx (79). Depreitere et al. applied the LAx method to a data set obtained from 21 patients and demonstrated that the median CPPopt over the first 48 hours was not significantly different between the PRx and LAx methods. Furthermore, the LAx method was able to

produce a CPPopt recommendation in 97% of the monitoring time versus 44% with the PRx method (78). The same paper reports a correlation between the time patients' CPP was in the LAx-based CPPopt range and survival (median (IQR) 9.1% in non-survivors versus 22.0% in survivors (p=0.006). It seems, based on this, that a method of analysing autoregulation using lower resolution data can produce comparable outputs to the PRx method and give CPPopt recommendations in a higher proportion of monitoring time. Santos et al. have also demonstrated that minute-by-minute ICP and MAP data can be used to generate a pressure reactivity index (called L-PRx) in intracerebral haemorrhage patients. Their method appears to compare well with the original PRx over a 20-minute period and is able to provide CPPopt recommendations in the same range (80). Therefore, high-frequency data sampling and processing may not be a limitation to the use of CPPopt to guide treatment. Sánchez-Porras et al. studied long pressure reactivity index (L-PRx), another method of low frequency (minute-by-minute) analysis of ICP and MAP using 20-minute averages. An initial study of 29 patients suffering from TBI found that L-PRx correlated strongly with 6-month outcome (81). Lang et al. went on to investigate outcome prediction using CPPopt in 307 patients after severe TBI using PRx and L-PRx. PRx was found to be a significant mortality predictor whilst L-PRx was not. The study suggested that mean CPP above and below CPPopt were associated with severe disability and mortality respectively. These findings were not statistically significant for L-PRx and the authors concluded that PRx and L-PRx cannot be used interchangeably (76).

CPPopt has been investigated in patients with TBI, SAH and intracerebral haemorrhage. However, most studies have been retrospective or prospective observational studies (69, 71, 75, 82, 83). I have found no published RCT data demonstrating improved outcomes in any of these groups where CPPopt has been utilised. There are a few difficulties with CPPopt. Firstly, continuous monitoring of CPPopt is difficult in the clinical setting. Data becomes missing due to patient transfers for CT; delays in having monitoring boxes attached; delays due to staff not appreciating the importance of certain monitoring (possibly due to lack of teaching); and technical faults due to broken monitoring leads which happens not infrequently. Whilst a tertiary referral centre with a team that has specific research interests in CPPopt may have good results without data loss, I suspect its application may be more difficult elsewhere (69, 70). These are obstacles to be overcome but should not limit its implementation. Secondly, it appears that the mean CPPopt (to achieve optimal reactivity) is slightly higher than the CPP currently recommended. It may be that a higher CPP does correspond to better autoregulation, but it may also be that better autoregulation comes at the cost of increased incidence of acute respiratory distress syndrome. Thirdly, Aries et al., identify that PRx is a global index and represents the mean of all intracranial vascular territories. The authors go on to acknowledge that certain areas (e.g. sites of injury) may require perfusion pressures that are different to the global index (70). LAx and CPPopt appear very promising but need to be applied to a larger group. If RCT evidence of improved outcomes existed one could imagine software developed to produce real-time imaging of individualised CPPopt with

automatic intervention recommendations being implemented. CPPopt Guided Therapy: Assessment of Target Effectiveness (COGITATE, Clinical Trials: NCT02982122) is a multicentre study due to start in 2017. The team aims to allocate TBI patients to either BTF management with CPPopt targets, or BTF management with CPP targeted between 60-70 mmHg. It will be interesting to read the results of this study, and any subsequent studies including patients with SAH.

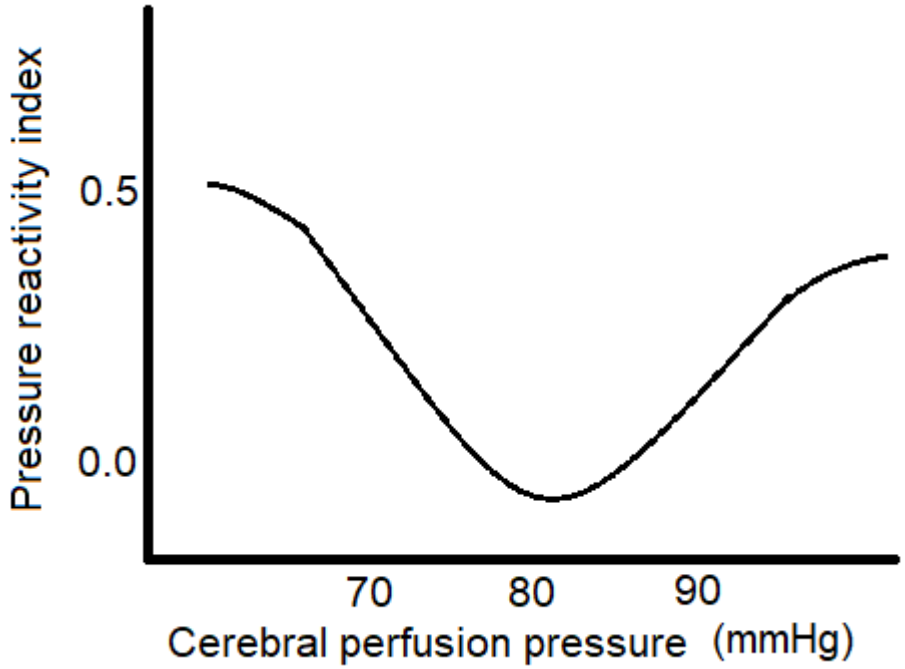
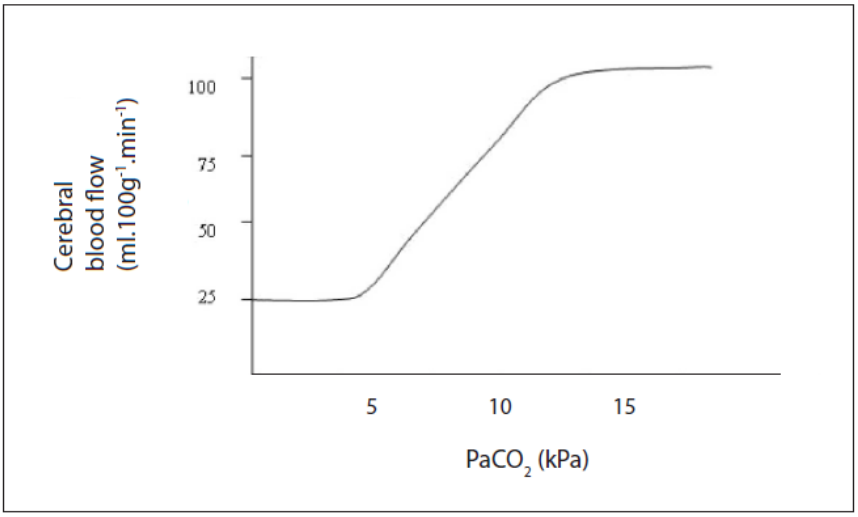


Figure 8: Optimal Cerebral Perfusion Pressure : example graph of PRx against CPP showing U-shaped relationship with CPPopt being approximately 80 mmHg.

Arterial Tension of Carbon Dioxide and Oxygen

Hyper- and hypocapnia cause cerebral vasodilation and vasoconstriction respectively (84). The mechanism appears to be regulated by changes in CSF pH rather than arterial pH, although there may be some direct arterial involvement (84-89). Hyperventilation causes arterial hypocapnia leading to cerebral vasoconstriction and decreased cerebral blood volume which is reflected by a decrease in ICP (90). Hyperventilation is said to be able to reduce ICP by a mean of 50% within two-to-30 minutes (20). The relationship between $PaCO_2$ and ICP does not appear to be linear and the greatest effect is between a $PaCO_2$ of 30-50 mmHg (4-6.5 kPa) (91). The vasoconstriction is also associated with a reduction in CBF. Hyperventilation has been reported to reduce CBF by over 30% in healthy volunteers (92). The increase in CBF appears to plateau above 10.6 kPa and there is no further reduction in CBF below 2.7kPa (Fig.8) (20, 41, 93). Brain extracellular pH decreases with an increase in brain $PaCO_2$. This results in mediators such as nitric oxide, prostanoids and cyclic nucleotides becoming active and intracellular calcium concentration decreasing leading to increased CBF (89). If patients are intentionally hyperventilated to induce hypocapnia, the vasoconstriction is said to only last approximately five hours before a gradual increase in CBF occurs (20, 94). Muizelaar et al., emphasise that any vasoconstrictive effect of hypocapnoea will be time-limited due to perivascular spaces normalising pH within 24 hours (95). Prolonged hyperventilation to reduce $PaCO_2$ causes cerebral vasoconstriction and decreased CBF, which not only reduces ICP but also causes ischaemia (96, 97). Considering this, hyperventilation to induce

marked hypocapnia is only advocated in the short-term during neurosurgery or acute neurological deterioration (54). If a patient has poor arteriolar tone due to severe hypotension, this relationship between $PaCO_2$ and CBF may be almost completely abolished (98).



Changes in cerebral blood flow with Arterial pCO_2

Figure 9: Cerebral Blood Flow Variation: Changes in Cerebral Blood Flow with Arterial Partial Pressures of Carbon Dioxide ($PaCO_2$)

Hypoxia appears to be an independent predictor of poor outcome after severe TBI (99). Over a range of partial pressure of oxygen (PaO_2) of 7.0 to 13.3 kPa, there is little change in CBF. This is thought to be because CBF responds to the arterial content of oxygen (CaO_2) rather than PaO_2 (100). CaO_2 is relatively constant with a PaO_2 between 7.0 to 13.3 kPa (101). Acute hypoxia significantly increases CBF: A decrease in PaO_2 of from 6.7 to 3.3 kPa (50 to 25 mmHg) causes enough cerebral vasodilation to double CBF (102). The response to hyperoxia (PaO_2 >15 kPa) is less clear but there is reported to be a variable decrease in CBF of 9-27% (101).

Partial Brain Oxygen Tension

Partial brain oxygen tension reflects the partial pressure of oxygen in the extra-cellular fluid of the brain and the amount of oxygen available for anaerobic respiration and ATP production. It acts as a marker for the balance between oxygen delivery and consumption, in a similar way that jugular venous bulb saturation is lower with increased oxygen consumption. The difference is that while jugular bulb venous oxygen saturations measure global cerebral oxygenation, $P_{bt}O_2$ can reflect venous partial pressure of oxygen from a focal area of brain tissue (103-105). The most commonly used sensors have been the Licox Integra system (GMS, Kiel-Mielkendorf, Germany), the Neurotrend sensor (Codman, Johnson & Johnson, Raynham, MA, USA, no longer commercially available) and the Neurovent sensor (Raumedic AG, Helmbrechts, Germany). The Licox system is comprised of three optical sensors in a microporous polyethylene tube with a diffusible membrane and can provide data on brain temperature, $P_{bt}O_2$ and ICP (Fig.9). After diffusing across brain tissue and a semi-permeable membrane, oxygen is reduced by a gold polarographic cathode creating an electrical current directly proportional to the oxygen concentration (106). The sensors are inserted under direct vision during surgery or via a craniotomy on the ICU using a triple-lumen cranial access device allowing monitoring and microdialysis (107). Readings are available immediately, although a reported period of 30 minutes to two hours is required for values to become consistent (103, 104). Most $P_{bt}O_2$ reading errors occur within the first four hours of placement (104). $P_{bt}O_2$ monitoring is reportedly safe, causing an iatrogenic haematoma in less than 2% of patients,

similar to parenchymal ICP monitoring, and safer than external ventricular drains (EVD) (108).

The 0.8 mm diameter probes are usually positioned in the sub-cortical white matter and can measure $P_{bt}O_2$ in an area of approximately 15-20 mm^2 from the probe (104). Following insertion, patients should undergo a repeat x-ray computed tomography (CT) head scan to confirm correct placement of the probe. The $P_{bt}O_2$ value relies on oxygen diffusing from the vasculature into brain tissue so correct location of the probe is essential for interpretation of $P_{bt}O_2$ values (109, 110). Values measured from healthy-appearing frontal sub-cortical white matter, where most probes are placed, may not reflect what is happening around focal injuries. Because of this, probes are sometimes sited adjacent to the focal injury, such as haematomas or haemorrhagic contusions, in patients following severe TBI. This is important when using $P_{bt}O_2$ to guide treatment. If the probe is sited in healthy tissue and reads 25 mmHg, then one might be falsely reassured and not alter management (see below for treatment thresholds). However, if that same probe had been sited in damaged tissue in the same patient it may read 15 mmHg and thus change management according to a predefined treatment algorithm. Furthermore, any research to assess a treatment threshold or association with functional outcomes may not demonstrate consistent results if different sites for $P_{bt}O_2$ monitoring are used. This leads to the following questions: Where is the best place to insert the $P_{bt}O_2$ probe? Is it in healthy tissue, adjacent to damaged tissue or directly inside the damaged tissue? Does $P_{bt}O_2$ guided management have an effect of functional outcomes? To my knowledge, after a non-systematic Medline

search, there are no RCTs assessing the impact of probe placement on $P_{bt}O_2$ readings and functional outcomes. There have been a small number of human studies assessing probe location but these have used different locations in different patients and thus inference is limited (36, 108, 111, 112). Perhaps the best of these is a single centre database review of prospectively collected data (n = 405) between 1995 and 2009. The $P_{bt}O_2$ sensor was placed in normal brain in 159 patients and abnormal brain in 246 patients. Mean $P_{bt}O_2$ readings in abnormal brain were 25.6 ± 14.8 mmHg compared with 30.8 ± 18 mmHg in normal brain. Patients with a favourable outcome had higher $P_{bt}O_2$ (32.2 ± 16.3 compared with 25.1 ± 13.5, $P < 0.001$) and patients with an unfavourable outcome were more likely to have a probe sited in abnormal brain tissue. However, patients were not randomised to probe placement and the authors note that the position of probe placement was significantly influenced by the type of injuries the patient had. 71.1% of patients with diffuse injuries had probes placed in normal appearing tissue compared with 11.5% of patients with mass lesions on CT (36). A subsequent porcine model assessing four probe placements in 12 swine has demonstrated that $P_{bt}O_2$ readings are dependent on the distance of the probe from the site of focal injury. Physiological interventions (e.g. hyperoxia) appeared to have little effect on $P_{bt}O_2$ at the site of injury but increased $P_{bt}O_2$ in healthy tissue (113).

It has been suggested that this ability of $P_{bt}O_2$ sensors to identify focal tissue hypoxia could be used to identify delayed cerebral ischaemia (DCI) after subarachnoid haemorrhage (SAH) (112). Due to the localised measurements I suggest this technique is likely to be limited and highly inaccurate.

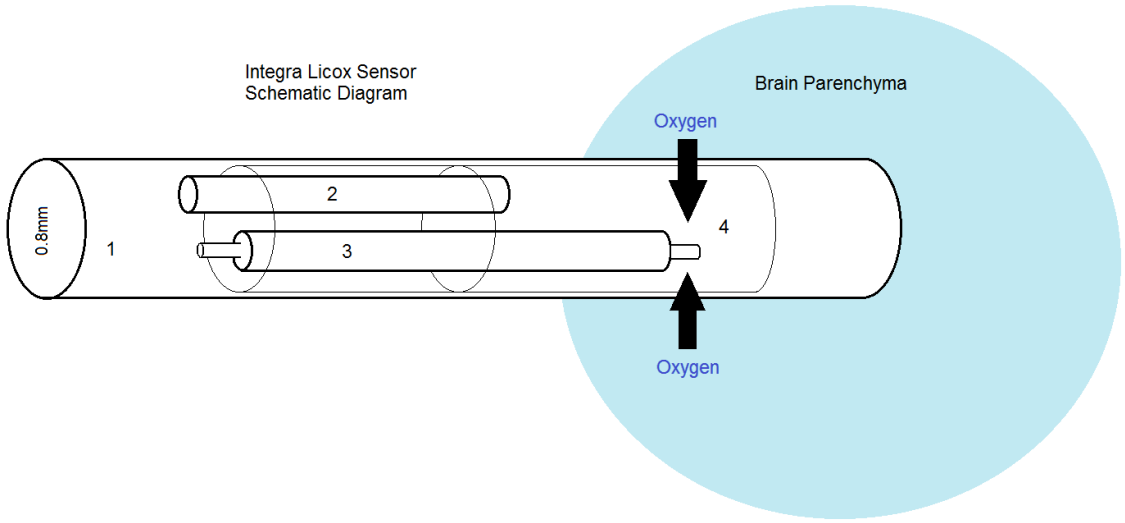


Figure 10: Schematic Diagram of Integra Licox Machine (Ingera, France). 1, Polythene tube with diffusable membrane encasing electrodes; 2, silver polarographic anode; 3, gold polarographic cathode; 4, electrolyte chamber

The significance of $P_{bt}O_2$ in TBI is not fully understood but it does correlate well with regional CBF and demonstrates the same autoregulation physiology discussed above. There is some evidence that $P_{bt}O_2$ can be increased by manipulation of the MAP and CPP, suggesting that $P_{bt}O_2$ could be a surrogate marker for CBF (114-116). $P_{bt}O_2$ may not be simply a marker of local cerebral ischaemia. Rosenthal et al., demonstrated a relationship between CBF and arterio-venous oxygen tension difference (110, 117). $P_{bt}O_2$ is not solely dependent on the CBF/CPP but also the amount of dissolved plasma oxygen passing through brain tissue. In this way, $P_{bt}O_2$ can be affected by other pathology, such as impaired lung function, which is not uncommon in trauma patients with severe TBI. This means that clinicians can improve $P_{bt}O_2$ by providing adequate oxygenation. $P_{bt}O_2$ is a promising tool and has been reported as being superior to jugular venous oxygen saturations, regional

transcranial oxygen saturations and near infrared spectroscopy in detecting cerebral ischaemia (103, 104, 118). Level I evidence of improved outcomes associated with $P_{bt}O_2$ monitoring or guided treatment is lacking.

Brain Trauma Foundation Guidelines

There are three physiological states that almost certainly worsen outcomes following TBI: hypoxia, hypotension and hyperventilation in actively ventilated patients. Due to the aetiology and clinical course of TBI, designing a prospective RCT to evaluate treatments for TBI is difficult. Few of the BTF recommendations are based on level I evidence (defined as that derived from good quality RCTs). Despite this, the authors note a significant reduction in severe TBI mortality from 50% to 25% since the 1980s following the implementation of evidence-based protocols (119). These protocols emphasise the importance of monitoring and adequate cerebral perfusion.

Analgesia, Sedation and Paralysis

The initial trauma and assessment, procedures undertaken by ICU and emergency department (ED) staff, surgical procedures, endotracheal intubation and mechanical ventilation and subsequent endotracheal suction can all be sources of pain. In addition to being unpleasant for the patient, pain can lead to increases in ICP. Morphine, fentanyl and remifentanil are all appropriate first-line therapies to provide analgesia, sedation and depression of airway reflexes in patients who are intubated. Reducing airway reflexes in

these patients is beneficial to prevent coughing and the associated increase in ICP (120).

The benefits of sedation include their synergistic action with analgesia, limiting ICP elevations related to coughing and agitation and aiding mechanical ventilation (120). By reducing agitation and muscle use they reduce oxygen consumption while also reducing $CMRO_2$ and CO_2 production. Propofol appears to be the ideal sedative agent for patients with severe TBI (121). It offers a quick onset, is easily titratable and quickly reversible to enable a predictable effect and intermittent neurological assessment on demand. Unfortunately, Propofol can cause significant systemic vasodilation and is best avoided in patients with refractory hypotension, especially with raised ICP which may further reduce the patient's CPP. In addition to this, long-term or high-dose administration of Propofol can also lead to rhabdomyolysis, metabolic acidosis, renal failure and bradycardia, a so-called 'Propofol-related infusion syndrome' (PRIS). Roberts et al., found that PRIS had an incidence of just over 1% in a study of 1,017 patients and that the mortality rates for these patients was 18% (122). Alternative sedative agents include benzodiazepines used as a continuous infusion or in intermittent boluses. Benzodiazepines can be beneficial in providing an anticonvulsant and amnesic effect. Neuromuscular blocking agents reduce ICP and can be considered for refractory intracranial hypertension, but are related to longer ICU inpatient stays and pneumonia. Their routine use is not recommended (18, 123).

Hypoxia

There are a number of studies associating short-term hypoxia with poor outcomes following TBI although many are also associated with hypotension (17, 124-126). The primary evidence to support worse mortality from hypoxia is derived from the retrospectively analysed data of the Traumatic Coma Data Bank (TCDB) (99). Data from 1,030 patients were analysed and demonstrated a significant increase in morbidity and mortality in the 22.4% of patients with hypoxemia. Cooke et al. also found that 27% of 131 patients admitted to an ED with TBI in Northern Ireland were hypoxic (PaO_2 <10 kPa or cyanosed) on arrival and this was associated with increased mortality. There was an association between severe TBI and increased rates of hypoxia and hypotension and increased mortality, but no causative relationship can be inferred from this study (125). Furthermore, Stocchetti et al., found that brief hypoxia (SaO_2 <60%) increased mortality from 14.3% to 50% when compared with patients who had no hypoxia. Of the 50% with hypoxia who survived to discharge, all were severely disabled. This was a small study of 50 patients who had episodes of hypoxia (SaO_2 <90%) whilst being transferred to hospital, and the results were not corrected for confounding factors (124).

The 2016 BTF guidelines no longer have a separate section regarding hypoxia above their section on hypotension (present in 2007 guidelines), most likely due to the interrelation with hypotension and the limited body of evidence (54). The 2007 BTF guidelines offered the following level III recommendation:

- *Oxygenation should be continuously monitored and hypoxia (defined as PaO_2 <60 mmHg or O_2 saturation <90%) avoided (123).*

Hypotension

Hypotension, defined as a systolic blood pressure (SBP) <90 mmHg (although more recently some argue <110 mmHg in TBI (127, 128)) or MAP <65 mmHg, following severe TBI is associated with increased morbidity and mortality, both from pre-hospital and in-hospital data (99, 127-131). Hypotension is reported to occur in up to 73% of patients in ICU and an isolated episode of hypotension has been associated with increased morbidity and a doubling of mortality in patients with TBI (99). Hypotension has been documented as an independent risk factor for increased mortality in severe TBI as demonstrated by studies from the TCDB (99). This fits with physiological data showing that CPP, and hence CBF, decreases significantly with a decrease in MAP and must be aggressively managed in the hypotensive patient. It is unlikely that haemodynamic instability is caused by TBI in isolation. There are several factors that can lead to hypotension in TBI patients. Trauma to long-bones, chest, abdomen, pelvis and blood vessels, as well as polyuria secondary to diabetes insipidus from brain injury are the most frequent causes of hypotension in this population. In addition to these causes, cardiogenic shock, spinal shock and anaesthetic agents can all cause hypotension (18, 132).

The 2016 guidelines have updated their SBP threshold based on two studies. The first is a retrospective database (Los Angeles Trauma System database) review by Berry et al. This review investigated 15,733 adult TBI patients

(moderate to severe) admitted to 13 centres in Los Angeles between 1998 and 2005 and allocated participants to three age categories (15-49, 50-69 and >70). Outcomes were in-hospital mortality and Glasgow Outcome Score at six months post-injury. Adjusted odds ratios (AOR) were used to determine increased odds in mortality for their defined optimal SBP. For ages 15-49 the optimal SBP threshold was 110 mmHg (AOR 1.98, CI 1.65-2.39, p<0.0001); for ages 50-69 it was 100 mmHg (AOR 2.20, CI 1.46-3.31, p=0.0002), and for patients ≥ 70 years it was 110 mmHg (AOR 1.92, CI 1.35-2.74, p=0.0003) (127). The second study is a small prospective (n=60) single-centre study of patients with severe TBI that assessed extended Glasgow Outcome Scale at six months. The investigators found that hypotension within the first 48 hours was predictive of mortality and functional outcomes at higher SBP thresholds than previously thought. The authors of the paper suggest that a SBP threshold of 120 mmHg may be more efficacious at minimising secondary insults after TBI (128). The BTF now recommend the thresholds from the Berry et al. study:

- *SBP should be maintained ≥100 mmHg for patients aged 50 to 69, and ≥110 mmHg for patients aged 15-49 and >70 (54).*

Management of Hypotension

The BTF guidelines do not give specific advice on how to maintain the recommended SBP thresholds. There have been a small number of reviews assessing fluid management in the pre-hospital treatment of hypotension after TBI (133-135). This includes assessment of nine RCTs and one cohort study of pre-hospital care which found no benefit from hypertonic or colloid solutions

versus isotonic saline (133). The aim of in-hospital management of crystalloid resuscitation is to achieve or maintain normovolaemia and colloid osmotic pressure. Excess fluid is associated with ARDS/acute lung injury and cerebral oedema (46, 48, 136-139) and insufficient fluid is associated with worsening hypotension and acute kidney injury (140, 141). Hypertonic saline solution (HSS) has been trialled as an alternative to typical cystalloids and does not appear to be beneficial for TBI or haemorrhagic shock. One RCT of 222 patients with haemorrhagic shock reported a reduction in mortality when comparing 250mls of HSS with 250mls of isotonic saline (ARR 9%, $p<0.03$) (142). Subsequent RCTs have not supported this finding (143, 144). A relatively recent RCT recruited 1,087 patients with TBI and allocated them to either TSS or 0.9% sodium chloride. The study was terminated early due to an inability to demonstrate improved neurological status or mortality at six months after the TBI (143). Colloids have been assessed as a choice of fluid resuscitation in trauma, burns and post-surgical patients and were not found to be superior in a 2012 Cochrane review (RR 1.01, 95% CI 0.92 to 1.09). As such, they are not recommended in TBI (145-147).

Patients not responding to fluid therapy, and those with congestive heart failure, may benefit from vasopressors. Noradrenaline is the recommended vasopressor and should be titrated through a central venous line (18). Noradrenaline is a catecholamine which acts as an alpha-receptor agonist causing increased vascular smooth muscle tone and increasing peripheral vascular resistance, thereby increasing SBP, MAP and CPP. In addition to its alpha-1 properties, noradrenaline exhibits alpha-2 properties and is not without

risk. Tschuor et al., reported that noradrenaline's alpha-2a properties may lead to increased platelet activation and microthrombosis (148). In addition to this, both noradrenaline and dopamine can cause arrhythmias, peripheral ischaemia, hypertension, dyspnoea and urinary retention (149). Dopamine causes cerebral vasodilation and increased ICP, so is best avoided in TBI unless necessary via a peripheral venous line until a central line is available for noradrenaline (150). In patients who are tachycardic, it has been suggested that phenylephrine, a pure alpha-agonist, may be more beneficial than dopamine and noradrenaline. Indeed, Sookpkung et al., found that phenylephrine resulted in a higher MAP and CPP than dopamine and noradrenaline (151). Other experimental models have reported better brain oxygenation with the use of noradrenaline versus phenylephrine (152). Both inotropes are used.

Intracranial Pressure

The 2016 BTF guidance acknowledges its advice to monitor ICP is based on "a low-quality body of evidence" (54). The 2007 indications to monitor ICP from the BTF were: all salvageable patients with a severe TBI and abnormal CT scan or those with a normal CT scan who are over 40 years of age, have motor posturing, or systolic blood pressure <90 mmHg (123). This is no longer a level II or III recommendation because it is based on descriptive studies, or studies that do not meet BTF inclusion criteria. However, the committee have re-stated the 3rd edition recommendations to highlight patients at risk of intracranial hypertension (54). Raised ICP (>20 mmHg) is associated with poor outcomes

following TBI, and extremely elevated levels can be life-threatening, resulting in brainstem compression and decreased CBF (153).

There are a number of studies indicating that ICP monitoring reduces mortality after TBI (154-159), yet some have shown no benefit and others have demonstrated worse outcomes (160-163). Shafi et al., analysed the US National Trauma Data Bank from 1994-2001 and included patients with severe TBI aged 20 to 50 with abnormal CT head scans and ICU stays of three or more days. Deaths within 48 hours of injury and patients with delayed admissions (over 24 hours from injury) were excluded. Patients who had ICP monitoring (n = 708) were compared with those who did not (n = 938). Interestingly, only 43% of patients meeting BTF criteria for monitoring received ICP monitoring (the databank was analysed pre-2006 guidelines). ICP monitoring was associated with a 45% reduction in survival (OR = 0.55; 95% CI, 0.39-0.76; $p < 0.001$) after adjusting for confounding factors such as admission GCS, age, BP and injury severity score (164). The BEST:TRIP trial was a multi-centre RCT in Bolivia and Ecuador which included 324 patients allocated to two groups. One group was allocated to ICP monitoring with an intraparenchymal monitor and had ICP maintained ≤20 mmHg using current BTF guidelines (n=157). The second group was managed according to imaging and clinical examination alone (n=167) (123, 160). The primary outcome was a composite of 21 components including survival time, duration of impaired consciousness and neuropsychological status at six months. There was no significant difference in the primary outcome between the two groups and the authors conclude that "*care focused on maintaining monitored*

intracranial pressure at 20 mmHg or less was not shown to be superior to care based on imaging and clinical examination". The study did report significant treatment regimen differences, most notably an increase in the use of barbituates in the ICP-monitored group. The chief criticisms of this study are that it was undertaken in countries with limited pre-hospital care and where the use of ICP monitors is infrequent so the external validity is questionable. The authors are careful to acknowledge that the findings do not support the monitoring-based interventional guidelines used in the study, rather than not supporting ICP monitoring per-se (160). A Cochrane review from 2015 looking at this single RCT echoes its findings (165), and a review from 2014 of two RCTs and seven cohort studies (n = 11,038) found no benefit from ICP monitoring (OR, 1.16; 95% CI, 0.87–1.54), and that there was a tendency toward longer ICU and hospital stay in those patients with ICP monitoring (165, 166). Both reviews note the importance of further large sample RCTs to confirm their findings. A number of authors have since argued that ICP monitoring is still beneficial in the management of patients with TBI and it is still recommended by the BTF 2016 guidance (54, 56, 167-169). The BTF describe "moderate-quality evidence" from four observational studies (n=13,164) that ICP-guided treatment leads to decreased in-hospital and two-week post-injury mortality (55, 170-172). It appears to be these studies which lead the BTF to continue to recommend ICP monitoring and ICP-guided treatment.

The current guidelines advocate treating ICP above 22 mmHg as a level IIb recommendation (previous threshold was 20 mmHg) (54, 123). Data for this

change comes from one cohort study from Cambridge which used a database of 459 patients with TBI (data collected from 1992 to 2009) and found a threshold of 22 mmHg was beneficial for both survival and favourable outcomes (173).

Intracranial Pressure Dose Response

As with CPP, it may be that the management of ICP is more complex than targeting a generalised ICP threshold. The effect of intracranial hypertension may not be based solely on its magnitude but could be based on duration and intensity. More simply, as an example, an ICP of 25 mmHg for ten minutes may be more predictive of poor outcome than an ICP of 20 mmHg for one hour. Furthermore, the amount that intracranial hypertension affects outcomes may be dependent upon whether cerebral autoregulation is intact and its relationship to CPP (174). The magnitude of intracranial hypertension times the duration, or the area under the curve of ICP versus time, has previously been termed “ICP dose” and appears to be related to mortality and neurological outcome (175). Güiza et al. assessed the impact of the duration and intensity of intracranial hypertension on six-month neurological outcomes in 261 adult and 99 paediatric TBI patients in a prospective multi-centre study (174). The team analysed minute-by-minute ICP and MAP data and demonstrated that higher ICP can be tolerated for shorter durations of time than lower ICP. In adults, ICP above 20 mmHg lasting longer than 37 minutes was associated with worse outcomes. In children this time was eight minutes. In addition to this, when the CPP was below 50 mmHg, all episodes of

intracranial hypertension, regardless of duration, were associated with worse outcomes. When graphed, intensity was measured from 10-40 mmHg on the X-axis and duration was measured from 5-360 minutes on the Y axis. The study also assessed 6-month mortality and neurological outcome as death, vegetative or severely disabled (Glasgow Outcome Score 1-3). A correlation coefficient, ranging from -1 to 1, was used indicating an ICP insult occurring more frequently with a lower or higher Glasgow Outcome Score respectively (i.e. -1 = correlated with worse outcomes, 1 = correlated with better outcomes, 0 = no correlation, defined as the transition point between -1 and 1). The LAx method of assessing cerebral autoregulation was also utilised. In both the adult and paediatric populations there was an approximately exponential relationship at the transition point of correlation with better outcomes to worse outcomes in relation to ICP duration and intensity (Figure 11). In adults, ICP thresholds of 15, 20, 25 and 30 mmHg were associated with worse outcomes at 223, 37, 12 and 8 minutes respectively. In addition, the cumulative effect of intensity and duration (the 'ICP dose') per patient, or the percent of time the patient spent to the right of the curve in Figure 11, was independently associated with mortality at six months. The authors also found that when autoregulation was impaired the curve shifted to the left and when intact the curve shifted to the right. CPP was also associated with a change in response. When CPP fell below 50 mmHg outcome was uniformly poor. However, the curve progressively shifted to the right from 50 mmHg to ≥70 mmHg.

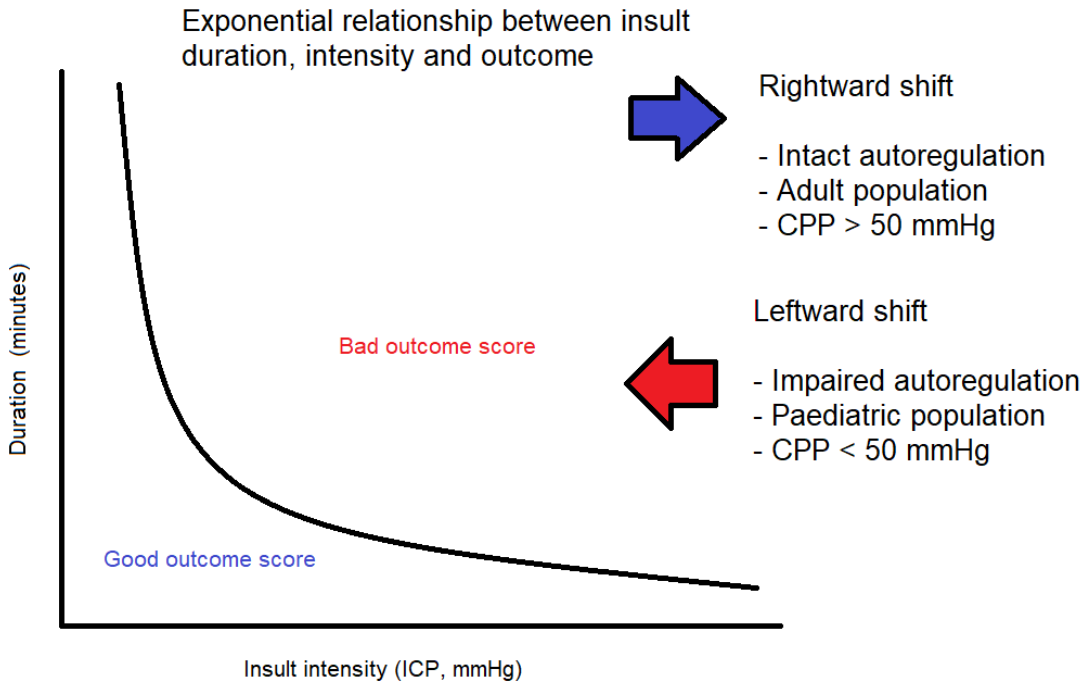


Figure 11: Intracranial hypertension duration and intensity with associated Glasgow Outcome Score (simplified from original). For original graph see Güiza et al.(174) The line represents an approximate exponential relationship between ICP dose and outcome. CPP, cerebral perfusion pressure; ICP, intracranial pressure.

At a basic level one could argue that this is an intuitive finding: intracranial hypertension is associated with worse outcomes and the higher the magnitude and longer the duration the worse the outcome will be. However, when combined with a measure of cerebral autoregulation and a measurement of CPPopt it may be that we can individualise treatments to remain to the left of the curve in Figure 11. The authors write that perhaps their findings may explain why previous RCTs using static ICP thresholds were “destined to fail” (174). Indeed, other authors have suggested that individualised ICP thresholds based on autoregulatory status are more predictive of mortality than generalised thresholds of 20 and 25 mmHg (176). Perhaps in the future we will see interventions measured on individually targeted CPPopt and ICP based

on cerebral autoregulation. When there exists some uncertainty regarding the positive role of ICP monitoring in ICU the real test will be whether CPPopt and individual ICP targets can be used to alter patient outcomes.

Osmotic Therapy: Mannitol and Hypertonic Saline

In a survey from 1996, 100% of neurosurgical units in the UK used mannitol as a treatment for intracranial hypertension (177). The mechanism by which mannitol reduces ICP is two-fold. The short-term effect of mannitol is to expand the circulating volume and decrease blood viscosity causing increased CBF and oxygen delivery. The increase in circulating volume also increases cardiac output. Because of this, one might expect mannitol to cause a rise in ICP. However, where cerebral autoregulation is intact, compensatory vasoconstriction occurs, reducing cerebral blood volume and ICP. After this initial response, cardiac output and BP may decrease due to peripheral vasodilation. Due to the fall in cardiac output and the subsequent osmotic diuresis, it is advisable to compensate the effects of mannitol with adequate fluid replacement. The second action of mannitol is to establish an osmotic gradient between the plasma and brain cells which pulls water from the cerebral extracellular space into vessels and reduces cerebral oedema, thereby reducing ICP. These osmotic properties of mannitol require 15-30 minutes to take effect (178). A prerequisite for this secondary slower action is an intact BBB. Cerebral oedema can be exacerbated by mannitol with a disrupted BBB (177). The long-term use of mannitol as a continuous infusion

may further exacerbate cerebral oedema as the mannitol molecules accumulate in the cerebral interstitial space (179).

There has been one RCT comparing 41 patients with moderate/severe TBI who were randomised to receive blinded solutions of either 20% mannitol or 0.9% sodium chloride. The study found a relative risk for death of 1.75 (95% CI 0.48 to 6.38) and an absolute increase in the risk of death of 11% (95% CI -14 to 35%) in the mannitol group (180, 181). This was an out-of-hospital study to determine the effect of mannitol on BP and was not an ICP-targeted study in ICU patients. 2007 BTF guidelines did not recommend the prophylactic use of mannitol, but did note that it is an effective method to reduce a raised ICP after severe TBI (123). The 2016 guidelines note that although hyperosmolar therapy may reduce ICP, there is insufficient evidence to support any level of recommendation. The committee acknowledge that HSS is increasingly used instead of mannitol and "is universal in its belief that hyperosmolar agents are useful in the care of patients with TBI" (54).

Many ICUs use HSS as a treatment for intracranial hypertension, either individually or in combination with mannitol. HSS is reported to expand the intravascular volume and extract water from the intracellular space, both increasing cardiac output and CBF and reducing ICP. Furthermore, HSS achieves this without the deleterious effects associated with hyperosmolarity, such as pulmonary oedema, central pontine demyelination or renal failure (182, 183). It may be that HSS is more beneficial than mannitol in treating raised ICP as Kamel et al., showed in their meta-analysis from 2011 (mean

ICP reduction was 2.0 mmHg greater for HSS versus mannitol, 95% CI -1.6 to 5.7) (184). HSS may be more effective at lowering ICP and ICU stay but does not appear to have a significant effect on mortality (185, 186).

Despite mannitol being widely used in ICU's, there appears to be poor consensus on the optimal dose to reduce ICP. In 2008, Sorani et al., published a retrospective analysis of mannitol use in 28 patients with TBI (187). The authors administered mannitol in doses of 50g or 100g with continuous ICP monitoring whilst aiming to maintain CPP > 60 mmHg. The mean ICP was 22.0 ± 10.6 mmHg prior to administering mannitol and decreased after mannitol was administered. The ICP continued to decrease for 30 minutes to 15.7 ± 8.1 mmHg in all patients. After 30 minutes, ICP was equal in the 100g group (15.6 ± 10.9) and the 50g group (15.7 ± 6.3). However, at 100 minutes, ICP remained decreased in the 100g group but had almost returned to its initial value in the 50g group (14.2 ± 6.7 versus 18.6 ± 7.6 mmHg; P = 0.001). Sorani et al., also published a meta-analysis of the ICP dose-response relationship of mannitol. The authors examined 18 studies and found a not statistically significant weak linear relationship between changes in ICP and dose (delta ICP = 6.6 x dose - 1.1; p = 0.27). There was, however, a decrease in ICP when the initial ICP was higher than 30 mmHg and non-linear regression suggested that the greatest ICP decrease occurred shortly after mannitol was given (188).

Neurosurgical Intervention

Surgical intervention in the form of decompressive craniectomy is a controversial intervention for intracranial hypertension refractory to medical

therapy. The procedure involves removing part of the skull to allow volume expansion with the aim of maintaining a stable ICP. Although decompressive craniectomy is associated with a decrease in ICP, outcomes are not necessarily improved. The DECRA trial (Decompressive Craniectomy in Diffuse Traumatic Brain Injury) found a decrease in ICP and length of ICU stay in 155 adult patients with severe TBI enrolled to a randomised controlled trial (189, 190). Patients with intracranial hypertension (20 mmHg for > 15 minutes) refractory to first-tier interventions were allocated to either bifrontotemporal decompressive craniectomies or standard care. The craniectomy group showed a reduction in ICP with fewer medical interventions and a shorter stay in ICU. Despite this, they also had worse outcomes (70% versus 51%; OR 2.21; 95% CI 1.14–4.26; $p = 0.02$) and rates of death at six months were similar between the groups (189, 190). RESCUEicp (Randomized Evaluation of Surgery with Craniectomy for Uncontrollable Elevation of Intracranial Pressure) was a prospective RCT running from 2004 to 2014 that allocated patients to surgical treatment (n = 202) or medical treatment (n = 196). The initial treatment was to maintain ICP ≤ 25 mmHg and CPP > 60 mmHg. Patients were managed with routine care, ventriculostomy, hyperosmolar therapy and TH (not < 34°C). Patients with refractory intracranial hypertension for one-to-12 hours despite these treatments were randomised to decompressive craniectomy and medical therapy or ongoing medical therapy without surgical intervention. In contrast to the DECRA trial, RESCUEicp demonstrated 22% less mortality in the surgical group at six months post-injury (191). However, those surviving in the surgical group were more likely to be in

a vegetative state or severely disabled. Cooper et al., argue that the use of the term “favourable outcome” is questionable in this paper. The proportion of patients with full independence at six months (moderate disability or better) is almost identical between the groups (26.6% medical, 27.4% surgical). They go on to state that all of the extra 22 patients who survived in the surgical group were in a vegetative state or severely disabled and none were independent. Furthermore, they point out that there was a high mortality amongst medical patients (48.9%) when compared with medical patients from the DECRA trial (18%) (192). 2016 BTF guidelines are yet to be updated to include evidence from the RESCUEicp trial.

Partial Brain Oxygen Tension to Guide Treatment

Normal values of $P_{bt}O_2$ have been reported as 23-35 mmHg from a study assessing awake patients undergoing functional stereotactic brain surgery for treatment of movement disorders (193) and is the value quoted by the Neurocritical Care Society (194). Both raised ICP and low $P_{bt}O_2$ have been shown to be independent predictors of poor prognosis in severe TBI, and 2007 BTF guidelines advocated initiating therapy to increase $P_{bt}O_2$ if it decreases below 15 mmHg (195-199). However, contemporary articles suggest that a threshold of 20 mmHg may be more beneficial (194, 200). The 2016 BTF guidelines state that the previous recommendation has “been revised”, and there is now no recommended threshold due to insufficient evidence. Five studies (n = 222) are included in their analysis and comprise of cohort and

observational studies observing worse outcomes with various thresholds of $P_{bt}O_2$ ranging from 15 mmHg to 25 mmHg (35, 195, 201-203).

Patient Condition	$P_{bt}O_2$ Value (mmHg)
Stable Neurosurgical Patients	23-35
Moderate Brain Hypoxia	20
Critical Brain Hypoxia	**15***
Severe Brain Hypoxia	10

Table 3: Brain tissue Oxygen Tension Thresholds. * $P_{bt}O_2$ threshold to start treatment as per Brain Trauma Foundation Guidelines (2007), there is no longer a threshold in the 2016 guidelines

Despite $P_{bt}o_2$ monitoring being generally advocated in the treatment of TBI, there is uncertainty about treatment thresholds and whether monitoring or treatment based on $P_{bt}O_2$ will improve outcomes. A single-centre retrospective analysis of 74 patients with severe TBI assessed patients with and without $P_{bt}O_2$ monitors (n = 37 in each group). There was no significant survival difference found between the two groups (64.9 vs. 54.1 %, p = 0.34) or difference in discharge GCS, or functional outcome. The $P_{bt}O_2$ group did have a significantly lower injury severity score and patients were placed on monitoring based upon the practices of the attending neurosurgeon rather than being randomised (204). There has been a prospective RCT from Taiwan that randomised patients with moderate to severe TBI to $P_{bt}O_2$-guided therapy and ICP monitoring ($P_{bt}O_2$ > 20 mmHg, n = 23) or ICP-guided therapy without $P_{bt}O_2$ monitoring (ICP < 20 mmHg and CPP > 60 mmHg, n = 27). Probes were sited in the 2-3cm margin of necrotic brain tissue from preoperative CT scans. Patients in the $P_{bt}O_2$ group had standard ICP guided treatment as per usual

care, but the primary focus was on $P_{bt}O_2$ guided treatment (i.e. if ICP was >20 mmHg and $P_{bt}O_2$ was <20 mmHg the priority was on treating $P_{bt}O_2$). The authors report significantly higher survival rates in the $P_{bt}O_2$ group (given as figures, no documented numbers) but acknowledge a generally low survival rate between both groups and a small sample size (205). The BOOST-2 trial (Brain Oxygen Optimization in Severe TBI Phase 2) was a ten-centre RCT of $P_{bt}O_2$ monitoring in severe TBI (ClinicalTrails.gov: NCT00974259). I can find no formal publication of this trial but the NETT (Neurological Emergencies Treatment Trials) website states that the trial enrolled 122 patients and demonstrated that a $P_{bt}O_2$-based treatment protocol reduced the mean hypoxia burden by 74% with a trend towards improved functional outcomes (206). The BOOST-3 trial aims to enrol patients with severe TBI and randomly allocate them to a treatment protocol based on ICP monitoring alone or ICP and $P_{bt}O_2$ monitoring. The primary outcome will be extended Glasgow Outcome Scale at six months post injury. There is a draft protocol for this study on the NETT website but it is not yet registered on ClinicalTrials.gov (207).

A few studies have investigated the optimal CPP required to maintain adequate $P_{bt}O_2$ in TBI and SAH patients (196-198, 208). $P_{bt}O_2$ appears to be variable across patients and a CPP of 60-100 mmHg is required to achieve satisfactory brain tissue oxygenation (196). Perhaps this reflects the multifactorial nature of $P_{bt}O_2$ and the different metabolic requirements of these patients. As mentioned earlier, CPP = MAP – ICP, so the CPP is dependent, in part, upon ICP. A raised ICP lowers CPP and is associated with worse outcomes in patients with TBI. Furthermore, patients who have a raised ICP

and lower $P_{bt}O_2$ have worse outcomes than those with an elevated ICP but a normal $P_{bt}O_2$ (199).

Geukins et al., have proposed three ways in which $P_{bt}O_2$ monitoring may improve patient outcomes and go on to suggest a treatment algorithm:

1. Early recognition of brain tissue hypoxia leading to earlier aggressive intervention e.g. decompressive craniectomy;
2. Directing osmotherapy (Oddo et al. found that hypertonic saline was superior to mannitol in reducing ICP whilst improving $P_{bt}O_2$ (209));
3. Targeting $PaCO_2$ to avoid hyperventilation reducing $P_{bt}O_2$ and preventing further cerebral ischaemia (104).

Nanguroori et al., in their systematic review of four studies (no RCTs, n = 491), found that $P_{bt}O_2$-centered care was associated with improved outcomes when compared with purely ICP/CPP-based therapy (OR = 2.1; 95% CI = 1.4-3.1) (210). Geukins et al., have advocated a step-wise approach to managing brain tissue hypoxia as follows:

1. Increase FiO_2 to 100% for two minutes to restore $P_{bt}O_2$ temporarily, check the probe and reduce elevated ICP if necessary;
2. Increase MAP/CPP with vasopressors e.g. noradrenaline or phenylephrine and assess response;
3. Increase FiO_2 to 60%, aspirate pulmonary secretions and increase positive end-expiratory pressures by increments of 2-4 cmH_2O if needed, whilst maintaining a normal ICP;

4. Reduce metabolic demand with good analgesia, sedation and maintain normothermia;
5. Transfuse patients with a haemoglobin concentration below 9 g/dl.

Given the evidence that $P_{bt}O_2$-directed therapy may improve outcomes, it seems appropriate to continue to monitor and correct low $P_{bt}O_2$ values as suggested by the Neurocritical Care Society's 2014 consensus summary statement (194).

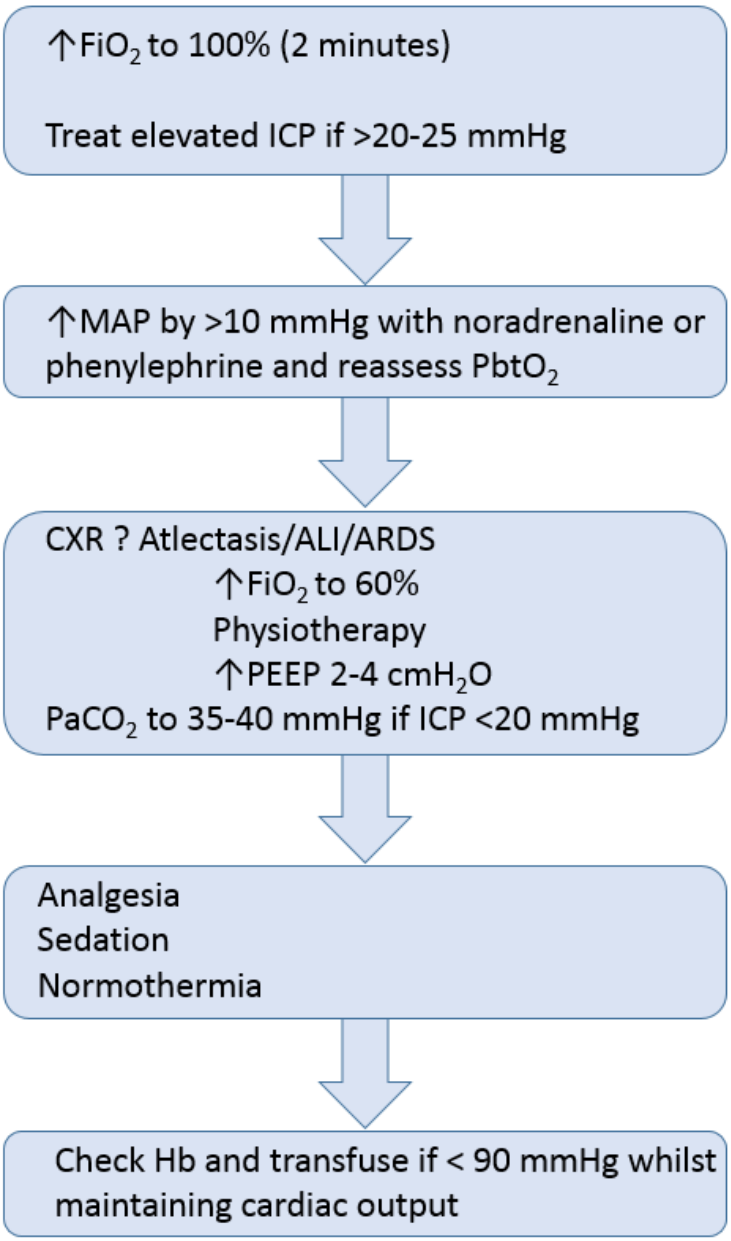


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Figure 12: Proposed Management of Low Partial Brain Oxygen Tension. FiO_2, fraction of inspired oxygen; ICP, intracranial pressure; MAP, mean arterial pressure; $P_{bt}O_2$, partial brain oxygen tension; CXR, chest x-ray; ALI, acute lung injury; ARDS, acute respiratory (104).

Therapeutic Hypothermia

Therapeutic hypothermia is defined here as the controlled reduction of core temperature below 36°C (211). The intentional cooling of patients is not a new

concept. Fay proposed cooling of patients after TBI in 1945 (212). In the 1950s TH was investigated in animal models of TBI (213, 214) and uncontrolled case series where it was thought to improve outcomes (212, 215-218). TH was also applied to patients following cardiac arrest (219). However, side effects such as pneumonia, cardiac arrhythmias and coagulopathy and difficulties in managing patients with hypothermia prevented its widespread use. Interest in TH resurged in the 1980s after experimental TBI models demonstrated decreased neuronal loss and improved outcomes in animals managed at 30-33℃ (220-223). Further benefit was suggested from a small number of clinical trials in the 90s leading to renewed interest (224-228). A 2003 systematic review stated that TH may reduce the risk of mortality and poor outcome after TBI but noted the many variables in treatment may influence results. These included the depth of hypothermia, rate of cooling, rate of rewarming and duration of hypothermia (229). TH is currently used as a treatment for hypoxic ischaemic encephalopathy (230), after cardiac arrest (231, 232) and remains a limited treatment option for severe TBI (54). In addition to TH for TBI, patients are often cooled to normothermia (therapeutic normothermia), which is the reduction and maintenance of an elevated core temperature to 36.0-37.5 °C (233). Targeted temperature management (TTM) refers to TH and therapeutic normothermia and the international critical care society recommends this term in preference to TH (234). For simplicity and because recent publications still use the term, I refer to TH throughout. The definitions of depths of hypothermia vary considerably in the literature so I have avoided terms such as mild, moderate and deep (211, 235).

Physiological Effects of Hypothermia

Temperature sensitive processes that occur after TBI such as excitotoxicity, free radical generation, neuroinflammation and programmed cell death have been reviewed extensively (236-241). A brief description of some of the mechanisms by which hypothermia is thought to alter the pathophysiology of TBI is discussed below.

Hypothermia has been associated with a reduction in $CMRO_2$. It has been reported that a 1°C reduction in body temperature causes a 5-7% reduction in $CMRO_2$ leading to reduced oxygen and glucose consumption (225, 242-244). When core temperature decreases to 32°C metabolic rate decreases to approximately 50% of normal with an associated decreased consumption of oxygen and production of carbon dioxide (245). This mechanism could enable adequate cerebral perfusion with a lower CBF. In 1954, Rosomoff and Holaday, in an animal study of ten dogs, found that deep hypothermia lowered CBF with a linear decrease in $CMRO_2$ from 35°C to 26°C (213). In contrast, Kuluz et al. observed, in a study of adult rats with selective brain cooling from normothermia to 30.9 ± 0.5°C, that CBF increased to 215 ± 25% of control levels. CBF then decreased during rewarming. This apparent cerebral vasodilatory response was thought to explain any potential beneficial effect from TH (246). More recently, Kawamura et al., have used positron emission tomography to demonstrate a reduction in CBF and $CMRO_2$ by 37+/-27% and 52+/-16% respectively in patients were cooled to 33-34°C (247). However, the pathophysiology of TBI is extremely complex and other temperature sensitive

processes that occur after TBI such as excitotoxicity, free radical generation, neuroinflammation and programmed cell death have also been reviewed extensively (236-241).

Another consequence of TBI is altered BBB permeability, resulting in movement of water, electrolytes and neurotoxic substances across the vascular system into the brain parenchyma (205, 248, 249). Experimental models have shown that extravasation of proteins through the BBB is reduced with mild hypothermia (248). Furthermore, hypothermia appears to significantly reduce brain water content with cerebral ischaemia and may also attenuate cerebral oedema, perhaps contributing to the decrease in ICP seen with hypothermia (250-252). In experimental models, hypothermia appears to reduce BBB permeability in TBI models by changing matrix metalloproteinases, extracellular enzymes that can disrupt the BBB (253). Limiting the pathological disruption in BBB permeability may be important in reducing vasogenic oedema and infiltration of inflammatory cells which are linked to adverse outcomes (254, 255).

In addition to attenuating BBB permeability, and therefore the circulating inflammatory response, there is experimental evidence that hypothermia can directly alter the inflammatory response of the central nervous system (CNS). Astrocytes and microglia appear to proliferate around the injury and release pro-inflammatory mediators as well as reactive oxygen species (256). Hypothermia has been seen to significantly reduce the activation of both astrocytes and microglia following TBI and attenuate increases in tissue

superoxide and nitric oxide (257-262). Hypothermia appears to cause this is by increased action of superoxide dismutase and action of nictric oxide synthase (263, 264). Hypothermia also appears to modulate neuronal necrosis and apoptosis in CNS injury models (265).

Patients suffering TBI, or cerebral ischaemia, can have changes to calcium-dependent signalling pathways that are mediated by disrupted signalling of protein kinases (266-269). Calcium/calmodulin-dependent protein kinase II (CaMKII), an important protein kinase mediating synaptic activity, is inhibited by cerebral ischaemia and this inhibition may be reduced by hypothermia (267). Protein kinase C (PKC) is inhibited after cerebral ischaemia where it is translocated to the membrane and hypothermia has reduced the translocation of PKC and its inhibition in experimental models (270).

The initial phase of TBI is associated with an efflux of excitatory amino acids and it has been suggested that hypothermia reduces this excitotoxicity (238, 244, 271, 272). Hypothermia of ischaemic areas (30°C and 33°C) has been seen to attenuate the rise in extracellular levels of dopamine and glutamate after generalised cerebral ischaemia (257). A number of studies have replicated this suggesting that hypothermia reduces excitotoxicity after cerebral ischaemia (273, 274).

Most of the evidence referred to above is from experimental models of TBI in animal studies. Pre-clinical studies have also demonstrated that hypothermia appears to not only improve histopathological outcomes but also reduce neurological deficits (223, 238, 275-278). As discussed below, most human

trials do not replicate these findings (279-281). Furthermore, clinical trials of interventions attempting to manage these sequelae, such as excitotoxicity, have generally failed (282).

Complications of Hypothermia

Intracellular shifts of electrolytes occur when patients undergo TH which can result in hypokalaemia, hypophosphataemia and/or hypomagnesaemia (283). Cold-induced diuresis is common and may be related to tubular dysfunction, hormonal changes and increased venous return due to the peripheral vasoconstriction associated with hypothermia (284, 285). This tubular dysfunction can further exacerbate the above electrolyte disturbances. Hypokalaemia can cause or exacerbate arrhythmias which are an independent effect of hypothermia (283). The increased metabolism of fat that occurs with hypothermia causes an increase in ketones and lactic acid which contribute to the mild acidosis commonly seen in patients with hypothermia. This is not usually significant and pH is rarely below 7.25 (286). Reduced insulin secretion and insulin resistance leading to hyperglycaemia have been associated with hypothermia (287-290).

There is a strong association between TH and cardiac arrhythmias, particularly bradycardia, with maintenance of blood pressure and stroke volume, meaning an overall decrease in cardiac output (approximately 25%) and an increase in systemic vascular resistance (243, 291-294). It is thought that the decrease in metabolic rate associated with hypothermia more than compensates for this decrease in cardiac output (295). Furthermore, there is evidence from a

retrospective cohort study (n=111) that patients who developed bradycardia when treated with TH for out-of-hospital cardiac arrest had better neurological outcomes (296). Maintaining euvolaemia is important in both the induction and maintenance of hypothermia. Peripheral vasoconstriction and cold-diuresis secondary to TH are associated with increased plasma noradrenaline, activation of the sympathetic nervous system and atrial natriuretic peptide and decreased antidiuretic hormone function as well as renal antidiuretic hormone (243). All of this can contribute to hypovolaemia and potentially decrease CPP leading to worse CBF. It is worth noting that patients are more likely to become hypovolaemic when treated with diuretics, such as mannitol. As mentioned earlier, both therapeutic and pathological hypothermia are associated with significant arrhythmias. This is usually seen at temperatures <30°C. An additional complication associated with hypothermia is reduced and altered platelet function as well as altered clotting enzymes at temperatures below 35°C and 33°C respectively (297, 298).

Pneumonia is often reported as a common side-effect of TH. A 2009 Cochrane review investigating the benefits of TH in TBI found that although there was a trend towards an increased risk of pneumonia, this trend was not significant and the level of evidence to support it was poor (299) .

Two of the most predictable side-effects of hypothermia are vasoconstriction and shivering. Studies in healthy humans have demonstrated that shivering starts at around 35.5°C and that vasoconstriction starts at around 36.5°C (235, 300). The effects of these two haemodynamic responses have been

investigated in post-operative patients and shivering is associated with increased cardiac events, especially in elderly patients (301-303). The increased metabolism and oxygen consumption associated with shivering leads to increased respiratory rate and tachycardia, further increasing oxygen demands (303, 304). This response is slightly different in the sedated patient on ICU because of mechanical ventilation. It is important to control shivering to reduce oxygen consumption and patient discomfort. This can be performed in the following ways:

- Drugs that lower shivering thresholds, such as paracetamol, non-steroidal anti-inflammatory drugs and opiates (e.g. pethidine);
- Paralysing agents and sedatives: A 2010 systematic review of sedation protocols from 68 ICUs found midazolam to be the most commonly used agent to suppress shivering, followed by Propofol (305) but muscle relaxants have also been used (306);
- Skin counter-warming.

Rewarming carries its own risks and is associated with rebound intracranial hypertension (229). Rapid rewarming may also lead to hypoglycaemia and electrolyte disturbances as insulin sensitivity returns to baseline and intracellular potassium is released (307). It is not clear what the ideal rate of rewarming is, only that rapid rewarming is associated with poor outcomes after TH (307-309). Rates of rewarming have varied from 1°C per day (310, 311) to 1°C per hour (312, 313), with many studies using 0.25°C per hour (308, 314).

Therapeutic Hypothermia in Traumatic Brain Injury: Clinical Evidence

Despite many animal studies demonstrating that TH at 32-35°C offers neuroprotection and may improve outcomes after TBI, there is limited clinical data to support its use (233, 238, 315, 316). A Cochrane review from 2009 examining 22 RCTs (n = 1,382) investigated TH as a treatment for TBI and demonstrated fewer deaths in patients treated with TH (OR 0.76, 95% CI 0.60 to 0.97). The review also found that patients treated with TH were less likely to have an unfavourable outcome (OR 0.69, 95% CI 0.55 to 0.86). However, the authors concluded that TH may be beneficial in reducing morbidity and mortality from TBI but that the only trials showing significant benefit were of low quality, with a tendency to overestimate the treatment benefit. As such, TH was only recommended for use in patients taking part in RCTs (317). A review in 2014 assessed 18 studies that provided mortality data and concluded that TH was associated with a significant reduction in mortality and poor outcome after TBI (318). The 2009 and 2014 reviews concluded that the evidence supporting TH for TBI was of low quality and suggested that more RCTs were needed. Two subsequent multicentre RCTs, Eurotherm3235 and B-HYPO (Brain Hypothermia), both published in 2015, appear to add to the growing consensus that TH is not beneficial after TBI (279, 281).

The B-HYPO trial rapidly cooled patients with TBI to 32-34°C within six hours of their injury or kept them normothermic (35.5-37°C). Patients were cooled for at least 72 hours and were rewarmed at a rate of <1°C per day. 150 of the

planned 300 patients were recruited and the trial was stopped due to low recruitment and likely futility. The trial found no differences in neurological outcomes or mortality between the groups. Criticisms of the trial include that it was relatively underpowered and that trials stopped early tend towards exaggerated treatment effects (281, 319, 320). Interestingly, fever control management (i.e. maintaining normothermia in patients with fever) was reported to significantly improve mortality. A 2015 systematic review of 16 RCTs investigating TH in TBI found that most studies () reported that avoidance of fever was associated with improved outcomes including decreased length of ICU stay and mortality (321).

Eurotherm3235 was a multi-centre RCT investigating TH (32-35°C) for ICP reduction after TBI. The trial enrolled patients with TBI who had ICP >20 mmHg for five minutes despite stage one treatments (Fig.11). Enrolment to the study was allowed up to 72 hours after TBI due to the greatest benefits of hypothermia appearing to be associated with early cooling. Patients were to remain cooled until their ICP was <20 mmHg and no longer temperature-dependent (322). 387 patients were enrolled at which time recruitment was suspended due to safety concerns. The authors concluded that in patients with ICP >20 mmHg after TBI, TH plus standard care did not result in better outcomes than standard care alone. Stage three (Fig.11) treatments were required to control ICP in 54% of patients in the control group and in 44% of patients in the hypothermia group. The adjusted common odds ratio for the GOS-E score was 1.53 (95% confidence interval, 1.02 to 2.30; p = 0.04), indicating a worse outcome in the hypothermia group (279). Criticisms include

that only 10% of patients were treated within 12 hours of injury. These patients may have had brain oedema at randomisation and one-to-two litres of cold saline to induce hypothermia could have been detrimental (323). Looking at the 10% of patients who received TH before 12 hours, mortality was halved in the intervention group (HR 0.54 [0.07-4.03]), although there are very large confidence intervals. Cooper et al. argue that a treatment threshold of 20 mmHg is too low and cite 25 mmHg as a more favourable threshold whilst still advocating TH in TBI (323).

The authors of these comments are investigators for POLAR-RCT (Prophylactic Hypothermia Trial to Lessen Traumatic Brain Injury). This study aims to randomise 500 patients to at least 72 hours of TH (33°C) initiated within three hours of injury, or standard normothermic management (324). The trial is currently recruiting patients (ClinicalTrials.gov NCT00987688). Another ongoing trial is LTH-I (Long-term Mild Hypothermia for Severe Traumatic Brain Injury), a prospective RCT aiming to recruit 300 TBI patients randomised to five days of TH (34-35°C) or normothermia (36-37°C) (325).

Recent reviews conclude that TH has not led to a decrease in mortality in clinical trials and that it might increase the risk of worse outcomes (320, 326). Given the positive results from animal studies of TH for TBI and the disappointing results from clinical trials, Hirst et al., are conducting a systematic review and meta-analysis of these animal studies to try to explain the disparity (327). I expect that there have been many small, heterogenous

pre-clinical studies which demonstrate risk of bias and experimental design flaws.

Summary: Difficulties with Clinical Research

In 1998 Roberts et al., in a systematic review, concluded that there was insufficient evidence to support or refute the existence of a real benefit from using hyperventilation, mannitol, CSF drainage, barbituates or corticosteroids in the management of TBI (180). In 2006, Adamides et al., conducted a review of treatments for TBI and concluded that there was no Class I evidence to support the routine use of any of the therapies they examined (hyperventilation, osmotherapy, cerebrospinal fluid drainage, barbiturates, decompressive craniectomy, TH, normobaric hyperoxia, and hyperbaric oxygen therapy) (328). In 2017, nearly 20 years on from the Roberts' paper, there is probably sufficient evidence to refute the existence of a real benefit from corticosteroids (329). We may be moving towards being able to refute TH and decompressive craniectomy as beneficial treatments. However, we can now add ICP, $P_{bt}O_2$ and CPP monitoring and their guided treatments to our list of uncertainties. The 2016 BTF guidelines have a single recommendation based on level I evidence: the use of steroids is not recommended for improving outcome or reducing ICP after TBI (54). This may seem cynical, but negative results, if based on good-quality trials that change practice for the better by stopping ineffectual or harmful interventions, may be as equally important as positive results.

Stein, in his comprehensive 2015 review of why trials fail in TBI, explains that there has been a 100% failure rate in identifying drugs that work in the acute phase of TBI (330). However, as he points out, this problem is not limited to TBI research and there is a high failure rate for phase III trials in sepsis, stroke, cancer, cardiology and orthopaedic research. Many positive results from experimental models and early clinical trials that do not translate to phase III trials, leading many to ask the question: Why do phase III trials fail (331-335)? Perhaps unsurprisingly, answers for TBI research include poorly conducted animal studies informing clinical trials; commercial and financial pressures to progress to phase III trials and complete trials on time; using an over-simplified definition of a complex disease; multiple variables masking positive signals and poor trial set-up or running (330, 336, 337). The authors of these suggestions provide clear advice to future trial developers about how to avoid similar pitfalls.

RCTs investigating TH <35°C suggest worse outcomes in TBI patients treated with TH (279). Unfortunately, due to the complex nature of TBI and TH, there remain questions about whether the timing of onset of TH, the rate and duration of cooling and rewarming, and the degree of hypothermia effect the results seen from trials of TH. Furthermore, one could question whether TH is beneficial in a subset of patients with TBI whilst harmful in others. Retrospective analysis of data from the Eurotherm3235 trial is ongoing. One area of interest is whether TH affected the $P_{bt}O_2$ of patients with TBI. This work makes up the first part of original research in my thesis and is discussed in the next chapter (338).

Chapter 2: Therapeutic Hypothermia Reduces Intracranial Pressure and Partial Brain Oxygen Tension

Introduction

This chapter describes the retrospective analysis of a subset of data from 17 patients enrolled into the Eurotherm3235 trial. Patient data included in this study were taken from patients enrolled at the Edinburgh site, which recruited 42 of 387 patients. As such, the enrolment and treatment methods are from Eurotherm3235. Professor Peter Andrews has kindly given permission for relevant parts of the methods to be reproduced here (339). Collection of $P_{bt}O_2$ data and its analysis is original research and was not part of the Eurotherm3235 trial protocol. Work from this analysis was published prior to completion of the Eurotherm3235 trial (338). Due to the early stopping of this trial, the total number of subjects was limited to 17.

As discussed in Chapter I, intracranial hypertension and decreased $P_{bt}O_2$ are targeted as potentially avoidable causes of morbidity after TBI. TH appears to reduce ICP but might adversely affect CBF. I report a retrospective analysis of prospectively collected data from 17 patients admitted to the Western General Hospital, Edinburgh. Patients with ICP >20 mmHg refractory to initial therapy were randomised to standard care (control) or standard care and TH (intervention, 32-35°C) to reduce ICP. ICP and $P_{bt}O_2$ were measured using the

Licox system, and core temperature was measured by rectal thermometer. Data were analysed at the hour before cooling, the first hour at target temperature, two consecutive hours at target temperature, and after six hours of hypothermia. There was a mean decrease in ICP of 4.3 ± 1.6 mmHg ($p<0.04$) from 15.7 to 11.4 mmHg, from precooling to the first epoch of hypothermia in the intervention group (n=9) that was not seen in the control group (n=8). All time periods demonstrated a decrease in ICP in the intervention group. There was a mean decrease in $P_{bt}O_2$ of 7.8 ± 3.1mmHg ($p<0.05$) from 30.2 to 22.4 mmHg, from precooling to stable hypothermia in the intervention group, which was not observed in controls. The findings support previous research in demonstrating that hypothermia decreased ICP, which might facilitate reduced hyperosmolar agent use. I go on to suggest that the decrease in $P_{bt}O_2$ is not below the 2007 BTF suggested treatment threshold of 15 mmHg, but might indicate a decrease in CBF in certain patients. Finally, individual patients demonstrated a significant reduction in $P_{bt}O_2$. The reason for this was unclear and further research assessing $P_{bt}O_2$ data is recommended in normothermic patients.

Materials and Methods

Patients were randomised to receive either standard care without TH (control) or standard care with TH (intervention). Hypothermia was initiated with 20-30 ml/kg refrigerated (4°C) 0.9% Sodium Chloride given intravenously and maintained using cooling blankets. The depth of hypothermia within the 32-35°C range was guided by the patient's ICP. Higher pressures resulted in a

colder target temperature. The aim was to lower the core temperature by the minimum required to achieve an ICP of ≤20 mmHg within the limits of 32-35°C. If target ICP was not achieved at 35°C, core body temperature was decreased by increments of 0.5°C until an ICP of ≤20 mmHg, or a core temperature of 32°C, was reached. Unit guidelines for the detection and treatment of shivering were applied to all patients. Therapeutic hypothermia was maintained for at least 48 hours in the treatment group and continued until ICP was maintained below 20 mmHg. Figure 12 outlines the study plan.

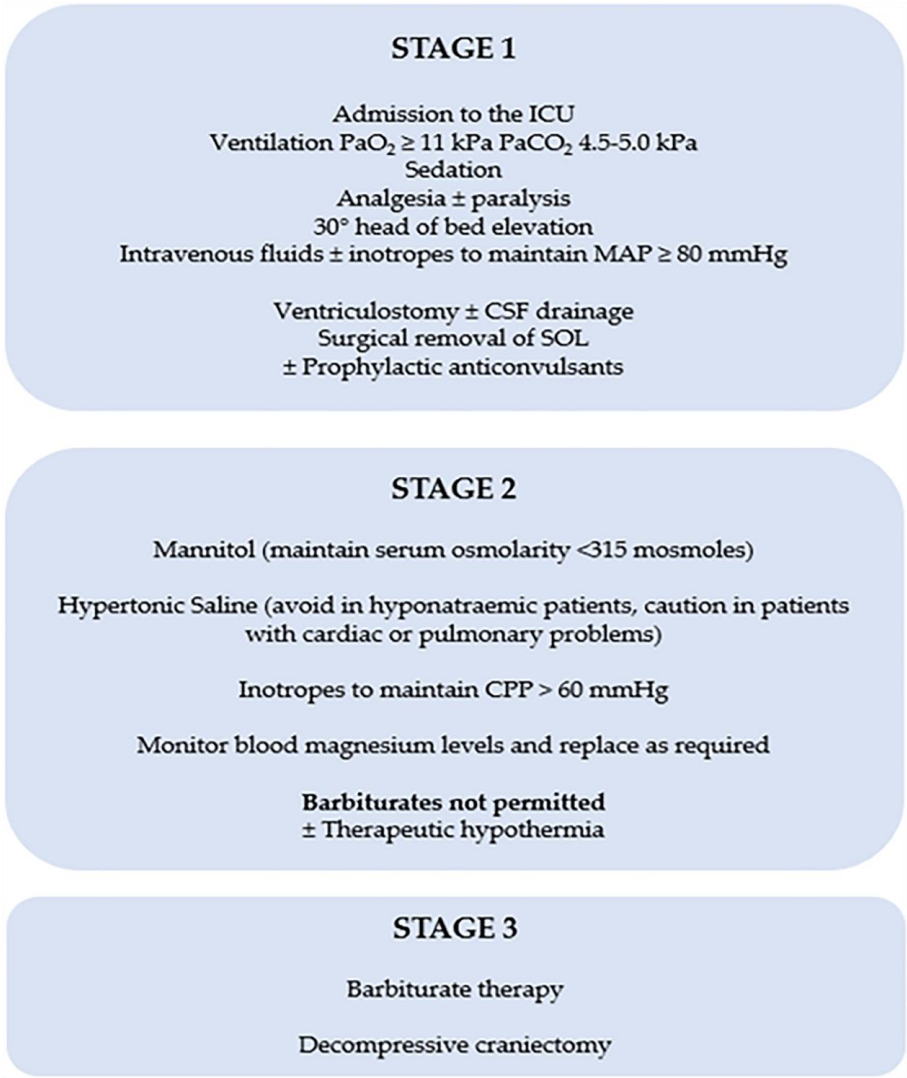

Figure 13: Stages of Management of Traumatic Brain Injury. Information for figure taken from BTF guidelines (Brain Trauma Foundation et al. 2007). CPP, cerebral perfusion pressure; CSF, cerebrospinal fluid; ICU, intensive care unit; SOL, space occupying lesion

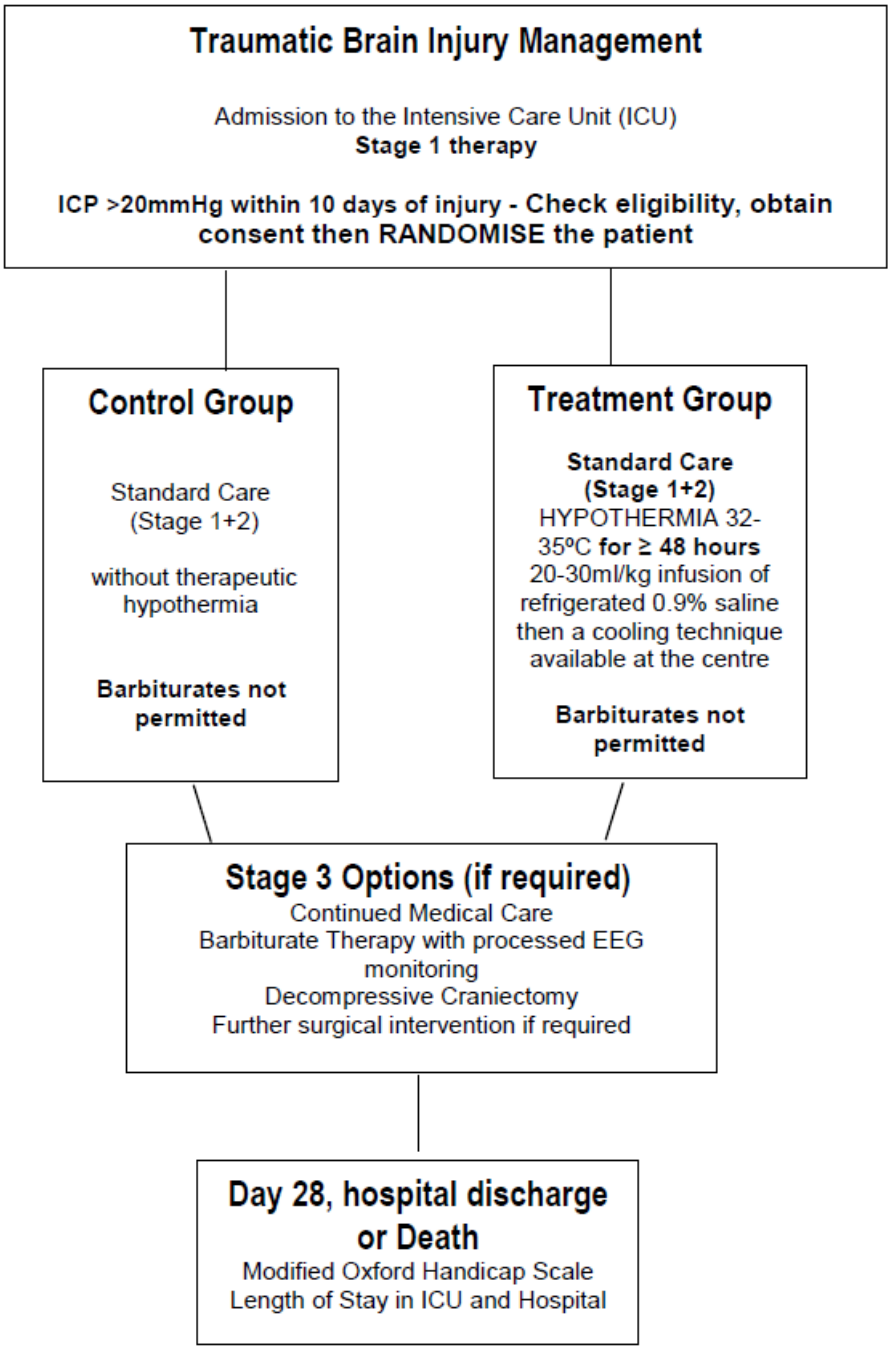

Figure 14: Study Flowchart . EEG, electroencephalogram; ICU, intensive care unit; ICP intracranial pressure. Adapted from Eurotherm3235 trial with permission (288). Stages are as per figure 11

Inclusion and Exclusion Criteria and Eligibility

Inclusion Criteria:

- Legal age for consent to take part;
- Primary closed TBI;
- Raised ICP > 20 mmHg for ≥ five minutes after first line treatments with no obvious reversible cause (e.g. patients position, coughing or inadequate sedation);
- ≤ 72 hours from the initial injury;
- Cooling device or technique available for > 48 hours;
- Core temperature ≥ 36°C at the time of randomisation;
- An abnormal CT head defined as one that shows haematoma, contusion, swelling, herniation or compressed basal cisterns.

Exclusion Criteria

- Patient already receiving TH;
- Administration of barbiturate infusion prior to randomisation;
- Patient unlikely to survive for the next 24 hours in the opinion of the ICU consultant or consultant neurosurgeon treating the patient;
- Temperature ≤ 34°C on hospital admission;
- Pregnancy.

Eligible patients for the study had raised ICP despite stage 1 therapy (Fig.11) for the management of TBI.

Randomisation and Treatment Allocation

Randomisation occurred after meeting inclusion criteria using a telephone randomisation service. Treatment allocation was minimised using the following baseline covariates:

1. Trial Centre;
2. Age < or ≥ 45 years;
3. Post-resuscitation GCS motor component 1-2 or 3-6;
4. Time from injury < or ≥ 12 hours;
5. Pupils both reacting, or one, or neither reacting.

Investigators were not blinded to treatment allocation because it was clinically obvious which patients were receiving TH.

Management of Patients in $P_{bt}O_2$ Study

$P_{bt}O_2$ has been recorded with the Integra Licox system in all patients admitted to the WGH ICU for ICP management since May 2010. At the time of analysis data were only available for 17 patients. Patients were managed as per the 2007 BTF guidelines, intubated and ventilated to achieve a $PaCO_2$ of 4.5 to 5.0 kPa, sedated and nursed with 30° head elevation. CPP was maintained at ≥60 mmHg by manipulating MAP with fluids and noradrenaline and limiting ICP ≤20 mmHg. The Licox probe was inserted into the brain parenchyma via a dedicated triple-lumen bolt that was sited via a burr hole for measurement of ICP, $P_{bt}O_2$, and brain temperature. Monitors were inserted into frontal white matter in the non-dominant hemisphere for diffuse injuries or on the site of

maximal trauma in focal injuries. The probe was not sited in non-viable tissue. Hourly core body temperature was recorded via rectal thermometer. The ICU Pilot software (CMA, Stockholm, Sweden) integrated minute data from the monitors to a bedside computer.

Refractory intracranial hypertension in either group led to escalation of therapy, including 125mls of 5% sodium chloride or 200mls of 20% mannitol as a bolus injection. Paralysis and further CT head scanning were also available. Neurosurgical intervention was available on site as required. Patients in the control group were cooled to normothermia (36.5-37.5°C) and pyrexia (>38°C) was managed with paracetamol and cooling blankets. All patients received seizure prophylaxis with a loading dose of Phenytoin (20mg/kg) and a maintenance dose (4-5mg/kg) for seven days post injury.

Data and Statistical Analysis

Cat Graham, a local Wellcome Trust Clinical Research Facility statistician, provided help with suggesting methods of statistical analysis. Data analysis was pre-specified and after determining the time to reaching target temperature was highly variable I decided not to perform an intention to treat analysis. Analysis was divided into four separate time periods coinciding with patient temperatures. The first two hours of $P_{bt}O_2$ data recorded were discarded from analysis to reduce artefact from the Licox monitor which has been previously documented and was present in this study (103, 104).

Data from 17 patients, nine intervention and eight control, were analysed. These were the first 17 patients enrolled to the Edinburgh site that had $P_{bt}O_2$ data recorded. The four time points used were: Time 0, the hour before randomisation (induction of cooling or control); Time 1, the first hour of hypothermia (<35°C); Time 2, the first episode of stable hypothermia (<35°C), defined as two consecutive hours of hypothermia; and Time 3, six hours of stable hypothermia. Values are given as the mean ± standard error of the mean unless otherwise stated. One-way repeated measures of variance (analysis of variance [ANOVA]) were performed to identify differences within each group and paired Student's t-tests were performed to identify differences within the groups at the set time points. Independent Student's t-tests were performed to identify differences between the groups. All statistical tests were performed using Statistical Package for Social Sciences 20.0 (SPSS version 20; IBM, Inc., Armonk, NY).

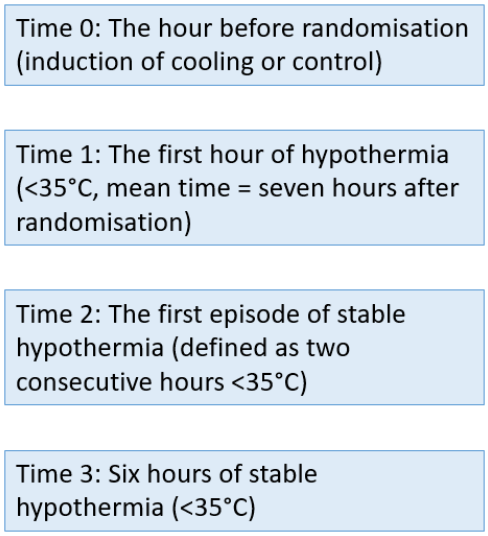

Figure 15: Times of Analysis

Following on from my viva in September 2017 I have added a section to the conclusion chapter which discusses the reasons for the tests used in more detail.

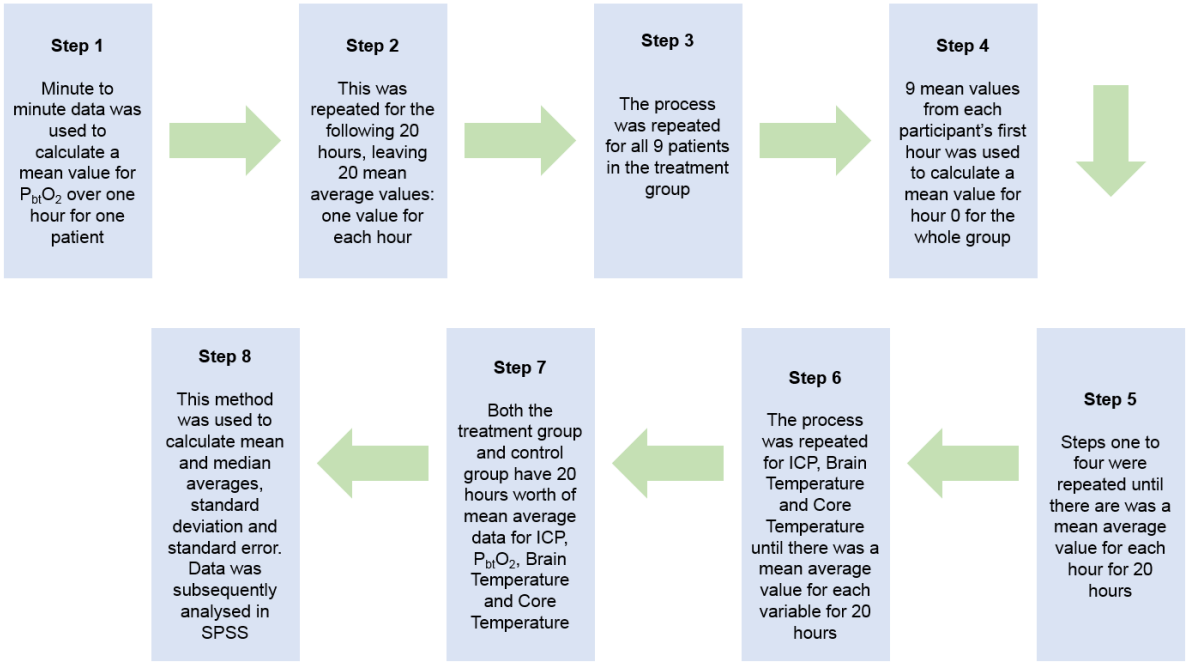

Figure 16: Flow Diagram of Data Analysis. $P_{bt}O_2$, partial brain oxygen tension; ICP, intracranial hypertension; SPSS, statistical package for social sciences

Results

Control Group Demographics

All eight patients were male and the mean age was 34. Three patients suffered extradural haemorrhages (EDHs) as their primary injury, two subdural haemorrhages (SDHs), two traumatic subarachnoid haemorrhages, and one patient suffered a diffuse injury. Three patients had non-depressed skull fractures. The median GCS on admission to the ED was seven (range 3–14, Table 4).

Intervention Group Demographics

Two participants were female, seven were male, and the mean age was 41. Three patients suffered EDH as their primary injury, three had diffuse injuries,

one had contusions, and one had diffuse injuries and a SDH. Five patients had non-depressed skull fractures and one had a depressed skull fracture. The median GCS on admission to the ED was seven (range 3–14, Table 4).

Missing Data

The first two hours of $P_{bt}O_2$ data recorded were discarded from analysis to reduce artefact from the Licox monitor. This artefact has been previously documented (103, 104). Half of the control group were not included in the Time 0 analysis of $P_{bt}O_2$ due to missing data (n=4/8, Appendix 1). In the remaining patients, $P_{bt}O_2$ recording was present over two hours before randomisation and the data was included from Time 0. ICP and $P_{bt}O_2$ data were also missing when monitors were detached to facilitate patient transfer. In addition, core temperature recordings were occasionally missing from patient charts. Due to the small sample size and relatively small amount of data missing, statistical models to analyse the effect of missing data were not performed. Raw data highlighting missing sections is included in Appendix 1 for ICP, $P_{bt}O_2$ and core temperature. I have added a section on management of missing data in the conclusion and reflective chapter.

TABLE 4. CLINICAL CHARACTERISTICS OF PATIENTS

Patient No.	*Age (year)/ sex*	*GCS score on admission*	*CT classification*
1	26/M	7	EDH
2	29/M	6	EDH
3	25/M	9	SDH
4	27/M	3	SAH
5	43/M	12	EDH
6	35/M	12	SDH
7	48/M	7	Diffuse
8	37/M	14	SAH
9	48/M	11	EDH
10	26/M	14	EDH
11	30/F	7	Diffuse
12	28/M	3	Diffuse
13	55/F	7	Contusions
14	55/M	8	EDH
15	55/M	6	SDH
16	25/M	3	Diffuse
17	49/M	6	SDH

Table 4: Clinical Characteristics of Patients. CT, computed tomography; EDH, extradural haemorrhage; F, female; GCS, Glasgow Coma Score; M, male; SAH, subarachnoid haemorrhage; SDH, subdural haemorrhage. Patients 1-8 were in the control group, patients 9-17 were in the intervention group.

Time to Temperature; Hb; FiO_2; PaO_2; and $PaCO_2$

The mean time to target temperature (<35°C) in the intervention group was seven hours (421 ± 72 minutes) after randomisation, due to long lead times to initiation of TH following pre-randomisation hypertonic therapy.

There was no significant change in FiO_2 from pre-cooling to target temperature in the intervention group (median 0.35 [0.30-0.55] vs. 0.30 [range 0.30-0.55] $p>0.05$) and there was no significant difference in FiO_2 between the two groups. PaO_2 and $PaCO_2$ values were not significantly different from pre-cooling to target temperature: Pre-cooling PaO_2 16.1 ± 0.9 kPa, target temperature PaO_2 16.3 ± 1.6 kPa, $p > 0.05$; pre-cooling $PaCO_2$ 4.5 ± 0.01, target temperature $PaCO_2$ 4.5 ± 0.02 kPa, $p > 0.05$. There was no statistically significant difference in PaO_2 or $PaCO_2$ values between the two groups. Haemoglobin values were not significantly different between the groups and Hb did not change from pre-cooling to target temperature in the intervention group (10.1 ± 0.4 vs. 9.7 ± 0.4, $p > 0.05$).

There was no significant difference in the number of osmotic agents used between the control and intervention groups (1, range 0-4 and 0-5 respectively, $p>0.05$).

Intracranial Pressure

A two-way repeated measures ANOVA revealed a difference between the two groups: $F(3,45)=4$, $p < 0.02$. A one-way repeated measures ANOVA demonstrated a statistically significant decrease in ICP in the intervention group: $F(3, 24)=6.13$, $p < 0.01$. Post-hoc analysis with t-tests show that in the intervention group, ICP decreased by 4.3 ± 1.6 mmHg ($p<0.04$), from 15.7 to 11.4 mmHg from Time 0 to Time 1 (Figs. 15 and 16). The decrease in ICP was maintained from Time 0 to Times 2 and 3. There was no statistically significant difference in ICP in the control group between these times.

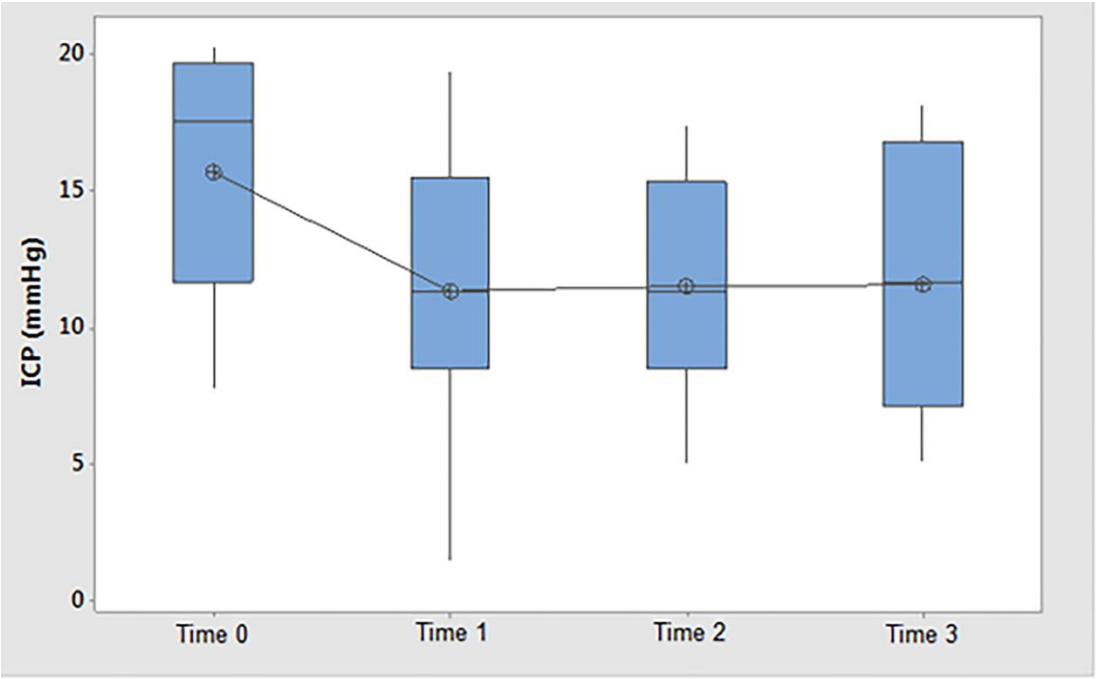

Figure 17: Boxplot of Intracranial Pressure versus Time in the Intervention Group. ICP = Intracranial Pressure. Time 0, Pre-Randomisation; Time 1, First hour of hypothermia (<35°C); Time 2, First episode of stable hypothermia; Time 3, Six hours of hypothermia.

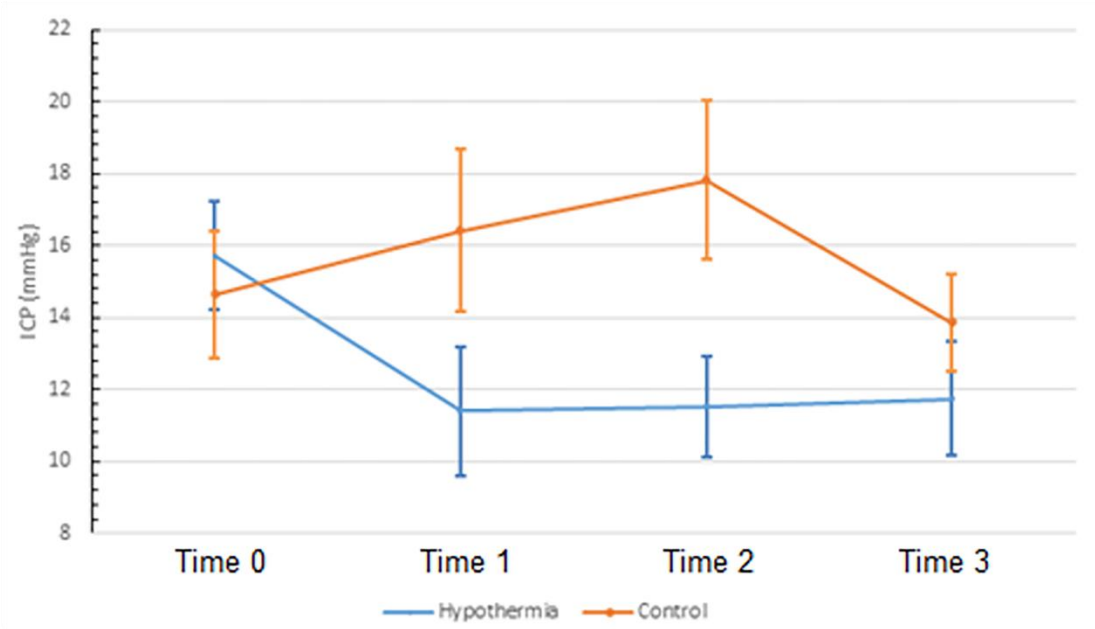

Figure 18: Changes in ICP with Time: intervention and control groups. Time 0, pre-randomisation; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). Two-way repeated measures ANOVA demonstrates a difference between the groups $F(3,45)=4$, $p < 0.02$. ANOVA, analysis of variance.

Partial Brain Oxygen Tension

Multiple imputation was applied to perform a two-way repeat measures ANOVA which revealed no statistically significant change in $P_{bt}O_2$ between the groups (Fig 19). A one-way repeat measures ANOVA was run in the intervention group which did suggest a difference in $P_{bt}O_2$ with time $F(3,18)$ 4.60, $p < 0.02$. Post-hoc analysis with paired t-tests demonstrated a statistically significant difference between Time 0 and Time 2 in the intervention group, where there was a decrease in $P_{bt}O_2$ of 7.8 ± 3.1mmHg ($p < 0.05$) from 30.2 to 22.4 mmHg (Fig. 17). However, Friedman's test was run after suggestion from my viva panel which did not suggest a statistically significant difference in the intervention group (P 0.12). See the thesis conclusion for a discussion about how the data was analysed. Furthermore, post-hoc analysis with a related-samples Wilcoxon signed rank test showed a not statistically significant decrease in $P_{bt}O_2$ from Time 0 to Time 2 (P 0.063). Three patients in the intervention group had a decrease in $P_{bt}O_2$ from >25 mmHg at Time 0 to <20 mmHg at Time 3. There was no statistically significant difference between Time 0 and any other times in the control group or a statistically significant difference between the two groups in terms of the change in $P_{bt}O_2$ (Fig. 19).

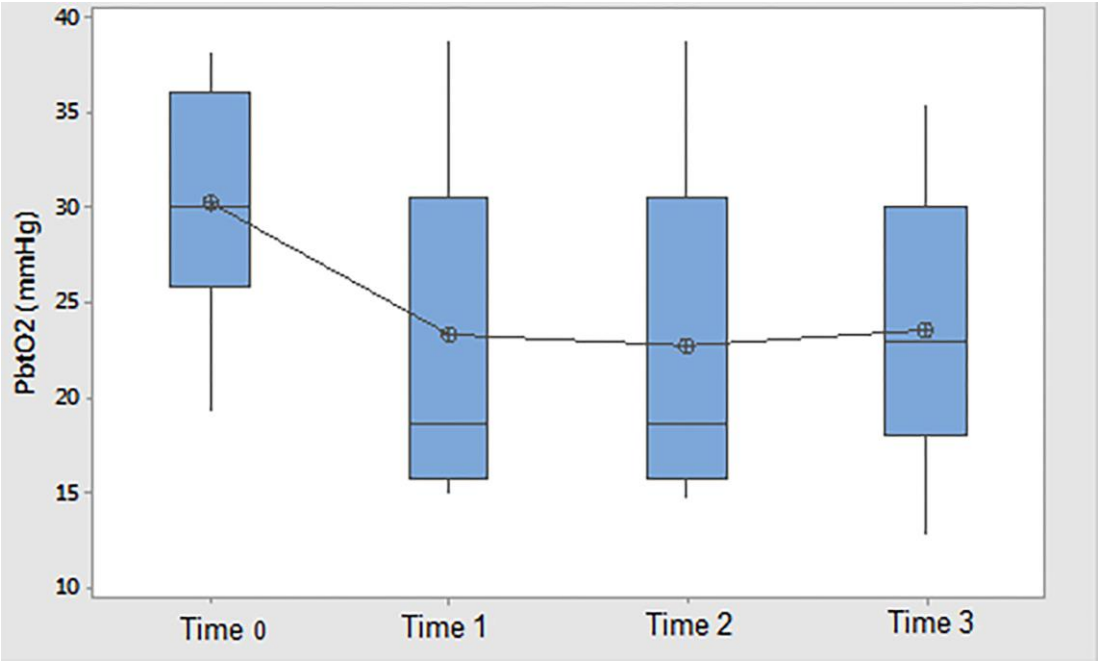

Figure 19: Boxplot of Partial Brain Oxygen Tension versus Time in the intervention group. $P_{bt}O_2$, partial brain oxygen tension; Time 0, pre-randomisation; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). Mean decrease in $P_{bt}O_2$ from Time 0 to Time 2 is 7.8 ± 3.1 mmHg from 30.2 to 22.4 mmHg.

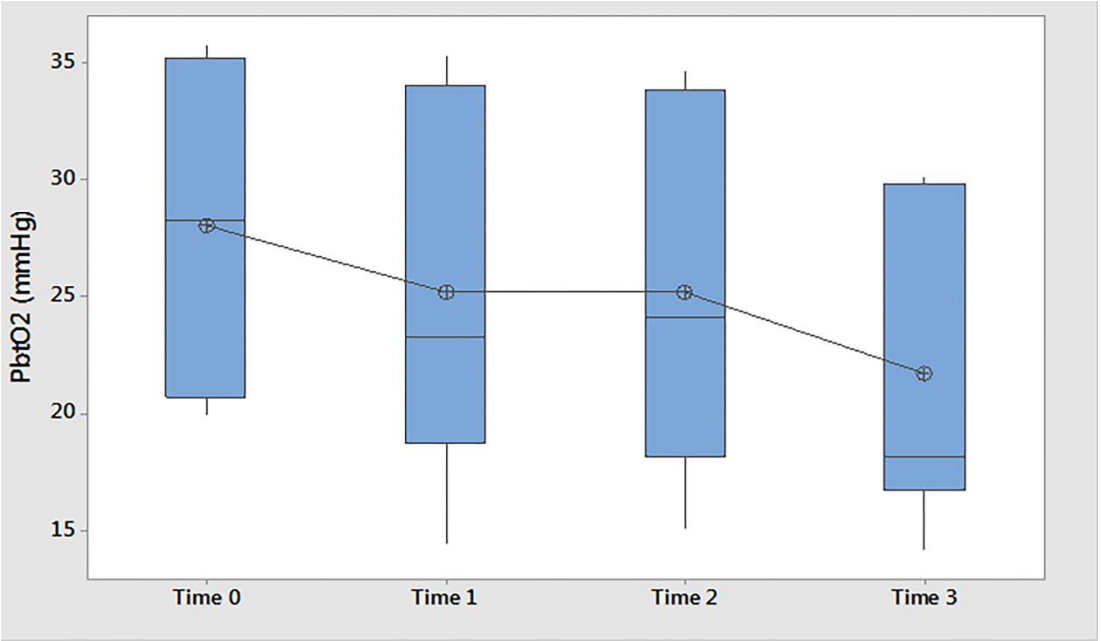

Figure 20: Boxplot of Partial Brain Oxygen Tension versus Time in the control group. Time 0, pre-randomisation; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). There was no statistically significant difference between Time 0 and the other time points.

Discussion

Intracranial Pressure

Starting ICP in the patients in this study was <20 mmHg because a single hypertonic treatment was given pending randomisation which also resulted in long lead times to initiation of cooling. TH reduced ICP from pre-cooling to the first episode of stable hypothermia (4.3 ± 1.6 mmHg, ~27%), despite pre-treatment with hypertonic therapy. The reduction in ICP was maintained at six hours, which is consistent with previous studies (Table 5) (227, 312-314, 340-348). There was no further decrease in ICP with prolonged hypothermia which is also consistent with prior studies (349). The reduction in ICP is comparable to the reduction caused by mannitol and HSS, which are reported to reduce ICP by 10–51% and have similar efficacy (183, 350-356). Reported adverse effects of mannitol include renal failure, electrolyte abnormalities, acidosis, hypotension, and congestive heart failure. Reported adverse effects of HSS include renal failure and electrolyte abnormalities in addition to other theoretical concerns (357). Mannitol causes a short-term reduction in ICP with diminishing returns and the prolonged use of HSS over 72 hours has been shown to increase mortality (354, 358). Therefore, TH could help to reduce the number of therapies required if used in combination.

TABLE 5. CHANGES IN INTRACRANIAL PRESSURE AND $P_{bt}O_2$ WITH TEMPERATURE IN PREVIOUS STUDIES

Study	n	*Mean decrease in ICP (mmHg)*	*Mean change in $P_{bt}O_2$*	*Target temperature (°C)*
Zhi *et al.* (2003)	396	4.1–6.6		32–33
Clifton *et al.* (2001)	392	1.75		33
Jiang *et al.* (2000)	87	9.23		33–35
Marion *et al.* (1997)	82	4.3		32–33
Qiu *et al.* (2007)	80	1.2–2.1		33–35
Liu *et al.* (2006)	45	5.33		33–35
Smrcka *et al.* (2005)	38	8.07		34
Gal *et al.* (2002)	30	6		32–34
Lavinio *et al.* (2007)	24	4.8		34
Sahuquillo *et al.* (2009)	23	7		32.5
Metz *et al.* (1996)	10	9.5		32.5–33
Gupta *et al.* (2002)[a]	30		Decrease 1.0 kPa	< 35
Zhang *et al.* (2002)	18		Increase 19.1 mmHg	31.5–34.9
Current study	17	4.3	Decrease 7.8 mmHg	32–35

Table 5: Changes in Intracranial Pressure and $P_{bt}O_2$ with Temperature in Previous Studies. Summary of change in ICP and $P_{bt}O_2$ with temperature from previous studies for comparison. [a] Estimate of results from graphed data. ICP, intracranial pressure; n, number of patients in each study; $P_{bt}O_2$, partial brain oxygen tension.

As discussed at the end of the Chapter I, TH is not a risk-free intervention, and is associated with arrhythmias, electrolyte disturbances, and pneumonia. This study supports others in finding that TH reduces ICP, but it did not evaluate mortality or neurological outcomes, and the use of hyperosmolar agents was not reduced.

Partial Brain Oxygen Tension

Both groups in this study demonstrated a decrease in $P_{bt}O_2$ with time. In the intervention group, there was a not statistically significant decrease of 7.8 ± 3.1 mmHg from 30.2 to 22.4 mmHg. In the control group, no statistically significant decrease was seen from Time 0 to any other time points. It is worth remembering that half of the control group were not included in the Time 0 analysis due to missing data (n=4/8). There was a decrease in $P_{bt}O_2$ in the

control group between Time 1 and 3 and Time 2 and 3 of 3.47 ± 1.02 and 3.48 ± 1.27mmHg, respectively. It could be that a statistically significant difference between would have been present if all data were included at Time 0. There was no statistically significant difference between the two groups.

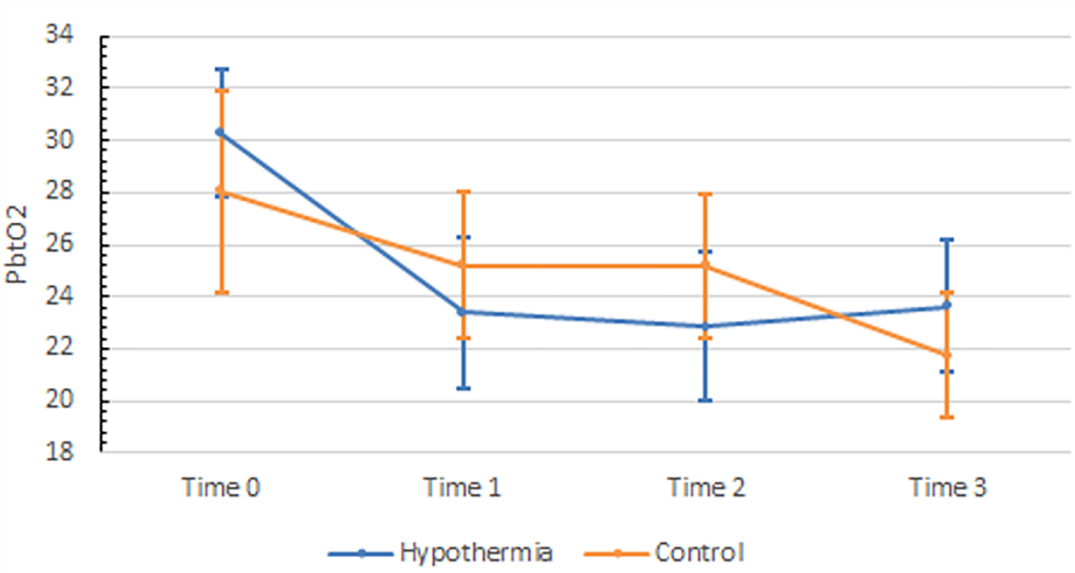

Figure 21: Changes in Partial Brain Oxygen Tension with Time: intervention and control groups. Time 0, pre-randomisation; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). There was no statistically significant difference between the two groups in terms of the change in $P_{bt}O_2$.

Previous studies have investigated the effect of hypothermia on $P_{bt}O_2$ with conflicting results. Gupta et al., in a single-centre, non-randomised study of 30 patients cooled to ≤33°C, observed a decrease in $P_{bt}O_2$ and concluded that decreasing brain temperature <35°C may impair brain tissue oxygenation (341). It has been argued that the sensor used to measure $P_{bt}O_2$ in this study (Paratrend 7, Diametrics Medical) was not corrected for temperature and did not reflect an accurate $P_{bt}O_2$. In contrast, Zhang et al., in a study of 18 patients

with severe TBI, found that hypothermia (31.5–34.9°C) increased $P_{bt}O_2$ from 9.6 ± 6.8 to 28.7 ± 8.8 mmHg (mean ± SD) (346). Patients in this study received TH within 20 hours following TBI and had $P_{bt}O_2$ recorded within the first 24 hours. Given the very low initial $P_{bt}O_2$ and subsequent increase within 24 hours following injury, it has been suggested that the increase was attributable to early changes in CBF and oxygenation rather than an effect of hypothermia (359).

Decreased $P_{bt}O_2$ is associated with poor outcomes independent of intracranial hypertension and the combination of raised ICP and decreased $P_{bt}O_2$ appears worse than isolated intracranial hypertension (195). Normal levels of $P_{bt}O_2$ in neurosurgical patients are reported as between 23 and 35 mmHg, with lower values coming from probes sited deeper in brain tissue or in focal lesions (108, 193, 360). While the decrease of $P_{bt}O_2$ in the intervention group is significant, the mean value did not decrease below the threshold of 15 mmHg recommended for intervention by the 2007 BTF guidelines, nor did it decrease below 20mmHg, the suggested threshold for compromised brain oxygen/moderate brain hypoxia (194). However, when looking at the individual value plots (Fig.20) for the intervention group, three patient's $P_{bt}O_2$ decreased from >25mmHg before cooling to <20mmHg after six hours of hypothermia. It is unclear why certain patients exhibited this decrease in $P_{bt}O_2$ while others did not and it may be unrelated to temperature. Further investigation is warranted to identify subgroups of patients demonstrating decreased $P_{bt}O_2$ to identify potential causes.

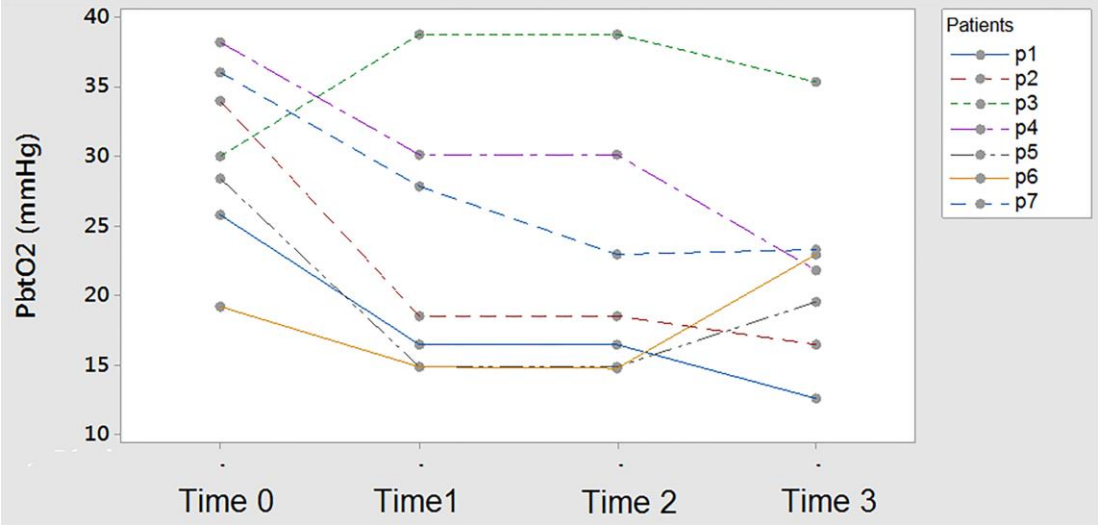

Figure 22: Individual Value Plots of $P_{bt}O_2$ versus Time.

Arterial blood gases were analysed using the alpha stat method to maintain autoregulation, which was consistent with current practice in the units that participated in the trial. This approach has been undertaken in other contemporary hypothermia trials, such as NABISH II and TTM (280, 361). There was no significant difference in the pre-cooling and target temperature $PaCO_2$ and PaO_2 values, which is important given the dependence of $P_{bt}O_2$ upon systemic oxygenation and transport (110). If the decrease in $P_{bt}O_2$ was due to changes in $PaCO_2$ and PaO_2 with temperature (Gay-Lussac's Law), I would expect to see changes in PaO_2 and $PaCO_2$. Therefore, the decrease in $P_{bt}O_2$ may be due to reduced CBF or a localised cause of decreased tissue perfusion.

Anaemia has been associated with a decrease in $P_{bt}O_2$ after TBI (362). There was no difference in Hb concentrations between groups, or in the intervention

group at pre-cooling or target temperature to account for the change in $P_{bt}O_2$. Two patients in the control group and three patients in the intervention group had a Hb concentration <9g/dL, the level at which $P_{bt}O_2$ might be compromised (362). However, these patients were anaemic before randomisation.

In a study of 31 patients with severe TBI cooled from ≥ 37°C to 33°C, Tokutomi et al. concluded that 35°C is the optimal temperature to reduce ICP whilst minimising unfavourable effects of hypothermia. Decreased ICP was most prevalent between 35°C and 36°C which coincided with a decrease in the incidence of jugular venous bulb oxygen desaturation. Oxygen consumption and delivery, measured through indirect calorimetry, also progressively decreased. Below 35°C oxygen delivery decreased more than the decrease in oxygen demand resulting in an overall deficit (349). A potential explanation for the decrease in ICP with hypothermia is a decrease in CBF ($PaCO_2$ related), which could also explain the decrease in oxygen delivery (227, 313). The decrease in $CMRO_2$ seen with hypothermia may be associated with decreased oxygen demand (363). Perhaps when patients are cooled below 35°C there is a decrease in ICP due to decreased CBF but there is a significant decrease in oxygen delivery causing an unwanted decrease in $P_{bt}O_2$.

Study Limitations

Due to the nature of the patient population and TH, it is not possible to blind researchers from the administration of TH. In addition, I was not blinded for the analysis of the two groups which could have led to bias of analysis.

I attempted to correct the time taken to reach hypothermia by comparing pre-randomisation values with the different time periods, but must acknowledge that the mean time to target temperature was ~7 hours. Given that early initiation of TH is considered beneficial in improving efficacy, it could be that a greater decrease in both ICP and $P_{bt}O_2$ would have been seen in the intervention group if the time to reach target temperature was reduced (364-366). In addition to this, it is likely that an intention to treat analysis would have given different results. I decided against an intention to treat analysis because of the small sample size and the majority of patients in the intervention group who would not have been hypothermic. Finally, there were missing data. The most apparent effect of the missing data is seen when analysing $P_{bt}O_2$ at Time 0 in both the control and intervention groups as described above (Appendix 1).

Conclusion and Recommendations

TH appears to be effective at reducing refractory intracranial hypertension and could potentially reduce the number of hyperosmolar therapies required but does not appear to lead to better outcomes. TH below 35°C might reduce oxygen delivery more than oxygen demand leading to reduced cerebral oxygenation (manifest in this study as a decrease in $P_{bt}O_2$). It is unclear why some patients exhibit a greater decrease in $P_{bt}O_2$ than others. Analysis of observational $P_{bt}O_2$ data from patients admitted to the Edinburgh site is ongoing.

The following recommendations for future research are based on information in the previous two chapters:

1. Emphasis on the importance of prospective multi-centre RCTs with a-priori plans of analysis and published protocols prior to patient enrolment over retrospective database analysis.
2. RCT investigating the effect of ICP monitoring versus no ICP monitoring in Western countries.
3. RCT investigating the effect of $P_{bt}O_2$ monitoring versus no monitoring in Western countries.
4. If a benefit is shown by either 2. or 3. then further RCTs analysing treatment thresholds are indicated. Equally, if harm is found then these monitoring modalities should be restricted to research.

Chapter 3: Delayed Cerebral Ischaemia after Subarachnoid Haemorrhage

Introduction

After reviewing changes in brain tissue oxygenation from traumatic brain injury I became interested in the effect of altered blood flow after acute haemorrhagic brain injuries. Specifically, my interest is in the vasoconstriction and delayed cerebral ischaemia (DCI) that occurs *after* subarachnoid haemorrhage (SAH), offering the possibility of early intervention. The remainder of this thesis concentrates on DCI after SAH and its potential causes and treatments. This chapter provides a brief explanation of the epidemiology and pathophysiology of SAH before explaining its relation to DCI. Aneurysmal SAH (aSAH) differs from TBI by having successful treatment options in the form of surgical clipping or interventional neuroradiological coiling. DCI, a complication which frequently occurs after SAH, shares many similarities with TBI: the pathophysiology underlying DCI remains elusive and there are limited treatment options. I outline current European and American guidelines on the management of SAH and DCI and discuss systematic reviews which highlight a lack of treatment for DCI. There are many potential treatments with some evidence from small, non-randomised trials, but conclusive evidence of effect is lacking. One such potential treatment, alpha calcitonin gene-related peptide (αCGRP), is alluded to in this chapter and is discussed fully in Chapter 4.

Subarachnoid Haemorrhage

Subarachnoid haemorrhage is a subtype of stroke that only accounts for about 5% of all strokes. However, because of its higher morbidity and mortality, its impact is said to equal that of ischaemic stroke, which accounts for 85% of all strokes (367, 368).

The overall incidence of SAH is reported as 6-11 per 100,000 people per year but is as high as 20 per 100,000 people per year in Finland and Japan and was reported to be as high as 21.8 by a recent study in the US (369-372).

SAH is the cause of one third of all stroke-related years of potential life lost before the age of 65. Of the 85-90% of patients who survive to reach hospital, 20-40% will die within one month of the SAH (373-377). Contemporary studies report in-hospital mortality as 18-20% but do not report mortality at one year (374-376). Approximately 70% of all people with SAH will either die or require help for activities of daily living at six months after the initial injury (367).

In addition to the clinical burden of SAH, in 2010 Rivero-Arias et al. reported an estimated economic burden of £510 million annually and a total loss of about 75,000 quality-adjusted life-years in the UK (378).

There is a female preponderance to SAH over the age of 40 (3:2 ratio), but the opposite is true below the age of 40 (379). Overall age-standardised incidence rates per 100,000 were greater in women [14.8 (13.4 to16.3)] than in men [9.4 (8.1 to 10.6)] throughout 1986 to 2005, with an overall female to male ratio of 1.59. A recent systematic review reported an overall incidence of 11.5 per

100,000 person-years in women and 9.2 in men with an overall female to male ratio of 1.24 (380).

Aneurysmal subarachnoid haemorrhage is the most common form of SAH, accounting for 85% (381). The remaining 15% are attributed to non-aneurysmal perimesencephalic haemorrhage (10%) and various rare conditions (5%) (382). Modifiable risk factors that roughly double the risk of aSAH include hypertension, alcohol excess, cocaine use and smoking (383). The inherited conditions, Ehlers Danlos Type 4, polycystic kidney disease, and familial intracerebral aneurysms are also associated with aSAH. Modifiable risk factors are thought to account for two out of every three aSAH, whilst genetic factors account for only one in every ten aSAH (384).

The Circle of Willis is the site of most aneurysms and these tend to occur at bifurcations. Ruptured aneurysms are most common at the anterior cerebral artery and the posterior communicating artery/internal carotid artery. Despite rupture risk increasing with the size of the aneurysm, most ruptured aneurysms are small (i.e. < 1 cm) (382).

There appears to be a seasonal influence on the incidence of SAH with a peak incidence in the winter and a nadir in the summer months. A 2013 systematic review of 48 articles found that "*SAH occurred less often in summer than winter (RR 0.89, 95 % CI 0.83-0.96), and was statistically significant more often in January than in the summer months of June-September*" (385). However, there are contemporary publications demonstrating no seasonal variation in

the US, an increase in incidence in the summer months in England, and winter and spring peaks in Australasia (386-390).

It is not clear why certain aneurysms rupture. An abrupt increase in transmural arterial pressure is associated in some ruptured aneurysms, but not all. Physical exercise, sexual intercourse and straining are only reported in about 20% of SAH (382).

Diagnosis

Clinical features of SAH include sudden onset headache, which occurs within seconds in 75% of patients and is the only symptom in 30% of patients presenting to general practice (391, 392). Although an instantaneous headache was traditionally thought of as the principal diagnostic feature of SAH, Linn et al. found that this was only present in 50% of patients, with nearly 20% reporting a gradual onset over five minutes (393). The headache can last for one-to-two weeks. Vomiting may be a concerning feature but it is also present in nearly half of all patients with non-haemorrhagic thunderclap headache. One in every 14 patients with SAH will have a seizure at onset, which is not a feature of non-haemorrhagic thunderclap headache or perimesencephalic headache (391, 394, 395). On admission to hospital, two-thirds of patients with SAH will have reduced consciousness and half of these will be comatose (396). Neck stiffness, which takes three-to-12 hours to appear, is a common symptom and is caused by inflammation secondary to blood in the subarachnoid space (382). In summary, clinical features cannot be relied upon to differentiate between SAH and other similar causes of

severe, acute headache. Therefore, radiological and laboratory investigations are often required to rule out SAH.

Traditionally, if a non-contrast CT head did not identify SAH then a lumbar puncture ≥12 hours after the onset of the headache, was performed to look for the presence of xanthochromia in the CSF. However, if performed within six hours of the onset of headache, a third generation non-contrast CT head has been reported as 98.5 -100% sensitive at identifying SAH (397, 398). Perry et al., in a multicentre prospective cohort study of 2,179 patients, found that of the 953 patients who had a CT head ≤6 hours after the onset of headache, all 121 with SAH were identified (397). Backes et al., in a single centre retrospective study of 250 patients, found that of the 69 patients with a negative CT head ≤6 hours from the onset of headache, only one had a SAH found on subsequent LP (398). Blok et al. describe a multicentre retrospective study of 760 patients who all had CT head ≤6 hours from onset of headache with a subsequent LP ≥12 hours (399). 52 of the 760 patients had bilirubin on LP but no SAH was identified on CT head. Only one of these 52 patients had a perimesencephalic nonaneurysmal SAH, none of them were found to have an aneurysmal SAH. In all three of these studies, there were no deaths or negative outcomes in patients who had a negative CT head performed ≤6 hours after the onset of headache. Furthermore, no SAHs were missed in patients with normal neurology and a normal CT head at ≤6 hours. A subsequent paper by Sayer et al., looking at 2,248 patients who underwent LP after a normal CT head, demonstrated that for every 250 LPs performed after a negative CT head, one cerebrovascular abnormality will be detected

(number needed to tap = 250) (400). Therefore, it has been argued that in patients with a normal CT head ≤6 hours after the onset of headache, no focal neurological deficit and a normal GCS, a decision to discharge the patient without performing a LP is reasonable. On positive identification of SAH on CT or LP, a CT angiogram is performed to identify the location and configuration of the aneurysm related to adjacent arteries.

		Sensitivity % (CI)	**Specificity % (CI)**	**NPV % (CI)**	**PPV (% (CI))**
Perry et al. 2011	Head CT Overall	92.9 (89.0-95.5)	100 (99.9-100)	99.4 (99.1-99.6)	100 (98.3-100)
	≤6 hours (953 patients)	100 (97-100)	100 (99.5-100)	100 (99.5-100)	100 (96.9-100)
	≥6 hours (2179 patients)	85.7 (78.3-90.9)	100 (99.8-100)	99.2 (98.7-99.5)	100 (96.3-100)
	119 Patients had an LP after a negative CT head, of these 17 had a positive LP. Of the 17, 6 had neurosurgical intervention (coil, clipping or ventricular drain); 10 had no cause for bleeding identified; and 1 was secondary to a brain tumour				
Backes et al. 2012	Head CT Overall	95.4 (89.5-98.5)	100 (97.4-100)	96.6 (92.2-98.9)	100 (96.5-100)
	≤6 hours (137 patients)	98.5 (92.1-100)	100 (94.8-100)	98.6 (92.3-100)	100 (92.3-100)
	≥6 hours (113 patients)	90 (76.3-97.2)	100 (87.2-98.6)	94.8 (87.2-	100 (90.3-100)
	1/69 patients in the ≤6-hour group had SAH from cervical AVM on LP. 5/76 patients in the ≥6-hour group had SAH (4 aneurysmal, 1 thoracic AVM)				
Blok et al. 2015	760 (all patients ≤6 hours)	-	-	99.9 (99.3-100)	-
	All 760 CT head scans were negative, every patient received an LP. 52/760 LPs had bilirubin, only 1/760 had a perimesencephalic non-aneurysmal haemorrhage.				

Table 6: Accuracy of CT Head at Identifying Subarachnoid Haemorrhage. NPV, negative predictive value; PPV, positive predictive value. CI, confidence interval; LP, lumbar puncture; CT, computed tomography.

Grading

The most commonly used SAH grading scales are the World Federation of Neurosurgeons Scale (WFNS) and Hunt and Hess scale, which are both clinical scales, and the Fischer scale, which is CT based (401-404). These grading systems offer a means of predicting prognosis and defining severity but suffer from observer variability.

Grade	WFNS (Clinical)	Hess and Hunt (Clinical)	Fisher (CT scan)
1	GCS 15	Asymptomatic or minimal headache	No evidence of blood
2	GCS 13-14	Moderate to severe headache with no neurological deficit other than cranial nerve palsy	Diffuse blood or thin layer of blood with all vertical layers <1mm
3	GCS 13-14 + motor deficit	Drowsy, confused or mild neurological deficit	Localised clots or vertical layers of blood ≥1mm
4	GCS 7-12	Hemiparesis, decerberate rigidity and vegetative disturbances	Diffuse SAH or non-subarachnoid blood but with intracerebral or interventricular clots
5	GCS 3-6	Deep coma, moribund appearance	

Table 7: Subarachnoid Haemorrhage Grading Scales. WFNS, World Federation of Neurosurgical Societies; GCS, Glasgow Coma Scale; CT, computed tomography.

Treatment of SAH

Comprehensive review of the treatment of SAH is beyond the scope of this thesis, but a summary is presented below.

Acute management of SAH focuses on cardiorespiratory stabilisation and cerebral perfusion following resuscitation council guidelines. If necessary, this can include intubation to maintain PaO_2 >13 kPa and $PaCO_2$ between 4.5-5.0 kPa. Traditionally, it was advised that hypertension should not be treated unless MAP exceeded 130 mmHg or there was clinical or laboratory evidence of end-organ damage (382). However, 2011/12 guidance suggests that a systemic arterial pressure of <160mmHg (MAP <110 mmHg) has a lower risk of re-bleeding and that this should be the aim with a titratable agent in patients with unsecured aneurysms (405-407). Connolly et al. acknowledge the risks associated with lowering CPP but report a cohort study demonstrating no reduction in $P_{bt}O_2$ after intravenous nicardipine in neurologically critically unwell patients (408).

Definitive management of the ruptured aneurysm is done by surgical clipping or endovascular coiling. Microsurgical clipping was the main treatment of intracranial aneurysms prior to Gugliemi's description of aneurysm occlusion by endovascular approach in 1991 (409). The ISAT trial, a multicentre RCT that randomised 2,143 patients with aSAH to microsurgical or endovascular repair, found a reduction in death at one year from 31% in the microsurgery arm to 24% in the endovascular arm (24% RRR, ARR 6-9% in favour of coiling) (410). Retrospective analysis demonstrated that aneurysmal neck diameter

and dome size were strong predictors of incomplete occlusion and subsequent aneurysm recurrence (409). As such, clipping is reserved for aneurysms with a wide neck or in locations such as the middle cerebral artery (MCA) (411).

Complications

In the 1980s The International Cooperative Aneurysm Study assessed the outcomes of surgical and medical management of 3,521 patients with SAH. The authors identified vasospasm and re-bleeding as the leading causes of morbidity and mortality after the initial bleed (412). Subsequently, hydrocephalus, intraventricular haemorrhage and medical complications have been implicated as additional contributors to worse outcomes (413, 414). More recently, Lantigua et al, in a prospective single centre trial, enrolled 1,200 patients between 1996 and 2009 to revaluate the causes and mechanisms of in-hospital mortality after SAH (375). The authors found that most deaths or severe neurological disability were secondary to the direct effect of bleeding, followed by re-bleeding, medical complications and vasospasm.

Re-bleeding

After the initial haemorrhage, re-bleeding is one of the most important preventable causes of death in hospitalised patients (375, 415). The maximum risk for re-bleeding occurs early after the initial haemorrhage and early re-bleeding is associated with worse outcomes than late re-bleeding (416). One third of rebleeds occur within three hours and up to half occur within six hours of the onset of headache (417, 418). Delayed aneurysm treatment, loss of

consciousness, decreased GCS and large aneurysms are all associated with an increased risk of re-bleeding (407).

Hydrocephalus

Acute hydrocephalus is reported to occur in 20-30% (411, 419), whilst chronic, shunt-dependent, hydrocephalus is reported to occur in roughly 10-50% of patients with aSAH (407). Hydrocephalus appears more commonly in those with poor grade SAH and is diagnosed on CT. Management is with CSF diversion (EVD or lumbar drainage) (407).

Medical complications

Severe non-neurosurgical complications of SAH occur in up to 40% during hospital admission (413). Fever and hyperglycaemia are independent risk factors of poor outcome and should be treated (407, 411). In addition to these complications, there is an increased risk of venous thromboembolism, hyponatraemia and pulmonary complications. Cardiac sequelae are common with poor-grade SAH secondary to increased sympathetic outflow and a large catecholamine release after brain injury. Independent of this, there is an autonomic neural stimulation from the hypothalamus. The hyperdynamic state and increased oxygen demands of the myocardium that this causes can result in a transient ischaemia. Subsequently many patients demonstrate short-lived ECG changes such as T-wave and ST segment abnormalities. These can mimic, or reflect, cardiac ischaemia depending on whether the changes are catecholamine dependent or related to hypothalamic stimulation (420). Up to

50% of patients develop clinically significant cardiac arrhythmias and it is necessary to keep such patients on cardiac monitors and undergo aggressive intervention to treat myocardial infarction and maintain a normal cardiac rhythm (421).

Delayed Cerebral Ischaemia

DCI has been stated as a major cause of morbidity and mortality in patients who survive the ruptured aneurysm (367, 407, 412, 422, 423). Lantigua et al., in the 2015 review discussed earlier, noted that “a robust association between vasospasm and in-hospital mortality is notably absent in the present study”. Furthermore, DCI from vasospasm was only confirmed in 5% of cases in their study and DCI as a complication was not an independent predictor of death. However, the authors go on to state that there is ample evidence that cerebral ischaemia from DCI is an important cause of morbidity after SAH (375, 424, 425). DCI is still described as “the most important preventable cause of mortality and poor neurological outcome [in patients who survive the initial bleed]” (426).

One of the difficulties in assessing the epidemiology and aetiology of DCI is the definition. Previous terms included DCI, delayed ischaemic neurological deficit (DIND), delayed neurological deficit (DND), secondary ischaemia and most commonly vasospasm. Contemporary articles suggest an associative, rather than causal, relationship between angiographic cerebral vessel narrowing and neurological outcome, and highlight the possibility of a multi-factorial aetiology (422, 427-429). When assessing the incidence of DCI and

association between radiological vessel narrowing and DCI, it is not always clear whether an author is referring to DCI (based on the definitions below) when they state 'vasospasm' or merely an arterial narrowing visualised radiologically. The importance of this becomes apparent when performing a meta-analysis and trying to calculate therapeutic efficacy and diagnostic accuracy. In 2010 a consensus statement was released proposing a definition of DCI. In addition to this, the Neurocritical Care Society released a consensus definition for DCI and a separate definition of vasospasm:

- DCI is a focal neurological impairment or decrease in GCS (≥2 points) that lasts for ≥1 hour, is not apparent immediately after aneurysm occlusion, and cannot be attributed to other causes by means of clinical assessment, blood tests or imaging.
- Vasospasm is radiologically demonstrated cerebral arterial vessel narrowing with corresponding clinical symptoms and signs (430, 431).

Between days three and ten after their initial haemorrhage, 30-40% of patients will suffer from DCI, and up to half of these patients will have a poor outcome (367, 396, 432). Risk factors for developing DCI include poor grade SAH, intraventricular haemorrhage, large amounts of subarachnoid blood, additional haematomas and smoking (423, 433, 434). There is moderate evidence (two high-quality studies as defined by the referenced systematic review) for an increased risk of DCI with hyperglycaemia, hydrocephalus, history of diabetes and early systemic inflammatory response syndromes (434).

DCI has an unclear aetiology which is thought to be multi-factorial. Potential causes include cerebral vessel narrowing, early brain injury (EBI) and cortical spreading depolarisation (CSD).

Cerebral Artery Narrowing

Cerebral artery narrowing after SAH has been a focus of research since it was demonstrated by Ecker et al. on angiography over six decades ago (435). A decade after their publication, focal neurology was associated with vessel narrowing (436). Vasospasm appeared to be localised to the vascular territory of the aneurysmal bleed and proportional to blood load (403, 437). The onset of vessel narrowing commonly occurs around day three, is maximal on days six-to-ten and lasts for up to two weeks (438-440). The density, duration and volume of subarachnoid blood were found to be key predictors of vessel narrowing (403, 441). Narrowing of cerebral arteries may cause reduction in CBF distal to the constricted vessel contributing to secondary ischaemia and result in the delayed neurological impairment (442). The cause of vessel narrowing is not clear but is thought to involve an inflammatory response, decreased nitric oxide levels, oxyhaemoglobin release and an increased concentration of endothelin-1 (ET-1). Much of the evidence supporting these theories is from experimental *in-vitro* studies.

Oxyhaemoglobin

Oxyhaemoglobin causes the activation of platelets and coagulation with a local inflammatory response when it enters the subarachnoid space (443-445).

Arterial narrowing occurs when oxyhaemoglobin comes into contact with the abluminal side of cerebral vessels and is known to induce cerebral artery vasoconstriction both *in vivo* and *in vitro* in primates, which is not seen with methaemaglobin or bilirubin (407, 446-448). The mechanism by which oxyhaemaglobin does this is thought to be by decreasing the production of prostacyclin, increasing prostaglandin E2 in vessel walls and inhibiting endothelial-dependant relaxation. Lipid peroxidation is also caused by the oxidation of oxyhaemoglobin to methaemoglobin and can lead to vasoconstriction (449). Attempts to exploit these mechanisms to inhibit vessel constriction and improve outcomes have not been successful (450).

Nitric Oxide

Nitric oxide (NO) causes direct and indirect vasodilation. The decrease in bioavailability of NO after SAH is two-fold: When Hb is released after the breakdown of blood in the subarachnoid space it scavenges up available NO due to the high affinity that NO has for Hb (451, 452). In addition to this, the morphology of arteries changes after SAH such that there is a down-regulation of endothelial and neuronal NO synthase thus decreasing the production of NO (453-455). NO is then unable to counteract the effects of the vasoconstrictor endothelin 1 (ET-1) (456).

Endothelin (ET)

Endothelin is fundamental to maintaining the normal vascular tone of blood vessel walls. The concentration of ET-1 in plasma and serum increases

beyond normal levels within minutes after SAH and peaks around days three-to-four. ET-1 is the most potent endogenous activator of vasoconstriction. There appears to be an excess release of ET-1 by astrocytes around the time of onset of ischaemic symptoms and concentrations are consistently elevated in patients with DCI (457, 458). However, ET-1 concentrations within the normal range have been reported in patients with radiological evidence of cerebral artery narrowing who do not have DCI (459-461). Increased ET-1 may, therefore, signify ischaemic damage rather than cause arterial vessel narrowing in DCI (428). Treatments focussing on endothelin receptor antagonists are discussed later in this chapter.

Alpha Calcitonin Gene-Related Peptide

Alpha calcitonin gene-related peptide (CGRP) is an endogenous neuropeptide and a potent vasodilator. αCGRP appears to be released, and is subsequently depleted, after aSAH to combat cerebral vasoconstriction which has led to the theory that exogenous αCGRP may be beneficial in managing DCI (462-464). CGRP is discussed in more detail in the next chapter.

Radiological Evidence

There is a strong correlation between radiological cerebral vessel narrowing and DCI (465, 466). Brown et al. found that 31% (17 of 54) of patients with moderate-to-severe vasospasm (determined by attending neuroradiologists but the severity is not defined in the publication) had delayed infarcts, compared with only 4% (three of 80) of those without vasospasm (465). It is

unclear whether this correlation represents causation or association. As many as 70% of patients will demonstrate cerebral vessel narrowing on angiography after SAH, but only 40% of these patients manifest neurological deficits and only 30% are diagnosed with DCI (467-470). There are several possible reasons for this. Cerebral vessel narrowing is not synonymous with DCI and hence the need for precise definitions (431). The patients who are most likely to develop DCI (WFNS Grades IV and V) are difficult to assess clinically due to being sedated and mechanically ventilated (423). As such, DCI is probably under-diagnosed in these patients. Furthermore, neurological deficits may not be directly proportional to the degree of radiological vessel narrowing (442).

Vasospasm, as identified by transcranial Doppler (TCD) and angiogram, only has a positive predictive value of 67% for identifying cerebral infarcts on CT (471). One might expect this to be higher if cerebral vessel narrowing was the primary cause of DCI. However, there are a few points to consider. Firstly, cerebral infarction on CT is not required for a diagnosis of DCI (431). Secondly, the rate of cerebral infarction on CT in patients with cerebral vessel narrowing ranges between 24-35% (472, 473) but is as high as 81% on MRI (474) suggesting that we may not be identifying cerebral ischaemia after DCI on CT images. Another inconsistency with correlating infarcts and vessel narrowing is that as many as 25% of delayed infarcts on CT either occurred in patients who had no evidence of vessel narrowing or occurred in separate territories to the narrowing (465, 475, 476). Angiogram and TCD are not 100% sensitive in identifying vessel narrowing and one study found that these imaging modalities only agreed on the diagnosis of vasospasm in 73% (95% CI, 63% to 81%) of

cases. Therefore, it may be that vasospasm was underdiagnosed in patients who were later found to have delayed infarcts on CT (471).

It is unclear whether microvascular constriction is the cause of DCI and there may be a stronger correlation between microvascular constriction and decreased regional CBF than there is with large vessel constriction (477-479). Pial arteries constrict on exposure to blood or microtrauma in animal studies (480) and constriction of intraparenchymal arterioles occurs after aSAH which may contribute to DCI (479). Spectral imaging has demonstrated constriction of small vessels within the first 72 hours after aSAH and cerebral arterioles have been directly observed contracting after aSAH (481, 482). Catheter angiography and TCD may not be identifying this microvascular vessel narrowing occurring in patients with DCI (483).

CT perfusion (CTP) and DSA (digital subtraction angiography, the gold standard for identifying cerebral vessel narrowing) are reported to have similar test characteristics according to one retrospective comparative study (484). CTP is reported to have an 84% sensitivity, 79% specificity and 88% positive predictive value for identifying DCI (485). The rate of CTP deficits is higher in patients with DCI and such patients exhibit worse cerebral perfusion (on CTP) than in those without DCI, even before they developed focal neurology (486). CTP has also shown that it is possible for areas of poor perfusion to recover, giving hope that DCI could be partially reversible (487).

Early Brain Injury

Damage that occurs to the brain within the first 72 hours after the haemorrhage is termed Early Brain Injury (EBI). Due to the timing of damage, most of the evidence supporting this theory is from experimental data suggesting physiological changes that occur after the haemorrhage. Following the haemorrhage there is intracranial hypertension and subsequent decrease in CPP which halts CBF and causes cerebral ischaemia and oedema (414, 488, 489). At ictus, the intracranial hypertension may exceed SBP and the rate of increase and peak ICP appears to be proportional to the amount of arterial blood extravasating into the subarachnoid spaces from the aneurysm (490-492). Subsequent hydrocephalus and CSF outflow obstruction further elevates the ICP (493, 494). Intracranial hypertension appears to occur in two ways after SAH. The most commonly observed form is an increase in ICP to about the arterial diastolic pressure which then decreases to just above the patient's baseline ICP (491). This usually reflects a small volume haemorrhage with cerebral oedema. The second form is a sustained increase in ICP secondary to acute hydrocephalus or a progressive haematoma (493, 494).

Cerebral oedema after SAH is a poor prognostic indicator. It is often present on admission CT scans and by day six it is present in up to 20% of patients (414, 495, 496). Cerebral autoregulation is frequently impaired after SAH (60, 497, 498) and can become dependent on CPP and blood viscosity. As such, any change in ICP or systemic arterial pressure can worsen oedema and ischaemia. Generalised cerebral ischaemia occurring shortly after the SAH

may cause disruption to the BBB and an inflammatory response, both of which contribute to cerebral oedema. In addition to this, cell death mechanisms are initiated and neuronal cell death appears to occur as early as 40 minutes after the haemorrhage (499-501). High concentrations of pro-inflammatory cytokines and vasoactive factors such as interleukin 6 and tumour necrosis factor-α in the CSF and serum correlate with increased frequency of DCI and negative outcomes after SAH (502, 503).

As noted above, oxyhaemoglobin may contribute to DCI and it has been argued that removing blood from the subarachnoid space may improve outcomes (449, 504). Intrathecal administration of thrombolytics and continuous cisternal drainage have been investigated with reports of success. Results of the EARLYDRAIN trial comparing continuous lumbar-CSF drainage with standard treatment are awaited (trial completed but results not yet published) (505, 506). A meta-analysis investigating the use of intrathecal thrombolytics suggested a reduction in the incidence of DCI but these findings were not statistically significant after excluding one study, which included intrathecal nimodipine in addition to thrombolytic therapy (507).

One of the limitations with the EBI theory is that it relies heavily on experimental data. Without evidence to support the theory in a clinical setting it is difficult to know how much of this data can be translated to humans (508, 509). Furthermore, the theory involves multiple complex pathways which are individually described but likely related. It may be that no treatment effect is observed when a single-target model is used (510). The results of a systematic

review and meta-analysis of intracranial in vivo animal studies of EBI and delayed cerebral arterial vessel narrowing after aSAH are awaited (511).

Cortical Spreading Depolarisation

Cortical spreading depolarisation as described by Leao in 1944, is a gradual depolarisation across grey matter at a rate of 2-5 mm/min (512). CSD has been investigated in TBI, migraine, malignant stroke, SAH and intracranial haemorrhage and is not a new theory (513, 514). The mechanism is thought to involve Na^{+} and Ca^{2+} pumps of a cellular membrane being exceeded by a cation influx. This is then followed by water entering the cell and decreasing the extracellular space by roughly 70%, resulting in depressed EEG activity (hence the alternative name, cortical spreading depression) (512, 515). MRI during migraines have shown what is thought to be a 'spreading oligemia', a wave of decreased blood flow propagating across the brain (516). To counteract the passive influx of cations across the membrane, the ATP-dependent Na^{2+} and Ca^{2+} pumps must expend more energy, which requires an increase in CBF. It is thought that in healthy tissue CSD causes a transient hyperperfusion, but when there is a dysfunction of the vasculature, microvascular spasm can occur causing 'cortical spreading ischaemia' (515). In an experimental model, Shin et al. demonstrated that peri-infarct spreading depolarisations caused vasoconstriction and reduced CBF in the ischaemic cortex and expanded infarct size (517). The timing of CSD episodes after SAH coincide with the chronology of DCI, occurring between days five-to-seven after the haemorrhage, suggesting that CSD is associated with the

development of DCI (518). There is limited clinical evidence that repeated CSD appears to occur before DCI with little evidence of 'vasospasm' on DSA, albeit in a small sample (13 patients) (519). The main difference between cerebral vessel narrowing and CSD as a cause for DCI appears to be that the vessel narrowing caused by CSD is microvascular (whereas most of the vessel narrowing originally attributed to vasospasm is large vessel narrowing seen on angiography and TCD). The biggest problem with both theories is that so far no treatment to ameliorate this vessel narrowing has been shown to be of any benefit.

Treatment of DCI

Single H Therapy: Is Induced Hypertension the Key?

Induced hypertension has been used for symptomatic DCI since the 1970s and was bundled together with hypervolaemia and haemodilution as 'triple H therapy' in the 1980s (520, 521). Induced hypertension may improve neurological symptoms, and the use of vasopressors to augment BP is still used as a treatment for DCI (522). Evidence to support hypervolaemia and haemodilution is lacking (521-524). Some small (n<25) clinical and experimental trials have observed an increase in CBF after a 0.9% sodium chloride bolus of 15ml/kg/hour, and vasopressor-induced hypertension has improved CBF, $P_{bt}O_2$ and neurological deficits (525-529). A systematic review from 2011 (ten publications, no RCTs) found that hypertension was associated with increased CBF and neurological symptom reversal was seen in two-thirds of treated patients (522). Unfortunately, like many of the interventions in SAH,

there has been no published RCT evaluating the efficacy of induced hypertension in the treatment of DCI. The HIMILAIA (hypertension induction in the management of aneurysmal subarachnoid haemorrhage with secondary ischaemia) trial, a multi-centre RCT investigating the effect of induced hypertension in the treatment of aSAH, was terminated early due to insufficient enrolment and it seems unlikely that there will be another such trial (530). Pilot data from the HIMILAIA trial showed that noradrenaline-induced hypertension was not associated with a statistically significant increase in CBF when compared with controls (CBF assessed by CT perfusion, mean increase in SBP 12 mmHg, n=25) (531). The American Heart/Stroke Association (AHA/ASA), in their 2012 guidelines, advise that hypertension is recommended in patients with DCI unless they are hypertensive at baseline or their cardiac status (no definition given) precludes it. They list this as a Class I recommendation (benefit thought to be greater than risk) with level B evidence (evidence from non-randomised studies) (407). The 2013 European Stroke Organisation guidelines acknowledge that there is no evidence from controlled studies for induced hypertension or hypervolaemia to improve outcome in patients with DCI (Class IV: uncontrolled studies, case series, case reports or expert opinion). Furthermore, they go on to state that induced hypertension is associated with increased cerebral oedema, haemorrhagic transformation in areas of infarction (532), reversible leukoencephalopathy (533), myocardial infarction and congestive heart failure (534). Despite this, hypertension is still used as a treatment for DCI (426).

Hypervolaemia may cause worse outcomes in patients with aSAH (526). Prophylactic hypervolaemia to maintain CPP was shown not to prevent DCI and had no improvement on CBF (535). It has also been associated with increased risk of pulmonary oedema, decreased $P_{bt}O_2$, and has been found to worsen outcomes (526, 536, 537). As such, the Neurocritical Care Society guidelines recommend isotonic fluids to maintain a euvolaemic state and avoiding hypervolaemia (430).

A restrictive strategy to maintain a haemoglobin level above 70 g/L after SAH is advocated based on evidence from an RCT on general critical care patients that was published in 1999 (538). Anaemia is present in up to half of all patients with SAH and is associated with brain tissue hypoxia and worse outcomes (539-541). Neurocritical care society guidelines advise a transfusion threshold of 80 g/L in SAH patients without DCI and 90-100 g/L in those with DCI unresponsive to first-line therapies (430). The ongoing RCT SAHaRA (Aneurysmal Subarachnoid Hemorrhage: Red Blood Cell Transfusion and Outcome) aims to evaluate red cell transfusion from 100 g/L down to 80 g/L in aSAH patients and determine the most appropriate target (542). The same research team are also conducting a systematic review and meta-analysis to summarise the existing evidence regarding red cell transfusions in aSAH patients (543).

Nimodopine

A 2007 Cochrane review assessed sixteen trials (n = 3,361) and found that calcium channel antagonists reduced the risk of poor outcome after aSAH

(relative risk 0.81, 95% CI 0.72 to 0.92, NNT 19) (544). However, the only statistically significant evidence was for oral nimodipine. Oral nimodipine remains the only intervention with evidence from RCT and meta-analysis that reduces the incidence of secondary ischaemia and improves outcomes after aSAH (RR 0.67, CI 0.55 to 0.81). The evidence for oral nimodipine is based on one large double-blind RCT, the British Aneurysm Nimodipine Trial of 1989. The trial enrolled 554 patients with aSAH who received surgical clipping between 1985 and 1987 and randomised them to placebo (n = 276) or 60mg oral nimodipine every four hours for 21 days (n = 278). No patients received radiological coiling. Treatment was started within 96 hours of SAH (main exclusion criteria was admission to the neurosurgical unit over 96 hours after SAH). In patients given nimodipine the incidence of cerebral infarction on CT was 22% (61/278) versus 33% (92/276) in the controls, a relative reduction of 34% (95% CI, 13-50%). Poor outcomes at three months were also reported to be reduced by 40% in the nimodipine group (relative risk given in paper, 95% CI, 20-55%) (25). Without data from this trial the results of the Cochrane review were no longer statistically significant (407). Oral nimodipine, 60mg every four hours for three weeks, is now standard care due to its possible benefit, modest risks and low cost. The Cochrane review found no statistically significant results to support the use of other calcium channel antagonists, magnesium sulphate or intravenous nimodipine (544).

Calcium channel antagonists were initially explored as an aid to post-ischaemic reperfusion because increased calcium in vascular smooth muscle cells was thought to cause vasospasm (545, 546). However, nimodipine's

action seems independent of any effect on large vessel narrowing as determined by angiography (547, 548). The mechanism of action of nimodipine also appears to be unique to aSAH as demonstrated by a 2006 systematic review which found no benefit from nimodipine after traumatic SAH (549). Furthermore, it was thought that nimodipine may exert its effect by stopping calcium influx at a neuronal level, but no beneficial effect has been seen from administration in patients after ischaemic stroke or TBI (550-552). There are two properties unique to nimodipine which could explain why it appears to improve outcomes whilst other calcium channel antagonists do not. Firstly, nimodipine may reduce the incidence of microthrombosis by increasing endogenous fibrinolytic activity (553). Secondly, there is experimental evidence that intravenous nimodipine decreased cortical spreading ischaemia induced by red blood cell products (554). It may be that these properties are responsible for the improved outcomes seen with nimodipine after SAH. Alternatively, it may be that the benefits from nimodpine seen in the British Aneurysm Nimodipine trial are not reproducible and are not reflective of patients receiving coiling.

Intrathecal Calcium Channel Blockers

Nicardipine, a dihydropyridine calcium channel blocker, has been administered via the intrathecal route with varying results. One case series of 177 patients examined the effects of intrathecal nicardapine (555). Patients with Fisher grade III aSAH undergoing aneurysmal clipping and cisternal drainage within 48 hours of the aSAH received 4 mg intrathecal nicardipine every 12 hours on

days three-to-14 post-operatively. 11.3% of these patients had radiographic evidence of vessel narrowing and 5.7% had clinical signs of DCI. The authors note a significant reduction in 'vasospasm' but also recognise that 18.6% of patients required a shunt operation. Shibuya et al. demonstrated a decreased incidence of DCI and angiographic vessel constriction by 20 and 26% respectively after prophylactic administration of 2mg IT nicardipine via a cisternal drain when compared with control patients (556). More recent trials also report positive findings but are limited to cases of refractory 'vasospasm' and have very small sample sizes (n=<10) (557, 558). However, nicardipine is associated with probable vasodilation-associated headaches, intracranial infections and hydrocephalus. Positive long-term outcomes from large RCTs are lacking. The NEWTON trial is a phase I/IIa multicentre RCT administering IT nimodipine microparticles in patients with aSAH (559). The trial uses EG-1962, a sustained delivery system of nimodipine in microparticles. These will be injected into the ventricles through an EVD in patients undergoing coiling or clipping of ruptured aneurysms. It is thought that systemic effects are less likely to occur as nimodipine concentrations are much lower in the plasma than CSF (560).

Magnesium

Hypomagnasaemia is common after SAH and has been associated with more cisternal and ventricular blood, a longer duration of unconsciousness and a worse WFNS score at admission. Hypomagnasaemia occurring between days two-to-12 also appears to predict DCI (561). Magnesium sulphate, a non-

competitive inhibitor of calcium channels, has been shown to attenuate cerebral vessel narrowing and reduce the size of ischaemic lesions in experimental models of SAH (562). Unfortunately, trials such as IMASH and MASH-2, which recruited 1,531 patients between them, demonstrated no benefit in clinical outcome from intravenous magnesium sulphate (563, 564). A subsequent meta-analysis concluded that there was no benefit from prophylactic magnesium sulphate in this group of patients and a post-hoc analysis of the IMASH trial reported worse clinical outcomes with high plasma concentrations of magnesium (565, 566). As such, the Neurocritical Care Society guidelines now advise against the routine use of magnesium in patients with aSAH (430).

Endothelin-antagonists

Endothelin antagonists have been shown to significantly reduce cerebral vessel narrowing. Unfortunately, they have not been shown to improve neurological outcomes (567-570). These results, and a 2011 meta-analysis of pharmacological interventions treating vasospasm, have led contemporary authors to question the relevance of vessel narrowing and look toward a multi-factorial aetiology for DCI. The 2011 meta-analysis examined 14 studies (n = 4,235) and found that despite a reduction in radiological vasospasm (RR 0.80, 95% CI 0.70 to 0.92), there was no statistically significant effect on adverse outcomes (RR 0.93, 95% CI 0.85 to 1.03) (567). The authors acknowledge difficulties with the variety of DCI definitions and conclude that the lack of improved outcomes could result from methodological problems, sample size,

insensitivity or poor clinical outcome measures (567). The CONSCIOUS trials (1, 2 and 3, Clazosentan to Overcome Neurological Ischaemia and Infarction Studies) were multicentre RCTs investigating the effect of the endothelin antagonist, clazosentan, on vasospasm after aSAH. CONSCIOUS-1 demonstrated that 15mg/h of clazosentan reduced moderate to severe angiographic cerebral vessel narrowing assessed by blinded evaluation of DSA (RRR 65%, 95% CI, 47% to 78%; P<0.0001) (570). Clazosentan was investigated further in patients receiving surgical clipping (CONSCIOUS-2) or coiling (CONSCIOUS-3) for aSAH. In CONSCIOUS-2, patients were randomised to clazosentan 5mg every hour (n = 768), or placebo (n = 389), for up to 14 days. The trial showed that clazosentan at 5mg/hour had no significant effect on morbidity or mortality, incidence of new infarcts or DCI. Furthermore, clazosentan was associated with an increased incidence of hypotension, pulmonary complications and anaemia (568). CONSCIOUS-3 was stopped early due to the results from CONSCIOUS-2 (569). However, before being stopped, CONSCIOUS-3 randomised 571 patients (189 to placebo; 194 to 5mg/h of clazosentan; and 188 to 15mg/h clazosentan). The 15mg/h group had a significantly reduced incidence of vasospasm-related morbidity and all-cause mortality compared with the placebo group (15 vs 27%; p = 0.007). No effect on clinical outcome was detected (as measured by the extended Glasgow Outcome Scale). The authors argue that, due to being stopped prematurely, the study was underpowered to detect a difference in clinical outcome (569).

A systematic review and meta-analysis, investigating endothelin receptor antagonists after experimental aSAH in animal studies, found no improvement in functional outcomes despite changes in cerebral vessel diameter (571). The review concluded that vasospasm is not a clinically relevant outcome measure in experimental aSAH studies and that there was insufficient animal data to support progression to the CONSCIOUS trials (571). The REVERSE (Evaluation of the Efficacy and Safety of Clazosentan in Reversing Cerebral Vasospasm in Adult Subjects with Aneurysmal Subarachnoid Haemorrhage) trial is an ongoing prospective, multicentre Phase II trial aiming to assess whether clazosentan can reverse angiographically-confirmed cerebral vasospasm in patients with aSAH (Clinical trials: NCT02560532). Outcome measures include successful reversal of angiographic vasospasm within three hours post-administration and reversal of global vasospasm within 24 hours post-administration.

Nitric oxide donors

Sodium nitroprusside and nitroglycerine (NO donors) have been shown to reverse cerebral artery narrowing (537, 572). However, these NO donors cause hypotension and expose NO to oxyhaemoglobin and deoxyhaemoglobin leading to the formation of methaemoglobin, S-nitroso-haemoglobin and ferrous-nitrosyl-haemogloin (453). Unfortunately, although intravenous, intra-arterial and intrathecal routes of administration have been tested, NO has a limited role in DCI treatment. Furthermore, the bulk of evidence to support NO donors is from animal studies investigating ischaemic

stroke and more research is needed (573). Inhaled NO is a selective pulmonary vasodilator and, contrary to intravenous use, does not cause hypotension. A reduction in ischaemia-reperfusion injuries in extrapulmonary organs after cardiac injury has been demonstrated in experimental studies of NO inhalation. These have also been supported by proof-of-concept human trials (574). As such, NO inhalation is being investigated as a post-cardiac arrest treatment and it has been suggested that investigation of NO as a treatment of secondary brain injury is warranted based on this evidence (575).

Endovascular Therapy

There is a large amount of conflicting evidence to dispute cerebral vessel narrowing as the primary cause, or predictor, of DCI (422). Evidence to support the involvement of vessel narrowing in DCI comes from endovascular therapy, one of the interventions for DCI that may show some promise. Endovascular therapy has become common practice in some centres (375, 426, 576-578) and can be divided into intra-arterial infusion of vasodilators (most commonly verapamil or nimodipine) and mechanical dilation (375, 426, 576-578). A 2015 review looking at the frequency and variability of endovascular treatments in 32 countries found that angioplasty was performed by 83% of non-US responders and 91% of US responders in the treatment of vasospasm (578). The AHA/ASA guidelines for the management of aSAH acknowledge endovascular intervention for patients with DCI not responding to haemodynamic augmentation, but do not offer guidance on its use (407). The success rate of percutaneous transluminal angioplasty (PCTA) for proximal

vessels has been reported as over 90% and the effect is persistent (579). There is documented improvement in CBF after PCTA and evidence from observational studies that intervention within two hours from neurological deficits results in better outcomes (580, 581). A 2011 systematic review and meta-analysis of 49 articles relating to interventional techniques to treat vasospasm did find an improvement in outcomes. 24 of the 27 publications (n = 1,028) reporting the use of transluminal balloon angioplasty demonstrated not only an improvement in vessel diameter, but also an improvement in neurological deficits. Twelve case series reported good angiographic and clinical results in those administered papaverine approximate to the region of vessel narrowing (582). Both techniques were associated with significant side effects and the quality of the studies was reported as very low to moderate (based upon the GRADE classification system) (583). There has been one published RCT which looked at PTCA as a treatment in patients with low-grade SAH and large amounts of cisternal clot and there have been no RCTs investigating the various intra-arterial vasodilators (584). There does appear to be weak evidence to support the use of endovascular therapy in the treatment of DCI with radiological evidence of cerebral vessel narrowing. However, robust RCTs are needed for both PCTA and intra-arterial vasodilators. These treatments are currently reserved for patients with DCI refractory to medical interventions, the benefit of which is not without doubt (426). Any treatment benefit of endovascular therapies may be masked by slow intervention times. Therefore, any future RCT must focus on timely diagnosis and delivery.

The evidence supporting endovascular therapies raises a few questions. Firstly, is the evidence strong enough to support endovascular intervention or are small sample studies exaggerating the treatment effect? High-quality RCTs are needed. Secondly, if there is a genuine beneficial effect from endovascular treatment, why is there not the same improvement in outcomes with pharmacological intervention? It has been suggested that there is an early acute peripheral vasospasm of the microvasculature, not detectable by conventional angiography, and a later, more localised vasospasm (585). It may be that early endovascular intervention, targeted to patients with radiological evidence of vasospasm, is beneficial because it is treating localised ischaemia. Pharmacological intervention, not targeted at patients with localised ischaemia, is not beneficial in those with global ischaemia. Alternatively, it may be that any benefit from pharmacological intervention is masked by negative side effects.

Thromboembolism

There appears to be increased levels of von Willebrand factor and platelet-activating factors after SAH, which may contribute to the increased thromboemboli seen in patients with DCI (586-589). An autopsy study of thromboemboli after aSAH in 28 patients demonstrated a significant increase in microclot burdens and histological evidence of ischaemia in those with DCI (589). The authors of that study argue the finding suggests that microthrombi may contribute to DCI. There is also evidence supporting this theory from trials of tranexamic acid in the treatment of rebleeding after SAH. The incidence of

post-aSAH rebleeding has been significantly reduced following tranexamic acid administration. However, tranexamic acid may have caused an increased incidence of DCI separate from large vessel narrowing, possibly by inducing microthrombosis (590-593). Trials of antiplatelet agents in the management of microthrombosis after aSAH have been largely negative, including those investigating prophylactic low molecular weight heparin (594, 595). The ULTRA trial (ultra-early tranexamic acid after subarachnoid haemorrhage) is a multicentre RCT randomising patients to standard treatment or standard treatment plus tranexamic acid within the first 24 hours (596). The primary outcome is functional outcome measured on the modified Rankin Scale. The trial is ongoing (NCT02684812).

Summary

The true aetiology and epidemiology of DCI is not yet understood (375). A comprehensive review of 223 publications in 2006 found 41 randomised trials investigating 16 different treatments for DCI. Perhaps unsurprisingly, the review demonstrated that the only proven therapy for DCI was oral nimodipine (450). A subsequent 2016 review reported 11 interventions which have not been demonstrated to improve neurological outcomes after SAH (426). Even the effect of oral nimodipine is not without doubt, although I suspect it is extremely unlikely that a further study to assess its efficacy will ever be performed. Another systematic review, also from 2016, assessed all relevant articles published between January 1st, 2010 and December 1st, 2015 (49 studies) and gives a more positive outlook. One of the findings from this review

was that only 18% (9/49) of the included studies had a sample size exceeding 100 patients, 53% had a sample size less than 50 (26/49) and the authors acknowledge that many of the treatments remain to be tested in larger RCTs (597). Ongoing trials not mentioned in the above text include those investigating oral sulforaphane (as SFX01, NCT 02614742); subcutaneous Kineret (an interleukin-1 receptor antagonist, ISRCTN 45931429); intraventricular injection of nimodipine (560); and intraventricular fibrinolysis (ISRCTN 13230264).

The radiological evidence for DCI is conflicting at best. Endovascular therapy strongly supports vessel narrowing as a cause of DCI but a large multi-centre RCT is needed which seems likely to face recruitment difficulties. The disease almost certainly has a multi-factorial aetiology. Of the three theories explaining the cause of DCI, it is my opinion that cerebral vessel narrowing is the most tangible and the most likely to yield a treatment modality that improves outcomes.

One intervention which showed promise in the early 90s was αCGRP. This peptide has been investigated intravenously in humans and administered into CSF in animal studies with some evidence of improved neurological outcomes. The following chapter discusses αCGRP in more detail.

Chapter 4: Calcitonin Gene-Related Peptide

In Chapter 3 I summarised previous research into treatments for DCI and emphasised that the only intervention with RCT/meta-analysis evidence of efficacy is oral nimodipine. There is a need, not only for a better understanding of DCI, but also an intervention that shows improvement in mortality and neurological outcomes. One potential intervention which interests me is alpha calcitonin gene-related peptide (αCGRP), an endogenous peptide and potent microvascular dilator. αCGRP was trialled in the early 90s and found to be effective at dilating cerebral vessels after SAH, both in animal studies and human trials. After a European RCT assessing its effect in 1992, further research has been lacking. In this chapter I describe the current understanding of the normal physiological role of αCGRP and give a brief outline of its role in disease. There is a systematic review and meta-analysis of the effect of αCGRP on cerebral vessel narrowing after SAH in animal studies and a summary of the three previous human trials of αCGRP after aSAH. I present my findings from a retrospective study which analysed CSF concentrations of αCGRP in patients with suspected aSAH undergoing LP to assess for xanthochromia. Finally, the protocol for a dose-finding and tolerability study in humans with aSAH is included.

Calcitonin Gene-Related Peptide

Calcitonin gene-related peptide is an endogenous 37-amino acid neuropeptide that is mainly associated with C and Aδ sensory fibres. These fibres have

nociceptive functions but also have a perivascular efferent function. In addition to the association with sensory fibres, CGRP is localised to non-neuronal tissues. The peptide has been investigated for its involvement in hypertension, atherosclerosis, heart failure, wound healing, migraines and SAH and there are multiple comprehensive reviews of its function (598-601). Despite this, the function of CGRP is still not fully understood.

CGRP was discovered in the early 1980s when alternative splicing of the calcitonin gene in the thyroid of ageing rats lead to CGRP production (602, 603). It was subsequently isolated in the plasma of patients with medullary thyroid cancer (604).

Key findings around this time included CGRPs presence in neuronal tissue, its potent vasodilatory properties and its expression and activation in the cerebral circulation (605, 606).

In humans, CGRP exists in α and β isoforms (occasionally referred to as CGRP I and II) which are synthesised from two genes on different sites of chromosome 11 (607). These genes are CALC (calcitonin) I and CALC II. CALC I undergoes alternative splicing to form either calcitonin or αCGRP. The CALC II gene is responsible for the production of βCGRP (608, 609). Aside from three amino acids, the α and β isoforms of CGRP are identical in humans and share similar biological properties (609, 610). Although there appears to be a degree of overlap, αCGRP is present throughout the central and peripheral nervous system, whereas the β isoform is expressed in the enteric nervous system. The β form was thought to be restricted to the enteric system,

and it is expressed up to seven-fold more than αCGRP in the intestine (611). However, βCGRP has been shown to be released with αCGRP in the vascular system to a lesser extent and has been found in the adventitia of mesenteric arteries in animal studies (612, 613). The detailed structure of CGRP with its amino acid sequence and its antagonist, $CGRP_{8-37}$, have been extensively covered elsewhere (598, 599).

Experimental models demonstrate upregulated CGRP synthesis following nerve damage and in tissues undergoing an inflammatory response (614). Capsaicin, the chili extract which causes pain and erythema on contact, is able to cause the release of CGRP and another neuropeptide, substance P (615, 616). It is thought that this occurs via capsaicin's action on transient receptor potential vanilloid 1 (TRPV1) receptors found on sensory C and Aδ-fibers. Capsaicin acts as a TRPV1 receptor agonist and can lead to the depletion of neuropeptides such as CGRP from nerve terminals (614). After such depletion, nerve growth factor (NGF) is required in the synthesis of new neuropeptides, such as CGRP. NGF is released from local inflammatory cells such as macrophages and keratinocytes and it is thought that NGF is the primary stimulant of CGRP synthesis following nerve damage and inflammatory responses (614, 617).

CGRP is stored in vesicles within the nerve terminal and is released after depolarisation by calcium-dependent exocytosis, a mechanism identified by the use of capsaicin, but other TRPV1 agonists, such as rutaecarpine and anandamide have also been shown to cause release of CGRP (618-621).

Rutaecarpine-mediated CGRP synthesis is associated with a hypotensive and cardioprotective response (620) whereas anandamide-mediated synthesis has been associated with mesenteric vasodilation (622). Other agents have been implicated in CGRP synthesis to a lesser extent, such as transient receptor potential ankyrin 1, angiotensin II and noradrenaline (598, 623).

αCGRP has a half-life of approximately seven-to-ten minutes in the human blood circulation, and there are multiple theories regarding its metabolism (624, 625). Substances implicated in the degradation of CGRP include tryptase (626), the endopeptidase neprilysin (627, 628), insulin-degrading enzyme (628, 629) and endothelin-converting enzyme 1 (629). In addition to these possible routes of metabolism, there is some evidence to support reuptake of CGRP into nerve terminals. An experimental study investigating CGRP-induced dilation of basilar arteries repeatedly administered capsaicin to cause CGRP release from perivascular terminals of capsaicin-sensitive nerve fibres. After three capsaicin challenges and a loss of vasodilatory response, the CGRP-depleted segments of basilar artery were incubated with CGRP. After incubation, there was a return to the previous levels of capsaicin-induced vasodilatory response suggesting that CGRP reuptake had occurred (630). Other experimental studies have found similar responses (631, 632).

The structure and function of the CGRP receptor is covered in-depth elsewhere (598, 599) but a brief summary is given here. G protein-coupled receptors (GPCR) are cell-surface proteins that interact with an extracellular stimulus and transduce the stimulus to produce a reaction inside the cell (633).

Calcitonin receptor-like receptor (CLR) is a type of GPCR and is the main functional part of the CGRP receptor. However, CLR requires receptor activity-membrane proteins (RAMP) to bind with CGRP and transduce a signal (634). There may be two, or more, distinct CGRP receptors, $CGRP_1$ and $CGRP_2$. The distinction between these two receptors is based upon their different responses to the CGRP antagonist $CGRP_{8-37}$. $CGRP_1$ is strongly antagonised, whilst $CGRP_2$ is not. $CGRP_1$ is made up of CLR and $RAMP_1$ and is thought to be responsible for cardiovascular effects, as evidenced by positive inotropic effects in rat and guinea pig atrium (635, 636). $CGRP_2$ is less well defined.

αCGRP is the most potent microvascular vasodilator known with an effect up to ten times higher than potent prostaglandins and up to 100 times greater than vasodilators such as ACh and SP (637). Picomole amounts of αCGRP cause increased blood flow and erythema in human skin which lasts between five-to-six hours (638). This dilatory effect has been demonstrated in kidney, coronary and cerebral vessels and is inhibited by CGRP receptor antagonists (600). CGRP causes a reduction in systemic BP in both animal and human studies with hypertensive and normotensive subjects (639-641). In what is thought to be a response to the hypotension caused by CGRP, there is a positive inotropic and chronotropic effect after intravenous administration in animal studies (642, 643). When CGRP is administered into the cerebral ventricles in animal studies it causes hypertension by activation of noradrenergic sympathetic nerves (644).

CGRP is thought to cause vasodilation by endothelium- and nitric oxide (NO)-dependent and -independent pathways. The independent pathway is most common. After administration of CGRP there is a rise in cyclic adenosine monophosphate (cAMP) and relaxation occurs without endothelium. This suggests that CGRP directly stimulates adenylate cyclase in smooth muscle to cause cAMP production. cAMP then causes phosphorylation and opening of K^+ channels leading to relaxation. This action is persistent in animal and human studies of cerebral vessels that have been stripped of endothelium (645, 646).

Dependent pathways involve CGRP acting on an endothelial receptor to increase cAMP and production of NO. NO enters smooth muscle cells and activates guanylate cyclase which promotes cGMP and ultimately vasodilation (647).

Involvement in Disease

CGRP has been investigated for its potential role in pulmonary hypertension, heart failure, atherosclerosis, sepsis, neurogenic pain, skin conditions, ageing, arthritis, diabetes and obesity (598). I have discussed the application of CGRP in hypertension, migraine and subarachnoid haemorrhage below as these appear to be the most widely documented. For a comprehensive review, Russel et al. offer an excellent description of the role of CGRP in diseases other than SAH (598).

Hypertension

The role of CGRP in hypertension has been evaluated in a number of experimental models and there is some evidence that CGRP actions are important in restricting the onset of hypertension (598). A worsening cardiovascular response is seen with the use of the CGRP receptor antagonist $CGRP_{8-37}$ in experimental models of hypertension. Such models include salt-induced hypertension, L-NAME-induced (N(G)-Nitro-L-arginine-methyl ester) hypertension and subtotal nephrectomy hypertension (648-650). CGRP and angiotensin II appear to work together to alter vascular tone and blood pressure in animal studies (639, 651). There are also human trials demonstrating that infusion of angiotensin II causes dose-dependent increases in plasma concentrations of CGRP combined with the increase blood pressure caused by angiotensin II (652). It has been argued that CGRP is released in response to hypertension but there is no evidence that this increase in plasma CGRP is functional. Furthermore, the association between CGRP and hypertension is not clear in experimental or human studies. CGRP receptor antagonists do not appear to affect blood pressure or resting heart rate, both in animal (653, 654) and human studies (655, 656). Lu et al. used knockout mice to assess the effect of αCGRP deletion and found that there was no difference in blood pressure at baseline, after exercise, or following a phenylephrine infusion when compared with wild-type mice (657). Therefore, the cardiovascular effects of CGRP reduction/antagonism may only become apparent in the diseased states discussed in experimental models above. From the current evidence, it has been stated that CGRP does not appear to

have a significant role in the regulation of normal blood pressure in humans (598).

Migraine

Migraine is characterised by primary episodic headaches associated with nausea, phono- and photophobia. The pathophysiology appears to be a primary neuronal dysfunction and the previous theory of vasodilation causing the headache and vasoconstriction causing the aura is no longer accepted (658-661). Cortical-spreading depolarisation, the self-propagating wave of neuronal depolarisation that spreads across the cortex discussed in Chapter 3, is theorised to cause the aura, alter BBB permeability and activate trigeminal nerve afferents (516, 662). It is the activation of trigeminal nerve afferents which is thought to cause inflammatory changes in the meninges causing the pain associated with migraines (663). The trigeminovascular system, which has sensory neurons innervating cerebral vessels, is thought to play a large role in the pathophysiology of migraine. Upper cervical nerve roots converge with the trigeminal nerve explaining why migraine headaches often include the anterior and posterior areas of the head and neck (664, 665). The trigeminal ganglion releases vasoactive neuropeptides on stimulation, such as substance P, neurokinin A and CGRP, the latter of which is said to be the main mediator of trigeminal pain (666, 667). CGRP is released in cortical tissue during CSD depolarisation and three different CGRP receptor antagonists have had a dose-dependent inhibitory effect on CSD, suggesting that CGRP has a role in this phenomenon (668). Furthermore, intravenous CGRP has been shown to

induce migraine symptoms in patients known to have migraines (669, 670). As such, CGRP antagonists may be a useful treatment for migraine, although no treatment has reached the market. There have been at least 11 varieties of CGRP receptor antagonists developed with studies of oral, intranasal, subcutaneous, and intravenous administration and assessment of acute and prophylactic treatment (598). An early CGRP receptor antagonist, Olcegepant, was shown to have no effect on haemodynamics and had a response to migraines that was comparable to available triptans in a small multicentre double-blind RCT (655, 656, 671-673). Unfortunately, due to the high molecular weight of the CGRP-antagonist, it was only able to be administered intravenously. An oral variant, telcagepant, was subsequently developed, showing good oral bioavailability and good efficacy in Phase II trials (620, 674). Telcagepant underwent multiple phase III trials, including a multi-centre RCT of 1,380 patients randomised to telcagepant, placebo or zolmitriptan. Telcagepant was found to be as effective as zolmitriptan with fewer adverse events (620). A subsequent trial of telcagepant as a prophylactic treatment of migraine was stopped early due to two patients developing elevations in serum transaminases (clinical trials NCT00797667). Merck have since stopped their development of telcagepant which appears to be due to the change in transaminases (675). There are several other oral variants of CGRP receptor antagonists still being developed and results of phase III trials are awaited but as yet there is no available CGRP antagonist or antibody available for the treatment of migraine (676, 677).

Subarachnoid Haemorrhage

Early experimental models investigating CGRP as a treatment for cerebral vasospasm after SAH were in the late 1980s. The hypothesis for treatment was that CGRP would dilate constricted cerebral vessels and thus improve neurological outcomes following DCI. Unfortunately, most of the models were limited to assessing vessel diameter. A few included neurological outcome as a secondary measure (678). The basis for this research was not limited to CGRP's potent microvascular vasodilatory properties. There is also an association with altered concentrations of CGRP after SAH. Experimental studies demonstrate that innervation of the cerebral circulation by CGRP-immunoreactive fibres is decreased after SAH which is not present in controls (based on estimations of the number of fibres present) (462, 464, 679, 680). Nozaki et al., in a single-injection model of SAH in dogs, assessed CGRP immunoreactivity in perivascular nerve fibres of pial arteries. Suppression of CGRP in cerebrovascular nerve fibres was first detected immunohistochemically on the third day after SAH, peaked on days seven-to-14 and recovered to normal by day 42 (464). It has been argued that the reduction in CGRP may be due to release of the peptide from perivascular nerve terminals caused by subarachnoid blood. The dilatory effect of CGRP on the basilar artery has also been observed to be greater in those animals with SAH as compared with controls (679). Observational studies of the MCA in posthumous humans have shown that patients dying from SAH had significantly lower levels of CGRP in these arteries when compared with arteries from patients who had died of myocardial infarction (681). In 1995 Juul

et al. measured concentrations of CGRP in plasma from the external jugular vein (n=20) and CSF (n=14) of patients with SAH who had their aneurysm clipped and received nimodipine. They compared these values with healthy controls (n=14 for external jugular vein, n=12 for CSF) and found that serum CGRP concentrations were significantly higher in the SAH group (30 ± 1 versus 19 ± 5 pmol/l, mean ± SEM, $p<0.05$). In contrast, the CSF concentration of CGRP was detectable in the SAH but undetectable in the controls (463). Endogenous CGRP appears to be released and subsequently depleted in response to cerebral vasoconstriction after SAH, suggesting that exogenous CGRP may be beneficial in managing DCI. There is some experimental evidence that CGRP may have another protective role separate from vasodilation. One animal study in rats (n=144) utilised a middle artery occlusion model to assess the effect of CGRP on neurobehavioural scores (measured on a five-point scale) and infarction size (observed by 2,3,5-triphenyltetrazolium chloride staining). The study demonstrated not only a reduction in infarction range but also an improvement in neurological function after CGRP administration. This effect was blocked by the CGRP antagonist $CGRP_{8-37}$ (682). The concentration of CGRP in neurons of brain tissue is increased following cerebral ischaemia and CGRP appears to have a protective function in neuronal cells (683, 684). The mechanism of action of CGRP in this role is unclear but appears to be related to mitogen-activated protein kinase (a serine/threonine/tyrosine-specific protein kinase that regulates cell functions) signal transduction pathways including the p38 and c-Jun N-terminal kinase pathways (685, 686). Specifically, Yang et al. suggest

that a reduction in c-Jun N-terminal kinase and p38 phosphorylation, and an increase in extracellular signal-related protein kinase phosphorylation (detected by western blotting), were responsible for the improved neurobehavioral scores and decreased infarct range seen in their study (682).

Systematic Review and Meta-Analysis of Animal Studies

The following systematic review and meta-analysis of animal data comes from a collaboration between myself, Dr Caroline Begg, Professor Peter Andrews and Professor Malcolm Macleod (see acknowledgements). An open-access protocol for the review is available online (Appendix 2) (678). The manuscript is under review.

At the time of writing there are 21 pre-clinical studies investigating the effect of αCGRP on cerebral arteries after SAH (599, 678). All 21 of these studies appear to demonstrate an increase in cerebral vessel size or amelioration of vessel constriction after administration of CGRP. A review of CGRP and its potential role in the treatment of DCI has been published but there has been no systematic review or meta-analysis of the effect of CGRP on cerebral vessel diameter or neurobehavioral outcomes in animals or humans (599). Systematic review provides a less biased assessment of findings from animal studies (687). This approach was used to summarise data from publications reporting *in vivo* animal studies that investigated the effects of αCGRP after

experimental SAH. The study protocol has been published elsewhere but is briefly outlined below (Appendix 2) (678).

Search Methods and Study Selection

In January 2015, two electronic databases (MEDLINE via PubMed Central, and EMBASE via OvidSP) were searched using the key words “alpha calcitonin gene-related peptide”, “αCGRP” and “subarachnoid haemorrhage” in combination using the Boolean operator [AND]. The search was restricted to “other animals”. Two investigators independently screened the abstracts and titles to identify those that met our inclusion criteria (Flynn and Begg). Any differences were resolved by discussion with a third reviewer (Andrews). We included *in vivo* animal studies describing the effect of αCGRP in animal models of SAH where outcome was reported as a change in arterial diameter and the articles were published in English. We also included studies which examined *in vivo* SAH and αCGRP administration with post-mortem *in vitro* measurements of artery diameter.

Data Extraction

Two investigators (Flynn and Begg) independently extracted data relating to species of animal and weight; method of inducing SAH (single injection, double injection or clot placement); whether the basilar artery (BA), MCA, anterior cerebral artery (ACA) or internal carotid artery (ICA) were measured; the method of measurement (angiography, *in vitro* measurement or direct *in vivo* visualisation); anaesthetic agent used; dose of αCGRP and time of administration from SAH; reporting and method of randomisation; reporting

and method of blinding; animal welfare guideline statement; statement of sample size calculation; whether there was a statement of potential conflicts of interest and the use of animals with comorbidities. Study quality was assessed using the CAMARADES 10-item quality checklist (688). One point was awarded for each of: (1) publication in a peer-reviewed journal, (2) statement of control temperature, (3) randomised intervention allocation, (4) intervention allocation concealment, (5) blinded assessment of outcome, (6) avoidance of anaesthetics with marked intrinsic neuroprotective properties (ketamine), (7) statement of *a priori* sample size collection, (8) statement of compliance with regulatory requirements, (9) conflicts of interest statement, and (10) use of animals with comorbidities.

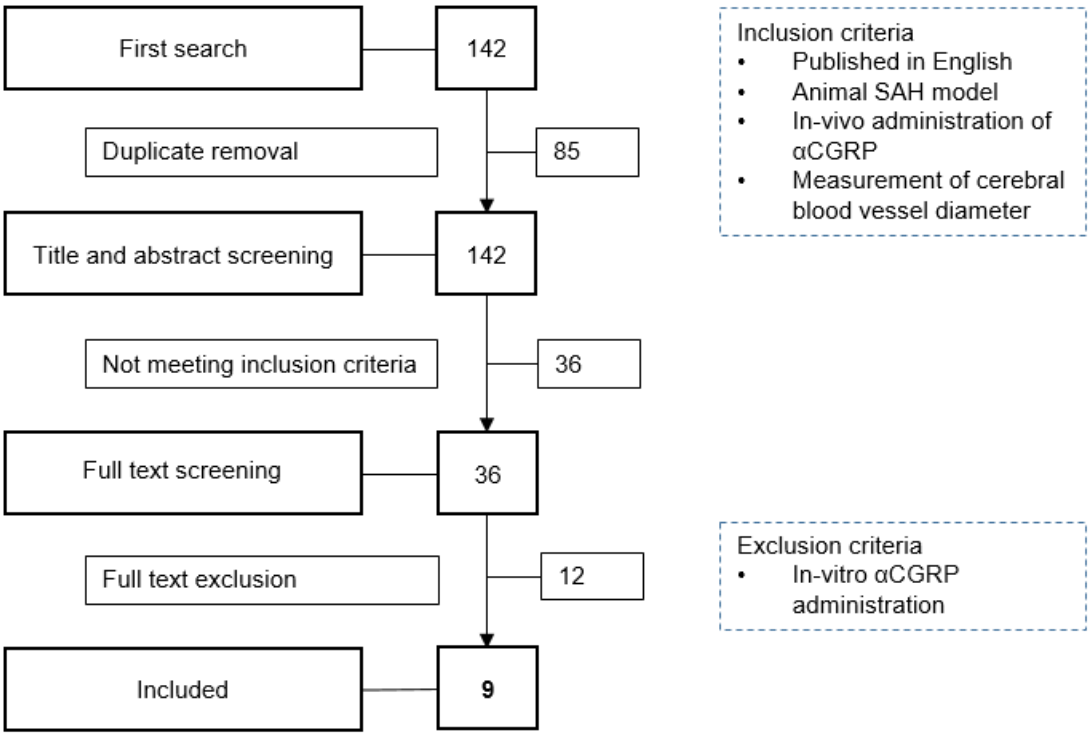

Figure 23: Flowchart of Study Selection. SAH, subarachnoid haemorrhage; αCGRP, alpha calcitonin gene-related peptide.

For meta-analysis, we recorded arterial diameter for intervention and control groups as a percentage from baseline (mean values and a measure of variance with the number of animals per group). Where a single control group was used for multiple treatment groups, we adjusted the size of the control group entered into the meta-analysis by dividing the size of the control group by the number of treatment groups served (689).

Two of the nine publications did not report quantitative data in their text, only presenting graphed data (690, 691). Mean values with standard error of the mean (SEM) were estimated from the graphs of these studies using Universal Desktop Ruler for Windows (AVPSoft).

Statistical Analysis

Meta-analysis was performed using normalised mean difference with a random effects model (689). Univariate meta-regression was used to explore associations of animal species, sex, strain and quality issues. Meta-regression of transformed data using a three-component cubic spline was used to assess dose-response. Where multiple experiments were performed in the same publication, these were treated as separate studies (689). Results are presented as the mean ± standard error unless otherwise stated. Statistical analysis was performed with STATA 14 (Statacorp LP).

Results

We identified 142 publications from our initial search. After combining MEDLINE and EMBASE results and deleting duplicates, 57 publications

remained. Titles and abstracts were then screened for eligibility by two authors (Flynn and Begg) resulting in 21 publications investigating αCGRP and cerebral vessel narrowing (690-710). After examining the full papers of these abstracts and removing those with *in vitro* αCGRP administration (n=12) and those that lacked SAH models, nine eligible publications remained (690-692, 696, 697, 701, 708-710). The nine publications included in the review were published between 1989 and 2013 (median year 1996). From the nine publications, 20 experiments were included in meta-analysis (Fig.19).

Characteristics of Studies

The total number of animals examined was 193 and the median number of animals used per experiment was nine (interquartile range (IQR) six-to-14). The median number of study quality checklist items scored was four (IQR two-to-six). No studies used animals with comorbidities; reported a statement of potential conflicts of interest or stated an *a priori* sample size calculation. 40% of studies reported control of body temperature. 20% described a randomised treatment allocation and 45% reported allocation concealment. Half of the experiments used a blinded assessment of outcome and half used an anaesthetic agent other than ketamine. All studies were published in peer reviewed journals and 70% reported compliance with local animal welfare guidelines. In 16/20 (80%) of the experiments, SAH was induced by autologous blood injection by either single or double injection methods, the remainder were induced with blood clot placement. Further study characteristics are presented in Tables 8 and 9.

Table 8. Study characteristics	
Publications, no.	9
Experiments, no.	20
Animals, no.	193
Median number of animals per experiment, median (IQR)	9 (6 to 14)
Species, no. (%) of experiments	
Rabbit	12 (60)
Dog	5 (25)
Rat	2 (10)
Monkey	1 (5)
SAH induction, no. (%) of experiments	
Autologous single injection method	12 (60)
Autologous double injection method	7 (35)
Autologous blood clot placement	1 (5)
Vessel examined	
Basilar artery	19 (95)
MCA, ICA and ACA	1 (5)
Method of visualising vessel diameter	
Angiography	16 (80)
In-vitro sections	3 (15)
Direct *in-vivo* visualisation	1 (5)
Preparation of αCGRP	
CGRP in solution	13 (65)
Gene transfer	4 (20)
Slow-release tablet	3 (15)
Timing of αCGRP administration	
Before SAH	3 (15)
< 1 day after SAH	1 (5)
1 – 3 days after SAH	16 (80)
Time of outcome assessment after administration of CGRP	
< 1 hour	4 (20)
1-3 days	12 (60)
5-7 days	4 (20)
Study quality (median (IQR))	4 (2-6)

Table 8: CGRP Study Characteristics. IQR, interquartile range; n, number; SAH, subarachnoid haemorrhage; CGRP, alpha calcitonin gene-related peptide

Table 9: Study Characteristics 2.

Reference	Animal	Model	n (control)	Experiments	Delivery route	Delivery time	Time of assessment	Dose	Assessment (diameter)	QS
Nozaki (1989)	Dog	SI and DI	6, 5, 5, 6 (11)	4	IC injection	Day 3 and Day 7	5m to 24h post-injection	10-10 to 10-12 mol/kg	BA, % of baseline	1
Toshima (1992)	Rabbit	SI	7, 10 (17)	4	IC injection	Day 2	2 hours after injection	100 ng/kg/min	BA, micrometers	2
Inoue (1996)	Monkey	Blood clot	5 (5)	1	IC tablet	Day 0	Day 0, Day 7, Day 14	400mcg	ICA, MCA, ACA, % of baseline	5
Ahmad (1996)	Rabbit	SI	7, 8, (7)	2	IC tablet	Day 2	Days 2, 3, 4, 5 and 6	24 and 153 mcg	BA, % of baseline	4
Imaizumi (1996)	Rabbit	SI	7, 7, 5, 5, 5 (36)	6	IC injection	Day 3	Pre-SAH to 24h post-delivery	10-10 to 10-12 mol/kg	BA, % of baseline	6
Toyoda (2000)	Rabbit	SI	8, 8 (14)	2	Gene transfer	5 days before SAH	Day 0 and Day 2	2 nmol/l	BA, % of control	2
Satoh (2002)	Dog	DI	6 (6)	1	Gene transfer	Day 2	Day 7	420 pmol/l	BA, % of mean baseline	6
Sun (2010)	Rat	DI	5 (5)	1	Intranasal	Day 3	Day 3	1mcg	BA, micrometers of baseline	4
Tian (2013)	Rat	DI	15 (15)	1	Gene transfer	Day 3	Day 7	35.4 ng/l	BA, % of control	4

SAH, subarachnoid haemorrhage; Model, model of SAH; n, number of animals in treatment groups (number of animals in control group); QS, quality score; SI, single injection method; DI, double injection method; IC, intracisternal; m, minutes; h, hours.

Treatment Effect

All 21 publications reporting *in-vivo* and *in-vitro* experiments demonstrated a dilation of cerebral arteries after αCGRP administration. Of the 20 eligible *in vivo experiments* included in meta-analysis, there was a 40.8 ± 8.2% increase in cerebral vessel diameter in the αCGRP group compared with controls ($p<0.0005$, 95% CI 23.7 to 57.9, I^2 96% Figure 2). There was also a significant dose-response to αCGRP in the ten experiments which administered a single dose into the cerebroventricular system (Fig 22).

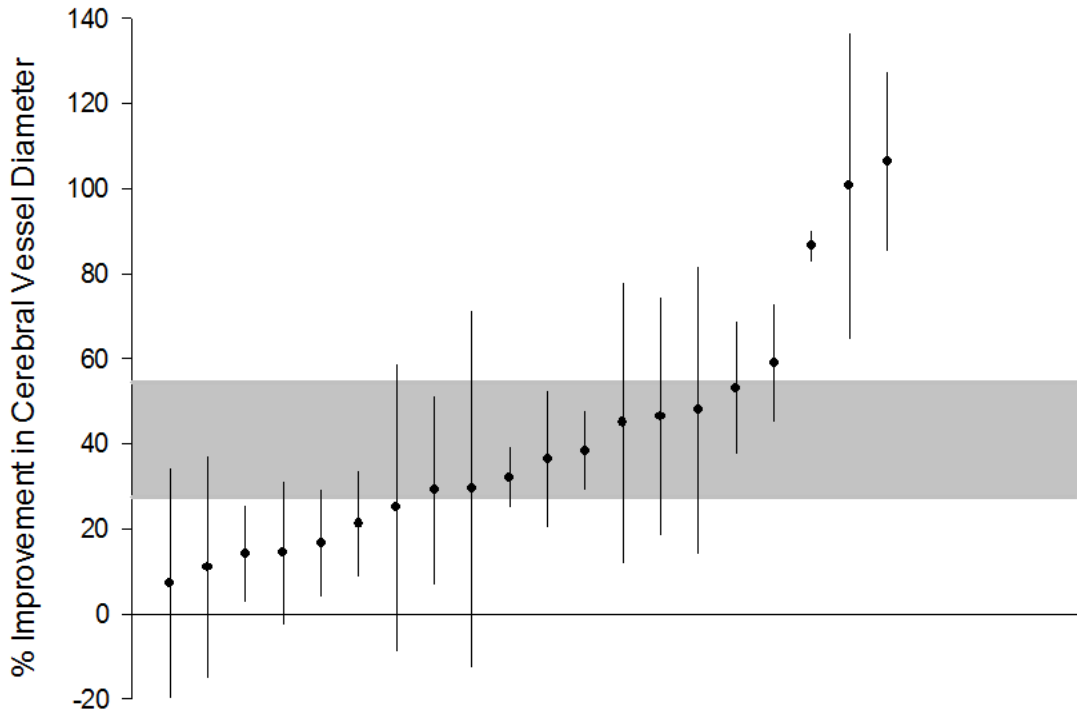

Figure 24: Change in Cerebral Vessel Diameter per Experiment. Experiments ranked by effect of alpha calcitonin gene-related peptide on change in vessel diameter. Error bars = 95% CI for individual estimates. The horizontal area

represents the 95% interval for the global estimate.

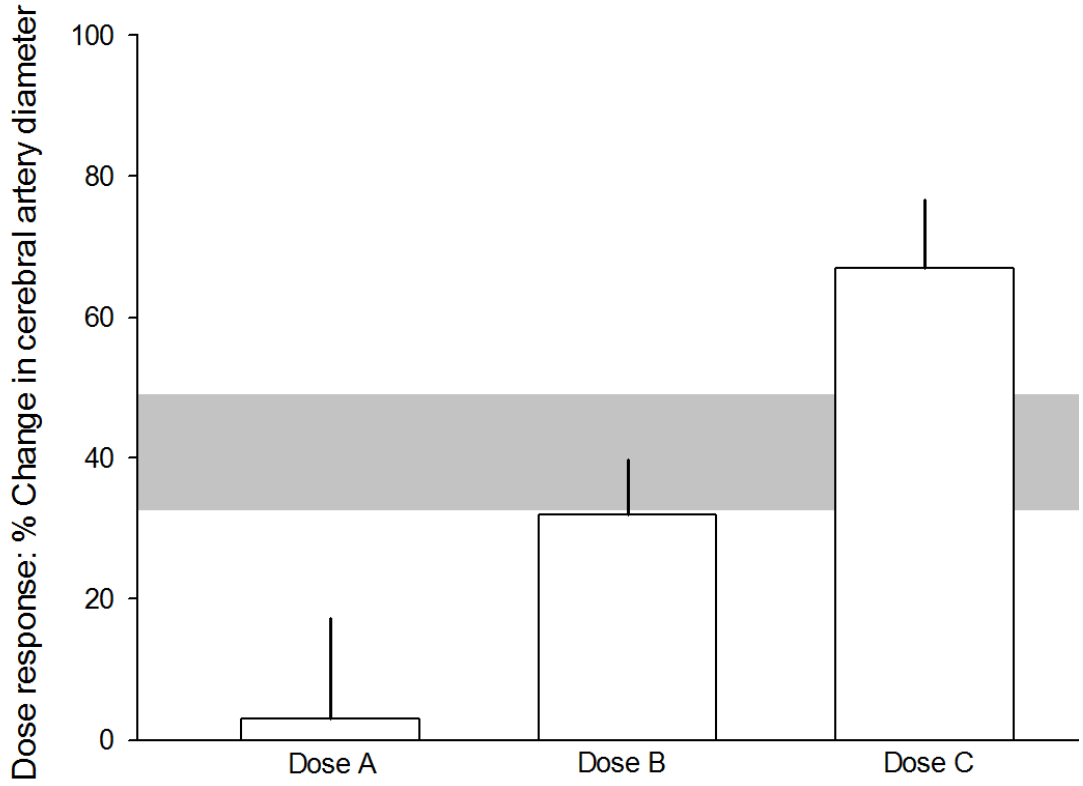

Figure 25: Dose-Response Relationship. Dose A = 10^{-12} mol/kg of alpha calcitonin gene-related peptide (αCGRP), 3.1 ± 14.2%. Dose B = 10^{-11} mol/kg αCGRP, 32 ± 7.8%. Dose C = 10^{-10} mol/kg αCGRP, 67 ± 9.7%. The error bars represent standard error whilst the shaded grey area represents the standard error of the global estimate. Differences were statistically significant (P <0.05).

The effect size tended to be lower in studies that reported randomisation, blinded assessment of outcome, blinded induction of SAH and use of an anaesthetic agent without intrinsic neuroprotective properties. However, none of these observations reached statistical significance. There was also a trend towards lower effect size in studies reporting compliance with more quality checklist items. This ranged from 57.3 ± 10.7% (p <0.05) from experiments with a quality score of one, to 28.1 ± 9.1% (p<0.01) from experiments with a quality score of six (Fig.22).

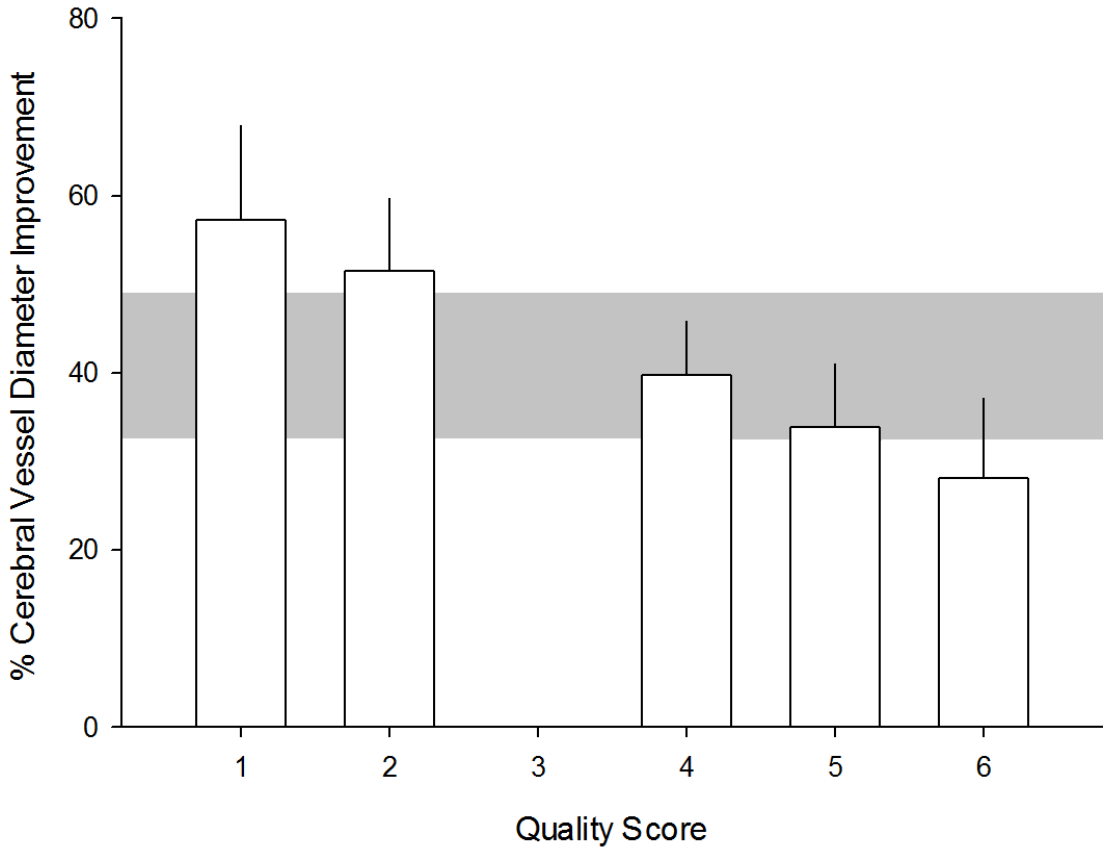

Figure 26: Study Quality Score versus Reported Efficacy: Relationship between quality score of studies and estimated efficacy of alpha calcitonin gene-related peptide at dilating cerebral arteries. Error bars represent standard error whilst the grey area represents the global estimate. Differences were not statistically significant (P >0.05).

Neurological Outcome and Adverse Effects

Four studies reported an effect on neurological outcome after αCGRP administration (692, 696, 697, 708). The standardised mean difference was 1.31 (95% CI -0.49 to 3.12, Q 40.5, n=65 animals) in favour of αCGRP. Tian et al. reported neurological outcome based on a comprehensive scoring system (0-48, 0 = best score, 48 = worst score) measured three times daily and based upon the assessment of four functions which has been used elsewhere (708, 711). The mean neurological outcome on day seven for the

αCGRP group was significantly better than for the control group (10.67 ± 1.16 versus 22.33 ± 2.08 respectively, $p < 0.001$). Imaizumi et al. assessed neurological outcome based on food intake and a slope tolerance test on days two, three and four post-SAH (696). No significant difference was found between the αCGRP and control groups for either assessment. Inoue et al. reported no significant difference in food intake, observable hemiparesis, consciousness disturbance or response to stimulation between the αCGRP and control groups (697). Ahmad et al. reported neurological outcome from grade I (normal) to grade III (unable to stand and presented abnormal posture) in addition to performing a slope tolerance test. Two rabbits in the control group were grade II (slow in response but able to walk) and III respectively, all other rabbits were normal and there was no statistical difference between the groups in the slope tolerance test. In all studies where it was measured, food intake was decreased after SAH but there was no significant difference between the αCGRP and control groups. Inoue et al. noted a significant decrease in weight in their αCGRP group compared with the control group on day 14, but noted no other adverse effects and were unable to explain this change in weight.

Eight publications reported physiological parameters which might be associated with adverse events. There was no significant difference in systemic arterial pressures or arterial blood gas results between the aCGRP or control groups. Imaizumi et al. observed that all animals tended to have an increased respiratory rate for approximately six hours after intrathecal injection of αCGRP or vehicle and demonstrated a high blood pH and low $PaCO_2$, but no difference between the groups (696). Both Nozaki and Toshima et al.

demonstrated a decrease in MAP when αCGRP was administered intravenously, which was not seen by intrathecal administration (709, 712). The study by Toshima et al. demonstrated a marked decrease in MAP following intravenous administration of αCGRP that was not seen with intracisternal administration (~70 mmHg versus ~40 mmHg at 30 minutes after αCGRP administration).

Other effects of αCGRP were also reported. Sun et al. observed a reduction in cortical cell death, decreased endothelial death and upregulated vascular endothelial growth factor with evidence of angiogenesis after αCGRP administration (691).

Discussion of Animal Data

Intrathecal administration of αCGRP dilates cerebral arteries in a dose-dependent manner in animal models and appears to be associated with fewer systemic effects than intravenous administration, chiefly the avoidance of hypotension. Furthermore, the effect of αCGRP on cerebral arteries appears to be more pronounced in the context of SAH, possibly because sensitivity of the artery to αCGRP may be greater owing to the depletion of endogenous αCGRP after SAH (704, 713). Alternatively, it may be that these arteries have a higher capacity for dilation after being constricted following SAH. In addition to the decreased systemic side effects seen by intrathecal administration, this route exposes αCGRP to the abluminal side of the blood vessel wall in a way more akin to its endogenous action. αCGRP is able to dilate cerebral arteries independently of endothelial cells, which are morphologically damaged after

SAH and so avoids a problem associated with endothelin antagonists (714, 715).

Laban et al., in their systematic review of experimental SAH studies of ERAs, demonstrated a 54% improvement in vessel diameter after administration of ERAs, but no significant effect on mortality, and no studies reported effects on functional outcomes (571). The authors concluded that there was no neurobehavioural data to support progression from pre-clinical to clinical trials for ERAs. In contrast to ERAs, some of the experiments in this review did examine neurobehavioural scores. Whilst there is not a large amount of data, there is a positive signal consistent with a substantial effect. Similarly, there is some human data to support improved outcomes with CGRP after SAH which will be discussed in the following section (640, 716).

Limitations of Studies

There were no female-only experiments and most experiments in this review used the single haemorrhage model of SAH (60%). The other forms were single injection and clot placement. Animals rarely develop a vessel narrowing-related ischaemic neurological deficit from any of these methods. Megyesi et al. note that this is probably because animal brains have a plentiful collateral blood supply (717). Whilst this is probably irrelevant for measuring the effect of αCGRP on vessel diameter, it becomes more problematic when trying to assess neurological outcomes. Another translational problem arises from the times of administration of αCGRP and assessment of neurobehavioural outcomes. In humans, DCI is said to occur most commonly between days

three-to-ten and cerebral vessel narrowing is maximal between days six-to-ten after ictus (718). The experiments assessed in this review administered αCGRP before and up to three days after SAH and assessed the response within hours to one week after administration (Tables 8 and 9). Megyesi et al. also note that the best model of vasospasm seems to be the primate model in which a blood clot is placed around a large cerebral vessel (717). Only one of the studies we analysed uses this model (719). More recently, Titova et al. in their systematic review, state that dog models of SAH are considered superior and that the ability of murine models to reflect human vasospasm is disputed (720). Finally, the median quality score of the studies was low (four) and an increased effect size was seen in studies with a lower quality score. This effect size may be expected to be lower with more high quality studies.

Human Trials

There have been three clinical trials of αCGRP administration in humans with SAH. All three of these trials have involved intravenous administration of αCGRP. To my knowledge, following a Medline search, there has been no intrathecal or intranasal delivery of CGRP in humans, although intranasal delivery of a CGRP-receptor antagonist may be investigated (721, 722).

Johnston et al. (1990)

In a multicentre RCT, Johnston et al. administered intravenous αCGRP to 15 patients (11 women, four men; mean age of 48 years; range 22-70) with neurological deficits after surgical clipping of ruptured aSAH (716). Patients

received αCGRP and placebo 24 hours apart in random order. 0.035 µg/min of αCGRP was administered and the infusion rate was doubled every ten minutes until either a clinical response was observed or the maximum dose (1.15 µg/min) was reached at one hour. If no side effects were observed, the maximum dose was continued for 20 minutes. Neurological deficits, as measured by the modified Glasgow Coma Scale, improved in nine patients with no adverse effects: one improved on both treatments, eight improved on αCGRP but not placebo and one improved on placebo but not αCGRP. Thus, of the nine patients who showed a treatment preference, eight favoured αCGRP.

Systolic BP decreased by 35.7 mmHg during αCGRP infusion ($p < 0.05$ compared with placebo; 95% CI 9.2 to 75.8 mmHg) and MAP decreased by 21.3 mmHg ($p < 0.01$ compared with placebo; 95% CI 9.8 to 46.6 mmHg).

Cerebral arterial vessel narrowing prior to administration of αCGRP was not measured, nor was the effect of αCGRP on cerebral diameter/flow velocities. However, the researchers excluded hydrocephalus, haematoma and those with additional aneurysms from their inclusion criteria and the patients demonstrated neurological deficits post-surgery consistent with DCI (716).

The European CGRP in SAH Trial (1992)

The largest of the three trials was the European CGRP in SAH Trial which examined 117 patients in a multicentre RCT. Patients with a post-operative focal neurological deficit or decrease in GCS >1 not attributable to a non-

ischaemic cause on CT were randomised to intravenous CGRP or standard care. 62 patients were randomised to an infusion of 0.6 µg/min of αCGRP for a minimum of four hours to a maximum of 10 days in those with a satisfactory neurological response (mean age 49.1, 63% female). If the patients developed hypotension, the infusion rate was decreased to 0.45 µg/min, then 0.3 µg/min if there was no improvement. The remaining 55 patients were assigned to standard care (mean age 48.4, 62% female). Outcome, measured on the Glasgow outcome score, at three months was good in 66% of those treated with αCGRP and 60% in the controls; the relative risk of a poor outcome in αCGRP treated patients was 0.88 (95% confidence interval 0.60 to 1.28).

The European CGRP in SAH Trial was limited by the large number of patients who had αCGRP discontinued. Hypotension was a common side effect of αCGRP. 41 patients in the CGRP group discontinued treatment. This was due to hypotension (19), lack of improvement at four hours (17) or later (four), or at the patient's request (one). The authors commented that "*systemic intravenous infusion of αCGRP is not the ideal route*" and that "*subarachnoid instillation may be more rational, since the peptide is normally found on the adventitial rather than the endothelial side of the cerebral vessel wall*" (640).

Juul et al. (1994)

In a non-randomised study, Juul et al. studied five patients (three males, two females; median age 44, range 40-77 years) after aSAH and surgical clipping of the aneurysm. Patients had Doppler sonography evidence of cerebral vasoconstriction in the postoperative period, as measured by haemodynamic

index (MCA velocity compared with internal carotid artery velocity on ultrasound) (723). Synthetic human αCGRP (CB 003, Celltech, UK) in 0.9% sodium chloride was infused at a rate of 0.6 µg/min. Patients were monitored with Doppler sonography before infusion; immediately after the start of the infusion; at regular intervals during the infusion; and one hour after stopping the infusion. Infusion times varied between 1.5 and 52 hours depending on clinical response. Patients demonstrating a larger change in haemodynamic index had shorter infusion times. A significant reduction was found in the haemodynamic index during the αCGRP infusion ($P < 0.05$) when compared with before infusion, suggesting amelioration of vasoconstriction. There was no change in the haemodynamic index on the contralateral side. No clinically or statistically significant changes were observed in BP or conscious level during the αCGRP infusion. A significant increase in heart rate was observed during the infusion as compared to before and after infusion (90 ± 4 (SEM) vs, 76 ± 5 beats per minute, $P <0.05$). Cardiac ultrasound data showed a mean cardiac output increase of 1.9 litres/min, and a mean decrease in total peripheral resistance of 538 dynes s/cm^5 consistent with systematic vasodilation.

This study was performed in a single centre with a small sample size, patients were not randomised and investigators not blinded and there is no clear indication from the publication why different times were used to stop the infusion. Because of this the total dose of αCGRP cannot be calculated.

Summary

Intrathecal αCGRP dilates cerebral arteries in a dose-dependent manner in animal models whilst showing a positive (but not statistically significant) signal towards improved neurological outcomes. This route also appears to be associated with fewer systemic effects than intravenous administration. In human trials, a non-statistically significant improvement in outcome for the CGRP group was seen in the European CGRP in SAH trial and Johnston et al. observed a statistically significant treatment preference for CGRP versus placebo in their small study (640, 716). All three human trials demonstrated systemic effects of intravenous infusion. αCGRP receptors of the perivascular nerve endings are located on the abluminal side (adventitia-media) of the arterial walls. The abluminal sides of the cerebral arteries lie in the subarachnoid space and are accessible via CSF administration. Moreover, αCGRP has a high molecular weight with poor penetration through the BBB (599). Systemic administration of αCGRP to treat cerebral vasospasm possibly did not result in adequate therapeutic concentrations in the cerebral subarachnoid space. It may be that the observed systemic effects (hypotension, tachycardia and facial flushing) occurred due to a high intravenous dose being required to achieve adequate CSF concentrations. It is my belief that repurposing αCGRP to CSF administration will reduce the volume of distribution and circumvent the first-pass effect, thereby reducing the effective dose and side-effects. Lumbar spinal administration of drugs results in greater variation of drug concentration in the cerebral subarachnoid space. Peak concentrations are achieved within minutes but there are some

data suggesting that rarely this may occur as late as three-to-four hours after administration and require a higher volume of study agent (724-726). Therefore, administering the peptide via an EVD/access device, which would be otherwise clinically indicated, is preferred to the lumbar spinal route. One of the challenges of translating animal data and methods to human trials is the invasive nature of the intrathecal route. Previous human trials with intravenous administration of αCGRP have used a continuous infusion owing to the short half-life of αCGPR in the systemic circulation (approximately seven to ten minutes) (625). However, when administered into the cerebrospinal fluid as a single dose in animal models, the effects of αCGRP have continued for four to six hours (690, 696). Therefore, a continuous infusion of αCGRP into the CSF may not be necessary. Furthermore, Toyoda and Sun et al. demonstrate novel approaches to administering αCGRP, one via gene-transfer and the other by intranasal delivery (691, 710). If intraventricular administration of αCGRP in humans ameliorates cerebral vessel narrowing and avoids the adverse effects seen with intravenous delivery, both gene-transfer and intranasal delivery offer potential alternatives without the difficulties associated with an intraventricular drain. However, the next step should be a dose-tolerability study of αCGRP administered into the cerebral ventricles.

Cerebrospinal Fluid Concentrations of CGRP

None of the human trials described above measured CSF concentrations of αCGRP or cerebral artery size before, during or after administration of αCGRP. Therefore, we cannot accurately determine dose-tolerability effects or the

efficacy of the peptide from these trials. There are four previous studies that examined the concentration of CGRP in the CSF of patients with SAH (463, 727-729).

The first of these is a 1991 study published in Chinese and has a translated abstract briefly outlining the concentrations of CGRP in the CSF of patients with cerebral vascular disease as measured by radioimmoassay. Unfortunately, a full translation was not available. As such, there is no information about the study participants, the timing of CSF analysis or the assay used. The authors report that αCGRP concentration in the CSF of patients with ischaemic cerebrovascular disease was 152 ± 60 pg/ml (mean ± no indication whether this represents SEM or SD), not significantly different from their control value of 45 ± 9 pg/ml. In patients with haemorrhagic cerebrovascular disease the concentration was 3,965 ± 680 pg/ml, significantly higher than in the ischaemic and control group (728).

Juul et al. (1995) observed significantly increased concentrations of αCGRP in patients with SAH but no correlation with cerebral haemodynamic changes (463). 34 patients were enrolled to this single-centre study, 14 for CSF sampling and 20 for external jugular venous blood sampling. 30 of the patients had surgical clipping of their aneurysm and the remaining four were not operated on. 14 healthy volunteers had blood sampling and 12 separate volunteers had lumbar CSF sampling. Doppler ultrasound was performed at one-to-three-day intervals to measure flow velocity at the MCA and ICA. CGRP was detected in low amounts in the CSF of SAH patients (4 ± 2 pmol/l, mean

± SEM) but was not detected in the CSF of controls. The highest level of CGRP was detected in a patient who had severe vasospasm and later died (14 pmol/l). The authors state that the highest venous CGRP levels were found in patients with the highest velocity index values on Doppler (used as a marker for vasospasm).

Schebesch et al. (2013) collected daily CSF from day one to day ten after onset of SAH in 12 patients with ventricular drains (727). All patients had an early CT, DSA and daily transcranial Doppler, targeting the MCA and ACA (cerebral vasospasm was considered present with a flow velocity >160 cm/s). 12 consecutive patients with spontaneous SAH were included (seven female, five male; mean age 49.3 years; median GCS 12). Five patients received surgical clipping whilst seven underwent coiling. Vasospasm, as identified by transcranial Doppler, was present in seven patients, and six patients developed an ischaemic stroke. 29 spinal CSF samples, obtained by lumbar puncture before spinal anaesthesia administration prior to orthopaedic and urological procedures, acted as a control group (15 female, 14 male; mean age 52.8 years, range 19-81 years). CSF samples were centrifuged for ten minutes at 2000g immediately after sampling and supernatants were stored at -80°C prior to enzyme immunoassay. Levels of αCGRP were significantly higher in the SAH group than the control group. The authors note that, in contrast to Juul et al., they could readily detect a baseline level of αCGRP in their control group and attribute this to the lower sensitivity of the radioimmunoassay used. Furthermore, CGRP levels were significantly higher from days one-to-four in patients who did not develop cerebral vasospasm and

patients without subsequent cerebral ischaemia. The authors did not present values of CGRP in their publication, only presenting a graph. Universal Desktop Ruler for Windows (AVPSoft) has been used to estimate values from the presented graph. Based on these estimates the mean value of CGRP for days one-to-ten, for patients with and without vasospasm was 0.39 ± 0.15 ng/ml (range 0.1 – 0.84 ng/ml; the authors do not state whether they use SD or SE). The control value was 0.1 ng/ml (no deviation given).

A further study by Schebesch et al. (2014) assessed the concentration of CGRP in plasma after SAH (729). 33 consecutive patients with SAH (Fisher Grade III or IV) had serum samples taken daily from day one-to-ten for CGRP analysis. Patients had DSA and Transcranial Doppler (cerebral vasospasm was considered present with a flow velocity >150 cm/s). Plasma CGRP levels appeared significantly higher in patients with anterior circulation aneurysms ($p < 0.001$), and higher levels of CGRP were observed in patients coiled as opposed to untreated or surgically treated patients ($p < 0.001$). Furthermore, higher levels of CGRP were found in patients with ischaemia as compared with those without ischaemia (p 0.038). The authors note that the concentration of CGRP in serum correlated with the anatomical location of the ruptured aneurysm and suspect that this could be caused by disruption of nerve fibres at the site of the ruptured aneurysm. It is also argued that the higher levels of serum CGRP in the coiled group could be due to mechanical manipulation of the aneurysm-bearing artery leading to excessive CGRP release. The authors go on to state that CSF CGRP levels were lower in controls than in patients with SAH (463, 727). On days one-to-four after SAH, CGRP levels in the CSF

were significantly lower in the vasospasm group than SAH patients without evidence of vasospasm. In the serum, higher levels of CGRP were correlated with a higher rate of ischaemia.

The studies report values with pg/ml, pmol/l and ng/ml. For comparison, the amount of CGRP in each study has been converted to nanograms (Table 10).

Concentration of CGRP in the CSF of patients with SAH versus controls			
Publication	Wang et al (1991)	Juul et al (1995)	Schebesch et al (2013)
Control Group (pu)	45 ± 9 (pg/ml)	Undetectable	0.1 (ng/ml)
Ischaemia Group (pu)	152 ± 60 (pg/ml)	-	-
SAH Group (pu)	3965 ± 680 (pg/ml)	4 ± 2 (pmol/l) *	0.39 ± 0.15 (ng/ml) †
Control Group (ng)	6.75 ± 1.35	Undetectable	15
SAH Group (ng)	595 ± 102	2.27 ± 1.14	58.7 ± 22.4
Highest recorded value (ng)	697	7.96 *	126

Table 9: CSF Concentrations of CGRP in SAH Patients: Outline of results from three publications reporting levels of CGRP in CSF of controls and patients with subarachnoid haemorrhage. Publication units (pu) have been converted to nanograms (ng) for comparison. SAH: subarachnoid haemorrhage; CGRP: calcitonin gene-related peptide; CSF: cerebrospinal fluid. * mean ± SEM. † mean values estimated from graph in publication using Universal Desktop Ruler for Windows (AVPSoft); the measure of variance was not defined in this study. A CSF volume of 150mls and 3789.36 for the atomic weight of CGRP have been used for conversion.

Due to the small number of samples and studies reporting the CSF concentration of CGRP in patients with SAH I analysed CSF samples in Edinburgh. I anticipated a concentration between undetectable and 15 ng in

the control samples and between 1.13 and 697 ng in the positive samples based on the above data (Table 10). This data could then be used for de-risking a proposed exogenous dose of CGRP. Furthermore, if the results were as expected, we would have confirmation that this method of analysis is suitable for use in a toxicity study.

In response to my PhD viva in September 2017 I have added a sample size estimation for the following CSF study. The question I am asking is 'how many CSF samples do I need to estimate the change in CSF concentrations of CGRP in patients after a subarachnoid haemorrhage with an α value of 0.05 and a β value of 0.20' (730). I have used Minitab to determine the sample size for a two-sample t-test, assuming the data produced is normally distributed. I have used data from Schebesch et al. to estimate this because it presents a control value and the difference between the control value and SAH value is smaller than that from the Wang et al. paper (727, 728). Therefore, it should provide a conservative estimate (i.e. larger sample size) than using the Wang et al. data. The calculation was based upon a standard deviation of 22.4ng. A 95% confidence interval and a minimum difference of 20ng were used in the calculation. The resulting estimated required sample size is 21 for each group (total sample size 42). The mean difference in the Schebesch et al. paper was 43.7ng. Using this as a minimum difference gives an estimated sample size of 6 for each group (12 in total). Given that the mean difference between the control and SAH groups in the Wang et al. paper was 588ng, I think using 20ng and a sample size of 42 is very conservative (727, 728).

Retrospective Analysis of CSF Concentrations of CGRP in Humans after SAH

Between September 2015 and August 2016, I retrospectively analysed diagnostic CSF samples prospectively collected from patients being investigated for aSAH at the Western General Hospital and Royal Infirmary of Edinburgh. This was arranged with NHS Lothian BioResource/Tissue Governance (Research Ethics Council reference: 13/ES/0126. Sample request reference: SR526). This route of obtaining samples for analysis was chosen due to time constraints and ease of access to samples without the need for a complex trial protocol and a comprehensive research ethics application and approval. The limitation to this form of study is that I have no knowledge of patient identifiable information and am unable to access patient records. Clinical information (but not patient identifiable information) has been provided by NHS Lothian upon request.

Methods

CSF samples were obtained by LP and stored at -20°C in NHS laboratories for up to six months. LP was undertaken as a diagnostic procedure in patients with suspected aSAH as part of routine clinical practice. The samples were allocated for research by the Western General Hospital and Edinburgh Royal Infirmary laboratories as they became available to laboratory staff.

Inclusion Criteria

- Males or females aged over 18 years;

- CSF samples taken as a diagnostic procedure in patients with suspected or identified aSAH.

Exclusion Criteria

- Intracranial pathology other than aSAH;
- Any severe or unstable concomitant condition or disease (e.g. known neurological deficit, cancer, haematological disease, coronary disease or psychiatric disorder) that is likely to affect the CGRP concentration in control patients.

CSF samples were stored for analysis between September 2015 and February 2016. CSF samples were analysed by enzyme immunoassay (Bertin Pharma Human CGRP Enzyme Immunoassay (EIA) kit, bought through Bioquote UK) in April 2016. Analysis was performed by Dr Forbes Howie at the Queen's Medical Research Institute, Edinburgh.

Results

22 CSF samples were analysed by EIA. 12 samples were positive for xanthochromia, 10 were negative. Of the 12 positive samples, none of the patients had positive CT imaging demonstrating SAH. αCGRP was not detected by EIA in any of the samples.

Sample No.	Xanthochromia	Sex	Age	CGRP	CT/CTA Result
1	Positive	M	74	Undetectable	Negative
2	Positive	M	53	Undetectable	Negative
3	Positive	F	48	Undetectable	Negative
4	Positive	F	35	Undetectable	Negative
5	Positive	M	79	Undetectable	Negative

6	Positive	M	57	Undetectable	Negative
7	Positive	M	70	Undetectable	Negative
8	Positive	F	44	Undetectable	Negative
9	Positive	F	38	Undetectable	Negative
10	Positive	F	31	Undetectable	Negative
11	Positive	F	52	Undetectable	Negative
12	Positive	F	67	Undetectable	Negative
13	Negative	F	44	Undetectable	Negative
14	Negative	M	59	Undetectable	Negative
15	Negative	F	71	Undetectable	Negative
16	Negative	F	63	Undetectable	Negative
17	Negative	M	63	Undetectable	Negative
18	Negative	F	68	Undetectable	Negative
19	Negative	F	47	Undetectable	Negative
20	Negative	F	33	Undetectable	Negative
21	Negative	F	55	Undetectable	Negative
22	Negative	M	52	Undetectable	Negative

Table 10: Study Demographics and Results. No., number; CGRP, calcium gene-relayed peptide; CT, computed tomography.

Discussion

The assay kit states that *"CSF may be assayed if diluted at 1:20 in EIA buffer"*. From the above literature, we anticipated a value between undetectable and 15 ng in the control samples and between 1.13 and 697 ng in the positive samples (463, 727, 728, 731). As such, the negative samples were diluted 1:5 with buffer and the positive samples were diluted 1:5 and 1:25. The standard curve for the EIA kit was as expected (Fig.23) and the control value was 153 pg/ml for a target value of 165 pg/ml. In contrast to previous studies, αCGRP was not detected in any of the samples.

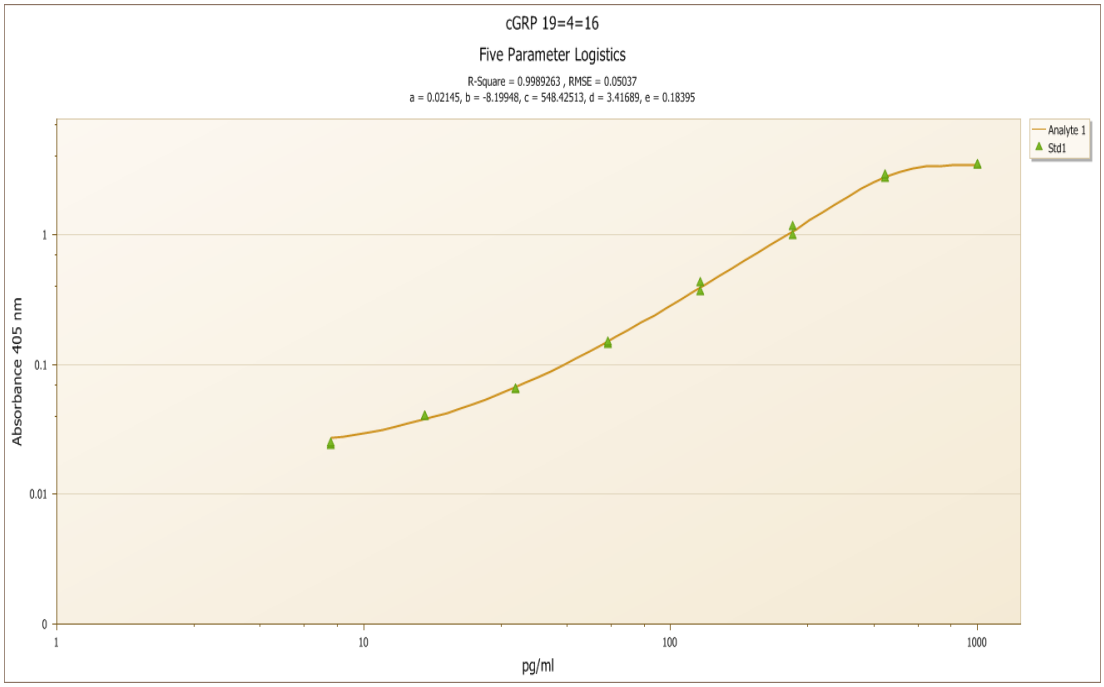

Figure 27: Standard Curve for CGRP Kit. CGRP, calcitonin gene-related peptide.

There are multiple unknowns associated with using samples from the BioResource in this study. Specific examples include: method of lumbar puncture; time for sample collection to storage; the number of times the sample was analysed prior to being placed in storage. The background history of the patients is unknown, including history of migraine, cluster headaches or depression (all of which have been associated with alterations in serum αCGRP concentrations (732-734). Potential reasons why CGRP was not detected in these samples include:

1. CGRP is an unstable compound in CSF and due to the time of storage and time at temperatures higher than -20℃ the peptide denatured:

After LP, CSF should be immediately transported for analysis as part of routine clinical care but I have no record of this. Furthermore, I do not know if the

sample was immediately frozen upon receipt and how long the sample was out of the freezer for initial laboratory analysis. The samples were subsequently transported from their initial laboratories to research laboratories in dry ice but were exposed to room temperature for a short time during transfer.

2. Insensitive RIA kit:

The RIA kit produced a control value (kit control, not CSF sample control) that was close to the target value and an expected standard curve. However, as Schebesch et al. suggest in their analysis of the 1995 Juul et al. study, it may be that failure to detect any CGRP was due to the sensitivity of the radioimmunoassay (463, 727). It seems prudent to use the same enzyme immunoassay as that used by Schebesh et al. for future analysis and comparison (727).

3. The concentration of CGRP was below recordable levels:

It is possible that the concentration of CGRP in the CSF was below detectable levels. I think this is unlikely given that there are multiple previous studies demonstrating detectable levels of CGRP in SAH patients and controls (463, 727, 728, 732). None of the positive CSF samples were associated with positive CT imaging. It could be argued that this is expected, given that patients would not routinely receive an LP for diagnosis if they already had a positive CT. One might expect subsequent CTA demonstrating an aneurysm. Based on previous results of CSF concentrations of CGRP in controls, I expected EIA to detect CGRP even in those patients without pathology (463, 727, 728).

4. Incorrect analysis technique and inappropriate dilution.

Dr Howie is an expert in peptide analysis with significant experience in this field. The choice of dilution was made in discussion with Dr Howie and seemed appropriate after reviewing previous evidence of concentrations (Table 10). It is possible that a flawed analysis technique led to these results, but seems unlikely.

Although this study was not helpful in de-risking our proposed dose of exogenous CGRP, it did highlight the importance of performing a pilot study assessing CSF concentrations of CGRP. I recommend that a reproducible method of analysis must be successful before starting a Phase II trial. Evidence for this could come from patients who routinely receive ventricular access devices for hydrocephalus where CSF would otherwise be discarded. I would recommend control samples coming from routine diagnostic lumbar puncture as part of a prospective trial instead of using the BioResource. Such a study could be organised through the Edinburgh EMERGE research team in the ED and the medical receiving units at the Western General Hospital and Royal Infirmary of Edinburgh. An extra 1ml of CSF could be taken during routine LP. I recommend immediate storage of samples at -40℃ and using Phoenix Pharmaceuticals EIA Kit, Burlingame, CA, USA. Furthermore, I would recommend seeking ethical approval for patient identifiable information in future research to prevent limitation of clinical information.

Chapter 5: Protocol for a Dose-Finding and Tolerability Study of Intraventricular αCGRP Administration in Patients with aSAH

The purpose of the systematic review and CSF concentration study presented in this thesis were to help develop a tolerability study investigating intrathecal administration of αCGRP. As part of that study, our research team also aimed to gather some dose-response data as evidence to support trial progression. An abridged version of the research protocol for this dose-toxicity study of exogenous CGRP administered into the cerebral ventricles, via ventricular access device or EVD, is included below. What is written here excludes introductory information already discussed in this thesis and regulatory information required in the formal protocol. The full trial protocol will be submitted to *BioMed Central Trials*.

The protocol was the basis for an invited full submission to the Medical Research Council's (MRC) Developmental Pathway Funding Scheme in 2016. The protocol was informed by the NHS Lothian's Research and Development team, the First in Human committee in Edinburgh and the Medicines and Healthcare Products Regulatory Agency (MHRA, see Appendix 3 for MRC application).

Study Objectives

The study objectives are to assess the dose tolerability response and the pharmacokinetics (plasma, CSF and urine) of αCGRP after CSF administration. This will provide information to design a therapeutic study of αCGRP given into the CSF for DCI after aSAH.

Research Questions

1. What is the dose-tolerability response for CSF αCGRP?
2. What is the CSF, plasma and urine concentration and half-life of αCGRP after CSF administration?
3. What is the dose-response for CSF αCGRP on cerebral vessels as measured by Doppler ultrasound and CT angiogram?

Endpoints

The primary endpoint is tolerability at each of the seven dose levels and we define tolerability by observing the following factors:

1. Haemodynamic instability including hypotension and tachycardia;
2. Reduction in GCS;
3. Clinical evidence of a seizure;
4. Death;
5. Spontaneous hyperventilation;
6. Facial flushing.

Pharmacokinetic endpoints:

1. CSF, plasma and urine αGCRP concentrations obtained throughout the course of the study;
2. Final dose administration.

Study Design

This is a clinical trial of an investigational medicinal product (CTIMP) to assess the pharmacokinetics and dose tolerability response of αCGRP after CSF administration using a three-plus-three traditional escalation rule, including seven increasing doses:

1. Two doses below the level at which an effect is expected (on vasoconstricted cerebral arteries after aSAH);
2. Three doses at the level an effect is expected;
3. Two doses above the level an effect is expected.

Blood, CSF and urine will be sampled at baseline and after drug administration (see ‘dosing and sampling’).

The patients will be those admitted to Critical Care at the Western General Hospital, Edinburgh, due to a ruptured aneurysm, confirmed by DSA or CTA, that has been secured by endovascular coiling or surgical clipping. Patients enrolled into the study will have a clinically indicated EVD/ventricular access device which will be used for administration of αCGRP. Patients will be continually monitored in Critical Care.

Number of Patients Recruited

The target recruitment rate is one to two patients per month. This is based upon recruiting a minimum of 21 and maximum of 42 patients within 18 months. Feasibility studies were carried out at the Western General Hospital in 2011 and 2015. These demonstrated that three patients per month were eligible of whom two of these would have consented to be in the study. This finding was consistent between patients and relatives giving consent in the case that patients lacked capacity. Assuming a recruitment rate of two per month and allowing a week between each dose level for analysis and trial meetings, we anticipate that the dose escalation can be completed within 18 months.

Justification of Dosing Schedule

The minimal anticipated biological effect level (MABEL) approach is taken in the absence of human data to allow determination of no observed adverse effect level (NOAEL). The best study to determine MABEL is Inoue et al. in monkeys (Macaca fascicularis) (719).

Inoue et al. created a tablet from microspheres containing αCGRP (αCGRP, molecular weight 3790; Bachem, Feinchemikalien, Buben) by using an in-water drying method through a water/oil/water emulsion before mechanically compressing the microspheres to form a tablet 2mm thick and 6mm in diameter (697). In Hartmann's solution, *in vitro*, there was no αCGRP release from the

tablet for the initial six days. During the next four days, there was a significant release of αCGRP; approximately 4mcg of αCGRP each day. Similarly, when the tablet was placed in the cisterna magna of rabbits, the maximal concentration of αCGRP in the CSF was seen on day six.

The same team then produced experimental SAH in ten cynomolgus monkeys by placing a clot around the internal carotid artery bifurcation on day zero using the method of Espinosa et al (735). The ten monkeys were split into three groups. Five of the monkeys (αCGRP Group) received αCGRP tablets containing 400mcg of αCGRP which were placed in the CSF. Two of the monkeys (Placebo Group) received placebo tablets placed in the same position. The last three monkeys (SAH Group) received no intervention after their experimental SAH.

The radioimmunoassay (RIA) analysis of the CSF for αCGRP concentration was performed before experimental SAH was induced and on day seven and 14 for each animal in the three groups. The non-αCGRP SAH Group showed no detectable αCGRP in the CSF at any time point. In the αCGRP Group 6.5 ± 5.4 nmol/L (mean ± standard deviation, n = five) was detected in the CSF on day seven. It was otherwise undetectable.

In the non-αCGRP groups (SAH + Placebo groups) arterial narrowing was present on day seven at the ICA, MCA and ACA, and was partially resolved by day 14. In the αCGRP group, arterial narrowing was significantly

ameliorated both at the MCA and ACA and on average (81.7, 81.1, and 75.7%, $P < 0.05$, 0.03, and 0.02, respectively).

	Changes in Diameter (%)[a]							
	Day 7				Day 14			
	IC[b]	MC	AC	Total[c]	IC	MC	AC	Total
SAH group (n = 3)	63.7 ± 3.5	53.8 ± 7.1	46.7 ± 10.6	55.8 ± 9.2	87.4 ± 3.6	91.3 ± 16.2	81.4 ± 20.4	87.3 ± 12.5
Placebo group (n = 2)	41.6 ± 19.7	74.9 ± 11.6	56.0 ± 20.0	57.5 ± 20.2	74.9 ± 27.1	78.1 ± 28.9	99.7 ± 17.5	84.2 ± 22.8
Non-CGRP group[d] (n = 5)	54.8 ± 15.8	62.3 ± 13.8	51.3 ± 14.0	56.5 ± 20.2	82.4 ± 15.4	86.0 ± 19.8	90.5 ± 18.8	86.0 ± 16.9
CGRP group (n = 5)	56.6 ± 14.7	81.7 ± 11.3[e]	81.1 ± 17.4[f]	73.1 ± 18.2[g]	88.7 ± 23.5	90.6 ± 13.5	84.0 ± 6.6	87.8 ± 15.2

[a] Values are mean ± standard deviation, expressed as percentage of vessel diameter on Day 0.
[b] IC, internal carotid artery; MC, middle cerebral artery; AC, anterior cerebral arery; CGRP, calcitonin gene-related peptide.
[c] Total represents the mean of IC, MC, and AC values.
[d] SAH and placebo groups are pooled into the non-CGRP group.
[e] $P < 0.05$ versus non-CGRP group.
[f] $P < 0.03$ versus non-CGRP group.
[g] $P < 0.02$ versus non-CGRP group.

Table 11: Change in Cerebral Artery Diameter: Inoue et al. 1996

Calculation of dosing schedule

The concentration of αCGRP in the CSF of humans appears to be higher after SAH and ranges from 0.10 to 0.84 ng/ml (15-125 ng assuming a CSF volume of 150 mls in adults) (727).

To achieve the minimum CSF concentration of 23 nanomoles/litre, that we predict will have biological effect, requires 3.45 nanomoles, assuming complete CSF distribution, or 0.575 nanomoles if retained within the lateral ventricle (unlikely to occur, but may reflect a peak concentration). The following information has been used to calculate the expected 'no effect' level of 10^{-13} mol /kg CGRP:

- The molecular mass of αCGRP is 3789.36g (~4000g)
- 4000g of αCGRP = ~1 mole

- 4 mcg of αCGRP = ~1 nanomole
- Macaca fascicularis have a CSF volume of 3ml/kg and weigh 4-8kg; CSF volume and body weight are approximately 10% of human values.
- Study dosing was approximately 4 mcg/day (1 nanomole) to give very low levels, measurable at day seven of 6.6 ± 5.4 nanomoles/litre.
- Prior studies from the same manuscript suggest that a minimum CSF concentration of 23 nanomoles/litre is the minimum effect level for αCGRP in CSF to cause vasodilation (26).
- Humans have a CSF volume of approximately 150mls (or 2mls/kg) and we assume complete CSF distribution.
- Therefore, the minimum effect level for αCGRP in CSF is found by 150/1000 = 0.15.
- 0.15 * 23 = 3.45 nanomoles (13.8 mcg).
- However, the concentration of αCGRP in the CSF of humans after SAH ranges from 0.1 to 0.84 ng/ml (15 to 125 ng assuming a CSF volume of 150 mls in adults) (727). The concentration in control patients is 0.1 ng/ml (15 mcg).
- Therefore, we have started at five doses below this effect level.

Doses (example doses in micrograms based upon a 75kg adult):

No effect level:

1. 10^{-13} mol/kg (0.03 mcg)
2. $3x10^{-13}$ mol/kg (0.09 mcg)

Possible effect level:

3. 10^{-12} mol/kg (0.3 mcg)

4. $3.0x10^{-12}$ mol/kg (0.9 mcg)
5. 10^{-11} mol/kg (3 mcg)

Effect level:

6. $3.0x10^{-11}$ mol/kg (9 mcg)
7. 10^{-10} mol/kg (30 mcg).

Anticipated effect size:

We anticipate little or no effect from our first three doses according to the MABEL approach. We expect partial to complete reversal of cerebral vessel narrowing from our sixth dose of CGRP, which would be consistent with previous animal studies.

Dosing and Sampling

- A maximum of one patient will be studied per day;
- Each patient will only receive one dose of αCGRP;
- The administration is a bolus dose, not a continuous infusion;
- The study will start with the lowest dose, which will be given to three patients, and will progress to the next, higher dose after review and approval by the Data Monitoring Committee (DMC);
- There are seven dose levels in total.

The study will start with the lowest dose, which will be administered to three patients. Each patient will receive a single dose of αCGRP given into the CSF. Escalation from the starting dose for the following patients will follow a traditional escalation rule using a 3+3 design. αCGRP will be administered as per the local site protocol for this route of administration. Those administering αCGRP will be on the intrathecal register and competent at performing this

role. CSF, blood and urine will be sampled at intervals for 24 hours.

Immediately after the intervention:

- Patients will continue to be monitored and managed in Critical Care with side effects documented.
- CSF drainage will be stopped for one hour, during which time transcranial Doppler flow velocities will be continually assessed from the MCA with a EZ-Dop scanner (Compumedics, Singen, Germany). Thereafter, transcranial Doppler measurements will be taken every 30 minutes up to six hours.
- Immediately after the drain is open (hour one), blood, urine and CSF samples will be taken. They will then be taken again each hour for five hours and once again 24 hours after the administration of the peptide. The samples will be anonymised with a study number, spun and frozen at -80°C in an alarmed freezer.
- A repeat CTA will be performed two to four hours after the intervention in an NHS CT scanner close to the Critical Care area which would be routinely used for patients if required. αCGRP will be given to patients during daytime hours to ensure staff are present to facilitate use of the scanner.

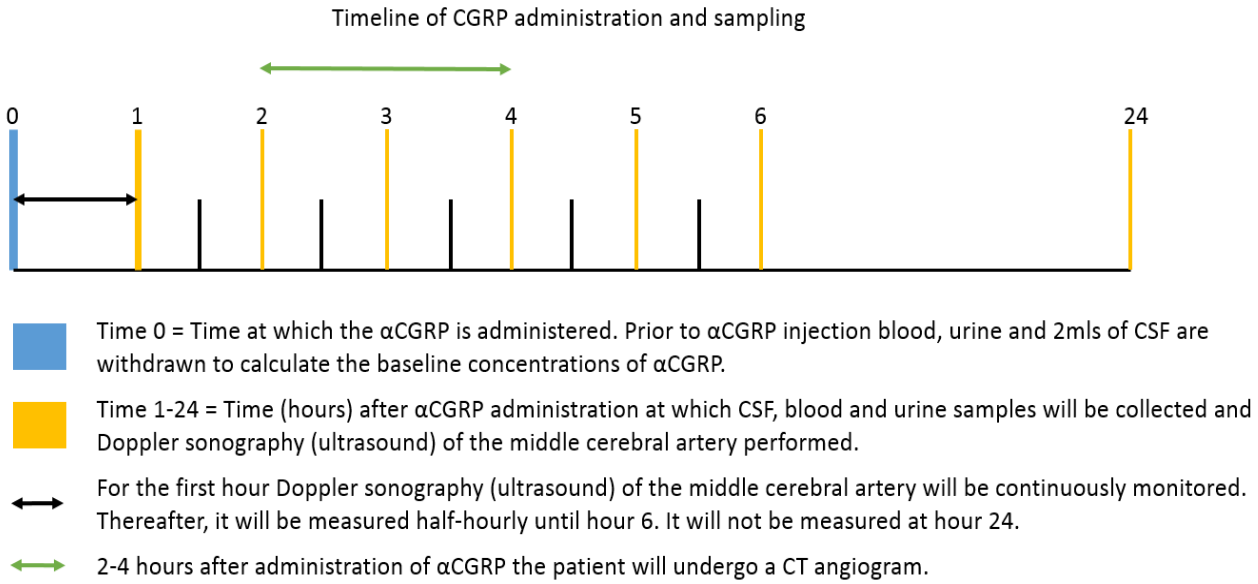

Figure 28: Sampling Schedule. Time in hours. Time 0 = the time at which CGRP is first administered. Doppler sonography of the MCA will be continuous for the first hour and then performed half-hourly until hour 6. It will not be performed at hour 24.

Stopping Rules and Decision Making

Recruitment will be suspended after any serious adverse event (SAE) and further dosing stopped. A DMC review will be undertaken to decide if further doses can be given to subsequent patients. Escalation to a higher dosing level will only occur after a DMC review.

The dose escalation will be stopped if cardiovascular SAEs are observed in two or more patients out of three at the same dose and associated with CSF administration of αCGRP. The study will not be stopped following a single cardiovascular SAE after administration as this may be unrelated to αCGRP. However, recruitment will be suspended and the DMC will review every SAE.

The study will only continue once the DMC are satisfied it is safe to do so. The study will be stopped should cardiorespiratory arrest, intracranial haemorrhage or death occur. In addition, if the DMC deems that the SAE is serious enough to suspend the study for any reason other than those listed above, it will be suspended. The justification for this is to balance the risks to the patients in this study against the potential information for future patient benefit gained by the research. Stopping the study due to one SAE potentially unrelated to αCGRP in a critically ill patient group, may unnecessarily deny future patients with this severe disease, who currently have few options for treatment, an intervention which has the potential to reduce morbidity and mortality.

A 3+3 approach will be taken, beginning at d_k=1. Each dose will be allocated to a minimum of three patients. If an SAE is reported at one dose, the DMC may request a further three patients be studied at that dose to assess tolerability before progressing to the next, higher dose (Fig.25).

- Assuming the study is not stopped early, a minimum of 21 patients will be needed for the study if the DMC does not request any repeat dosing prior to progression from one dose level to the next. Calculation: 7 doses x 3 patients at each dose = 21 patients.
- A maximum of 42 patients will be needed for the study if every dose is required to be repeated in a further three patients prior to progression due to one SAE at each dose level. Calculation: 7 dose levels x 6 patients = 42 patients.

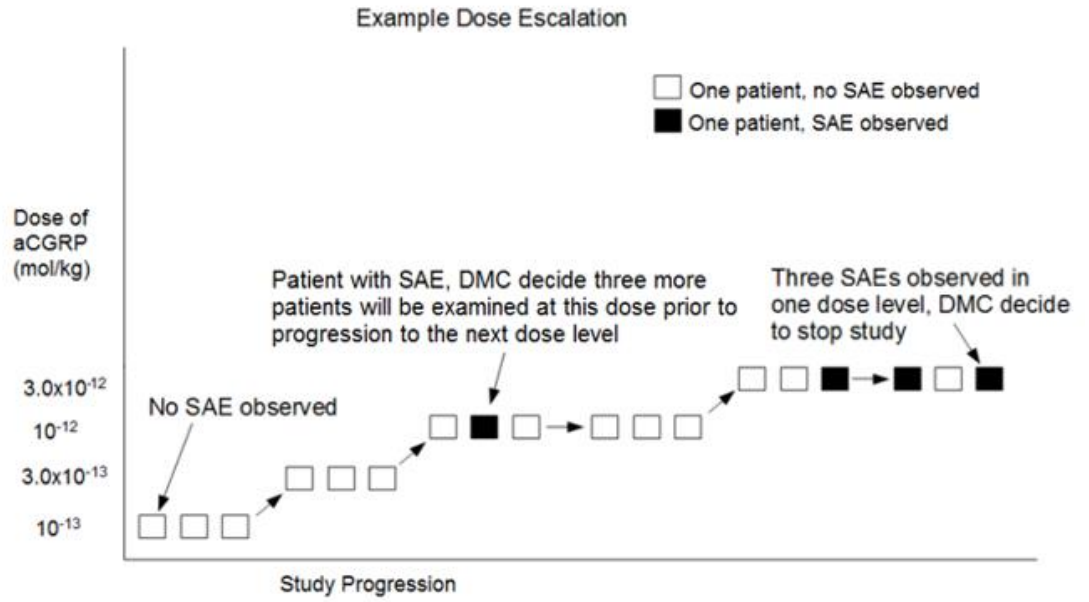

Figure 29: Study Progression Based on DMC Review

Inclusion Criteria

- Males or non-pregnant females aged over 18 years;
- Patients with a ruptured aneurysm, confirmed by DSA or CTA which has been secured by endovascular coiling or surgical clipping;
- World Federation of Neurological Surgeons (WFNS) grade I-IV measured prior to the patient undergoing endovascular coiling and which does not worsen to grade V after the patient has undergone the procedure (Grade V = GCS <7);
- CSF access device or EVD clinically indicated;
- Written informed consent to participate in the study must be obtained from the closest relative or welfare guardian prior to initiation of any research procedures.

Exclusion Criteria

- SAH due to causes other than saccular aneurysm;
- Multiple aneurysms among which the ruptured one cannot be identified and/or all are not secured during coiling;
- No end-of-procedure DSA;
- Refractory hypotension (BP < 90mmHg);
- Multiple organ failure;
- Any severe or unstable concomitant condition or disease (e.g. known significant neurological deficit, cancer, haematological disease, coronary disease or psychiatric disorder), which would affect assessment of the safety or efficacy of the intervention (investigator's opinion).

Informing Potential Patients about the Trial

The clinical team responsible for patient care will identify potential patients. Specifically, this will be a neurovascular clinical nurse specialist who has expertise in aSAH. Often, patients who require EVD/access devices after aSAH do not have capacity. Because of this, a clinical nurse specialist will describe the study to relatives of the patient, or their welfare guardian, and provide verbal information with the information leaflet. If the relative/welfare guardian would like the patient to take part or would like further information at this stage, a member of the research team will discuss this with them. These researchers will confirm eligibility, explain the study further and obtain consent. Particulars of the staff informing the patients and the date and time of this will

be recorded in the subject pre-screening log, including a record of further discussion with a member of the research team.

αCGRP Manufacturer

The synthesis of αCGRP is carried out using standard solid phase chemistry at a Good Laboratory Practice (GLP) compliant manufacturing site, Bachem AG, Hauptstrasse 144, 4416 Bubendorf, Switzerland. No materials at risk of transmitting transmissible spongiform encephalopathy are used in the manufacturing process. αCGRP manufacture and filling, under aseptic conditions, and Qualified Person release are performed by Bachem according to Good Manufacturing Practice (GMP). The quality of the product is controlled by ensuring compliance to internal specifications set with due regard to European guidance on the control of Investigational Medicinal Products (IMPs). Once delivered, the pharmacy team at the Western General Hospital, Edinburgh, will dilute αCGRP to specific doses.

αCGRP is to be stored frozen at -20°C (+/- 5^{o}C). Stability studies are being conducted to confirm the stability of αCGRP formulation over the proposed study duration. αCGRP will be stored in the pharmacy at the Western General Hospital, Edinburgh.

Prohibited Medications

- Drugs with anti-cholinergic side effects: e.g. Atropine, Benztropine (Cogentin), Biperiden, Ipratropium (Atrovent), Oxitropium (Oxivent), Tiotropium (Spiriva), Glycopyrrolate (Robinul), Oxybutynin (Ditropan, Driptane, Lyrinel XL), Tolterodine (Detrol, Detrusitol), Chlorphenamine (Chlor-Trimeton), Diphenhydramine (Benadryl, Sominex, Advil PM), Dimenhydrinate (Dramamine), Orphenadrine, Trihexyphenidyl, Dicyclomine (Dicycloverine);
- Angiotensin Converting Enzyme inhibitors (to be omitted on the day of surgery and for the duration of the study);
- Angiotensin Receptor Blocking Agents (to be omitted on the day of surgery and for the duration of the study);
- Drugs that may cause facial flushing. E.g. Nitroglycerin, Nifedipine, Niacin, alcohol and Vancomycin.

Proposed Analysis

Administration of αCGRP into the cerebral ventricles via the ventricular access device/EVD best fits a single compartment model. The ventricles are the central compartment wherein the drug-in-study is administered and this is also the bio-phase or effect compartment. Systemic circulation, where CSF (along with the dissolved αCGRP) will be transported, is the peripheral compartment and is not context sensitive. Some αCGRP undergoes cleavage at the Leu16-

Ser17 bond through neutral endopeptidase present in CSF, leaving behind inactive metabolites. Metabolism of αCGRP from the systemic circulation is of no relevance to CSF concentrations because transport into the systemic circulation is not context sensitive and there is no redistribution of αCGRP back into the cerebral ventricles. Therefore, the volume of distribution (Vd) of αCGRP after administration in the CSF will be given by 'Vd = amount of αCGRP administered in ventricular CSF / concentration of αCGRP measured in CSF'. The clearance (CL) of αCGRP from ventricular CSF will be given by 'CL = amount of αCGRP administered in ventricular CSF / area under the curve (AUC)'.

AUC can be integrated from the graph of decaying concentration of αCGRP over time. Elimination half time (t½) of αCGRP administered in CSF will be given by 't½ = 0.693 x Vd / CL', where 0.693 is the natural logarithm of 2. From the known values of Cmax, Vd and CL the loading dose and maintenance infusion rate for a phase II trial can be derived as 'loading dose = Cmax x Vd' and 'Maintenance infusion rate = Cmax x CL'.

Descriptive analysis will be presented including any AE, SAE or withdrawals. Where available, this AE/SAE/withdrawal information will be presented in relation to the timing of doses. We will analyse dose response based on CTP and TCD results. Dose response curves will be produced after each cohort, showing the levels of αCGRP found in blood, CSF and urine for all subjects. Pharmacokinetic and pharmacodynamic analysis will be ongoing throughout

months seven-to-24 whilst we collect data from each group of patients. For the pharmacokinetic/pharmacodynamic modelling we expect a steady-state condition in the CSF and a fixed-effect model. The questions we will attempt to answer are:

1. What is the dose-response?
2. What is the minimally effective dose?
3. How many adverse events are expected at higher doses?
4. What is the half-life of αCGRP in the CSF?
5. How much αCGRP crosses the BBB?
6. Is αCGRP renally excreted?

Definitions of SAEs/AEs

The following events are considered life-threatening and/or likely to prolong existing hospitalisation and are therefore SAEs:

1. Haemodynamic instability including: SBP decrease >25 mmHg; a MAP decrease >20 mmHg; heart rate increase to >150 bpm.
    - A BP SAE will be recorded after three confirmed non-invasive BP measurements with change assessed from baseline (time zero), or any symptomatic hypotension (a reduction in BP associated with symptoms, including lightheadness);

- A heart rate SAE will be a heart rate greater than 150 bpm, sustained for more than five minutes, or any symptomatic tachycardia (an increase in heart rate associated with symptoms including chest tightness).

2. Reduction in GCS >2 points or one motor point; change in pupil response or size
3. Clinical evidence of a seizure.

The following will be recorded as Adverse Events (AEs)

1. Spontaneous hyperventilation; $PaCO_2$ < 4.0 kPa, monitored using a TOSCA device (Radiometer Medical, Åkandevej 21, DK-2700 Brønshøj, Denmark). This is possible after cerebral ventricular system injection (736).
2. Facial flushing. If observed, an assessment of the involvement one-to-four facial quadrants will be made and an assessment of intensity of rubor made (mild, moderate, intense).

End of Study

The end of the study is defined as 24 hours from the administration of αCGRP after the final set of samples have been taken, or 24 hours after an SAE. The Investigators and/or the trial steering committee and/or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons. The end of the study will be reported to the Research Ethics Council and Regulatory Authority within 90 days, or 15 days if the study is terminated

prematurely. The investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. A summary report of the study will be provided to the Research Ethics Council and Regulatory Authority within one year of the end of the study.

Response to Protocol and Summary

The proposed study has been presented to Edinburgh University Research Governance (ACCORD) and is supported pending funding. Unfortunately, the research proposal was not successful at the full application stage for Medical Research Council funding, (panel score 21/30: 4, 5, 4, 4, 2). The responses were mostly positive and included well-reasoned comments which are discussed below:

> *"The Schebesch et al. study from 2013 demonstrated a clear difference between vasospasm and non-vasospasm patients' CSF CGRP levels in the first four days (727). However, the study only measured CGRP in CSF up to ten days in aSAH patients. Given the risk of DCI persists up to 14-21 days, it would be useful to measure CGRP levels up to these time points or until the external ventricular drain/access device is removed."*

The reviewer makes a valid suggestion, and one that would be appropriate in a separate study of changes in αCGRP concentrations in patients with aSAH and EVDs or VADs. There are a few problems with applying this to our proposed study. The half-life of αCGRP is four-to-six hours in the CSF (690,

696) and seven-to-ten minutes in the systemic circulation (624, 625, 736, 737). Therefore, any change in αCGRP concentration from a single intrathecal bolus of αCGRP is unlikely to alter the CSF concentration for as long as 14-21 days. The risk of ventriculitis will increase with repeated CSF sampling. Finally, the cost of the project will increase significantly with continued monitoring.

> *"There should be a specified time point for recruitment (e.g. 48 hours from ictus). This is based on the Schebesch et al. paper from 2013 suggesting that the difference in CGRP levels persisted for only four days (727). This is likely to affect recruitment targets."*

I agree that there should be criteria to exclude patients whose ictus is greater than ten days. However, as the reviewer has already pointed out, the risk of DCI can persist up to 14-21 days. Therefore, although the CGRP levels have been shown to be higher in the first four days in patients who did not go on to develop vasospasm in the 2013 Schebesch et al. paper, this does not mean that patients already with DCI, or at risk of developing DCI, (i.e. patients beyond day four) would not benefit from CGRP.

> *"In addition to GCS and seizures, new neurological deficits should also be part of the AEs. In addition, an indication of ICP and draining from the EVD should be examined as changes in the blood flow can affect ICP."*

I agree with the first part of this comment. New neurological deficits should be included as AEs. It remains unclear whether monitoring ICP has any genuine

beneficial impact on outcomes in TBI patients (see Chapter 1). Patients with an EVD do not routinely have their ICP measured at the Western General Hospital and a drain set to allow CSF outflow in the case of hydrocephalus would not be demonstrating a genuine physiological ICP (i.e. if the patient's ICP rises above the level of the drain then CSF will flow out of the drain to lower ICP). The study could include a measure of CSF drainage from the EVD. In the case of patients with a ventricular access device, it may not be in the patient's best interest to have an ICP monitor inserted (see evidence of ICP monitoring benefit/risks of infection in Chapter 1).

> *"Outcome assessment: Having recruited the patients, it would be sensible to collect some preliminary outcome data – in my view, although CT perfusion will be helpful, the main outcome measure that should be collected in this preliminary study is daily or twice-daily TCD measures for as long as clinically possible. Additional data on the presence/absence of clinical DIND will be useful to collect as well."*

The proposed study is a dose-toxicity study, but I agree that evidence of efficacy would be beneficial to progress to the next phase, which is why we have proposed CTP and regular TCD. A clinical neurological score assessment should also be included. This would not only be beneficial for evidence of efficacy but also as a measure of incidence of DCI and its clinical course in this group of patients.

"The frequency of CSF sampling produces a high risk of introducing CSF infection in these patients, with potentially devastating consequences. There must be a clear protocol to reduce infection associated with drug administration and CSF sampling in these patients. CSF infection should also be monitored as part of the safety. Is there any indication of the effect of CSF infection on CGRP levels?"

The reviewer raises a valid point, which is why CSF sampling is limited. To my knowledge, there is no data on the effect of CSF infection on CSF CGRP concentrations. It would be worthwhile doing a subgroup analysis if any patients did develop a ventriculitis to determine if there is a change in CGRP concentrations with infection. There are both intrathecal drug administration and CSF sampling protocols which are included in the full trial protocol.

"Intrathecal use limits the population it can be applied to. Typically, only a third of treated SAH patients will have this access. The question arises if the anticipated future application is for this same patient group who are having an EVD sited for other reasons or another group. It is also unclear if it is expected to be used prophylactically or as a treatment of established vasospasm? If repeated doses are planned for prophylactic treatment this will clearly require further study. If CGRP is proposed as a single treatment for established vasospasm, then it will further limit the treatment to a very select group of patients. One could imagine if successful that this might be expanded to the two-thirds of SAH patients without EVD by administration by lumbar drain although it is uncertain with SAH in situ if the drug distribution within the CSF in that scenario will be the same. It would be helpful to have more clarity on

what the expected ultimate mode of use of the drug would be to understand what percentage of SAH patients this will really be applicable to. Furthermore, while vasospasm represents a contribution to outcome the degree is unknown but sample size calculations from Loch MacDonald make rather depressing reading for following this approach." (738).

The reviewer raises a good point, especially regarding the potential limitations of the lumbar intrathecal route in SAH patients. The purpose of our proposed trial is to assess dose response and toxicity. I think if we are able to establish a safe dose of CGRP and demonstrate a degree of efficacy at this stage then we can start to think about how we can apply CGRP as a treatment. If effective, CGRP could be used as a treatment of established vasospasm in patients with EVDs, although as the reviewer correctly states, this limits the intervention to a small group of patients. There are multiple ways that CGRP could be altered to change administration:

1. A slow release preparation could be developed similar to the microspheres developed by Inoue et al. and Ahmad et al. (692, 697). Ideally, this would be administered at the time of surgical/radiological intervention but could potentially be administered via EVD.

2. A continuous infusion could be administered via EVD/VAD (with the associated limitations already mentioned).

3. There is animal data from Sun et al. that intranasal delivery of CGRP attenuated vasospasm in rats. Intranasal delivery offers a mode of non-invasive delivery suitable for both prophylactic treatment and intervention in established DCI (691, 739).

4. There are a small number of animal studies successfully demonstrating amelioration of vessel narrowing after gene transfer of CGRP. While this route of administration appears a long way from human trials, it offers another potential route in the future (708, 740, 741). Interestingly, Toyoda et al., demonstrated some prophylactic benefit from gene transfer of CGRP in rabbits that were administered a viral-encoded gene five days prior to experimental SAH. Basilar artery contraction was 23% (±11%, $p<0.02$) less than those who had received vehicle (710).

The referenced paper of Loch MacDonald, which quotes the incidence of DCI as 12-30% and a required sample size of over 5,000, assumes a small treatment effect and is more relevant to a future Phase III trial than an initial dose-toxicity study. I agree that it does make depressing reading but this is not a reason to stop seeking better treatments for DCI. The paper also highlights the importance of a unified definition of DCI as mentioned in Chapter 2.

- *"The applicants make passing reference that CGRP dilates small vessels of the microcirculation but do not provide any evidence for this or explain its importance. The reason it is important is that the existing drug studies have*

clearly established that treating large vessel vasospasm does not ameliorate outcome. Clazosentan was highly successful at resolving large vessel vasospasm with extremely impressive dose response curves reducing from 66% to 23% but ultimately did not alter outcome. Conversely Nimodipine had a small effect on outcome (33% vs 20% poor outcome) but its action was not through large vessel dilation as evidenced by rates of vasospasm. The implication from statements that CGRP acts on small vessels is that it will not fall into the same trap as Clazosentan in acting as a highly effective large vessel vasodilator but not altering outcome, but no evidence is provided that it acts on the microcirculation specifically. This may be available and would be highly beneficial to the rationale for the study."

The evidence I have provided in Chapter 3 is to highlight that cerebral vessel narrowing may be involved in the pathogenesis of DCI despite the findings of the CONSCIOUS trials. DCI may also be related to microvascular vasoconstriction not visualised by DSA. I cannot find any animal models of SAH and DCI assessing the microvascular effects of αCGRP. All *in vivo* studies included in our systematic review assessed large vessel changes (most commonly basilar artery changes, one measured ICA/MCA/ACA changes). However, Kobari et al. have demonstrated that αCGRP dilates small parenchymal vessels through a specific αCGRP receptor in a non-SAH model in cats (742). In addition to demonstrating its dilatory effects, Clazosentan was also associated with pulmonary complications, anaemia and hypotension which may have contributed to its apparent lack of efficacy. It will be interesting to read the results of the REVERSE trial (Clinical trials NCT02560532).

αCGRP has not been associated with such side effects in animal studies or human trials.

The two factors that are likely preventing a CGRP trial going forward are:

1. The CONSCIOUS trials appear to have demonstrated no outcome benefit from an agent that ameliorates cerebral vessel narrowing, thereby leading many to question its relevance (571).
2. The proposed route of delivery appears high-risk in a critically unwell patient group.

The arguments for evaluating the effect of intrathecal αCGRP include:

1. It acts on the abluminal side of blood vessels and so is still able to dilate morphologically damaged vessels after SAH.
2. Systemic effects are likely to be limited from this route of delivery.
3. Unlike endothelin receptor agonists, there was a positive signal towards improved neurological outcomes in the meta-analysis of animal studies.

Recommendations

- If nimodipine is beneficial in the treatment of aSAH then understanding how it exerts its beneficial effects is crucial. This could help explain the pathophysiology of DCI and lead to improved treatment.

- It is not uncommon to observe patients with suspected DCI receiving both nimodipine and inotropic support to reach a target SBP. The use of an inotropic agent, or fluid therapy, to achieve a SBP is without a

strong evidence base. Small, non-randomised trials do support the use of induced hypertension in the management of DCI. However, there is no guidance on whether SBP or MAP should be targeted or what the target should be. The use of inotropes and fluid therapy to achieve a SBP is not without risk. The only Cochrane review of an aspect of triple H therapy was of volume expansion (hypervolaemia) in 2004 (743). The last systematic review of haemodynamic therapies was conducted in 2011 (522). Therefore, an updated systematic review and meta-analysis would be useful. A trial randomising patients with DCI (as per the definition in Chapter 3) to induced hypertension with multiple targets or normotension is required. The difficulty, apart from recruitment failure as experienced by the HIMALAIA trial, is choosing how any target is maintained, with fluid therapy or inotropic support, and whether patients would still receive nimodipine or not. Designing a prospective RCT to maintain euvolaemia and use inotropic support to achieve two target temperatures versus no induced hypertension with retrospective analysis of fluid therapy is one option. Such a trial is likely to need to be multi-centre to achieve adequate recruitment.

- A trial examining the effects of intrathecal αCGRP in patients with DCI should be undertaken for the reasons discussed above. It may be that such a trial is only possible through commercial routes.

Conclusion and Reflection

This thesis, in part, summarises the preparatory work involved in clinical research and is an example of the difficulties associated with delivering a clinical trial. I have submitted two applications to the First in Human (FIH) committee with associated meetings and revisions to our application based on comments. Our research team has had three meetings with ACCORD (NHS Lothian/University of Edinburgh research sponsor); a teleconference and an advisory meeting with the MHRA; and I have submitted three separate applications to the Medical Research Council (MRC, two provisional, one full application). Each of these applications takes a significant amount of work (see appendix 3 for MRC summary application) and includes a detailed cost-analysis in addition to a full trial protocol. To complete an application involves not only the primary investigators, but is also dependent upon the aid of people in university and NHS finance departments, NHS research and development staff, commercialisation staff, laboratory staff (both NHS laboratory for storage and private laboratories for RIA), statisticians, senior clinicians included in the research team who are not directly involved in the application or initial stages of research and commercial pharmaceutical representatives. In addition to this, two review papers were published and original research (requiring ethical approval) was undertaken to support these applications.

An argument against our proposed trial which was put forward by the First in Human committee and ACCORD was that investigating CGRP in a vulnerable

patient group was associated with two problems. Firstly, that side effects in critically unwell intubated and sedated patients may be missed. Secondly, that these critically unwell patients would be more likely to have serious adverse events than healthy volunteers. As such, it was recommended that a study involving spinal administration of CGRP in healthy volunteers as a dose-tolerability study should be conducted instead. My argument against this is that it is unethical to administer CGRP to healthy volunteers for tolerability analysis when these patients would not even receive a theoretical benefit from its administration and would receive the risk of hypotension and headache. In contrast, patients with poor-grade SAH who have developed DCI have an extremely poor prognosis with little or no treatment options. In these patients, CGRP would offer a potential treatment benefit. Furthermore, poor-grade SAH patients with DCI are routinely managed on ICU with invasive monitoring and one-to-one nursing care where physiological effects of CGRP are unlikely to go unobserved. The external reviewers for the MRC did not raise similar concerns. There appeared to be three main limitations to our applications being successful:

1. Administering a pharmacological agent directly into cerebral CSF appears inherently unsafe despite our attempts to provide evidence that other agents are delivered by this route;
2. The cost of the peptide was prohibitively expensive (see Appendix 3 for costs);

3. There was perceived to be insufficient evidence to support a clinical trial of intrathecal CGRP. I think that this is chiefly due to results from the CONSCIOUS trials.

Outcomes after SAH have improved in the last 40 years despite a lack of innovative treatments and a wealth of unsuccessful interventions (426, 470, 597). I think this is due to improved adherence to guidelines and basic clinical care in addition to increased management in specialist centres. Although SAH outcomes have improved, DCI remains a significant cause of morbidity and mortality after aSAH and an effective treatment is lacking. There are ongoing clinical trials as discussed in Chapter 3 (e.g. NEWTON, ULTRA, SAHaRA, REVERSE and EARLYDRAIN) and the results of these are keenly awaited (506, 542, 560, 596). Unfortunately, I do not think I will be able to add to this list by investigating intrathecal CGRP as a treatment option for DCI unless a commercial partner is interested and willing to fund the research. I will, however, undertake a systematic review of studies investigating hypertension as a treatment for DCI. This could form the basis for a RCT investigating induced hypertension in patients with DCI.

Having now finished writing this thesis and having attempted to conduct a clinical trial I realise that the bulk of the work may not be in running the trial and the write-up. The hardest part of conducting clinical research might be in convincing investors to provide funding and convincing regulatory committees to approve the trial.

Reflection on PhD

The following section has been included in response to the thesis viva in September 2017. It is intended to describe what I have learnt from undertaking a PhD, including study design and data analysis. I have tried to present a chronological account of my PhD and in doing so respond to some points made in the viva. I first discuss the analysis of $P_{bt}O_2$ data and the difficulties I had with missing data and statistical analysis. I go on to outline the process of applying for local committee and funding approval. Finally, I discuss my experience of undertaking a PhD at this stage of medical training.

In 2013 I started a Masters in Medical Sciences (MMedSci) degree led by Dr Richard Weller at the University of Edinburgh. I had finished my Foundation Year 2 training and wanted to undertake a postgraduate research degree and thought that this would be a good introduction to research. I approached Professor Andrews, at the Western General Hospital ICU, and enquired about potential research opportunities in neuro-intensive care. My interest was in clinical research and I was aware that Professor Andrews was the Chief Investigator for the Eurotherm3235 trial. We discussed various research projects and agreed that I would investigate two. Firstly, I would conduct a subgroup analysis of $P_{bt}O_2$ data from patients in the Eurotherm3235 trial (Chapter 2). Secondly, I would investigate CGRP as an intervention for delayed cerebral ischaemia after subarachnoid haemorrhage (Chapters 3-5).

The proposal was that I would develop a clinical trial whilst also analysing original data for the MMedSci.

Eurotherm3235 Data

This work was conducted in collaboration with Professor Andrews and Dr Rhodes at the Western General Hospital, Edinburgh. The benefit of conducting this part of my PhD was that it allowed me to carry out original analysis of the effect of temperature on $P_{bt}O_2$ in patients with TBI. The research involved patients in an unblinded multi-centre, randomised-controlled trial where funding and regulatory milestones had been achieved. Furthermore, my analysis was not post-hoc, but was instead prospective analysis of data from the Edinburgh centre. Our results were presented at the 4th International Hypothermia Conference prior to the New England journal publication by Andrews et al (279, 744). Whilst Eurotherm3235 was investigating outcome data from the use of hypothermia in TBI, I was interested in the changes in $P_{bt}O_2$ values. The reference to ICP changes supported previous findings rather than contributing new information (227, 312-314, 340-348).

With regards to study design, Eurotherm3235 serves as an example of a multi-centre, randomised controlled trial that successfully recruited 387 patients based upon a sample size calculation with an a priori statistical analysis plan. However, my analysis of data from patients recruited to Eurotherm3235 revealed a problem with the implementation of study design. Specifically, the

mean time to reaching target temperature was approximately seven hours from randomisation (Chapter 2). Furthermore, this delay was seen in the Edinburgh trial centre, where one might expect to find the least delay in reaching target temperature due to the centre's prior experience in inducing hypothermia. In our Therapeutic Hypothermia publication it was proposed that long lead times to induce hypothermia were due to pre-randomisation hypertonic therapy (745). As discussed in Chapter 2, I attribute the delay in reaching target temperature to the realities of conducting clinical research. It would be interesting to see if there was a difference between the time of randomisation and the time recorded for the administration of cold saline. There are many possible causes for delays:

- Staffing: an example is staff breaks where 1:1 nursing care (one nurse to one patient) becomes 1:2 or 1:3. It is possible that patients met criteria for randomisation and were randomised but there was a delay in starting cooling due to staff waiting for the end of a break. This may sound trivial or inappropriate, but many staff members working on wards will be aware that patient care can be affected by staff breaks. Furthermore, it is not difficult to imagine that at the end of a break the patient may be due intravenous antibiotics, fluid changes etc. It may be that initiating cold fluids came at the end of a list of interventions. This can be mitigated with research nurses prioritised to randomisation and ensuring the intervention begins quickly. However, the reality of clinical work is that predicting when patients will become eligible is challenging.

Furthermore, the times that patients meet criteria for cooling may be outside the hours of 0800 to 1700.

- Equipment preparation: Here I refer to the storage and administration of cold saline. Ideally this would be stored within close reach and be administered rapidly to achieve target temperature.
- Other interventions interfering with administration of cold fluids: e.g. patients going to CT scan shortly after randomisation.
- Variables that may affect the rate of cooling such as fevers, body mass, seizures etc.
- Education: I believe this is key to overcoming some of the issues mentioned above. In an environment where patients and research participants will be managed by a non-research clinical team there needs to be some incentive for staff to facilitate research without compromising clinical care. I suspect that if staff members understand the importance of why different variables are being recorded, or why interventions are being instigated, then there would be more of an incentive for these to happen. Furthermore, the unblinded nature of the study allows for bias and complacency. By this I mean that the urgency of instigating an intervention may become less important the less clinical staff believe in its efficacy.

Eurotherm3235 prospectively enrolled patients. My own analysis was retrospective and was not blinded. Because I was analysing brain and core temperatures in addition to $P_{bt}O_2$ and ICP, I could establish which patients

were in the control or intervention groups. If another investigator had been included to analyse the data we could have separately examined changes in these variables, although the investigator examining core temperature would always know which patients were in which group.

One aspect of joining a clinical trial that was already being delivered that I found difficult was defending a study that I had not designed. This is not meant as a criticism of the trial, nor is it necessarily problematic. The act of responding to criticisms was a positive experience. I believe that the peer review process in its purest form should be a positive, thought-provoking exercise used to better our understanding of research. However, one difficulty I experienced was that my understanding of the study design was not as strong as it would have been had I been involved in the study design process from conception. I may have been able to answer criticisms more readily had I been involved in the initial decisions. For example, the decision to use cold saline to induce hypothermia and the associated potential risk of pulmonary oedema or increased CPP with an increased MAP. Had I been involved in this decision I might have better understood the practical applications and the previous evidence supporting its use and the alternatives available (Chapter 1) (339). This is just one example, there were many other small areas of study design that perhaps took me longer to understand than it would have taken if I had been involved in the study's conception. There was also a benefit to me experiencing this difficulty. When I came to write the αCGRP protocol, I was aware that what appears obvious to the research team is not always clear to

someone outside of the research team reading the protocol. This became especially apparent when writing grant applications.

Statistical Analysis

ICP, $P_{bt}O_2$, and chart data were recorded from TBI patients enrolled in the Eurotherm3235 study. Patient data from the Licox monitor was recorded and timestamped on excel spreadsheets. I looked through all excel spreadsheets to identify accurate Licox recordings and excluded inadequate data for $P_{bt}O_2$, ICP and brain temperature (e.g. erroneous negative values, those without the analysis time period). I also examined ICU charts for each patients' ICU stay and recorded core temperature, blood gas analysis, ICP treatments, whether patients were paralysed and whether patients received NMDA receptor antagonists. Each patient's hour data was averaged (see Appendix 1 for example of raw data). Prior to analysis, the research team met with a local research statistician at the Welcome Trust Clinical Research Facility, who directed statistical analysis. It was decided that due to the small sample size, correcting for non-parametric data was not required. This analysis was questioned by reviewers of the published work and our response was accepted:

> *"A repeat measures ANOVA was used to take into account measurements at different time periods from the same subjects after discussion with our local statistician. We do not have multiple covariates*

for analysis and therefore have not used the mixed methods approach. While we acknowledge that there may be a relationship with our outcome and other variables, due to the sample size of the study and lack of multiple covariates for analysis we are unable to examine this further."

I have summarised the statistical tests and results here for easy reference. For exact values see Chapter 2:

Intracranial Pressure

Within Group Control Tests: One-way repeat measures ANOVA: no statistically significant difference with time.

Within Group Intervention Tests: One-way repeat measures ANOVA: statistically significant difference with time. T-tests run to determine when the difference was.

Between Groups Tests: Two-way repeat measures ANOVA: statistically significant difference between the groups with time.

PbtO2

Within Group Control Tests: One-way repeat measures ANOVA: no statistically significant difference with time. T-tests documented to demonstrate that there was also a decrease in $P_{bt}O_2$ in the control group.

Within Group Intervention Tests: One-way repeat measures ANOVA: statistically significant difference with time. T-tests run to determine when the difference was.

Between Groups Tests: Unable to run two-way ANOVA due to missing data. No statistically significant difference between the two groups based on unpaired t-tests.

Based upon my PhD supervisors, Professor Andrews and Dr Rhodes, a local statistician (Cat Graham, see publication acknowledgements) and journal peer review accepting this method of analysis, the work has been published and presented in this viva (please see Appendix 2 for the publication) (745). A repeat measures ANOVA was used because it accounts for measurements from the same subject repeated over multiple time points without treating the data from different times as though they are from independent subjects (746). I thought that the ideal test would have been a two-way repeat measures ANOVA if the data was normal. However, I could not run a two-way repeat measures ANOVA in Minitab or SPSS for $P_{bt}O_2$ due to data missing in comparison fields (see Appendix 1 for example of missing data). Therefore, I performed two one-way repeat measures ANOVAs (once for the intervention group and once for the control group) to determine if there was a statistically significant difference within the two groups for $P_{bt}O_2$. Then, to check between the groups I performed post-hoc analysis with t-tests. This seemed to be the best I could do with repeat measure data and did not seem controversial to me. I do acknowledge that not performing intention-to-treat analysis is controversial. However, I do not think that intention-to-treat analysis would have reflected the effect of hypothermia on $P_{bt}O_2$ because of the delay in reaching target temperature (Figure 30).

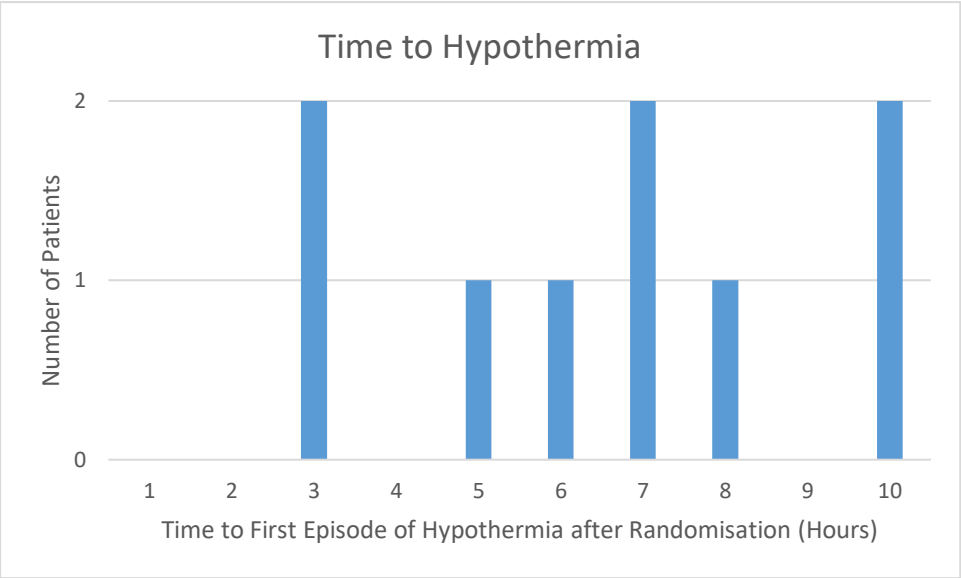

Figure 30: Time to first episode of hypothermia. Time taken for patients to reach the first episode of hypothermia (≤35°C) measured in hours (<35C)

The principal arguments discussed in the viva regarding the statistics used in the second chapter of the thesis were, as I recall them:

1. Based on the graphs presented (Figures 16 to 18, Chapter 2), the data did not appear to be parametric and therefore performing ANOVA and t-tests was not appropriate. It was suggested that the Kruskal-Wallis test may have been more appropriate.

2. Performing multiple t-tests was incorrect as it will have produced a seemingly statistically significant result if performed enough times.

With regards to the first point, the original intention to treat data, when looking at the hour prior to randomisation to 19 hours after, appears parametric for

both the ICP and $P_{bt}O_2$ data (Figures 30 to 33). Furthermore, the F-test in ANOVA has been shown to be robust when assuming normality in terms of a Type I error (747-750). However, I agree that the graphs presented in Chapter 2 do suggest that the data does not look normally distributed. I had discussed this with a statistician, Cat Graham, and had been advised to perform parametric tests.

I performed probability tests to look at the data prior to running the statistical tests in chapter two. I have reproduced the graphs of these tests below. The first four graphs (Figures 30 to 33) demonstrate that the data points appear normally distributed but also that the Anderson Darling test gives a P value of greater than 0.05, suggesting that we cannot assume the data is not normally distributed. It is important to note that this is for the intention to treat mean data for the hour prior to randomisation to twenty hours after (hour -1 to hour 19).

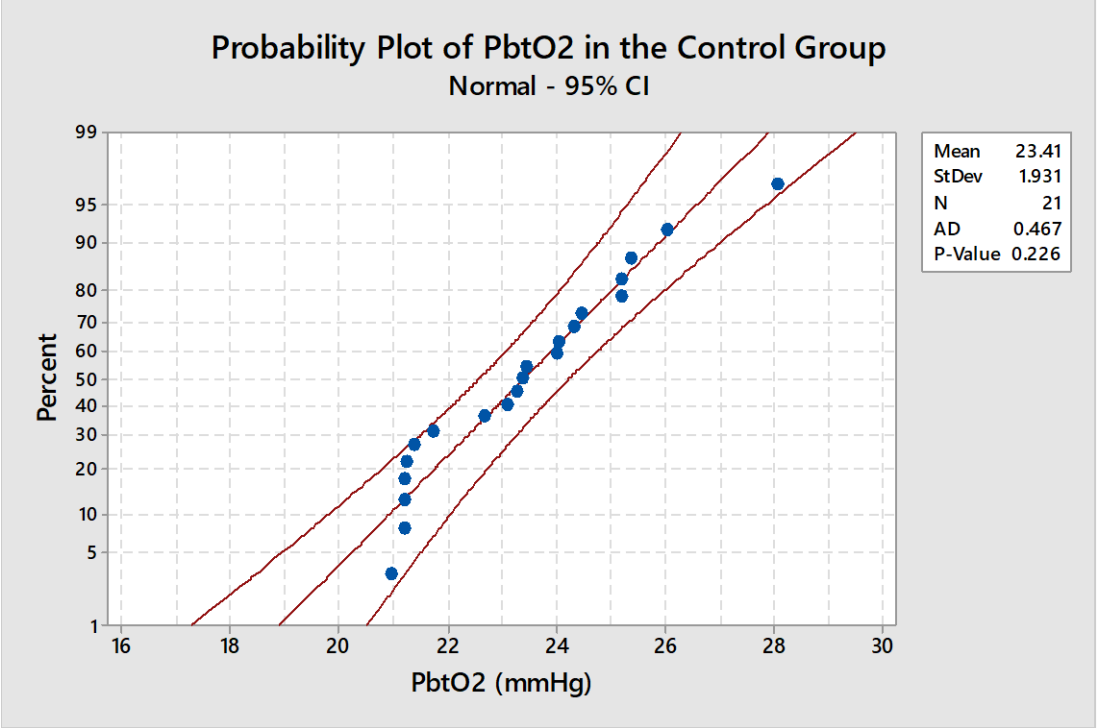

Figure 31: Probability Plot for partial brain oxygen tension ($P_{bt}O_2$) in the control group. Data points look approximately normally distributed within the confidence interval and Anderson Darling test P value > 0.05.

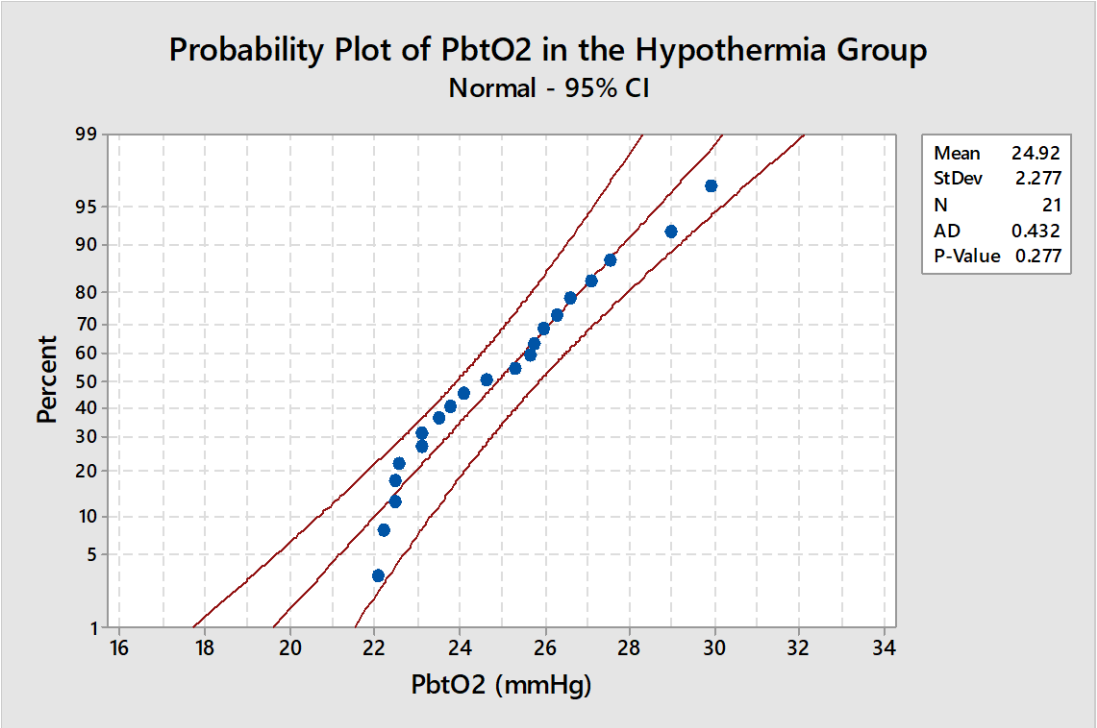

Figure 32*:* Probability Plot for partial brain oxygen tension ($P_{bt}O_2$) in the intervention group. Data points look normally distributed and Anderson

Darling test P value > 0.05.

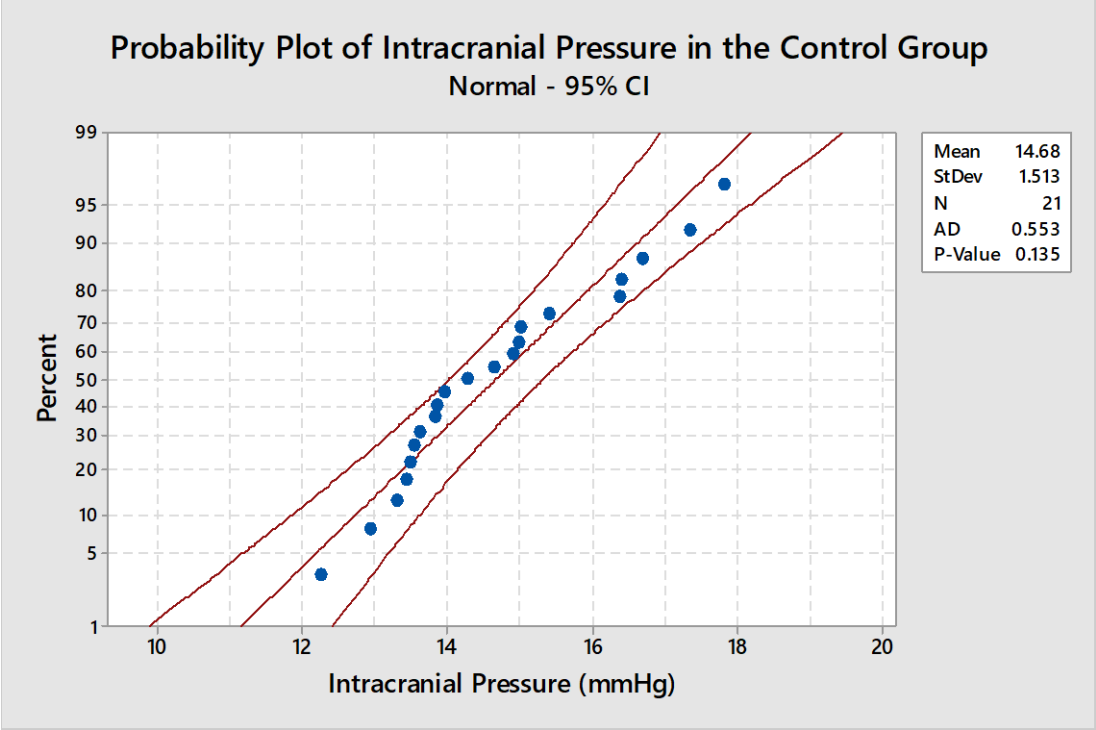

Figure 33: Probability Plot for ICP in the control group. Data points look normally distributed and Anderson Darling test P value > 0.05.

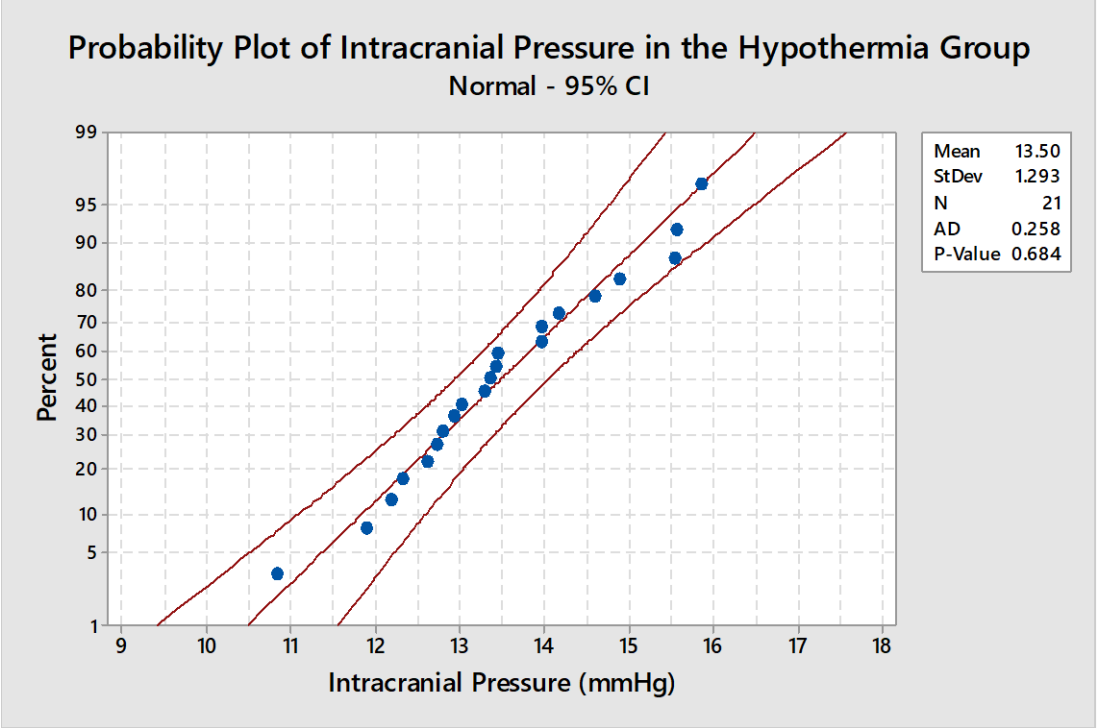

Figure 34: Probability Plot for ICP in the intervention group. Data points look normally distributed and Anderson Darling test P value > 0.05.

The data from the four different time points that were used in Chapter 2 (the hour prior to randomisation; the first hour of hypothermia; the first episode of stable hypothermia (defined as two consecutive hours); finally, six consecutive hours of hypothermia) also appears generally normal and non-normality cannot be assumed based on the Anderson Darling test (Figures 34 to 37). Indeed, the only graphs which do not demonstrate normal data are those concerning $P_{bt}O_2$ and ICP in the control group (Time 3 in Figure 35 and Time 2 in Figure 37).

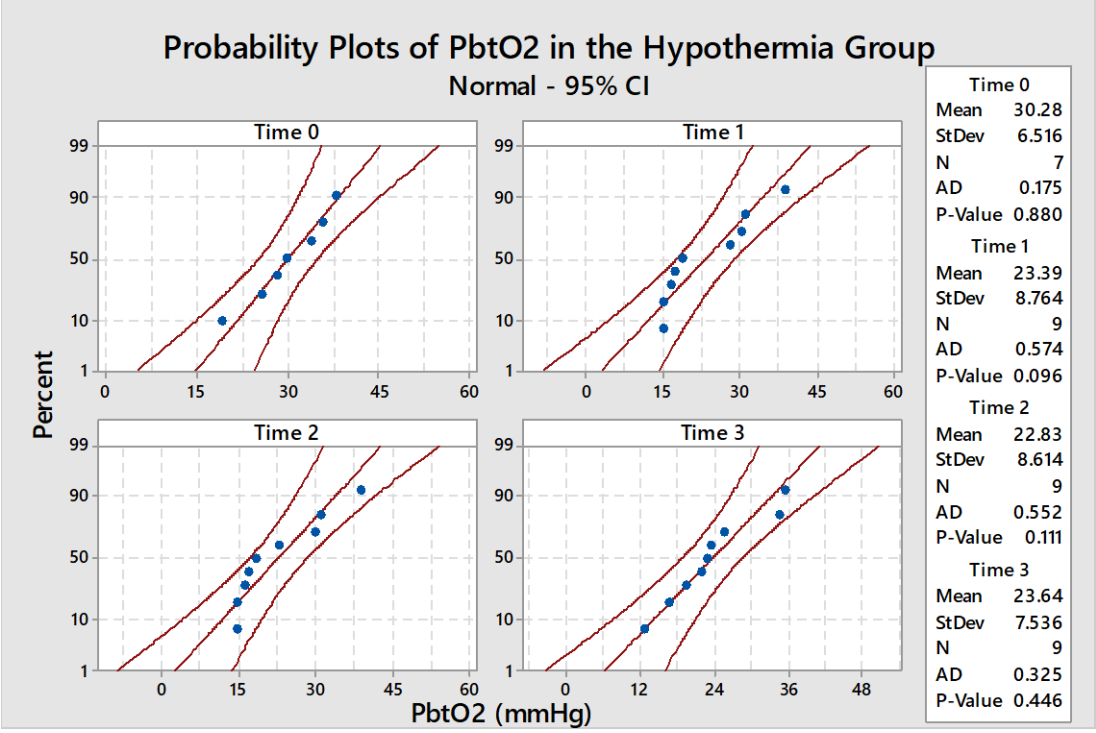

Figure 35: Probability plots for partial brain oxygen tension ($P_{bt}O_2$) in the intervention group. Time periods as per Chapter 2 Data.

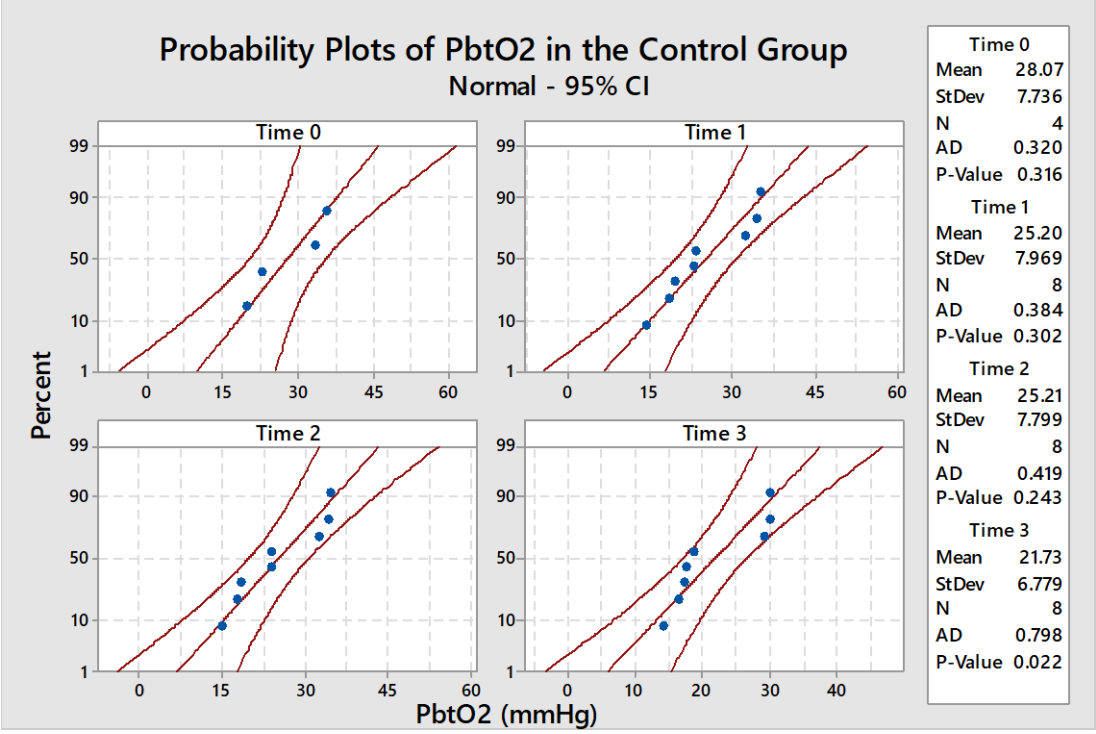

Figure 36: Probability plots for partial brain oxygen tension ($P_{bt}O_2$) in the control group. Time periods as per Chapter 2 Data.

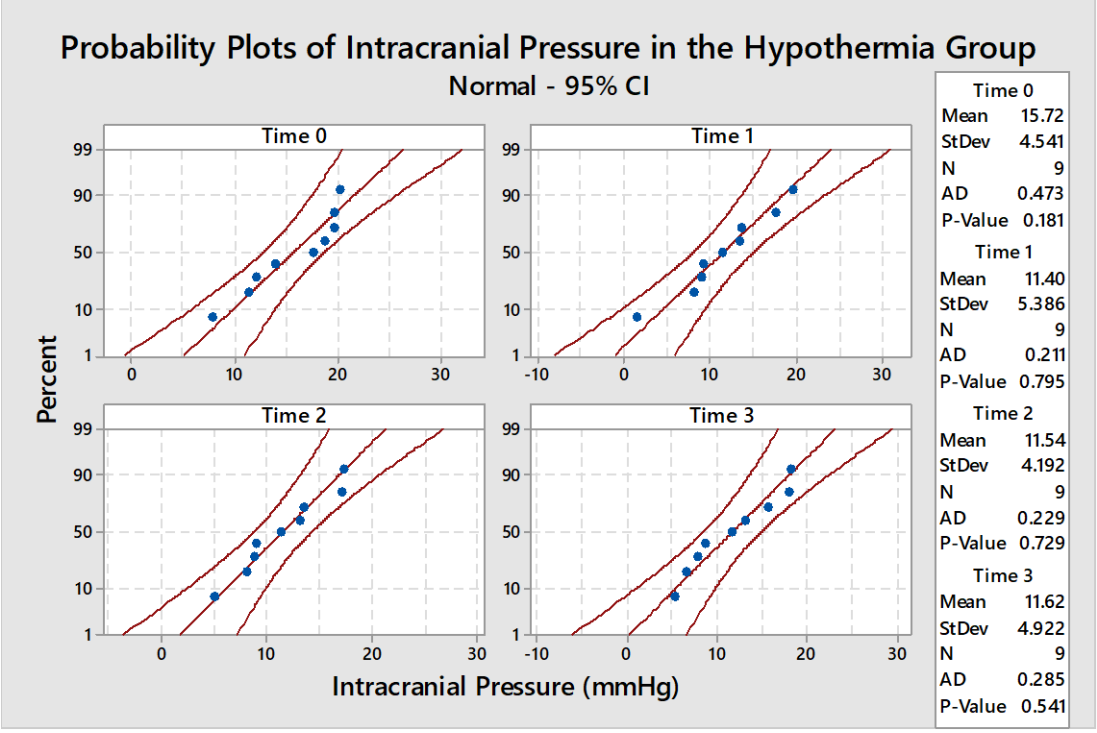

Figure 37: Probability plots of intracranial pressure in the intervention group. Time periods as per Chapter 2 Data.

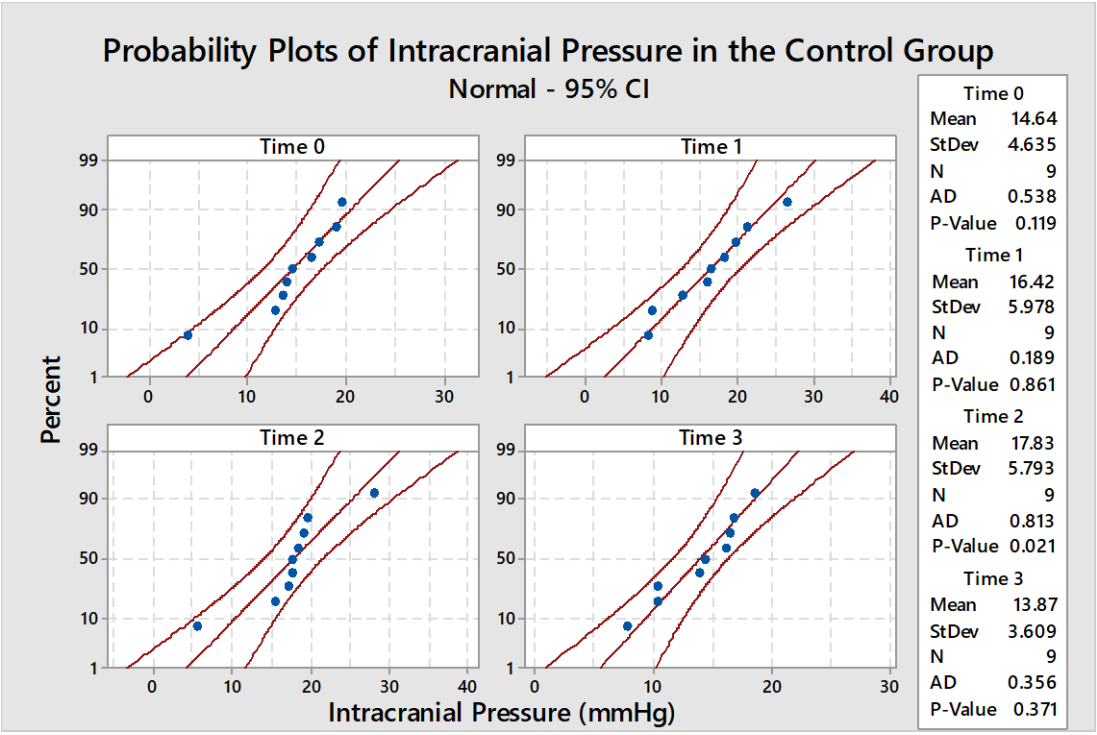

Figure 38: Probability plots of intracranial pressure in the control group. Time periods as described in Chapter 2.

To summarise, the data can be assumed to be normally distributed apart from one block of data in the control group for $P_{bt}O_2$ and one block of data in the control group for ICP (751). Being pragmatic about this, the risk of performing a parametric test with non-parametric data is of having a Type I error (incorrectly rejecting the null hypothesis). In Chapter 2 I reported that, in terms of $P_{bt}O_2$, "*there was no statistically significant difference between the two groups"* and that *"there was no statistically significant difference in ICP in the control group".* Therefore, concern regarding a type I error is unnecessary because the null hypothesis is accepted. The following is taken from a wonderful paper by Geoff Norman which appears to be inspired by a paper highlighting the importance of "*inappropriate statistical dogmatism*" (752). The main point taken from this publication is that, despite their use being a common criticism of papers, parametric tests assuming normality are very robust.

"For the standard t tests ANOVAs, and so on, it is the assumption of normality of the distribution of means, not of the data. The Central Limit Theorem shows that, for sample sizes greater than 5 or 10 per group, the means are approximately normally distributed regardless of the original distribution. Empirical studies of robustness of ANOVA date all the way back to Pearson (1931) who found ANOVA was robust for highly skewed non-normal distributions and sample sizes of 4, 5 and 10. Boneau (1960) looked at normal, rectangular and exponential distributions and sample sizes of 5 and 15, and showed that 17 of the 20 calculated P-values were between .04 and .07 for a nominal 0.05. Thus, both theory and data converge on the

conclusion that parametric methods examining differences between means, for sample sizes greater than 5, do not require the assumption of normality, and will yield nearly correct answers even for manifestly nonnormal and asymmetric distributions like exponentials." (748, 749, 753)

With regards to performing a Kruskal-Wallis test, there is another reason, apart from the data being generally normal: the Kruskal-Wallis test assumes independence of observations and does not account for repeat measures and as such may actually increase the chance of a type I error (754). As mentioned earlier, the reason to use a non-parametric test in non-normal data is to reduce the risk of a type I error. Thus, it would be counterproductive to use the Kruskal-Wallis test for this data. An example of a non-parametric test allowing for repeat measures is Friedman's test. However, Friedman's test is equivalent to a non-parametric one-way ANOVA and so could not be used to test differences between the groups. Both the Kruskal-Wallis test and Friedman's test are omnibus tests and so require post-hoc analysis.

I have stated that the concern about chosing the wrong tests lay in the potential for making a type I error and that I had accepted the null hypothesis is the data which failed the Anderson Darling test. However, I did report three statistically significant ANOVAs in chapter 2. These included a within groups effect on $P_{bt}O_2$ in the hypothermia group; a within groups effect on ICP in the hypothermia group and a between groups effect on ICP. For completeness, I have repeated these tests with non-parametric analysis using SPSS. For this

I used Friedman's test, as it is able to account for repeat measures. This test demonstrated a within group difference in ICP for the hypothermia group (P 0.04) but no statistically significant difference in the change in $P_{bt}O_2$ in the hypothermia group (P 0.12). I have used the related-samples Wilcoxon signed rank test for post-hoc analysis. This showed that the decrease in $P_{bt}O_2$ from Time 0 to Time 2 in the intervention group was not statistically significant (P 0.06). It is worth pointing out that the risk of using non-parametric tests in normal data is of having a type II error. If the new analysis is accepted then the summary is:

- Statistically significant decrease in ICP seen within the hypothermia group and between the groups.
- No statistically significant decrease in $P_{bt}O_2$ either within or between the groups. A non-statistically significant trend towards decreased $P_{bt}O_2$ in both.

This analysis does not change my ICP discussion points in Chapter 2 as non-parametric testing is consistent with parametric testing, nor does it change my $P_{bt}O_2$ discussion.

The second point raised was regarding using t-tests across multiple time points (although the same argument can be applied to almost any test with an α of 0.05). Indeed, the argument made was that if I had run 20 t-tests I would inevitably have a seemingly statistically significant result (I believe this was

based upon a p value of 0.05 x 20 = 1). In response to this: multiple t-tests were not run to achieve a statistically significant result. The ANOVA was run to identify whether there was a statistically significant difference within the groups. The ANOVA is an omnibus test and requires a post-hoc test (I chose the t-tests in this instance) to identify where (in this case, when) the statistical difference might be. The concern of using multiple t-tests is that it increases the chance of making a Type I error (the incorrect rejection of a true null hypothesis). Whilst that risk might be 5% with one t-test, it is not quite 15% with three t-tests (it is approximately 14.26%) (755, 756). This is why a repeat measures ANOVA was performed in the first instance rather than multiple t-tests. However, the one-way repeat measures ANOVA was not able to determine where the significance lay within the groups or time points, only that there is a significant difference. I think I did not explain this clearly in Chapter 2. With any repeat measures data there will exist this problem. To identify at what point there was a statistically significant result requires multiple tests. A single test (the ANOVA in this case) can be run to determine if there was any statistically significant difference between the groups, but it cannot state when. This is likely to occur often in clinical trials because we measure changes over time, not solely ‘before and after’. One way to counteract this is to use Bonferroni correction, which divides the α by the number of hypotheses being tested. This correction reduces the statistical power (increases the chance of a false negative) and can be conservative if the statistics are positively correlated.

Managing missing data

Missing data is likely to occur in a multi-centre trial involving the recording of multiple variables by different staff members with multiple recording devices. Missing data reduces the power of a trial and the effect of missing data is larger in smaller studies with less data. Increasing the target sample size can partially compensate for this and as such perhaps more attention should be paid to missing data in study design (i.e. increasing sample size, educating staff regarding the importance of data recording and collection) (757).

Missing data can be classified based on the following assumptions first described by Rubin (758, 759):

- Missing completely at random (MCAR)

The missing data is not related to the value being obtained or the observed responses. An example of this would be missing $P_{bt}O_2$ or ICP data due to equipment failure where values were not recorded onto the software program. This occurred, and still does occur on the intensive care unit due to faulty cables. Another example would be loss of blood results due to samples being lost in transit. The benefit of MCAR data loss is that the analysis remains unbiased. Power may still be lost in the design, as mentioned above.

- Missing at random (MAR)

This is said to be a more realistic assumption and occurs where missing responses are dependent upon observed responses, but are not related to the specific missing values. For example, that a $P_{bt}O_2$ value is missing may be independent of the value of $P_{bt}O_2$ but it might be dependent upon the ICP that led to the patient being transferred for a CT scan. One could argue that in this example ICP and $P_{bt}O_2$ are not independent variables and so it is not MAR, but the important thing about MAR is that the missing variable is not directly responsible.

- Missing not at random (MNAR)

Missing not at random data refers to data that is missing specifically because of that variable. An example would be missing ICP data due to a patient being taken to CT because of refractory intracranial hypertension.

There are different methods of handling missing data, some of which I have discussed below. The best solution is to prevent data loss by good study design or retrieve missing data. If this is not possible, or data are lost despite good study design one must rely on data imputation, replacing missing values with substitute values. It is not always clear which method of imputation is best each is associated with limitations (757):

1. Prevent data loss through good study design.

2. Try to obtain missing data: this is possible with missing values which can be obtained from paper charts or laboratory recordings but is not possible when the initial record keeping is at fault.

3. Listwise deletion: delete all data from participants with any missing data. This is only practical if there is a very large sample and if the data is missing at random.

4. Averaging: replace missing data with the sample mean as a conservative estimate. The problem with this method is that it artificially reduces the variability of the data.

5. Attempt to estimate the missing data based on observed data. This is essentially making 'educated guesses' based on previous values. This is easier when there are a limited number of variables, but with multiple possible values and large variability it becomes more problematic.

6. Common point imputation: this can be used with scaled data where one uses the middle point or the most commonly chosen point (e.g. on a scale of 1 to 5, choosing 3 or the most commonly chosen point).

7. Regression substitution (imputation): multiple regression analysis can be used to estimate a missing value. Using this method relies on having enough data to be able to accurately create stable regression equations. The existing variables are used to make a prediction on what the missing value would be. However, no novel information is added. Therefore, the sample size is increased and the standard error is reduced.

8. Multiple imputation: perhaps the most popular approach. Statistical packages such as SPSS have multiple imputation calculators that can create plausible values based upon any correlation between missing data. In addition to this, it incorporates random errors into the predictions. The method resembles regression substitution but instead of a single value being produced, a series of plausible values are produced containing a degree of variability. Not only is a missing value produced, multiple imputed data sets are produced and are used for standard analysis. The user receives multiple analysis results to produce a single overall result. The advantage of multiple imputation is that it restores the natural variability of the missing values and incorporates uncertainty into the analysis. It can be used with small sample sizes or large samples with a large amount of missing data.

One option for my missing $P_{bt}O_2$ was to perform multiple imputation on the data thus enabling a two-way repeat measures ANOVA in SPSS. Performing multiple imputation may also make interpreting normality from a probability plot easier due to increasing the sample size. I have performed multiple imputation for the $P_{bt}O_2$ data to create five versions of the data (five complete data sets, six including the original with missing data). A two-way repeat measures ANOVA of this data showed no statistically significant difference between the two groups with time (Wilk's Lambda 0.808, P 0.411).

Summary of Statistical Analysis

I found it difficult to analyse the data in this study for the following reasons:

- There are missing data.
- There are repeat measures in the same patients. Variables were measured over different time points from the same patient. This becomes difficult when running ANOVA because the tests often assume data is not linked. As such, repeat measures ANOVA was used. Because of missing data, I could not run a two-way repeat measures ANOVA in SPSS without correcting for missing data.
- The data appears grossly normal, but not completely adherent to the normal distribution line as discussed above. After discussion with a statistician, it was decided that I should perform parametric analysis.
- There are multiple variables to be tested, which may be linked.

- There were several outliers in the raw data, some of which were physiologically impossible.

I was advised to perform these statistical tests by a professional statistician, the data were reviewed by my supervisors, two intensive care consultants with prior experience of publishing their own work, and the data was subsequently published in a journal after peer-review. I had attended a University of Edinburgh course that taught basic statistics aimed at postgraduate students studying for masters and doctoral degrees. How to perform a two-way repeat measures ANOVA with multiple imputation for missing data was not covered in this course and so the practical aspects of running these tests was self-taught. The most difficult aspect of statistical analysis for me was not identifying the correct tests to perform, but learning how to practically apply them in the software packages. There are so many options available that it is very easy to make the wrong choice. Furthermore, some packages provide so much information after running a test that it can be difficult to establish which piece of information is actually useful.

My advice to other PhD students would be to make the most of the University courses available, because they are very good, but also to acknowledge that a professional statistician is likely to be needed for complex statistics. A learning point, and suggestion to others writing a thesis, is to clearly defend the use of statistical methods in the thesis to avoid having to do so in the viva. Or to at least have a defence in your thesis that may aid your viva discussion.

Alpha Calcitonin Gene-Related Peptide Study

Kokkorris et al., in their review of αCGRP and aSAH, noted that there has been a lack of further research after the intravenous administration of αCGRP in patients with SAH by the European CGRP in Subarachnoid Haemorrhage Study Group in 1992 (see chapters 3 and 4) (599, 640). As the authors of that paper suggested, the next logical step appears to be to investigate the effect of αCGRP after intrathecal administration. I completed a study protocol for the intrathecal administration of αCGRP in humans and an application to submit to the First in Human committee in Edinburgh (Appendix 4). Initially, we had attempted to investigate intrathecal αCGRP as a Phase I trial in patients receiving spinal anaesthesia for urological procedures. We planned to administer αCGRP via spinal and sample CSF via spinal catheter in patients undergoing day case urological procedures who would have otherwise received spinal anaesthesia. The First in Human committee in Edinburgh decided that because αCGRP had previously been administered to humans (albeit as an intravenous administration, never intrathecal), they were not the correct regulatory body to approve such a study. I decided to re-write the protocol and change our study population from healthy volunteers to patients with poor-grade aSAH requiring an IVD or EVD that could be used for αCGRP administration and CSF sampling. In this way, patients would be on the ICU and would have continuous monitoring and the ability to receive CT scans and frequent Doppler imaging of their MCA. Although this study was, to my mind, a dose-testing and tolerability study, we had aimed to collect some evidence

of efficacy to facilitate progression to the next phase. After re-writing the protocol, aspects of it were reviewed by Professor Andrews, Dr Harris (research nurse), Cat Graham (statistician), Dr Downer (interventional radiology) and Ioannis Fouyas (neurosurgery). This took us into the second year of the PhD.

The design of our proposed CGRP study has been discussed fully in Chapter 4, along with my thoughts on why the study did not receive funding at the beginning of Chapter 5, so I have not repeated this here. Evidence of study design can be seen both in appendices 3 and 4 and in the above chapter. The main difficulties that I experienced whilst writing the protocol were in explaining it clearly to others. Aspects of the protocol that seemed obvious to me were causing confusion for others. For example, there was confusion regarding the 3x3 escalation rule, which appeared simple to many people but had to go through different iterations before it was clear to everyone. There was also some concern regarding the predicted sample size and duration of the study. The difficult here is identifying a duration that will enable enough recruitment without being prohibitively expensive. To help facilitate this I discussed the study with patients and the relatives of patients who were seen in follow-up clinics after having had SAH. This was done in coordination with a specialist nurse (Karen Briggs) involved with the coiling service in Edinburgh. Patient and relative information leaflets were included in the trial protocol appendices. We determined three-quarters of patients would have consented/been consented to be part of the study. This, together with the number of patients

admitted with poor-grade SAH, gave us an estimate of how many patients we expected to be able to recruit. I projected a conservative study duration (see Appendix 5 for Gantt Chart of proposed study milestones).

In March 2015 I submitted a grant application to the MRC, which was rejected based upon insufficient supporting evidence being available. In addition to this, the MRC thought that commercial exploitation could be difficult and that potential industry collaborations were vague. I subsequently submitted a further grant application to the MRC in July 2016. Since the first application I had, with the help of named colleagues, completed a systematic review and meta-analysis of animal studies investigating intrathecal αCGRP as a treatment for 'vasospasm' after SAH and looked at CSF concentrations of αCGRP in patients with SAH. The second application was accepted to the full application stage. After the first application it became apparent that it would be useful to have some evidence of CSF concentrations of αCGRP to help de-risk the study. I had performed a PubMed search of studies that had investigated αCGRP in animal studies as part of the meta-analysis. This search was duplicated by Dr Begg, a co-author of the meta-analysis but did not identify the publication by Schebesch et al. (727).

The learning point from this experience for me was to acknowledge that research is fundamentally a financial endeavour. Having acknowledged this, I should try to identify what the weaknesses of the proposed study are and combat these. For example, with αCGRP the scientific uncertainty is that some

authors no longer think that vasospasm plays a large role in DCI. Other authors have provided evidence to suggest that DCI does not actually contribute to mortality in a significant way (see chapters 3 and 4 for referenced discussion). There is little evidence that αCGRP can improve neurological function in animal studies (our meta-analysis suggests a potential benefit). Finally, previous studies have demonstrated an intervention that is able to dilate arteries without improving outcomes (see Clazosentan discussion in Chater 3). The financial weakness was that our application was based solely upon obtaining orphan status.

Personal Learning Points

I have been approached by a number of postgraduate medical doctors wanting advice regarding research. Some have been specifically asking about the MMedSci programme and some have been asking about undertaking a PhD. My first question tends to be “Why do you want to do research?” Sometimes it is because of a specific area of interest and sometimes because the medical specialty they are employed in has an expectation of research. On an individual level I think both of these are acceptable reasons, but perhaps they should be tackled differently. One dour surgical colleague, when I asked why he undertook a PhD, replied “nobody in Edinburgh takes you seriously unless you’ve tortured some mice for three years”. Unfortunately, I think there is some truth to his comment. If conducting a research degree purely for progression and to make the acquisition of a consultant post less arduous, I would suggest

trying to find a supervisor who has a background of high-profile publications in journals with a high impact factor. More important than this is to find one with funding available and ideally someone who has already submitted for ethical approval. Alternatively, some of my colleagues have become PIs for research led by pharmaceutical companies, who are able to provide funding and employ staff to tackle ethical applications. If undertaking research because of a specific interest or because of a desire to further understanding, ones' approach might be different. It may be more difficult because the barriers to research appear to be money and regulatory approval. Both of these barriers can lead to compromises on study design and quality. An example of this is sample size: an adequate sample size can be limited both ethically and financially. Before starting my PhD, I had thought that hard work and organisation would mitigate most problems. However, ultimately money seems to be the most important thing in research. Some may argue that good research is the most important thing. I would argue that it is impossible to conduct 'good' research without enough money. High impact factor publications lead to a higher chance of getting more acclaim and subsequently a higher chance of a successful grant. The learning point here is: be organised. Look for a study that has funding and ethical approval. If a good idea has neither of these things consider looking for something else, and if adamant that this is what you want to do, immediately start applying for a research grant. Nine out of twenty of the MRC's outline application questions and 30% of the allocated words are dedicated to funding. The full application includes expanded sections on 'Downstread Project Support' and 'Intellectual Property'.

The RCT I proposed at the end of Chapter 5, to investigate the effect of induced hypertension in patients with DCI, is worthwhile. I think this not only because we currently do not know whether we are causing harm, or whether we may be able to provide better care with more specific targets, but because I think we are currently applying a blind intervention based upon unproven theory. The problem is that it will be difficult for me to argue a financial case for such a study. The potential financial benefits are:

- Decreased patient hospital stay (reduced direct costs and indirect from decreased risk of infection etc.);
- Reduced time off work and therefore increased gross domestic productivity (GDP);
- Reduced cost of current ineffective interventions.

The first two can apply to any successful intervention for any disease. The difficulty is quantifying and justifying any proposed benefit. If I propose that we are causing harm with induced hypertension in patients with DCI, how could I quantify that harm? I have little evidence to suggest that it is causing more harm than benefit. In terms of cost of the current intervention, the cost of noradrenaline and an associated arterial line is low. Similarly, with my recommendation to repeat a nimodipine trial that I suspect would show no benefit. What is the financial incentive to perform such a trial? The cost of nimodipine is minimal, and as the Cochrane review states, the potential harm is also minimal (see chapter 3 for full discussion) (544). The important point here is to try and be pragmatic and think about how the research one proposes

to do will benefit society and how that can be argued in a grant application. With an intervention such as CGRP, if it was suggested to significantly reduce morbidity or mortality then it is potentially financially viable.

Timing of Research in a Career

I suspect that most UK clinicians who have not undertaken a PhD prior to studying medicine do so during their registrar years. I have no reference to support this, it is speculation and from my experience of speaking to colleagues who have completed postgraduate degrees. Many colleagues have finished a masters degree or diploma after their first two years of postgraduate clinical work, but few seem to have done an MD or PhD. Certainly, the Edinburgh Clinical Academic Track (ECAT) targets clinicians at the registrar level (a requirement for acceptance is to hold a national training number, which is typically awarded at Specialist Training year 3). The ECAT programme appears ideal for those with an interest in clinical academia. It offers two routes:

1. A clinical lectureship: successful applicants are employed until six months after completion of clinical training. The first year offers the opportunity to plan for a PhD and is thus called a 1+3 year model to obtain a PhD. After this time, trainees return as clinical lecturers with protected academic time until completion of training (CCT).

2. ECAT PhD fellowships: candidates do not have to hold national training numbers, although it is strongly encouraged. The degree is 3 months + 3 years, with the first 3 months used to facilitate the development of a PhD proposal. After this time, trainees may take up the opportunity to become clinical lecturers.

With regards to the knowledge or intellectual capability required, stage of training should not be a barrier to research. There is no greater intellectual complexity involved than was required at medical school, and I believe this is true throughout medical training. There is a great breadth of knowledge required which may have been acquired in some specialties with prior knowledge. However, many colleagues undertaking laboratory research have stated that they were required to learn a great deal of basic science knowledge, which their non-medical peers had learnt as undergraduates and which they did not learn, and would not need to learn as senior clinical trainees or consultants. The greatest challenge I have faced doing a PhD during my stage of training has occurred since returning to full time training. Returning to training has been much more difficult than I had anticipated. This is chiefly because my level of training is not congruent with the number of years that I have been practicing as a doctor. This is perhaps another reason I would encourage anyone considering a PhD to go through the ECAT programme if wanting to do so in Edinburgh. I suspect that undertaking a PhD after finishing postgraduate exams would fit more easily into a training schedule. I have had the opportunity since finishing this work to investigate other research proposals but have declined because of training commitments. Therefore, I think in

retrospect I would encourage others to undertake a PhD at a later stage, if only for these practical reasons.

Conclusion

I have learnt a great deal from undertaking this PhD separate from the academic work presented in the first four chapters. I have learnt about study design and statistical analysis. I have learnt about the practical aspects of undertaking research and the huge amount of work that must be performed in setting up a trial. I have come to appreciate that even small trials, such as my investigation of CSF samples, involve a larger number of people than I had previously thought. Perhaps one of the best skills I have gained is to readily question interventions and our reasons for implementing them. I not only refer to data from recent publications promoting a new intervention, or repurposed interventions, but also the treatments that we currently administer as part of routine care. The examples I give are nimodipine and induced hypertension but I could equally include many of the drugs that are routinely given in ICUs and theatres.

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Appendix 1: Raw Data for Chapter 2

Hour	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
p17		37.9	38.1	38.1	38.2	38.1	38.4	38.4	38.8		39.1	39	39	37.8	37.1	36.7	36.3	36.2	36.6	37
p19	36.8	37	37.2	37	37	37.2	37.1	37	37.1	37	37.2	37.2	36.8	36.9	37	37	37.2	37.1	36.8	36.9
p25	37.9	38.2	38.7	38.9	38.3	37.9			39.3			40	39	38.8	38.2	38		38.4	38	
p26		37.6	37.6	37.2	37	37.2	37.2	36.9	37	37.4	37.4	37.5	37.5	37.6	37.6	37.5	37.5	37.3	37.3	37.3
p27		37	37	37	37	36.8	37.2	37.4		37.3			36.6		36.3	36	35	35	35.2	
p34	37.5	38.4	38.5	38.3		38.2		37.3			36.5			37.2	37		36.8	37.3	37.3	37.1
p43	37	37.6	37.9	37.5	37.5	37.5	37.5	37.3	38.2	37.5	37.5	37.8		37.5				37.5		
p68	38.6							37.8		37.6	38	38.1		38.1			37.5		38.2	
p20			37.6	37.1	36.8	36	35.5	35	34	33	32.7	32.1	32.2	33.1	33.5	33.5	33.2	32	32.6	32.6
p28	36.9			38.2	37.8	37.6		37.3	37.3	37.1	37	37.1	36.2	35.9	35.5	34.6	33.5	32.7		32.5
p36	38.3	38.5	38.6	38.5	38.3	37.9	37.1	36.8	36.1	35.2	34.7	35	35	35	35	35.3	35.1	35.7	35.7	35
p51	36.8	35.8	35.6	35.7	35.4	35	34.7	34.3	35.2	35	34.5	33.8	34.3	34.7	35.8	36.2	35.4	34.3	33.2	33.5
p53	36.7	36.5	35.5	34.8	34.2	34.8	34	34	34	34		34	33.9	33.5	33.6	33.9	34	34		
p55		36.3					36.1	36	36	36	34.4	34.3	34.3	33.9	33.5	33.5	34.2	34.3	34.2	33.9
p56	35.1	35.9			34.9	36.9	34	34.3	34	34.2	34	34.5	34.5	34.6	33.5	34.9	35.1	34.4	34.3	34.9
p61	37.6			38.2	36.9	35.9	35.2	34.8	35.8	35.3	35.3	34.8	34.9	35	34.9	34.8	34.9	35.3	35.5	35.8
p70	36.2	35.7	36	36.4	35.8	35.5	35.2	34.8	35	35.2	35	35	35	35.2	35.1	34.9	34.9	35	35.2	34.9

Table 12: Raw Core Temperature Data. Missing data highlighted in grey. Hour = time from randomisation. First group = normothermia group. Second Group = hypothermia group.

Hour	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
p17			17.54	19.22	19.69	20.72	16.27	18.46	24.12		13.47	14.99	14.85	14.72	14.24	14.02	11.41	13.26	12.55	13.7
p19			4.08	5.23	6.55	9.2	13.77	14.44	18	18.58	19.78	21.25	16.84	16.65	17.76	15.05	14.01	14.37	13.82	14.92
p25	35.74	34.84	34.2	32.73	33.56	33.6	34.15	35.32	34.23	32.93	32.63	34.04	34.01	33.17	30.14	28.96	30.85	30.9	32.77	30.97
p26			24.39	23	21.79	23.62	24.73	19.58	15.09	12	15.45	14.66	16.78	16.46	16.54	17.2	19.2	22.04	23.87	23.01
p27			17.19	13.18	13.35	14.09	16.13	23.14	18.68	19.45	19.51	18.29	17	19.53	17.37	16.8	17.1	19.74	23.34	26.85
p34	33.51	33.67	32.98	32.19	30.92	31.25	32.18	32.53	34.63	33.53	33.42	32.88	35.27	32.02	29.14	30.2	29.46	29.74	29.46	28.81
p43	23.07	23.99	23.1	22.9	22.56	31.16	31.25	34.61	32.72	39.29	35.54	31.33	31.15	29.66	30.02	29.99	28.44	31.42	30.16	29.08
p68	19.94	11.69	17.61					23.51	24.19	22	22.26	20.25	20.44	19.2	18.64	17.39	17.37	23.36	28.61	28.56
p20	25.88	21.39	25.37	25.85	27.29	23.09	18.93	16.56	15.3	13.73	10.76	9.14	11.4	12.7	14.78	14.15	12.66	11.61	11.78	11.57
p28	33.99	27.83	31.13	38.71	33.73	27.75	26.53	29.05	24.73	36.78	33.02	26.08	14.25	17.21	20.6	18.63	18.15	18.4	21.6	18.41
p36	30.06	32.33	32.96	34.85	39.07	44.41	36.9	43.09	42.05	38.01	38.85	34.91	39.22	39.95	39.49	35.15	35.46	36.26	36.25	28.69
p51	38.21	28.46	32.45	28.48	33.91	30.21	22.4	28.06	27.15	24.55	25.9	21.86	19.03	16.87	12.55	17.1	27.98	16.28	13.24	15.49
p53	28.47	29.77	18.18	14.96	18.26	10.13	10.98	15.57	14.28	19.55		16.67	14.6	11.47	12.19	19.66	18.48	21.42	18.69	19.14
p55			37.54			30.6	48.06	38.35	35.88	36.69	31.12	33.37	33.38	29.07	29.12	36.26	34.65	35.91	35.5	33.31
p56	19.25	18.54		16.36	14.97	14.91	14.83	19.62	22.75	24.59	23	21.57	24.71	25.84	25.88	23.33	21.33	21.25	20.56	19.75
p61	36.08	26.48	47.35	51.97	33.69	29.46	25.92	27.94	29.93	28.76	26.53	23.04	23.15	23.35	26.86	22.95	25.54	28.19	31.73	31.88
p70		15.47	15.5	16.63	19.42	19.61	17.93	17.3	18.05	20.27	22.09	23.21	25.45	25.56	23.88	23.92	22.48	22.5	24.94	25.09

Table 13: Raw $P_{bt}O_2$ Data. Missing data highlighted in grey. Hour = time from randomisation. First group = normothermia group. Second Group = hypothermia group.

Hour	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
p17	19.6	17.7	16.2	20.9	25.5	20.7	22.1	26.6	28.4		16.2	15.2	15	16.5	18.5	20.9	20.9	18.2	13.2	14.9
p19	12.9	15.2	17.3	19.3	18.2	14.7	15.6	16	17.4	19.5	17	16.2	16.1	16.8	17	16.4	14.1	13.1	11.8	9.98
p25	19	13.8	12.9	9.58	9.03	12.6	15.9	18.3	17.9	17	16	16	15.1	14.5	14.2	13.6	14.2	14	16.3	14.7
p26	17.3	16.6	17.1	17	18.5	19.6	20.4	21.2	19.2	12.8	11.8	11.7	11.1	10.3	10.4	11.1	11.2	12.4	12.4	11.4
p27	13.6	16.6	20.5	14.3	19.3	19.9	20	8.61	5.72	6.53	9.15	9.9	10.6	10.4	8.5	10.9	9.22	12.5	11.9	12.2
p34	16.5	18.1	18.7	17.9	18.5	18	18.6	19.8	19.8	17.3	16.2	16.7	17.2	16.2	14.5	16.7	12	11.8	12.7	11.9
p43	14.1	12	11.5	6.87	5.68	11.5	8.97	8.17	18.6	4.95	2.58	4.23	7.32	7.83	7.95	11.8	2.25	11	8.52	7.78
p68	4	10.2	5					12.6	15.8	16.5	18.9	19.3	19.4	18.5	17.5	19	19.9	21.6	19.9	15.3
p20	18.8	20.5	22.4	20.9	18	18.7	18.8	17.5	18.1	17.9	18.3	16.5	17.2	18	16.3	15	16.6	13.8	17.3	17.7
p28	11.3	11.4	13.8	12	12.1	5.78	10.5	12.3	7.18	10.4	13	8.44	11.9	13.2	9.63	8.08	5.65	5.8	4.32	6.57
p36	17.6	13.9	9.05	9.48	14.5	22.7	15.3	11.7	11	9.98	9.08	8	8.82	8.98	8.25	8.15	8.57	8.73	8.72	7.43
p51	7.82	5.15	6.9	9.37	12.6	8.88	9.83	15.1	15.4	14.5	8.77	7.75	10.7	14.7	16.2	17.7	15.1	12.6	12.9	16
p53	19.8	24.7	21.8	11.3	18.2	21.3	14.7	23.3	13.1	18.2		13.4	15	16.6	19.6	23.1	21.8	19.2	22.1	21.9
p55	20.3	18.3	18.2			-1.62	6.05	14.8	15.3	11.5	13.3	17.8	12.3	12.9	16.6	15.7	11.7	11.3	13.1	13.8
p56	19.8	21.2		13.4	19.5	18	17.1	16.1	15.6	15.5	15.6	18.5	17	17.1	18.3	17.6	17.2	13.5	18.1	20.6
p61	13.9	21.1	14.7	6.85	4.45	2.28	2.88	1.45	-1.17	-1.1	2.6	5.03	4.47	5.13	20.9	11.8	11.1	18.4	11.7	4.35
p70	12.2	9.28	9.63	9.43	12.5	12.8	13.2	13.6	13	13.1	12.4	12.7	11.1	13.1	14.5	12.8	13.2	14.1	12.9	11.3

Table 14: Raw ICP Data. Missing data highlighted in grey. Hour = time from randomisation. First group = normothermia group. Second Group = hypothermia group.

Appendix 2: Publications

THERAPEUTIC HYPOTHERMIA AND TEMPERATURE MANAGEMENT
Volume 00, Number 00, 2015

DOI: 10.1089/ther.2015.0002

Clinical Research

Therapeutic Hypothermia Reduces Intracranial Pressure and Partial Brain Oxygen Tension in Patients with Severe Traumatic Brain Injury: Preliminary Data from the Eurotherm3235 Trial

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Traumatic brain injury (TBI) is a significant cause of disability and death and a huge economic burden throughout the world. Much of the morbidity associated with TBI is attributed to secondary brain injuries resulting in hypoxia and ischemia after the initial trauma. Intracranial hypertension and decreased partial brain oxygen tension ($P_{bt}O_2$) are targeted as potentially avoidable causes of morbidity. Therapeutic hypothermia (TH) may be an effective intervention to reduce intracranial pressure (ICP), but could also affect cerebral blood flow (CBF). This is a retrospective analysis of prospectively collected data from 17 patients admitted to the Western General Hospital, Edinburgh. Patients with an ICP >20 mmHg refractory to initial therapy were randomized to standard care or standard care and TH (intervention group) titrated between 32°C and 35°C to reduce ICP. ICP and $P_{bt}O_2$ were measured using the Licox system and core temperature was recorded through rectal thermometer. Data were analyzed at the hour before cooling, the first hour at target temperature, 2 consecutive hours at target temperature, and after 6 hours of hypothermia. There was a mean decrease in ICP of 4.3 ± 1.6 mmHg ($p < 0.04$) from 15.7 to 11.4 mmHg, from precooling to the first epoch of hypothermia in the intervention group ($n = 9$) that was not seen in the control group ($n = 8$). A decrease in ICP was maintained throughout all time periods. There was a mean decrease in $P_{bt}O_2$ of 7.8 ± 3.1 mmHg ($p < 0.05$) from 30.2 to 22.4 mmHg, from precooling to stable hypothermia, which was not seen in the control group. This research supports others in demonstrating a decrease in ICP with temperature, which could facilitate a reduction in the use of hyperosmolar agents or other stage II interventions. The decrease in $P_{bt}O_2$ is not below the suggested treatment threshold of 20 mmHg, but might indicate a decrease in CBF.

Introduction

Traumatic brain injury (TBI) is a significant cause of disability and death and a huge economic burden on our society. The incidence of TBI is rising throughout the world and the World Health Organization estimates that TBI will become a primary cause of death by the year 2020 (Hyder *et al.*, 2007). In the US, ~1.7 million people suffer a TBI each year. Approximately 52,000 of these die and of those that survive to discharge, 43% have an ongoing disability 1 year after injury (Langlois *et al.*, 2006; Corrigan *et al.*, 2010). The financial cost of TBI in the US in the year 2000 was estimated to be $406 billion (Corso *et al.*, 2006). In Europe there is an annual incidence of about 235 per 100,000 people and there are ~7.7 million people suffering with disabilities due to TBI (Tagliaferri *et al.*, 2006).

Much of the morbidity of TBI is attributed to secondary injuries, which occur after the initial injury and are associated with a failure to maintain adequate oxygen delivery to the injured brain (Chesnut *et al.*, 1993; Chesnut, 1995). As part of the management of secondary brain injury, the Brain Trauma Foundation (BTF) guidelines from 2007 advocate intracranial pressure (ICP) monitoring and maintaining an ICP below a threshold of 20–25 mmHg (Brain Trauma Foundation *et al.*, 2007). This is based upon evidence demonstrating worse outcomes in patients with intracranial hypertension above this threshold (Brain Trauma Foundation *et al.*, 2007; Romner and Grande, 2013; Zeng *et al.*, 2014).

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In addition to monitoring ICP, many centers monitor partial brain oxygen tension ($P_{bt}O_2$), a marker of the oxygen available in the brain for adenosine triphosphate production, which also reflects the balance between oxygen delivery and consumption (De Georgia, 2014). Both raised ICP and low $P_{bt}O_2$ have been shown to be independent predictors of poor prognosis in severe TBI, and BTF guidelines advocate initiating therapy to increase $P_{bt}O_2$ if it falls below 15 mmHg (Jaeger *et al.*, 2006; Jaeger *et al.*, 2007; Chang *et al.*, 2009; Jaeger *et al.*, 2010; Oddo *et al.*, 2011). However, contemporary articles suggest that a threshold of 20 mmHg may be more beneficial (Longhi *et al.*, 2007; Chang *et al.*, 2009; Oddo *et al.*, 2011; Le Roux *et al.*, 2014; Oddo and Bosel, 2014).

Therapeutic hypothermia (TH), the controlled lowering of core body temperature below 36°C, is currently used as a treatment modality for neonatal hypoxic ischemic encephalopathy and postcardiac arrest (Arrich *et al.*, 2009; NICE, 2011). There is also emerging evidence that it may be beneficial in the management of ischemic stroke (van der Worp *et al.*, 2010).

Despite conflicting evidence, TH is often used in intensive care units (ICUs) to manage patients following severe TBI (Sydenham *et al.*, 2009; Hutchinson *et al.*, 2013; Crossley *et al.*, 2014). Neither the 2007 BTF guidelines nor a Cochrane review from 2009 support the use of TH in the management of severe TBI (Brain Trauma Foundation *et al.*, 2007; Sydenham *et al.*, 2009). However, a recent systematic review by Crossley *et al.* (2014) found some evidence to suggest that TH may be of benefit in the management of TBI. Both the Cochrane review and the review by Crossley *et al.* (2014) note that the evidence to support TH comes from low-quality trials, which have a tendency to overestimate the treatment effect, and state the need for more high-quality trials.

The Eurotherm3235 trial is a pragmatic multicenter randomized controlled trial investigating the effects of TH (32–35°C) on the outcome after TBI. TH is titrated to reduce ICP in patients following TBI who have an ICP >20 mmHg refractory to stage one treatment (Fig. 1) (Andrews *et al.*, 2011; Andrews, 2012). Participants are randomized to either a control or intervention group and receive standard care without TH or standard care with TH, respectively as per the Eurotherm3235 trial protocol (Andrews *et al.*, 2011).

This study reports the retrospective analysis of prospectively collected data from the first 17 patients enrolled on the Eurotherm3235 trial in Edinburgh who were monitored using the Licox system. We examined the effect of induction of TH on ICP and $P_{bt}O_2$.

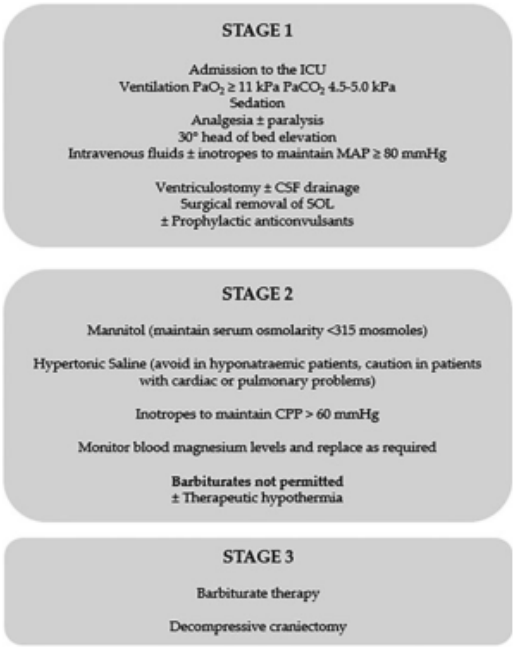

FIG. 1. Stages of management of traumatic brain injury. Information for figure taken from BTF Guidelines (Brain Trauma Foundation *et al.*, 2007). CPP, cerebral perfusion pressure; CSF, cerebrospinal fluid; ICU, intensive care unit; SOL, space-occupying lesion.

Materials and Methods

Ethical approval and consent

Ethical approval for the Eurotherm3235 trial was granted by the Scotland A Research Ethics Committee. Full consent was obtained from/for all patients, copies of which are retained within the patient's hospital notes and the Welcome Trust Clinical Research Facility, Edinburgh. The trial has been conducted in accordance with Good Clinical Practice guidelines.

Study design and patient selection

The study was a retrospective analysis of prospectively collected data from the first 17 patients enrolled into the Eurotherm3235 trial at the Western General Hospital, Edinburgh. This is a subgroup analysis of patients who received Licox monitoring from a single center, hence the small study number. Analysis of the Eurotherm3235 trial is ongoing but does not include multicenter analysis of $P_{bt}O_2$.

Patients were randomized to standard treatment based on the 2007 BTF guidelines or standard treatment and TH by an online randomization service (www.eurotherm3235trial.eu) as soon as possible after meeting the inclusion/exclusion criteria. Inclusion criteria lead to recruitment of patients less than 72 hours after TBI with a primary closed brain injury and an ICP >20 mmHg refractory to stage one treatment without obvious reversible causes and with an abnormal computed tomography (CT) head scan (Fig. 1). We excluded hypothermic patients (<36°C), those already receiving cooling therapy, those receiving barbiturates before randomization, and patients unlikely to survive the next 24 hours in the opinion of the admitting neurosurgeon.

Patients were managed according to the 2007 BTF guidelines, intubated and ventilated to achieve a $PaCO_2$ of 4.5–5.0 kPa (at 37°C), sedated and nursed with 30° head elevation. Cerebral perfusion pressure was maintained at ≥60 mmHg by manipulating mean arterial pressure with fluids and noradrenaline and limiting ICP ≤20 mmHg. Hypothermia was initiated in the intervention group with 20–30 mL/kg of refrigerated 0.9% saline given intravenously and maintained with cooling blankets to an initial target temperature of 35°C.

If ICP was not maintained below 20 mmHg, the depth of cooling was increased in 0.5°C increments to a maximum depth of 32°C. TH was maintained for a minimum of 48 hours and continued until ICP was no longer dependent upon hypothermia.

Refractory intracranial hypertension in either group lead to an escalation of therapy, including the use of 125 mL of 5% sodium chloride or 200 mL of 20% mannitol (approximately equimolar) as a bolus injection. Paralysis, further CT imaging and surgical intervention were also available.

Pyrexia in the control group (>38°C) was managed with paracetamol and cooling to normothermia (36.5–37.5°C). All patients received seizure prophylaxis with a loading dose of Phenytoin (20 mg/kg) and a maintenance dose (4–5 mg/kg) for 7 days postinjury. A comprehensive protocol was used to prevent and treat shivering in the cooled patients. Regular paracetamol was administered to patients and their peripheries were wrapped. Countercurrent surface warming and clonidine were both available according to the protocol and persistent shivering was not common in the Edinburgh center.

A Licox monitor (Integra, Lyon, France) recorded ICP, $P_{bt}O_2$, and brain temperature through fiber optic pressure catheter, oxygen electrode, and thermistor, respectively. The Licox® probe was inserted into the brain parenchyma through a dedicated triple-lumen bolt which was placed by a burr hole. The bolt was placed so that the monitors were inserted into the frontal white matter, in the nondominant hemisphere for diffuse injuries or on the side of maximal injury in focal injuries. The probe was not placed in nonviable tissue. Core body temperature was measured by rectal thermometer. The ICU Pilot software (CMA, Stockholm, Sweden) integrated data from the monitors to a bedside computer each minute until ICP monitoring was considered no longer required. See Figure 2 for study flow chart.

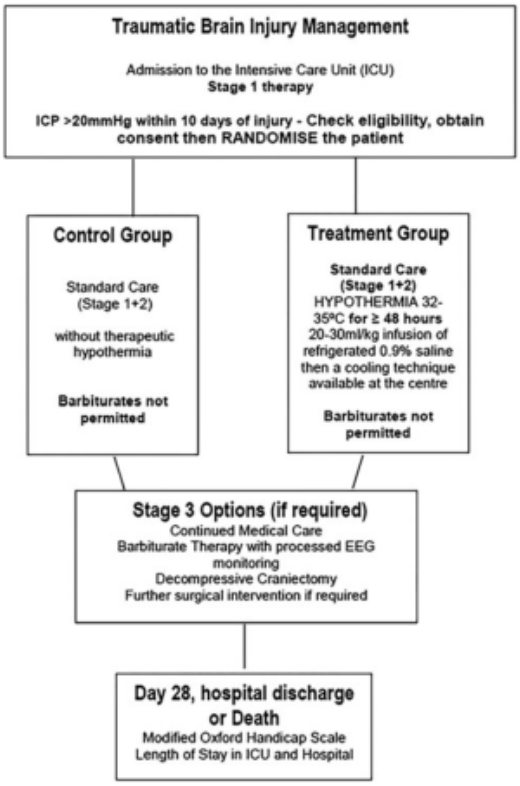

FIG. 2. Study flowchart. Adapted from Eurotherm3235 trial (Andrews, 2012). EEG, electroencephalogram; ICP intracranial pressure.

Data and statistical analysis

The first 2 hours of $P_{bt}O_2$ data recorded were not included in the analysis to reduce artifact from the Licox monitor (Geukens and Oddo, 2012; De Georgia, 2014). Data from 17 patients, 9 intervention and 8 controls, were analyzed at four time points: Time 0, the hour before randomization (induction of cooling or control); Time 1, the first hour of hypothermia (<35°C); Time 2, the first episode of stable hypothermia (<35°C), defined as 2 consecutive hours of hypothermia; and Time 3, 6 hours of stable hypothermia. Values are given as the mean ± standard error of the mean unless otherwise stated. One-way repeated measures of variance (analysis of variance [ANOVA]) were performed to identify differences within each group and paired Student's *t*-tests were performed to identify differences within the groups at the set time points. Independent Student's *t*-tests were performed to identify differences between the groups. All statistical tests were performed using Statistical Package for Social Sciences 20.0 (SPSS version 20; IBM, Inc., Armonk, NY).

Results

Control group demographics

The mean age of the participants was 34 years. All eight participants were male. Three patients suffered extradural hemorrhages (EDHs) as their primary injury, two subdural hemorrhages (SDHs), two traumatic subarachnoid hemorrhages, and one patient suffered a diffuse injury. Three participants had nondepressed skull fractures. The median Glasgow coma score (GCS) on admission to the emergency department was 7 (range 3–14, see Table 1).

Intervention group demographics

The mean age of the participants was 41 years. Two participants were female, seven were male. Three patients suffered EDH as their primary injury, three had diffuse injuries, one had contusions, and one had diffuse injuries and a SDH. Five patients had nondepressed skull fractures and one patient had a depressed skull fracture. The median GCS on admission to the emergency department was 7 (range 3–14, see Table 1).

Time to target temperature, hemoglobin, FiO_2, PaO_2, and $PaCO_2$

The mean time to target temperature (<35°C) in the intervention group was 7 hours (421 ± 72 minutes) after randomization, due to long lead times to initiation of TH following prerandomization hypertonic therapy.

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS

Patient No.	*Age (year)/ sex*	*GCS score on admission*	*CT classification*
1	26/M	7	EDH
2	29/M	6	EDH
3	25/M	9	SDH
4	27/M	3	SAH
5	43/M	12	EDH
6	35/M	12	SDH
7	48/M	7	Diffuse
8	37/M	14	SAH
9	48/M	11	EDH
10	26/M	14	EDH
11	30/F	7	Diffuse
12	28/M	3	Diffuse
13	55/F	7	Contusions
14	55/M	8	EDH
15	55/M	6	SDH
16	25/M	3	Diffuse
17	49/M	6	SDH

CT, computed tomography; EDH, extradural hemorrhage; F, female; GCS, Glasgow coma score; M, male; SAH, subarachnoid hemorrhage; SDH, subdural hemorrhage.

There was no significant change in FiO_2 from precooling to target temperature in the intervention group (median 0.35 [0.30–0.55] vs. 0.30 [range 0.30–0.55] $p > 0.05$) and there was no significant difference in FiO_2 between the two groups. PaO_2 and $PaCO_2$ values were not significantly different from precooling to target temperature: Precooling PaO_2 16.1 ± 0.9, target temperature 16.3 ± 1.6 kPa, $p > 0.05$; precooling $PaCO_2$ 4.5 ± 0.1, target temperature 4.5 ± 0.2 kPa, $p > 0.05$. There was no statistically significant difference in PaO_2 or $PaCO_2$ values between the two groups. Hemoglobin values were not significantly different between intervention and control groups and were not different at precooling to target temperature in the intervention group (10.1 ± 0.4 vs. 9.7 ± 0.4, $p > 0.05$).

There was no significant difference in the number of osmotic agents used between the control and intervention groups (1, range 0–4 and 0–5, respectively, $p > 0.05$).

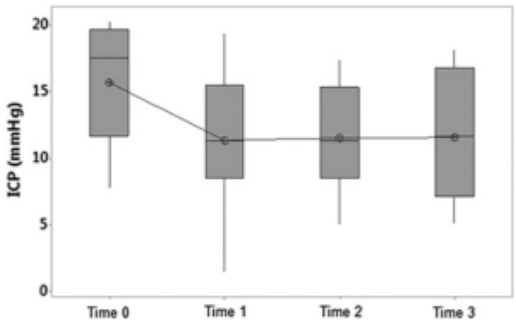

FIG. 3. Boxplot of ICP versus time in the intervention group. Time 0, prerandomization; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). Mean decrease in ICP from Time 0 to Time 1 = 4.3 ± 1.6 mmHg ($p < 0.04$).

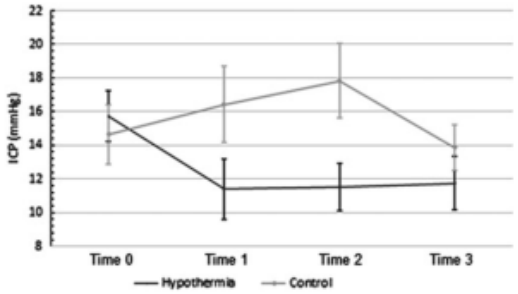

FIG. 4. Changes in ICP with time: intervention and control groups. Time 0, prerandomization; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). Two-way repeated measures ANOVA demonstrates a difference between the groups $F(3,45) = 4$, $p < 0.02$. ANOVA, analysis of variance.

Intracranial pressure

In the intervention group, the ICP decreased by a mean of 4.3 ± 1.6 mmHg ($p < 0.04$) from 15.7 to 11.4 mmHg from Time 0 to Time 1 (Figs. 3 and 4). This decrease in ICP was maintained from Time 0 to Times 2 and 3. A one-way repeated measures ANOVA in the intervention group was consistent with these results: $F(3, 24) = 6.13$, $p < 0.01$. There was no statistically significant difference in ICP in the control group between these times. A two-way repeated measures ANOVA reveals a difference between the two groups at these time points: $F(3,45) = 4$, $p < 0.02$.

Partial brain oxygen tension

Paired *t*-tests demonstrated a statistically significant difference between Time 0 and Time 2, where there was a mean decrease in $P_{bt}O_2$ of 7.8 ± 3.1 mmHg ($p < 0.05$) from 30.2 to 22.4 mmHg (Fig. 5). A one-way repeated measures ANOVA

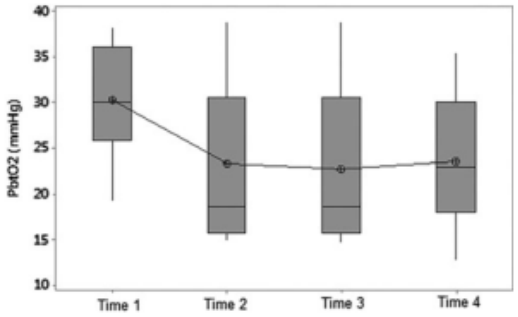

FIG. 5. Boxplot of partial brain oxygen tension versus time in the intervention group. $P_{bt}O_2$, partial brain oxygen tension; Time 0, prerandomization; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). Mean decrease in $P_{bt}O_2$ from Time 0 to Time 2 of 7.8 ± 3.1 mmHg ($p < 0.05$) from 30.2 to 22.4 mmHg.

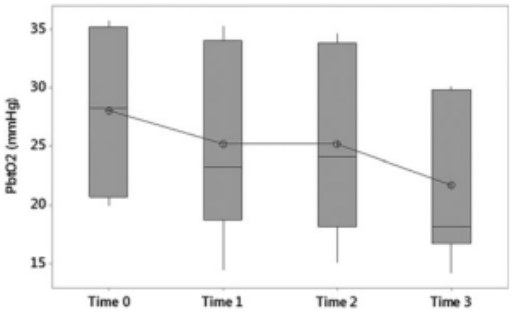

FIG. 6. Boxplot of partial brain oxygen tension versus time in the control group. Time 0, prerandomization; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). There was no statistically significant difference between Time 0 and the other time points.

for the intervention group also showed a difference in $P_{bt}O_2$ with time $F(3,18)$ 4.60, $p < 0.02$. Three patients in the intervention group had a decrease in $P_{bt}O_2$ from >25 mmHg at Time 0 to <20 mmHg at Time 3.

There was no statistically significant difference between Time 0 and any other times in the control group. However, paired *t*-tests demonstrated a mean decrease of 3.47 ± 1.02 mmHg ($p < 0.02$) between Time 1 and Time 3, and a mean decrease of 3.48 ± 1.27 mmHg ($p < 0.03$) between Time 2 and Time 3 in the control group (Fig. 6). There was no statistically significant difference between the two groups in terms of the change in $P_{bt}O_2$ (Fig. 7).

Discussion

Intracranial pressure

Starting ICP in this report was less than 20 mmHg (ET3235Trial protocol) because a single hypertonic treatment was given pending randomization which also resulted in long lead times to initiation of cooling. TH reduced ICP by a mean of 4.3 ± 1.6 mmHg from precooling to the first episode

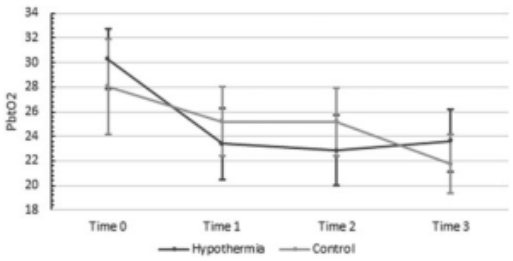

FIG. 7. Changes in partial brain oxygen tension with time: intervention, and control groups. Time 0, prerandomization; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C). There was no statistically significant difference between the two groups in terms of the change in $P_{bt}O_2$.

of stable hypothermia (~27%), despite pretreatment with hypertonic therapy. The reduction in ICP was maintained at 6 hours and there was no further decrease with prolonged hypothermia, which is consistent with previous studies (Shiozaki *et al.*, 1993; Schwab *et al.*, 1998). This is a comparable reduction to mannitol and hypertonic saline, which are reported to reduce ICP by 10–51% and have similar efficacy (James *et al.*, 1977; James, 1980; Freshman *et al.*, 1993; Berger *et al.*, 1995; Qureshi *et al.*, 1998, 1999a, 1999b; Sorani *et al.*, 2008).

Neither of these therapies is without risk. Reported adverse effects of mannitol include renal failure, electrolyte abnormalities, acidosis, hypotension, and congestive heart failure. Reported adverse effects of hypertonic saline include renal failure and electrolyte abnormalities in addition to other theoretical concerns (Torre-Healy *et al.*, 2012). Furthermore, hyperosmolar therapy is limited in that mannitol leads to a short-lived reduction in ICP with diminishing returns and the prolonged use of hypertonic saline over 72 hours has been shown to increase mortality (James *et al.*, 1977; McGraw, 1978). Therefore, TH may be beneficial in providing a persistent reduction of ICP. It is not suggested that TH is used as an alternative to hyperosmolar therapies, but rather that it may help to reduce the number of therapies required when used in combination.

TH however, is not a risk-free intervention either, and can be associated with a number of adverse effects, including arrhythmias, electrolyte disturbances, and pneumonia. While pneumonia is often reported following TH, a Cochrane review from 2009 found that the trend toward an increased risk of pneumonia was not significant and the quality of evidence supporting it was poor (Sydenham *et al.*, 2009).

This study supports others in finding that TH reduces ICP, but it does not suggest that outcomes are improved or that the use of hyperosmolar agents is reduced (Table 2). We await the full analysis of the Eurotherm3235 trial that will conclusively demonstrate whether TH improves outcomes or not.

Partial brain oxygen tension

Both groups in this report demonstrated a decrease in $P_{bt}O_2$ with time. In the intervention group there was a significant decrease of 7.8 ± 3.1 mmHg from 30.2 to 22.4 mmHg. In the control group, no statistically significant decrease was seen from Time 0 to any other time points. However, it is worth noting that half of the control group were not included in the Time 0 analysis due to missing data ($n = 4/8$). The first 2 hours of data from randomization were not included in the analysis because it coincided with the time we allowed for the oxygen electrode to stabilize. In the remaining patients, $P_{bt}O_2$ recording was already in place before randomization and the data were able to be included.

There was a statistically significant decrease in $P_{bt}O_2$ in the control group between Time 1 and 3 and Time 2 and 3 of 3.47 ± 1.02 and 3.48 ± 1.27 mmHg, respectively. It could be that a statistically significant difference between Time 0 and the other time periods would have been seen if all data were present at Time 0. There was no statistically significant difference between the two groups.

Previous studies have investigated the effect of hypothermia on $P_{bt}O_2$ with conflicting results. Gupta *et al.* (2002) saw a decrease in $P_{bt}O_2$ in a study of 30 patients cooled to

TABLE 2. CHANGES IN INTRACRANIAL PRESSURE AND $P_{bt}O_2$ WITH TEMPERATURE IN PREVIOUS STUDIES

Study	n	*Mean decrease in ICP (mmHg)*	*Mean change in $P_{bt}O_2$*	*Target temperature (°C)*
Zhi *et al.* (2003)	396	4.1–6.6		32–33
Clifton *et al.* (2001)	392	1.75		33
Jiang *et al.* (2000)	87	9.23		33–35
Marion *et al.* (1997)	82	4.3		32–33
Qiu *et al.* (2007)	80	1.2–2.1		33–35
Liu *et al.* (2006)	45	5.33		33–35
Smrcka *et al.* (2005)	38	8.07		34
Gal *et al.* (2002)	30	6		32–34
Lavinio *et al.* (2007)	24	4.8		34
Sahuquillo *et al.* (2009)	23	7		32.5
Metz *et al.* (1996)	10	9.5		32.5–33
Gupta *et al.* (2002)[a]	30		Decrease 1.0 kPa	< 35
Zhang *et al.* (2002)	18		Increase 19.1 mmHg	31.5–34.9
Current study	17	4.3	Decrease 7.8 mmHg	32–35

Summary of change in ICP and $P_{bt}O_2$ with temperature from previous studies for comparison.
[a]Estimate of results from graphed data.
ICP, intracranial pressure; *n*, number of participants in each study; $P_{bt}O_2$, partial brain oxygen tension.

a minimum of 33°C and concluded that decreasing brain temperature below 35°C may impair brain tissue oxygenation. However, it has been argued that the Paratrend 7 (Diametrics Medical, High Wycombe, United Kingdom) sensor used to measure $P_{bt}O_2$ in this study was not corrected for temperature and so did not reflect an accurate $P_{bt}O_2$. In contrast, Zhang *et al.* (2002) found that mild hypothermia (31.5–34.9°C) increased the mean (SD) $P_{bt}O_2$ from 9.6 (6.8) to 28.7 (8.8) mmHg in 18 patients with severe TBI. Patients in this study received TH within 20 hours following TBI and had $P_{bt}O_2$ recorded within the first 24 hours. Given the very low initial $P_{bt}O_2$ and subsequent increase within 24 hours following injury, it has been suggested that this increase was attributable to early changes in cerebral blood flow (CBF) rather than an effect of hypothermia (Andrews and Gupta, 2003).

Decreases in $P_{bt}O_2$ have been associated with poor outcomes independent of raised ICP and the combination of both a raised ICP and decreased $P_{bt}O_2$ appears worse than intracranial hypertension in isolation (Chang, 2009). Normal levels of $P_{bt}O_2$ in neurosurgical patients are reported as between 23 and 35 mmHg, with lower values coming from probes sited deeper in brain tissue (Hoffman *et al.*, 1996; Dings *et al.*, 1998; Pennings *et al.*, 2008). While the decrease of $P_{bt}O_2$ in the intervention group is significant, the mean value did not decrease below the threshold of 15 mmHg recommended for intervention by the 2007 BTF guidelines (Brain Trauma Foundation *et al.*, 2007), nor did it decrease below 20 mmHg, the suggested threshold for compromised brain oxygen/moderate brain hypoxia (Le Roux *et al.*, 2014). However, when looking at the individual value plots (Fig. 8) for the intervention group, one can see that three participants demonstrated a change in $P_{bt}O_2$ from above 25 mmHg before cooling to less than 20 mmHg after 6 hours of target temperature. It is unclear why certain patients exhibit this decrease in $P_{bt}O_2$ while others do not, but further investigation is warranted to identify those patients likely to benefit most from targeted temperature management.

Arterial blood gases were analyzed using the alpha stat method to maintain autoregulation, which was consistent with current practice in the units that participated in the trial. This approach has been undertaken in other contemporary hypothermia trials, such as NABISH II and TTM (Clifton *et al.*, 2011; Nielsen *et al.*, 2013). Despite this, there was no significant difference in our precooling and target temperature $PaCO_2$ and PaO_2 values, which is important given the dependence of $P_{bt}O_2$ upon systemic oxygenation and

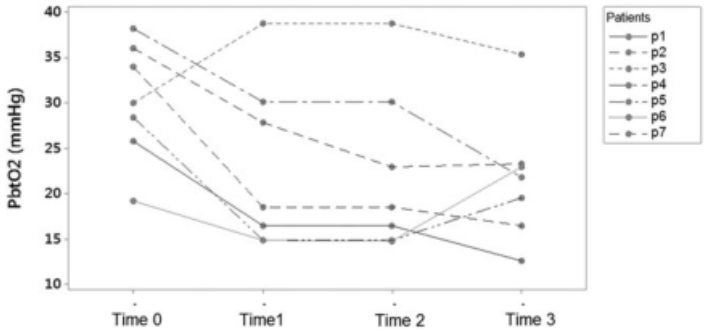

FIG. 8. Individual value plots of $P_{bt}O_2$ versus time. Time 0, pre-randomization; Time 1, first hour of hypothermia (<35°C); Time 2, first episode of stable hypothermia (<35°C); Time 3, 6 hours of hypothermia (<35°C).

transport (Rosenthal *et al.*, 2008). If the decrease in $P_{bt}O_2$ was due to changes in $PaCO_2$ and PaO_2 with temperature (Gay-Lussac's Law), we would expect to see changes in PaO_2 and $PaCO_2$. Therefore, the decrease in $P_{bt}O_2$ may be due to reduced CBF.

Anemia has been associated with a decrease in $P_{bt}O_2$ following TBI (Oddo *et al.*, 2012). The current study found no difference in hemoglobin levels between either the two groups, or in the intervention group at precooling or target temperature to account for the change in $P_{bt}O_2$. The hemoglobin of two patients in the control group and three patients in the intervention group was <9 g/dL, the level at which $P_{bt}O_2$ is thought to be compromised (Oddo *et al.*, 2012). However, in those randomized to the intervention group, the decreased hemoglobin was present before the patients reached target temperature.

Balancing oxygen delivery with demand

Tokutomi *et al.* (2003) have previously studied the optimal temperature to reduce ICP while resulting in minimal unfavorable outcomes in patients with TBI and concluded that this temperature is about 35°C. In their study from 2002, decreases in ICP were most noticeable between 35°C and 36°C and the incidence of jugular venous bulb oxygen desaturation was also decreased at these temperatures. However, oxygen consumption, measured through indirect calorimetry, and oxygen delivery also progressively decreased. Below 35°C Tokutomi *et al.* (2003) found that oxygen delivery decreased more than the decrease in oxygen demand resulting in an overall deficit.

A potential explanation for the decrease in ICP with hypothermia is a decrease in CBF ($PaCO_2$ related), which could also explain the decrease in oxygen delivery (Marion *et al.*, 1993; Shiozaki *et al.*, 1993). Hypothermia is thought to decrease cerebral metabolic rate for oxygen ($CMRO_2$) and there may be an associated decrease in oxygen demand (Keller, 2000). While we believe we can reduce ICP and $CMRO_2$, perhaps we are yet to find the balance between decreasing CBF/ICP and maintaining adequate oxygen delivery and are causing unwanted decreases in $P_{bt}O_2$ because of this.

Limitations of the study

The present study has some limitations that need to be considered. Due to the nature of the patient population and TH, it is not possible to blind researchers from the administration of TH. In addition, the researchers were not blinded for the analysis of the two groups which could potentially lead to a bias of analysis.

Although we have attempted to correct the time taken to reach hypothermia by comparing prerandomization values with the different time periods, we must acknowledge that the mean time to target temperature was ~ 7 hours. Given that early initiation of TH is considered beneficial in improving efficacy, it could be that a greater decrease in both ICP and $P_{bt}O_2$ would have been seen in the intervention group if the time to reach target temperature was reduced.

Finally, it should be noted that there were missing data. For example, data were not recorded due to a faulty connection attached to the $P_{bt}O_2$ monitor or a connection accidentally detached. Some of the data were missing in a nonrandom manner. An example is missing ICP and $P_{bt}O_2$ data when patients were transferred to the CT scanner because monitors were detached to facilitate patient transfer. Due to the small sample size and relatively small amount of data missing, statistical models to analyze the effect of missing data were not performed. The most apparent effect of the missing data is seen when analyzing $P_{bt}O_2$ at Time 0 in both the control and intervention groups as described above.

Conclusion

TH is an effective addition to the management of intracranial hypertension and could potentially reduce the number of hyperosmolar therapies required. It remains to be seen whether the use of TH, titrated to reduce ICP, will result in improved outcomes in patients following TBI. TH below 35°C could reduce oxygen delivery more than oxygen demand leading to reduced cerebral oxygenation and it is unclear why some patients exhibit a greater decrease in $P_{bt}O_2$ than others. Further analysis of patients enrolled on the Eurotherm3235 trial is required to assess the effects of TH on $P_{bt}O_2$.

Acknowledgments

The authors would like to thank Cat Graham for her help with designing the methods of statistical analysis. The Eurotherm3235 trial is funded by the NIHRH's Technology Assessment Program and J.R. has a Chief Scientists Office NHS research fellowship. Informed written consent was received from participants/their representatives to take part in the Eurotherm3235 trial and subsequent publication of information thereafter. Copies of the consent form are held in the patients' clinical records and in the Welcome Trust Clinical Research Facility, Edinburgh, and are available for review by the Editor-in-Chief upon request.

Author Disclosure Statement

P.J.D.A. is the Chief Investigator of the Eurotherm Trial, which is funded by the National Institute of Health Research's Health Technology Assessment Program. J.R. is the Principal Investigator of the Eurotherm Trial. J.R. and P.J.D.A. are on the speakers' panel for Integra and have participated in educational meetings for Bard Medical. Both of these companies manufacture cooling devices. L.M.C.F. has no competing interests to declare.

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F1000Research F1000Research 2015, 4(F1000 Faculty Rev):1200 Last updated: 02 NOV 2015

REVIEW

Advances in the understanding of delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage [version 1; referees: 4 approved]

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v1 **First published:** 02 Nov 2015, **4**(F1000 Faculty Rev):1200 (doi: 10.12688/f1000research.6635.1)

Latest published: 02 Nov 2015, **4**(F1000 Faculty Rev):1200 (doi: 10.12688/f1000research.6635.1)

Abstract

Delayed cerebral ischaemia has been described as the single most important cause of morbidity and mortality in patients who survive the initial aneurysmal subarachnoid haemorrhage. Our understanding of the pathophysiology of delayed cerebral ischaemia is meagre at best and the calcium channel blocker nimodipine remains the only intervention to consistently improve functional outcome after aneurysmal subarachnoid haemorrhage. There is substantial evidence to support cerebral vessel narrowing as a causative factor in delayed cerebral ischaemia, but contemporary research demonstrating improvements in vessel narrowing has failed to show improved functional outcomes. This has encouraged researchers to investigate other potential causes of delayed cerebral ischaemia, such as early brain injury, microthrombosis, and cortical spreading depolarisation. Adherence to a common definition of delayed cerebral ischaemia is needed in order to allow easier assessment of studies using multiple different terms. Furthermore, improved recognition of delayed cerebral ischaemia would not only allow for faster treatment but also better assessment of interventions. Finally, understanding nimodipine's mechanism of action may allow us to develop similar agents with improved efficacy.

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How to cite this article: Flynn L and Andrews P. **Advances in the understanding of delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage [version 1; referees: 4 approved]** *F1000Research* 2015, **4**(F1000 Faculty Rev):1200 (doi: 10.12688/f1000research.6635.1)

Grant information: The author(s) declared that no grants were involved in supporting this work.

Competing interests: The authors declare that they have no competing interests.

First published: 02 Nov 2015, **4**(F1000 Faculty Rev):1200 (doi: 10.12688/f1000research.6635.1)

Physiological responses to brain tissue hypoxia and blood flow after acute brain injury

Introduction

Aneurysmal subarachnoid haemorrhage (aSAH) has an incidence of 6–11 per 100,000 people per year and accounts for only 5% of all strokes[1–4]. Despite this, aSAH is the cause of one third of all stroke-related years of potential life lost before the age of 65[5]. Approximately 70% of all people with aSAH will either die or require help with activities of daily living at six months after the initial injury[5]. The mean age of onset of aSAH is 55 years and, when combined with its poor morbidity and mortality, it causes an enormous socioeconomic burden[6,7]. The significant morbidity attached to aSAH can be attributed to rebleeding, delayed cerebral ischaemia (DCI), hydrocephalus, and other medical complications, despite successful treatment of the ruptured aneurysm. Of these complications, DCI is the most important cause of morbidity and mortality in patients who survive the ruptured aneurysm[5,8,9]. Between days 3 and 10 after the initial aSAH, 30–40% of patients will suffer DCI and half of these will have a poor outcome[5,10,11].

Our understanding of DCI is meagre at best. Conventionally, DCI was thought of as a neurological deficit observed at least three days after aSAH with radiological confirmation of large vessel narrowing and was often termed "vasospasm". However, more contemporary articles question whether the relationship between angiographic cerebral vessel narrowing and neurological outcome is associative rather than causative and have highlighted the possibility of a multifactorial aetiology[12–15]. One of the problems with the disease and research surrounding DCI is the terminology applied. Terms include DCI, delayed ischaemic neurological deficit (DIND), delayed neurological deficit, secondary cerebral ischaemia, and vasospasm. In 2010, a consensus statement was issued defining DCI as a focal neurological impairment or decrease of ≥2 points on the Glasgow Coma Scale which lasts for ≥1 hour, is not apparent immediately after aneurysm occlusion, and cannot be attributed to other causes by means of clinical assessment, blood tests, or imaging[16]. The Neurocritical Care Society's consensus definition was similar for DCI and also defined vasospasm as radiological evidence of cerebral vessel narrowing with corresponding neurology[17].

Cerebral artery narrowing

Over six decades ago, cerebral vessel narrowing was demonstrated by angiography after aSAH[18]. A decade later, a link was found between cerebral vessel narrowing and focal neurology[19]. Then in the late 1970s, it appeared that vessel narrowing was not only localised to the vascular territory of the aneurysmal bleed but also proportional to blood load and occurred between days 3 and 12 after the aSAH[20,21]. More contemporary authors found the onset of vessel narrowing started on day 3, was maximal by days 6–10, and lasted for up to two weeks[22–24]. The density, duration and volume of subarachnoid blood are key predictors of vessel narrowing[21,25]. Narrowing of cerebral arteries may cause a reduction in cerebral blood flow distal to the constricted vessel and contribute to secondary ischaemia[26]. The cause of vessel narrowing after aSAH is unclear but is thought to involve oxyhaemoglobin release, an inflammatory-mediated response, decreased nitric oxide levels, and an increased concentration of endothelin-1 (ET-1)[14].

Oxyhaemoglobin

Oxyhaemaglobin induces cerebral artery vasoconstriction *in vitro* and *in vivo* in primates, which is not seen with methaemoglobin or bilirubin[27–29]. It is thought that oxyhaemoglobin decreases the production of prostacyclin and increases prostaglandin E2 in vessel walls, thereby causing vasoconstriction. It can also inhibit endothelial-dependent relaxation. The oxidation of oxyhaemoglobin to methaemoglobin, which occurs spontaneously, causes lipid peroxidation and vasoconstriction[30]. It is plausible that oxyhaemoglobin causes vasoconstriction by some or all of these mechanisms but attempts at modulating them have not completely reversed vessel narrowing or, importantly, improved outcomes.

Nitric oxide

Nitric oxide, which is responsible for the relaxation of vascular smooth muscle cells, appears to be depleted after aSAH. This may be due to a number of reasons, one of which is that nitric oxide is scavenged by haemoglobin, released during the breakdown of subarachnoid blood, due to nitric oxide's high affinity for haemoglobin[31,32]. In addition to this, the production of nitric oxide may also be decreased due to the down-regulation of endothelial and neuronal nitric oxide synthase, which occurs in spastic arteries after aSAH[33–35]. Both of these mechanisms will lead to a decrease in the bioavailability of nitric oxide, which is then unable to counteract the effects of the vasoconstrictor ET-1[36]. Furthermore, exogenous donors of nitric oxide, such as sodium nitroprusside and nitroglycerin, although associated with systemic side effects, have been shown to ameliorate cerebral artery narrowing[37,38]. In addition to the hypotension seen with these exogenous donors, there is also a concern that exposing nitric oxide to oxyhaemoglobin and deoxyhaemoglobin will lead to the formation of methaemoglobin, S-nitrosohaemoglobin and ferrous-nitrosyl-haemoglobin[33]. Interestingly, Kida *et al.* note in their comprehensive review that inhaled nitric oxide acts as a selective pulmonary vasodilator and avoids the hypotension seen with intravenous administration. Animal studies have demonstrated a reduction in ischaemia-reperfusion injuries after nitric oxide inhalation in extrapulmonary organs after cardiac injury. These have also been supported by proof-of-concept human trials[39]. The research discussed is used to support post-cardiac arrest ischaemia but Garry *et al.* also encourage further investigation of nitric oxide as a treatment of secondary brain injury in their review with reference to aSAH[40].

Endothelin

Endothelin is key to maintaining the vascular tone of blood vessels, with ET-1 being the most potent endogenous activator of vasoconstriction. The amount of ET-1 in serum and plasma increases within minutes after the aSAH and peaks around days 3–4, the time at which DCI starts to occur. There also appears to be an excessive release of ET-1 by astrocytes around the time of onset of ischaemic symptoms[41,42]. ET-1 concentrations appear consistently elevated in patients with DCI. However, there are conflicting reports of ET-1 concentrations within the normal range in patients with radiological evidence of cerebral artery narrowing who do not have DCI[43–45]. Authors have questioned whether increased ET-1 marks ischaemic

damage rather than arterial vessel narrowing in DCI[14]. Therefore, there are a number of different mechanisms that could be contributing to the arterial narrowing commonly seen after aSAH.

Alpha calcitonin gene-related peptide

Alpha calcitonin gene-related peptide (CGRP) is an endogenous neuropeptide and a potent vasodilator. CGRP exhibits its vasodilating properties by two mechanisms: one is nitric oxide and endothelium-dependent and the other is cyclic adenosine monophosphate mediated and is endothelium-independent[46]. Endogenous CGRP appears to be released, and is subsequently depleted, after aSAH to combat cerebral vasoconstriction which has led to the theory that exogenous CGRP may be beneficial in managing DCI[47–49]. Because CGRP can act independently of endothelial cells, which are morphologically damaged after aSAH, it may be successful in treating DCI. A number of animal studies and three human trials have investigated the effect of CGRP on cerebral arteries after aSAH. All animal studies appear to show either a reversal or improvement in cerebral artery narrowing[46]. The largest human trial, the European CGRP in aSAH study, demonstrated little improvement in morbidity or mortality from intravenous administration but noted that systemic side effects, such as hypotension, were limiting and suggested that intrathecal administration may be more beneficial, as endogenous CGRP acts on the abluminal side of vessel walls[50]. A trial investigating the effect of CGRP after intrathecal administration is still awaited.

Radiological evidence

An often-cited argument against cerebral vasoconstriction being a causative factor of DCI is that, whilst up to 70% of patients demonstrate cerebral vessel narrowing on angiography, only 40% of these will manifest neurological deficits and only 30% develop DCI[51–54]. However, it must be acknowledged that even the consensus definition of DCI provided in the introduction has its limitations[16]. Patients with poor grade aSAH (World Federation of Neurosurgical Societies Grades IV and V), the group of patients most likely to develop DCI, are often sedated and mechanically ventilated and are particularly difficult to assess clinically[55]. Therefore, it is likely that we are under-diagnosing and under-treating DCI in this group of patients. Furthermore, it may be that the degree of large cerebral vessel narrowing does not correlate well with symptom severity[26].

Following a review of current tests available for the diagnosis of delayed cerebral ischaemia, Rodriguez *et al.* advise clinical examination and transcranial Doppler (TCD) in the screening and diagnosis of "vasospasm". The authors reserve multi-modal magnetic resonance imaging (MRI) and computed tomography (CT) for specific situations, and acknowledge digital subtraction angiography (DSA) as the gold standard for diagnosis (Figure 1)[56]. Rabinstein *et al.* found that TCD and angiogram demonstrating cerebral vessel narrowing (termed vasospasm) only had a positive predictive value of 67% for cerebral infarction on CT[8]. We would expect this to be higher if cerebral vessel narrowing was the primary cause of DCI. Rates of cerebral infarction in patients with evidence of cerebral vessel narrowing range between 24 and 35% using CT[57,58], but have been found to be as high as 81% in some studies using MRI[59]. In addition to this poor correlation between cerebral vessel narrowing

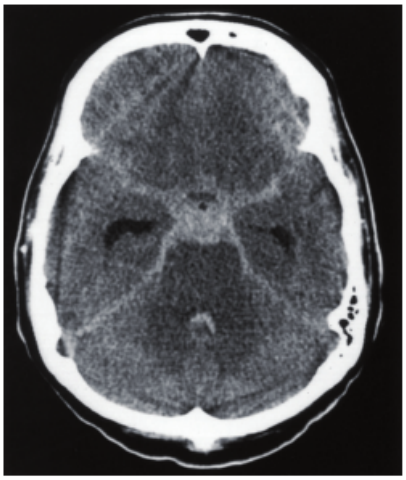

Figure 1. CT image of subarachnoid haemorrhage. Non-contrast CT scan of brain showing subarachnoid haemorrhage in classical "star sign" distribution with blood distributed along basal vessels.

and infarction, there is clinical evidence that up to 25% of delayed infarcts on CT are not in the same territory as the vessel narrowing, or are found in patients that did not demonstrate vessel narrowing at all[60–62]. Rabinstein *et al.* note that TCD and angiogram only agreed on the diagnosis of "vasospasm" in 73% of cases and so it could be that vessel narrowing simply wasn't identified in patients who were later found to have evidence of infarcts on CT[8]. Despite these conflicting messages, clinical studies do report that those patients with radiological evidence of cerebral vessel narrowing are at greater risk of DCI[62,63].

Herz *et al.* directly visualised pial artery constriction after application of blood or microtrauma to pial arteries in animal studies[64]. Further *in vitro* research has suggested that constriction of intraparenchymal arterioles occurs after aSAH and may contribute to DCI[65]. Maximal luminal narrowing has been seen between days 3 and 7 and repeated *in vivo* in mouse studies. The correlation between decreased regional cerebral blood flow and microvascular constriction appears stronger than that seen with large vessel narrowing[65–67]. Uhl *et al.* identified constriction of small vessels in surgical patients within the first 72 hours after aSAH by spectral imaging, and Pennings *et al.* later directly observed cerebral arterioles contracting after aSAH[68,69]. Therefore, it may be that vessel narrowing is consistently occurring with DCI but that we are not visualising it because it is microvascular and not readily visible on catheter angiography or TCD[56].

CT perfusion scanning (CTP) may provide haemodynamic evidence to support the diagnosis of DCI. Dankbaar *et al.* evaluated the diagnostic value of CTP for DCI and reported 84% sensitivity, 79% specificity, and 88% positive predictive values[70]. Sanelli *et al.* found that more CTP deficits occurred in patients with DCI than in those

without[71]. Dankbaar *et al.* later suggested that patients with DCI exhibit worse cerebral perfusion (measured on CTP) than patients without DCI even before focal signs occurred. Encouragingly, they demonstrated partial recovery in areas of poor perfusion, suggesting that DCI could be partly reversible[72]. However, Killeen *et al.* concluded from their retrospective comparative study that CTP and DSA had similar test characteristics for identifying DCI in aSAH patients[73].

Endothelin-antagonists

A shift in theory from cerebral vessel narrowing to a multifactorial aetiology occurred after the CONSCIOUS trials and a recent meta-analysis of pharmacological treatments for delayed cerebral ischaemia[74–76]. The meta-analysis demonstrated that, despite a reduction of cerebral vessel narrowing, no statistically significant effect on poor outcome was observed[74]. However, the authors note that the dissociation between a reduction in cerebral vessel narrowing but not poor outcomes could result from methodological problems, sample size, and insensitivity of outcome measures, in addition to a multifactorial aetiology of DCI. The CONSCIOUS trials were multicentre randomised controlled trials (RCT) investigating the effect of clazosentan, an endothelin-A (ET-A) antagonist, on "vasospasm" after aSAH. The first of these trials, CONSCIOUS-1, demonstrated that, despite a significant reduction in angiographic cerebral vessel narrowing, there was little evidence to support its use to improve morbidity and mortality and it was associated with increased rates of pulmonary complications, hypotension and anaemia[76]. CONSCIOUS-2 demonstrated no benefit from clazosentan in patients treated with surgical clipping, which led to the early termination of the trial[75]. Laban *et al.* recently published a review of animal studies investigating endothelin receptor antagonists after experimental aSAH and found no improvement in functional outcomes[77]. Perhaps more importantly, the review described insufficient animal data supporting endothelin receptor antagonists to warrant progression to a human trial. The authors also suggest that cerebral artery diameter, or "vasospasm", is not a clinically relevant outcome measure in experimental aSAH studies[77].

The example of clazosentan appears to provide evidence that cerebral artery narrowing is not the sole cause of DCI. However, there is conflicting evidence as more invasive methods of reducing vessel narrowing can improve outcomes (Figure 2). Kimball *et al.* reviewed 49 articles relating to interventional techniques to treat "vasospasm". A total of 24 of the 27 publications (1,028 patients) reporting the use of transluminal balloon angioplasty noted an improvement in vessel diameter and neurological deficits. Twelve case series reported good angiographic and clinical results for patients who received papaverine (a vasodilator) administered approximate to the site of vessel narrowing[78]. Both techniques were associated with significant side effects and the quality of the studies was reported as very low to moderate (based upon the GRADE classification system)[79]. Nevertheless, the review does provide evidence that cerebral artery narrowing is likely to be strongly involved in the pathology of DCI.

Nimodopine

The calcium channel antagonist nimodipine is the only proven intervention to reduce the incidence of DCI and improve outcomes after aSAH. Nimodopine was initially investigated as a vasodilator in the hope that it would aid post-ischaemic reperfusion, as it was thought that an increase in calcium in vascular smooth muscle cells led to "vasospasm"[80,81]. In 1989, the British Aneurysm Nimodipine Trial subsequently demonstrated a significant reduction in cerebral infarction rates and improved neurological outcomes at three months after aSAH[82]. A Cochrane review in 2007 concurred with these findings but noted that the supporting evidence was based mainly on one large study. This led to oral nimodipine becoming standard care for patients after aSAH[83]. Interestingly, the review found no statistically significant results to support the use of other calcium antagonists, magnesium sulphate, or intravenous administration of nimodipine.

Magnesium sulphate is a non-competitive inhibitor of calcium channels and has vasodilatory and neuroprotective properties, similar to nimodipine. Hypomagnesaemia is common in patients after aSAH, appears to be proportional to the severity of the bleed, and is predictive of DCI[84]. Magnesium sulphate has also been shown to reduce cerebral artery narrowing and the size of ischaemic lesions after aSAH in animal models[85]. However, the Neurocritical Care Society guidelines advise against the routine administration of magnesium in patients with aSAH[17]. This is supported by data from the intravenous magnesium sulphate for aneurysmal subarachnoid haemorrhage (IMASH) and MASH-2 trials and a recent meta-analysis demonstrating no beneficial effect of magnesium in this group of patients[86–88]. A *post hoc* analysis of the IMASH trial reported an association between high plasma levels of magnesium and worse clinical outcomes[89].

In summary, one calcium channel antagonist, nimodipine, has been shown to be effective in the prevention and treatment of DCI after aSAH whilst other calcium channel antagonists and a non-competitive inhibitor of calcium channels appear to have little effect on, or worsen outcomes.

It remains unclear how nimodipine exerts its neuroprotective effects but its action seems independent of any effect on large vessel narrowing[90,91]. It was thought that nimodipine may exert its effect by stopping calcium influx at a neuronal level, but no beneficial effect has been seen from administration in patients after ischaemic stroke or traumatic brain injury[92–94]. In addition to this, a recent systematic review found no benefit from nimodipine after traumatic SAH, suggesting that the mechanism of action of nimodipine is unique to aSAH[95]. Nimodipine has two properties that it does not share with other calcium channel antagonists. Firstly, it increases endogenous fibrinolytic activity, which may reduce the incidence of microthrombosis[96]. Secondly, it antagonises cortical spreading ischaemia in rats, which may be one of the culprits in DCI and is discussed in further detail below[97].

F1000Research 2015, 4(F1000 Faculty Rev):1200 Last updated: 02 NOV 2015

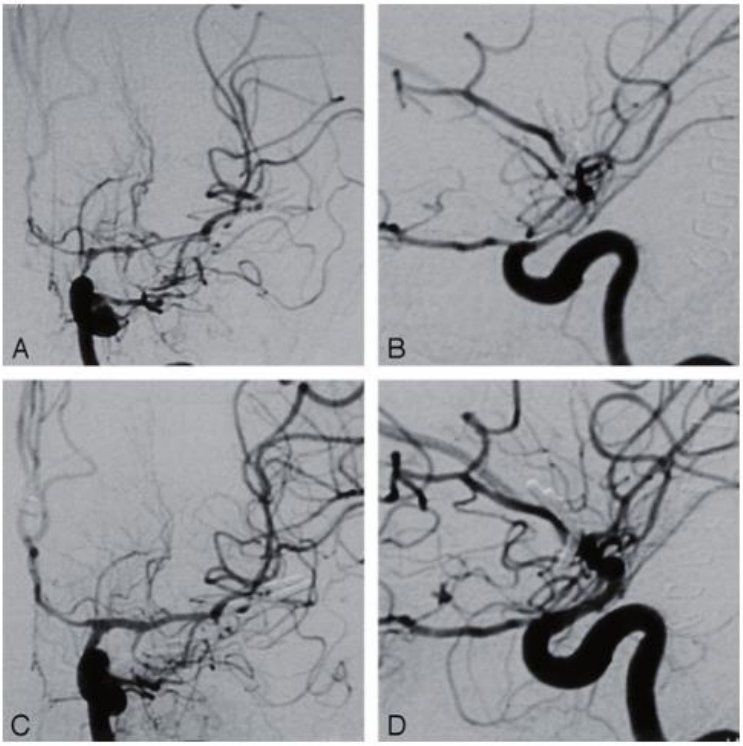

Figure 2. Angiograms demonstrating cerebral vessel narrowing after subarachnoid haemorrhage. A and **B**: Anteroposterior (**A**) and lateral (**B**) angiograms of the left internal carotid artery demonstrate vessel narrowing at the level of the carotid siphon, the terminal internal carotid artery, the A1 segment of the anterior cerebral artery and the middle cerebral artery. **C** and **D**: Anteroposterior (**C**) and lateral (**D**) angiograms obtained after intra-arterial injection of nimodipine.

Contemporary hypotheses

Early brain injury

Early brain injury (EBI) refers to damage to the brain in the first 72 hours after the haemorrhage. There are a number of pathophysiological events in this time period that could influence later complications, such as DCI, and much of our understanding is derived from experimental data. One of these changes is a severe rise in intracranial pressure leading to decreased cerebral perfusion pressure, cessation of cerebral blood flow and ultimately global ischaemia and oedema[98–100]. The intracranial hypertension at ictus is often greater than systolic blood pressure, and the rate of increase and peak intracranial pressure appears to be proportional to the amount of arterial blood extravasating into the subarachnoid spaces from the aneurysm[101–103]. Cerebral spinal fluid outflow obstruction, in addition to hydrocephalus, further exacerbates intracranial hypertension[104,105]. However, the increase in intracranial pressure is not uniform and there are two distinct groups of patients in terms of their intracranial hypertension. The first, more common, scenario is an increase in intracranial pressure to the arterial diastolic pressure which then decreases to just above the patient's baseline intracranial pressure[102]. These patients typically have a small volume haemorrhage with cerebral oedema. The second type of increased intracranial pressure is sustained due to either a progressive haematoma or acute hydrocephalus[104,105].

The cerebral oedema seen after aSAH is often present on admission CT scans and becomes more common, being present in up to 20% of patients by day 6[98]. Cerebral oedema is itself a poor prognostic factor after aSAH[98,106,107]. The global cerebral ischaemia that occurs during the initial aSAH may lead to the disruption of the blood-brain barrier, and initiate cell death mechanisms and inflammatory responses which all contribute to cerebral oedema. Regulated and

unregulated neuronal cell death appears to occur within 24 hours after aSAH and as early as 40 minutes after the initial injury[108–110]. Serum and cerebrospinal fluid (CSF) levels of pro-inflammatory cytokines and vasoactive factors, such as tumour necrosis factor-α, interleukin-6, and interleukin-1 receptor antagonist, correlate with DCI and poor outcomes[111,112].

In addition to these inflammatory responses, blood degradation products are thought to contribute to DCI and perhaps removing blood from the subarachnoid space may improve outcomes[30,113]. Continuous cisternal drainage and intrathecal administration of thrombolytics have been trailed with reports of success, and results of the EARLYDRAIN trial comparing continuous lumbar-CSF drainage with standard treatment are awaited[114,115]. A meta-analysis of the use of intrathecal thrombolytics suggested a reduction in the incidence of DCI but these findings were not statistically significant after excluding one study, which included intrathecal nimodipine in addition to thrombolytic therapy[116].

Cerebral autoregulation, the ability of blood vessels to maintain constant cerebral blood flow (CBF) with arterial blood pressures between ~60 and 150 mmHg, is impaired after the aneurysm rupture[117–119]. Once impaired, autoregulation starts to rely on cerebral perfusion pressure and blood viscosity. Because of this, any change in intracranial pressure or systemic arterial pressure can potentially worsen oedema and ischaemia.

A limitation to many of these theories is that the majority of data comes from animal studies of experimental aSAH models. Some authors have questioned whether we can reliably translate data derived from this model to human studies[120,121]. We await the results of a systematic review and meta-analysis of intracranial *in vivo* animal studies of EBI and delayed cerebral arterial vessel narrowing after aSAH[122]. The review aims to analyse aSAH models and define standard experimental parameters and endpoints for the study of EBI after aSAH and aSAH models of delayed cerebral arterial vessel narrowing.

Cortical spreading depolarisation

Cortical spreading depolarisation (CSD), also termed cortical spreading depression, reflects a wave of depolarisation that spreads across grey matter at 2–5 mm/min. CSD is not a new theory, nor is it limited to aSAH, and has been implicated in brain injuries and migraine[123]. It occurs when a cation influx across cellular membranes exceeds the Na^+ and Ca^{2+} pump action and is followed by water and shrinkage of the extracellular space by ~70% causing depression of EEG (electroencephalography) activity[124,125]. Because the Na^+ and Ca^{2+} pump is ATP-dependent, to counteract the passive influx of cations across the membrane energy consumption increases, which leads to increased regional blood flow requirements. When there is a dysfunction of the vasculature in the region, as occurs after aSAH, severe microvascular spasm can occur, rather than vasodilation, causing "cortical spreading ischaemia"[125]. There is evidence that CSD occurs after the initial aneurysm rupture from both animal and human studies, and it is thought that after each depolarisation hypoperfusion of the cortex occurs due to vasoconstriction[126]. Furthermore, up to 75% of all CSD episodes occur between days 5 and 7 after the aSAH, which matches DCI chronology[127]. Another link between CSD and DCI comes from the CoOperative Study on Brain Injury Depolarisations (COSBID), which demonstrated that repeated CSD preceded DCI with little evidence of "vasospasm" on digital subtraction angiography (DSA), albeit in a small sample (thirteen patients)[128].

Microthrombosis

Increased levels of procoagulants have been seen prior to DCI, specifically an increased von Willebrand factor 72 hours after aSAH and increased platelet-activating factors on day 4[129–132]. Microthrombi have also been identified at the autopsy of patients after aSAH, suggesting that they are involved in aSAH pathology[132]. The rate of rebleeding following aSAH has been significantly reduced following tranexamic acid administration. However, it may have led to an increased incidence of DCI separate from large vessel narrowing, possibly because the antifibrinolytic therapy caused microthrombosis and promoted DCI[133–136]. Unfortunately, the results of studies investigating antiplatelet agents in the treatment of microthrombosis after aSAH have been largely negative, including those investigating prophylactic low-molecular-weight heparin[137,138].

Therapies

Intrathecal therapies

Intrathecal administration of nicardipine, a dihydropyridine calcium channel blocker, has been demonstrated in a number of clinical studies with varying results. Susuki *et al.* examined a series of 177 patients with Fisher grade III aSAH undergoing aneurysmal clipping and cisternal drainage within 48 hours of the aSAH[139]. Patients received 4 mg intrathecal therapy nicardipine every 12 hours on days 3–14 postoperatively. Of these patients, 11.3% had radiographic evidence of vessel narrowing and 5.7% had clinical signs of DCI. The authors note a significant reduction in "vasospasm" but also recognise that 18.6% of patients required a shunt operation. Shibuya *et al.* demonstrated a decreased incidence of DCI and angiographic vessel constriction by 20 and 26% respectively after prophylactic administration of 2 mg intrathecal therapy nicardipine *via* a cisternal drain when compared with control patients[140]. More recent trials also report positive findings, but are limited to cases of refractory "vasospasm" and have very small sample sizes[141,142]. However, nicardipine is associated with probable vasodilation-associated headaches, intracranial infections and hydrocephalus, and positive long-term outcomes from large RCTs are lacking. The NEWTON trial is a phase I/IIa multicentre RCT administering intrathecal nimodipine in patients with aSAH[143]. The trial uses EG-1962, a sustained delivery system of nimodipine in microparticles. These will be injected into the ventricles through an external ventricular catheter in patients undergoing coiling or clipping of ruptured aneurysms. It is thought that systemic effects are less likely to occur as nimodipine concentrations are much lower in the plasma than CSF[144]. We await the results of this trial and subsequent progressive trials with interest.

Pleiotropic interventions

Statins have been investigated as a potential treatment for DCI due to their multiple effects, although a recent meta-analysis of the four single-centre RCTs demonstrated no benefit from statins after

aSAH[145]. Despite evidence that statins can reduce the duration of impaired autoregulation after aSAH, two more recent multicentre RCTs found no benefit from statin administration after aSAH[146–148].

Another potential agent in the treatment of DCI is cilostazol, a phosphodiesterase 3 inhibitor and platelet aggregation inhibitor that affects smooth muscle cells. A meta-analysis of two RCTs and two quasi-RCTs demonstrated amelioration of cerebral vessel narrowing and a benefit on outcome at discharge, even after excluding the lower quality studies[149–151]. A subsequent trial has echoed these findings, but only one study has reported long-term outcomes and did not demonstrate improved outcomes with cilostazol[151,152].

Conclusion

In summary, cerebral vessel narrowing is consistently seen after aSAH, but its location and severity is not predictably linked to DCI. There is no conclusive evidence to support the treatment of vessel narrowing in the management of DCI, despite some studies reporting improved outcomes, specifically after more invasive techniques. Nimodipine is the only effective treatment for DCI but we still do not understand how nimodopine exerts its neuroprotective effect, although it does not seem to work by reversing cerebral artery narrowing, at least not in large vessels. It is possible that we are not detecting microvascular vasoconstriction or ischaemia on CT and TCD and so our understanding of the pathology is limited. Furthermore, improved recognition of DCI clinically, from imaging and/or biochemical markers would not only allow for quicker treatment but also better assessment of interventions. DCI almost certainly has a multifactorial aetiology and it may be that only by combining interventions will we see improved outcomes, but first we must understand the aetiology. Understanding how nimodipine, the only drug with proven efficacy, exerts its effect may be the key to creating new interventions with improved efficacy. There remains a large amount of work to be done in understanding DCI and investigating future potential treatments.

Author contributions

Both authors were involved in the writing and revision of the manuscript and have agreed to the final content.

Competing interests

The authors declare that they have no competing interests.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Evidence-based Preclinical Medicine

Open Access

Evidence-based Preclinical Medicine ISSN 2054-703X

SYSTEMATIC REVIEW PROTOCOL

Efficacy of alpha-calcitonin gene-related peptide in dilating cerebral arteries: protocol for a systematic review and meta-analysis of *in vivo* animal studies

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(2016) Evidence-based Preclinical Medicine 3, 1, 1–3, e00013, DOI: 10.1002/ebm2.13

ABSTRACT

Aneurysmal subarachnoid haemorrhage (SAH) is a type of stroke causing up to 70% of patients to either die or be dependent on others at six months. Delayed cerebral ischaemia (DCI) is the most important cause of poor outcome after the initial injury. Oral nimodipine is the only pharmaceutical treatment with proven benefits and alternative therapies are needed. There are strong associations between DCI and cerebral vessel narrowing but interventions to treat DCI by improving vessel narrowing have been largely ineffective. Alpha-calcitonin gene-related peptide (αCGRP) is a potent microvascular vasodilator which may be effective in treating DCI by preventing cerebral vessel narrowing after SAH. This protocol outlines the methodology for a systematic review and meta-analysis investigating the effect of αCGRP on cerebral vessel narrowing and neurological outcomes after SAH from *in vivo* animal studies.

Keywords: subarachnoid haemorrhage, delayed cerebral ischaemia, calcitonin gene-related peptide

Introduction

Aneurysmal subarachnoid haemorrhage (SAH) is a subtype of stroke with an incidence of 6–11 per 100,000 people per year and a significant morbidity and mortality.[1–4] Of the 85–90% of patients who survive to reach hospital, 40% will die within 1 month of the SAH and approximately 70% of all patients will either die or require help with activities of daily living at 6 months.[5]

After successful treatment of the ruptured aneurysm and reduction of the risk of rebleeding, 30–40% of treated patients develop a syndrome of neurological deficits due to a syndrome known as delayed cerebral ischemia (DCI). DCI typically occurs between days 3 and 10 after the aSAH and half of all patients with DCI will have a poor outcome.[5–7] Nimodipine is the only pharmaceutical treatment with a proven, but modest, benefit and additional therapies are needed.[8] Despite conflicting evidence, there is a strong correlation between cerebral vessel narrowing and DCI and it is thought that reduced blood flow distal to the constricted vessel contributes to secondary ischaemia.[9–11] Efforts to prevent or treat DCI by treating cerebral vessel narrowing have so far been disappointing.[12,13]

Alpha calcitonin gene-related peptide (αCGRP) is an endogenous 37-amino acid neuropeptide and a very potent microvascular vasodilator. Several animal studies and three human trials have investigated the effect of αCGRP on cerebral arteries with a view to using αCGRP as a treatment for DCI.[14] However, there has been no systematic review assessing the efficacy of αCGRP in reducing cerebral vessel narrowing or neurobehavioural outcomes after SAH in animal models.

The aim of this review is to assess all *in vivo* animal studies which have investigated a measure of the effect of αCGRP on cerebral artery diameter after SAH using a systematic approach.

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Objectives

- To identify all animal studies investigating the effect of αCGRP in models of SAH;
- to systematically review the literature describing the effect of αCGRP on cerebral blood vessels after SAH in animal models;
- to perform meta-analysis;
- and to provide empirical evidence of the effect of αCGRP on cerebral blood vessels after experimental SAH in animals.

Outcome Measures

- The primary outcome measure will be change in cerebral artery diameter after αCGRP administration
- The secondary outcome measure will be neurological outcome.

Search Strategy

Two electronic databases were searched (MEDLINE via PubMed and EMBASE via OvidSP) for controlled *in vivo* animal models of experimental SAH reporting the effect of αCGRP on cerebral arteries from 1969 to August 2015 using the key words "alpha calcitonin gene-related peptide", "αCGRP" and "subarachnoid haemorrhage" in combination using the Boolean operator [AND]. The search was restricted to "other animals". Two investigators independently screened the abstracts and titles to identify those that met our inclusion criteria and provided the full text of selected studies. Any differences were resolved by discussion with a third reviewer.

[(alpha calcitonin gene-related peptide) OR (α-CGRP)] AND [(subarachnoid haemorrhage) OR (SAH)].

Eligibility Criteria

We included *in vivo* experimental mouse, rabbit, rat, cat, dog, pig, goat and primate SAH studies investigating the effect of αCGRP on cerebral blood vessel diameter. We did not evaluate and include studies published in languages other than English, *in vitro* experiments, studies on isolated extracranial vessels or studies on humans. Studies published in languages other than English were recorded and the number of these will be stated and referenced in the review.

Data Analysed

Dual-collection of the following data will be undertaken from eligible studies:

- Reference of paper analysed including, authors, journal name, publication date and the nationality and institute details
- Total, intervention and control sample sizes
- SAH-induction technique and injection site
- Species, sex, age and weight of animal used
- Vehicle used
- Method of measuring arterial diameter, for example cerebral blood flow, velocity, angiography, digital subtraction angiography, computed tomography, magnetic resonance imaging, transcranial Doppler or direct visualization methods
- The cerebral artery measured
- Dose and route of intervention administered
- Type of anaesthetic agent
- Intervention regimen (time from SAH to intervention, administration route and number of administrations)
- Arterial blood gas status (pH, PCO_2, pO_2, SO_2)

Where a single publication reports multiple experiments the data will be treated as independent experiments. Where only graphed data are available and we are unable to contact the authors we will estimate the data using Universal Desktop Ruler for Windows.

Methodological Quality of Studies

The methodological quality of studies will be evaluated based upon the CAMARADES checklist.[15]

1. Publication in peer-reviewed journal
2. Statement of control temperature
3. Randomized intervention allocation
4. Intervention allocation concealment
5. Blinded assessment outcome
6. Avoidance of anaesthetics with marked intrinsic neuroprotective properties (ketamine)
7. Statement of *a priori* sample size calculation
8. Statement of compliance with regulatory requirements
9. Statement regarding possible conflicts of interest
10. Use of animals with comorbidities

Statistical Analysis

Where meta-analysis is not performed we will write a descriptive summary. Otherwise data will be combined using normalized mean difference and random effects meta-analysis. Using univariate meta-regression we will explore associations of animal species, sex and strain and quality issues (random allocation to group; allocation concealment; blinded assessment outcome; sample size calculation; compliance with regulations; statement of conflict of interest; avoidance of neuroprotective anaesthesia;

statement of control temperature and publication in a peer-reviewed journal). We will assess the presence of a dose–response relationship using meta-regression of transformed data using a three component cubic spline. As a sensitivity analysis the impact of study characteristics will be explored using partitioning of heterogenicity. The critical value of α will be 0.05 for study design and quality issues. If there are more than 20 outcomes we will use Funnel plotting, Egger regression and Trim and Fill analysis.

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Appendix 3: Full Medical Research Council Application

BMC:DPFS – CASE FOR SUPPORT

Section 1: Project Summary

1.1 Title (max 150 characters) [same as Je-S Project Title]
Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

1.2 Technical Summary (max 2000 characters) [same as Je-S Technical Summary]
Subarachnoid haemorrhage (SAH) accounts for 5% of strokes in the UK. However, because of its higher morbidity and mortality, its impact is said to equal that of ischaemic stroke, which accounts for 85% of all strokes. Of the 85-90% of patients who survive to reach hospital, 40% will die within one month of SAH. Aneurysmal SAH (aSAH) accounts for 85% of all non-traumatic SAH. Part of the severe associated morbidity and mortality can be attributed to delayed cerebral ischaemia (DCI) that occurs several days after the haemorrhage and is often fatal. DCI is substantially due to cerebral arterial vasoconstriction, occurs in 30-40% of patients and is the major cause of death from aSAH. Alpha calcitonin gene-related peptide (CGRP) is one of the most potent microvascular vasodilators known. There is a substantial treatment gap for DCI, and CGRP is a potential candidate agent. A number of studies have demonstrated that DCI following SAH is associated with a decrease in CGRP levels in nerves and an increase in CGRP levels in draining blood, suggesting that CGRP is released from nerves to oppose vasoconstriction. It is an almost immediate dilator of pre-constricted arteries and the arterial dilatory effect of CGRP is to greater than 90% of the pre-constriction levels. Tachyphylaxis or tolerance does not develop with CGRP treatment and the dilatory effect of CGRP on cerebral arteries is independent of endothelial cells, which are damaged after SAH. This evidence has led to the concept that exogenous CGRP may be beneficial in the treatment of DCI. Previous research has suggested that administration of CGRP into the cerebrospinal fluid (CSF) after aSAH will be more effective than intravenous administration. CGRP is a naturally occurring peptide within the CSF but we do not know how well tolerated this route of administration will be. We will administer CGRP into the CSF of patients following aSAH, assess the tolerability of ascending doses and measure cerebral perfusion.

1.3 Project Duration and Cost [same as in Je-S submission]	
Proposed start date (dd.mm.yyyy)	16.01.2017
Proposed duration of award (Months)	25
Project fEC (£000s)	808,194.24
RC contribution (£000s)	693,582.40
Project Partner Contribution (£000s)	0

Section 2: Investigator Details

2.1 Principal Investigator [same as Je-S Principal Investigator]	
Name	Professor Peter Andrews
Post Held	Consultant in Anaesthetics and Critical Care
Department	Centre for Clinical Brain Sciences
Institution	University of Edinburgh

2.2 Co-Investigators [same as Je-S Co-Investigators]	
Name	**Institute/Organisation/Company**
Professor David J Webb (DJW)	University of Edinburgh
Dr Ioannis Fouyas (IF)	University of Edinburgh

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Section 1: Project Summary

1.1 Title (max 150 characters) [same as Je-S Project Title]
Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

1.2 Technical Summary (max 2000 characters) [same as Je-S Technical Summary]
Subarachnoid haemorrhage (SAH) accounts for 5% of strokes in the UK. However, because of its higher morbidity and mortality, its impact is said to equal that of ischaemic stroke, which accounts for 85% of all strokes. Of the 85-90% of patients who survive to reach hospital, 40% will die within one month of SAH. Aneurysmal SAH (aSAH) accounts for 85% of all non-traumatic SAH. Part of the severe associated morbidity and mortality can be attributed to delayed cerebral ischaemia (DCI) that occurs several days after the haemorrhage and is often fatal. DCI is substantially due to cerebral arterial vasoconstriction, occurs in 30-40% of patients and is the major cause of death from aSAH. Alpha calcitonin gene-related peptide (CGRP) is one of the most potent microvascular vasodilators known. There is a substantial treatment gap for DCI, and CGRP is a potential candidate agent. A number of studies have demonstrated that DCI following SAH is associated with a decrease in CGRP levels in nerves and an increase in CGRP levels in draining blood, suggesting that CGRP is released from nerves to oppose vasoconstriction. It is an almost immediate dilator of pre-constricted arteries and the arterial dilatory effect of CGRP is to greater than 90% of the pre-constriction levels. Tachyphylaxis or tolerance does not develop with CGRP treatment and the dilatory effect of CGRP on cerebral arteries is independent of endothelial cells, which are damaged after SAH. This evidence has led to the concept that exogenous CGRP may be beneficial in the treatment of DCI. Previous research has suggested that administration of CGRP into the cerebrospinal fluid (CSF) after aSAH will be more effective than intravenous administration. CGRP is a naturally occurring peptide within the CSF but we do not know how well tolerated this route of administration will be. We will administer CGRP into the CSF of patients following aSAH, assess the tolerability of ascending doses and measure cerebral perfusion.

1.3 Project Duration and Cost [same as in Je-S submission]	
Proposed start date (dd.mm.yyyy)	16.01.2017
Proposed duration of award (Months)	25
Project fEC (£000s)	808,194.24
RC contribution (£000s)	693,582.40
Project Partner Contribution (£000s)	0

Section 2: Investigator Details

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Name	Professor Peter Andrews
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2.2 Co-Investigators [same as Je-S Co-Investigators]	
Name	**Institute/Organisation/Company**
Professor David J Webb (DJW)	University of Edinburgh
Dr Ioannis Fouyas (IF)	University of Edinburgh

Dr Jonathan Downer (JD)	NHS Lothian
Dr Liam Flynn (LF)	University of Edinburgh

2.3 Industrial Project Partners [Project Partners in Je-S]	
Name	**Institute/Organisation/Company**
	N/A at present - see section 7

2.4 Non-Industrial Project Partners (Collaborators) [Project Partners in Je-S]	
Name	**Institute/Organisation/Company**
	N/A at present - see section 7

2.5 Sub-contractors	
Name	**Institute/Organisation/Company**

Section 3: Host Institute Technology Transfer Office Contact

3.1 Host Institute Technology Transfer Office Contact	
Name	Giles Dudley

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Section 4: Need and Proposed Solution

4.1 What is the health, clinical or product development need you are seeking to address? (max 150 words)

Prevention and treatment of DCI after aSAH.

Despite successful treatment of the aneurysm, up to 40% of treated patients develop a syndrome of cognitive deficits known as DCI four to ten days after the initial haemorrhage. DCI is described as the single most important cause of mortality and morbidity in patients whose ruptured aneurysm is successfully treated. It results in a poor outcome in half of the patients with this complication. Cerebral arterial vessel narrowing is associated with a 1.5-3 fold increase in mortality in the first two weeks after aSAH and, despite recent reductions in case fatality, DCI remains a major problem. There is a substantial unmet clinical need in this group of patients. Patients with SAH receive only modest benefits from nimodipine and are unlikely to have their clinical need met by other means before our proposed solution is available (1).

4.2 What is your proposed solution to this need and how long will it take to develop? (max 150 words)

Administration of CGRP into the cerebrospinal fluid (CSF) of patients after aSAH.

CGRP is an endogenous neuropeptide expressed in neurons in the dorsal root, trigeminal and vagal ganglia. CGRP is distributed in sensory fibres connected to blood vessels and is a powerful vasodilator. It is the potent reversal of cerebral arterial vessel narrowing by CGRP in patients after aSAH that interests us. This effect is independent of vascular endothelial function which is important because endothelial cells are reported to be functionally and morphologically damaged after aSAH. CGRP does not cross the blood-brain-barrier freely and CSF administration is likely to reduce, or even avoid completely, the systemic side effects seen with intravenous administration.

After completion of this trial we anticipate progression to a Phase II trial with an industrial partner. We anticipate 10 years to market from this Phase I trial.

4.3 Who are the end users of your proposed solution, how many of them are there, and what benefits does your solution provide them? (max 250 words)

The end users of the proposed solution are patients who suffer aSAH. The incidence of aSAH in the EU and USA is approximately 6 to 11 patients per 100,000. Annually this equates to approximately 50,000 to 90,000 patients (based upon a combined population of 830 million). Up to 40% of patients with aSAH go on to develop DCI after the initial haemorrhage. DCI is substantially due to cerebral arterial vasoconstriction and is a major cause of death from aSAH. The primary benefit offered by administration of CGRP is partial or complete reversal of vessel narrowing with improved neurological outcomes in those patients with DCI. The secondary benefit is prevention of DCI. Therefore, if CGRP is found to be beneficial only as treatment for DCI it will benefit the 40% of patients with aSAH who develop DCI and who currently have no treatment option (20,000 to 36,000 patients annually). However, if CGRP is found to be preventative it may be beneficial for the whole population of patients who suffer aSAH. Patients who develop DCI have an unmet clinical need that is unlikely to be met by the time our proposed solution is available (see 4.7) (1). DCI is said to remain the single most important cause of mortality and morbidity in those patients who survive to definitive aneurysm treatment. Meeting this need would substantially reduce disease burden.

4.4 Are there further needs that could be addressed by your proposed solution and/or by components of your proposed solution (i.e. is it a platform technology)? (max 100 words)

CGRP has previously been investigated for its involvement in hypertension, heart failure, ischaemia and vessel remodelling, migraines, neurogenic inflammation and arthritis. There is also some pre-clinical evidence that CGRP may be beneficial in the treatment of pulmonary hypertension (2). Data regarding the concentration of CGRP in CSF of patients

after administration and the presence or absence of headache may be beneficial to those investigating CGRP antagonists in the treatment of migraine. However, we do not anticipate our proposed use of CGRP to be a platform technology for these diseases.

Section 4: Need - Competitiveness

4.5 Who (in academia or industry) is developing/has developed competing solutions? (max 75 words)

1. Evgen Pharma: David Howat, Diederik Bulters: SFX01 after subarachnoid haemorrhage. ClinicalTrials.gov Identifier NCT02614742;
2. Manchester University: Nanveet Singh, Pippa Tyrell: Kineret in patients with subarachnoid haemorrhage. ISRCTN45931429;
3. MacDonald R: Clazosentan (CONSCIOUS trials). ClinicalTrials.gov NCT00940095;
4. Edge Therapeutics: Etminan: NEWTON trial investigating nimodipine microparticles. Clinical Trials ID: NCT01893190
5. Hanggi: intraventricular fibrinolysis and low-frequency rotation after subarachnoid haemorrhage. ISRCTN13230264.

4.6 What are the competing solutions and what is their developmental status? (max 75 words)

1. A synthetic variant of sulforaphane as an oral treatment for patients with SAH. Phase II, recruiting participants.
2. A study of the cerebrospinal fluid pharmacokinetics of intravenous Kineret (interleukin-1 receptor antagonist) in patients with subarachnoid haemorrhage. Results published.
3. Clazosentan was investigated in the CONSCIOUS trials, results published.
4. Intrathecal nimodipine microparticles. Study completed, results awaited.
5. Intraventricular fibrinolysis and low-frequency rotation after severe subarachnoid hemorrhage. Results published.

4.7 What are the shortcomings of competing solutions and what is the advantage of your proposed solution? (max 150 words)

1. Sulforaphane may reduce oxidative stress from free haemoglobin released after the initial haemorrhage but will have systemic effects. This reflects a minor part of the pathology after SAH and may be absent by the time of DCI.
2. Kineret may impact the inflammatory process after SAH but is unlikely to affect vasoconstriction. This solution has had a long gestation.
3. Clozasentan had no improvement in outcomes because of systemic side effects.
4. Oral nimodipine therapy is only modestly effective and intravenous nimodipine is ineffective, hence ongoing efforts to find alternatives (1).
5. Intraventricular fibrinolysis had no improvement on outcomes after SAH.

The advantage of intrathecal CGRP is that it acts on the abluminal side of vessel walls and is endothelium independent, working despite the vessel wall injury that occurs after SAH. CGRP crosses the blood-brain barrier poorly, limiting side effects. There is evidence that it reduces neurogenic inflammation, improves vessel remodelling and wound healing.

4.8 What is the anticipated cost of your proposed solution both at launch and at scale? How does this compare with competing solutions? If the cost is anticipated to be greater than competing solutions, why will your solution be favoured? (max 100 words)

CGRP will be classed as an orphan drug and typically the median cost for such treatments is £50,000 (3). With a conservative price of £10,000 per treatment the market in the EU & USA is £6 – 10bn. There is no competing solution on the market. The only treatment that is currently used is oral nimodipine, which is only very modestly effective and costs £100 for 21 days (based on British National Formulary costs). We envisage CGRP being used in combination with nimodipine rather than in direct competition to nimodipine. There are

currently no other competing solutions and it is difficult to anticipate the cost of other therapies being investigated.

Section 5: Rationale and Evidence

5.1 What is the rationale and supporting evidence for why your proposed solution will meet the targeted need? (max 1250 words)

- Radiological vessel narrowing is consistently seen after SAH in patients with DCI.
- Mechanical dilation of vasospastic arteries with transluminal angioplasty has been effective at treating DCI suggesting that vessel narrowing plays a substantial role in the pathology of DCI.
- There have been three clinical trials with intravenous CGRP and 21 pre-clinical trials.
- In contrast to Clozasentan, which was subsequently noted to have insufficient animal data to warrant progression to a human trial, there is evidence to suggest improved neurological outcomes in pre-clinical studies.
- We propose that repurposing CGRP to CSF administration will reduce systemic effects whilst maintaining and enhancing its effect on vessel diameter.

A recent review notes that cerebral vessel narrowing is consistently seen after aSAH and that patients with radiological evidence of vessel narrowing are at greater risk of DCI (1). Up to 70% of patients with SAH demonstrate large cerebral vessel narrowing on angiography and angiographic vasospasm is strongly correlated with cerebral infarction after SAH.

The onset of vessel narrowing appears to start around day 3, is maximal by days 6–10, and lasts for up to 2 weeks (4-6). The density, duration and volume of subarachnoid blood are key predictors of vessel narrowing (7, 8). Narrowing of cerebral arteries may cause a reduction in cerebral blood flow (CBF) distal to the constricted vessel and contribute to secondary ischaemia (9).

Radiological evidence for an association between vessel narrowing and DCI is strong. In a meta-analysis of 9 studies the overall range of reported sensitivity and specificity of CT angiogram (CTA) for detecting vasospasm in patients with aSAH was 63%–98% and 90%–98%, respectively. For CT perfusion (CTP) this was 58%–95.1% and 86%–100%, respectively (10). CTP has a reported 88% positive predictive value for identifying DCI and shows worse hypoperfusion in those patients with DCI (11). Patients with DCI also exhibit worse cerebral perfusion on CTP prior to the onset of focal neurology than patients who do not develop DCI (12). Data from the CONSCIOUS I trial demonstrates that the degree of radiological vessel narrowing (categorised as mild, moderate, severe) is highly associated with cerebral infarction (odds ratio 9.3; 95% confidence interval, 3.7-23.4) (13).

It is likely that small vessel narrowing also contributes to DCI. Pial artery constriction was directly visualised after application of blood or microtrauma to pial arteries in animal studies (14). Further in vitro research has suggested that constriction of intraparenchymal arterioles occurs after aSAH and may contribute to DCI (15). Maximal luminal narrowing is seen between days 3 and 7 and repeated in in vivo mouse studies. The correlation between decreased regional CBF and microvascular constriction appears stronger than that seen with large vessel narrowing. Uhl et al. identified constriction of small vessels in surgical patients within the first 72 hours after aSAH by spectral imaging, and Pennings et al. later directly observed cerebral arterioles contracting after aSAH (16, 17). Therefore, it is plausible that vessel narrowing is consistently occurring with DCI but not detected because it is microvascular and not readily visible on catheter angiography or transcranial Doppler (18).

Kimball et al. reviewed 49 articles relating to interventional techniques to treat vessel narrowing in patients with DCI. A total of 24 of the 27 publications (1,028 patients) reporting the use of transluminal balloon angioplasty noted an improvement in vessel diameter and neurological deficits. Twelve case series reported good angiographic and clinical results for patients who received papaverine (a vasodilator) administered close to the site of vessel narrowing. Both techniques were associated with significant side effects and the quality of the studies was reported as very low to moderate (GRADE classification

system). Nevertheless, the review provides evidence that cerebral artery narrowing is likely to be strongly involved in the pathology of DCI (19).

There have been 21 pre-clinical studies investigating the effect of CGRP on cerebral arteries after SAH (20, 21). Nine of these have been controlled in vivo studies of the effect of CGRP on cerebral arteries after experimental SAH. All 21 of these studies have demonstrated either an increase in cerebral artery size or an amelioration of vessel narrowing after administration of CGRP. We performed a systematic review and meta-anaylsis of the in vivo studies with CSF administration of CGRP (20 experiments). The review demonstrated a 41±8% (SE) increase in cerebral vessel diameter ($p < 0.0005$, 95% CI 23.7 – 57.9). In addition to this, four of the publications reported an effect on neurological outcome after CGRP administration. The standardised mean difference was 1.31 in favour of CGRP.

The CONSCIOUS trials demonstrated that Clozasentan (endotehlin-receptor antagonist) was able to dilate cerebral arteries after SAH. However, it did not improve outcomes. Laban et al. reviewed experimental studies of endothelin receptor antagonists and noted no neurobehavioural data to support progression to clinical trials (22). In contrast to this, in our review there is a positive signal consistent with a substantial effect on neurobehavioural scores after CGRP was administered to animals after SAH (see attached pdf). Furthermore, a non-statistically significant improvement in outcome for the CGRP group was seen in the European CGRP in SAH clinical trial (RR 0.88, CI 0.60 to 1.28); Johnston et al. observed a statistically significant 89% treatment preference to CGRP versus placebo, in those patients who showed a treatment preference, in their clinical trial (23, 24).

There have been three clinical trials of CGRP administration in patients who have suffered aSAH. All three have investigated systemic intravenous administration of CGRP. Unfortunately none have measured CSF concentrations. However, all demonstrate the potential role of CGRP in dilating constricted cerebral vessels compared with best standard care or placebo. Furthermore, Schebesch et al. measured the concentration of CGRP in 12 patients with SAH and compared this to the concentration in 29 patients without CNS disease (25). Not only was the concentration of CGRP higher in patients with SAH than those without, but it was also significantly higher in patients who did not develop DCI. The significantly elevated levels of the CGRP during the first days after onset of the SAH in the non-DCI group indicate a potential protective role of CGRP.

The largest clinical trial was the European CGRP in SAH trial which examined 117 patients who developed a focal neurological deficit after surgery for aSAH (23). Sixty-two patients were randomised to an intravenous infusion of 0.6 mcg/min of CGRP for 4 hours to a maximum of 10 days. The remaining 55 patients were randomly assigned to standard best management as controls. Outcome at 3 months (Glasgow Outcome Scale) was good in 66% of those treated with CGRP and 60% in controls; the relative risk of a poor outcome in CGRP-treated patients was 0.88 (95% confidence interval 0.60 to 1.28). 41 patients in this group discontinued treatment due to hypotension (19), lack of improvement at 4 hours (17) or later (4), or patient's request (1 patient).

We propose that repurposing CGRP to CSF administration will reduce the volume of distribution and circumvent the first-pass effect because CGRP is a large molecule and crosses the blood-brain barrier poorly. It is expected that this will reduce the effective dose and the side effects previously seen with systemic intravenous administration [1, 2].

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Section 6: Deliverability - Objective and Approach

6.1 What is the project's primary objective/deliverable? In the case of applications involving Institutes, Units or Centres with existing core funding, including those funded by MRC and NIHR (i.e. BRCs & BRUs), applicants need to describe how the proposed research and associated request for funds builds on, but is distinct from, their agreed programmes of research. (max 150 words)

The primary objectives of the project are to assess:

- the tolerability of ascending doses and the pharmacokinetics (plasma, CSF and urine) of CGRP after CSF administration;
- a surrogate for clinical response with cerebral perfusion studies.

The project data will be analysed and a report written before progressing to a Phase II trial.

- Current status: Bachem will supply clinical grade CGRP and an IMP dossier. A teleconference was held with the MHRA to provide regulatory guidance. The study was supported and the minutes of the meeting are provided. The trial protocol has been peer-reviewed (and was informed by NHS Lothian first-in-human committee) and the trial has support from critical care, neurosurgery and interventional neuroradiology at the Western General Hospital, NHS Lothian. The protocol has been risk assessed by NHS Lothian and the University of Edinburgh, who have agreed to support the study.

6.2 Please give details of previous awards relevant to the project, capturing the funder, time awarded, grant period, grant title and £s awarded alongside a short summary of how each one supported the current application. (max 600 words)

The largest clinical trial of intravenous CGRP administration was supported by Celltech Ltd in 1992. aSAH epidemiological studies and biological studies of CGRP have received funding from Researcher Development awards from the Department of Health and the Health Economics Research Centre. However, we are unaware of any awards in the past 20 years that are relevant to our proposed project.

6.3 What is the project's starting point and what approach is proposed to reach the objective? Why have you chosen this approach over alternatives? How have you engaged end-users and/or downstream intermediaries in the development of your plan? For clinical studies, please include details of and rationale for (1) study design, (2) study participants, (3) study endpoints, (4) dose, (5) anticipated effect size and (6) analysis plans. (max 1250 words)

Research Questions:

1. Is CGRP tolerated when given into cranial CSF?
2. What is the lowest dose with efficacy?
3. What is the dose-response?
4. What is the half-life of CGRP in the CSF?
5. How much CGRP crosses the blood-brain barrier?
6. Is any CGRP renally excreted?

The starting point is application for ethical and MHRA approval. Subsequently we will begin recruiting patients. Based on a recruitment feasibility study, we anticipate this to take a maximum of 18 months. After administering the final dose of CGRP we will complete the project report. If we demonstrate tolerability we will start to contact industrial partners prior to the final dose with a view to partnering and a Phase II trial. End-users were consulted as part of two separate feasibility studies in December 2011 and January 2015. On both occasions the trial was looked upon favourably (see 6.14).

Study design:

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The proposed study is a Phase I trial involving the administration of CGRP into the CSF of patients who have suffered an aSAH. Participants will receive one dose of CGRP and be monitored for pre-specified adverse and serious adverse events (AEs/SAEs). Changes to their cerebral vessel diameter will be assessed by transcranial doppler ultrasound and cerebral perfusion by CT scanning. CSF, blood and urine samples will be taken to analyse changes in the concentration of CGRP over time. We plan to test seven doses of CGRP in total. The study will start with the lowest dose, which will be administered to three patients. Escalation from the starting dose will follow a traditional escalation rule using a 3+3 design:

• Three patients will receive one administration of the first dose of CGRP into their CSF.

• After these three patients have received CGRP the Data Monitoring Committee (DMC) will review tolerability and safety information.

• Based on this information the DMC will decide:

-to progress to the next dose in a further three patients;
-repeat the same dose in a further three patients;
-or to stop the trial.

• If the DMC decide it is safe to progress to the next dose, another three patients will receive one administration of the next dose of CGRP and the DMC will again review the tolerability and safety information from this dose.

• If the DMC decides a further three patients should be studied using the same dose, another DMC review will occur after those three patients have received CGRP before progressing to the next dose.

• This process will continue until the final dose has been assessed or the study is stopped. In this way the study will progress to milestone 3. Please see below for more information on SAEs.

The DMC will make their decision based on the following rules: If one SAE is observed in the first group of patients then the same dose will be examined in a further three patients. If no SAEs are observed in this second group then the study may progress to the next dose level. If a further SAE is observed in this second group then the study will be stopped. If two or more SAEs are observed in the first group then the study will be stopped.

Two scenarios will lead to the end of the trial:

1. The DMC committee stops the trial based on the stopping criteria OR
2. The final dose has been administered and 28-day acute hospital mortality/discharge data has been collected from the last patient.

Study participants:

- Male or non-pregnant female patients aged 18 to 80;
- Ruptured aSAH grade I-IV (World Federation of Neurosurgeons) confirmed on digital subtraction angiography;
- Aneurysm secured by endovascular or surgical technique;
- Requiring a clinically-indicated CSF external ventricular drain (site of CGRP delivery).

Study endpoints – tolerability primary endpoint:

The primary endpoint is quantified by AEs/SAEs occuring at any dose. The study will end when all seven doses have been tested or when the study is stopped by the DMC as above. We define tolerability by observing the following factors:

SAEs

1. Haemodynamic instability including:

a. systolic blood pressure decrease >25 mmHg
b. a mean blood pressure decrease >20 mmHg;
c. heart rate increase to >150 bpm.

A blood pressure SAE will be recorded after an invasive measurement or three confirmed non-invasive blood pressure measurements with change assessed from baseline (time zero), OR any symptomatic hypotension (a reduction in blood pressure associated with symptoms, including light-headedness).

A heart rate SAE will be a heart rate greater than 150 bpm, sustained for more than 5 minutes, OR any symptomatic tachycardia (an increase in heart rate associated with symptoms including chest tightness) which was not present prior to administration of CGRP.

2. Reduction in Glasgow Coma Score >2 points or 1 motor point; change in pupil response or size
3. Clinical evidence of a seizure.

AEs

1. Spontaneous hyperventilation; PaCO2 < 4.0 kPa. This is possible after CSF injection.
2. Facial flushing. If observed, the extent will be assessed over 1-4 facial quadrants based on degree of rubor (mild, moderate, intense).

Pharmacokinetic endpoints:

1. CSF, plasma and urine GCRP concentrations obtained throughout the course of the study.
2. Final dose administration.

Dose response endpoints:

1. Change in cerebral vessel diameter measured by CT perfusion before and after CGRP administration.
2. Measurement of cerebral vessel flow velocities measured by transcranial Doppler before and after CGRP administration.

Dose:

The minimal anticipated biological effect level (MABEL) approach is taken in the absence of human data to allow determination of a no observed adverse effect level (NOAEL). The best study to determine MABEL used a CGRP tablet containing 400 mcg of CGRP which was placed in the cranial CSF of monkeys (Macaca fascicularis) and released 4 mcg/day (26). The radioimmunoassay (RIA) analysis of the CSF for CGRP concentration in the CGRP group showed 6.5 ± 5.4 nmol/L CGRP (mean ± standard deviation, n = 5) was detected in the CSF on day 7. It was otherwise undetectable.

The concentration of CGRP in the CSF of humans is higher after SAH and ranges from 0.1 to 0.9 ng/ml (15-135 ng assuming a CSF volume of 150 mls in adults).

The following information has been used to calculate the expected 'no effect' level of 10^-13 mol /kg CGRP:

- The molecular mass of α-CGRP is 3789.36g, or approximately 4000g
- 4000g of α-CGRP = ~1 mol
- 4 mcg of α-CGRP = ~1 nanomole

- Macaca fascicularis have a CSF volume of 3ml/kg and weigh 4-8kg; CSF volume and body weight are approximately 10% of human values.
- Study dosing was approximately 4 mcg/day (1 nanomole) to give very low levels, measurable at day 7 of 6.6 ± 5.4 nanomoles/litre.
- Prior studies from the same manuscript suggest that a minimum CSF level of 23 nanomoles/litre is the minimum effect level for CGRP in CSF to cause vasodilation (26).
- Humans have a CSF volume of approximately 150mls (or 2mls/kg) and we assume complete CSF distribution.
- Therefore, the minimum effect level for CGRP in CSF is found by 150/1000 = 0.15.
- 0.15 * 23 = 3.45 nanomoles (13.8 mcg).
- However, the concentration of CGRP in the CSF of humans after SAH ranges from 0.1 to 0.9 ng/ml (0.015 to 0.135 mcg assuming a CSF volume of 150 mls in adults). The concentration in control patients is 0.1 ng/ml (0.015 mcg).
- Therefore, we have started at 5 doses below this effect level.

Doses (example doses based upon a 75kg adult in micrograms in brackets, negative numbers superscript):

No effect level:

1. 10-13 mol/kg (0.03 mcg)
2. 3x10-13 mol/kg (0.09 mcg)

Possible effect level:

3. 10-12 mol/kg (0.3 mcg)
4. 3.0x10-12 mol/kg (0.9 mcg)
5. 10-11 mol/kg (3 mcg)

Effect level:

6. 3.0x10-11 mol/kg (9 mcg)
7. 10-10 mol/kg (30 mcg).

Anticipated effect size:

We anticipate little or no effect from our first three doses according to the MABEL approach. We expect partial to complete reversal of cerebral vessel narrowing from our sixth dose of CGRP, which would be consistent with previous animal studies.

Analysis:

Tolerability - Descriptive analysis will be presented including any AE, SAE or withdrawals. Where available this AE/SAE/withdrawal information will be presented in relation to the timing of doses.

We will analyse dose response based on CT perfusion and transcranial Doppler results. Dose response curves will be produced at the conclusion of each cohort showing the levels of CGRP found in blood, CSF and urine for all subjects.

Pharmacokinetic and pharmacodynamic analysis will be ongoing throughout months 7-24 whilst we collect data from each group of patients.

For the pharmacokinetic/ pharmacodynamic modelling we expect a steady-state condition in the CSF and a fixed-effect model.

6.4 Please identify the extent to which your approach is timely and innovative. For example, does it push boundaries over and beyond current leading-edge

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approaches, or is it applying existing technologies in new areas? (max 100 words)
Our approach involves repurposing an existing drug to a new route of administration. CGRP has previously been administered intravenously in three clinical trials. These trials demonstrated the effect of CGRP in ameliorating cerebral vasoconstriction but also demonstrated severe systemic effects of CGRP. CGRP has never previously been administered into the cerebral ventricles, so this is an entirely novel approach for this agent, avoiding systemic adverse effects, and supported by pre-clinical studies.

6.5 Where appropriate, please justify the use of animals or patients and the numbers of animals, samples, patients, etc tested. (max 150 words)
A clinical trial in patients is justified based upon the animal safety and efficacy data, which includes evidence of improved neurological scores after this route of administration in animal models of aSAH. We will recruit a minimum of 21 and a maximum of 42 patients depending upon dose escalation tolerability, based upon a 3+3 dose escalation strategy to determine an effective dose. If no SAEs occur and the DMC decides the study may progress without repeating any dose level, then the minimum number of patients recruited will be 21. If the DMC decides that each dose level needs to be repeated, the maximum number of patients recruited will be 42 (see section 6.3). Patient samples include blood, urine and CSF samples for ELISA. We are interested in what the half-life of CGRP is in the CSF; how much CGRP crosses the blood-brain barrier; and if any CGRP is renally excreted.

6.6 What are your plans for disseminating the results of the research? Are there any restrictions on this dissemination and, if so, what are these? (max 100 words)
There are no binding restrictions on the dissemination of results from this study. A confidential report of the results will be written but not published in an open-access journal. This is to protect the data produced by the study, which will be used for further development of the peptide as a patentable therapeutic (see section 8.8).

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Section 6: Deliverability - Project and Risk Management

6.7 Who will the project manager be? If already identified, please provide details of their experience in managing projects similar to that proposed. If not yet identified, please provide a job specification for the project manager role and your recruitment strategy. (max 150 words)

The project will be managed by Louise Sinclair, an experienced senior trial manager (6 years managing critical care trials). Louise will prepare and oversee the trial management plan and milestones; convene trial committee meetings; liaise with the team and internal and external collaborators; help prepare reports for MRC, regulators and ethics in addition to budget maintenance. She will be supported by PA as overall lead. Together they are part of an existing team experienced in managing a varied portfolio of critical care trials (single- and multi-centre).

6.8 Please provide details of the track record of the project team in delivering projects similar to that proposed to include, where relevant, details of who will manage outsourced relationship(s) and what experience they have of managing relationships of this kind? (max 250 words)

PA's team have delivered two multicentre RCTs, one a CTIMP, and have 25 years' experience of critical care research. The team have recently completed the Eurotherm3235 trial, a multicountry RCT involving neurosurgical patients in critical care, similar to our patient cohort. PA is a critical care consultant and will supervise the clinical aspects of the trial. The study will be supported by LF who has authored contemporary reviews of CGRP and DCI, and has co-authored the trial protocol. LF, in collaboration with PA, will manage outsourced relationships. DJW is President-elect of the British Pharmacological Society and a clinical pharmacologist with expertise in translational programmes, including developing endothelin antagonists for aSAH. He has been a cardiovascular clinical investigator for over 30 years, from early Phase I exploratory research to large phase III studies. DJW has contributed to study design, specifically dose levels, and supports this trial. The project team includes IF, a consultant neurosurgeon and senior lecturer with an interest in neurovascular surgery, and JD, a consultant neurointerventionalist with substantial acute vascular research experience. A clinical research fellow (to be appointed) will be responsible for peptide delivery and monitoring of patients thereafter in addition to patient transfer to and from the CT scanner.

Patients will be identified by Karen Briggs, a Nurse Specialist who has contact with all patients with subarachnoid haemorrhages at the Western General Hospital. Karen will coordinate the clinical service.

Commercialisation support will be given by Sunergos Innovations Ltd, who have demonstrable success in attracting partnership funding, including CIC, DCS and DPFS.

6.9 What are the key risks to delivering the project, how likely are these to occur and what would their impact be? How will these risks be managed? (max 250 words)

1. Recruitment failure: Risk = low. Impact = moderate to high. Prior feasibility studies put this at a low risk (see 6.14). We have managed this risk with 'no-go' criteria in milestone 2 (see milestone form).
2. Trial stopped early due to decision by DMC: risk = moderate. Impact = moderate. We have started the dose escalation with a low dose from which we expect to see little to no efficacy and no serious adverse events (see 6.3).
3. Failure to obtain ethical approval: Risk = low. Impact = high. The team have previous experience of obtaining ethical approval in a similar trial with a similar patient population. We have also sought informal advice from a member of Scotland A REC. The catheter for delivery will have been inserted on clinical grounds, so CGRP delivery is the issue under consideration (see 9.1). Six months are allocated to milestone 1 to allow edits to our application if necessary.
4. Failure to obtain MHRA approval: Risk = low. Impact = high. The team has previous experience of applying for MHRA approval in similar trials and we have discussed the trial at

a regulatory teleconference where support was given for the proposed study. The impact of this risk would be high. Six months are allocated to milestone 1 to allow edits to our application if necessary.

Section 6: Deliverability – Resource Requirements and Environment

6.10 What resources (materials, methods, data, people, infrastructure, outsourced tasks etc) are needed to undertake the proposed project? Please specify the need, the costs and the timelines of usage/employment with respect to achievement of the stated milestones? (max 300 words)

Materials: Total costs for consumables (pregnancy tests, syringes, needles, sample tubes, printer paper etc.) needed for Milestones 1 to 3 (M1-3) is £2,020. A laptop is required for entry of encrypted data/information at the bedside. This will also be used for regulatory approvals, data analysis and write up (M1-3). Equipment costs are £1,450.

The CGRP costs 348,000 CHF. It is needed for M2. We also require an IMP dossier from Bachem for MHRA approval (M1), which will be provided upon ordering the peptide.

Methods: ELISA is required for analysing pK samples (used during M2, data from this needed for M3). Kits have been sourced and previously used successfully at Edinburgh University. The cost for storage and analysis, including consumables and technician time is £32,256. Pharmacy costs for handling the peptide and preparing vials for drug administration will be £4,595. These costs relate to M2.

People: All Co-Is work through M1-3. PA will supervise the clinical aspects of the project. He will be supported by an interventional neuroradiologist (JD) and a neurosurgeon (IF). Patients will be identified by a nurse specialist (Karen Briggs) coordinating the clinical service (M2). LF will complete the trial protocol, regulatory applications and the trial report. DJW provides pharmacological advice. Cat Graham, the trial statistician, will be employed to carry out analysis (see attached justification). Ruaridh Buchan, Senior Research Pharmacist, supports the trial, including protocol and feasibility review (M2). Louse Sinclair will be the trial manager. Please refer to the attached full justification for breakdown of staff costs according to milestones.

Infrastructure: There is an on-site trial office with secure storage currently used by members of the proposed trial team (M1-3). CT scanning is available on site and has been arranged with NHS Lothian (M2). Staff training, MHRA, trial registration and archiving costs come to £4,793 (M1-3).

6.11 Are these resources in hand? If not, what gives you confidence that they will be available when required? Include manufacture of novel therapeutics where appropriate (max 150 words)

The required resources are in hand. The trial has been discussed with the relevant clinical services including pharmacy, critical care, neuroscience nurse specialist, neuroradiology and neurosurgery who have agreed to support the trial. CT scans, blood tests and consumables are available through NHS Lothian. ELISA of CGRP has been carried out at Edinburgh's Medical Research Institute and ELISA kits are available. Dr Howie (University of Edinburgh Head of Specialised Assay Service) has agreed to storage and analysis of samples. Bachem have agreed to supply the peptide and have provided a quote with timescales. Upon a successful grant application, we will order the peptide from Bachem allowing time for the peptide to be manufactured prior to the start of M2.

6.12 Please provide a high-level justification for the requested resources, in terms of the overall needs of the project (a more detailed explanation can be given in the "Justification of Resources" document). (max 300 words)

Requested consumables are those required for delivery of the peptide and regulatory approvals. We have costed consumables for 42 patients, although note that if the trial is stopped early or progresses without repeating any dose levels we will only require enough for 21 patients. In addition, if repeat dosing is not requested then the cost of sample analysis will be reduced. CGRP needs to be supplied to GMP standards for intrathecal patient administration. We have investigated and obtained quotes from alternative sources, including local and international pharmaceutical companies. No cost savings were achievable without compromising on quality, time to production or experience of making

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the peptide. Local laboratories were unable to make CGRP to GMP standards. Sample storage and analysis (see 6.5) will be undertaken by a technician who offers storage and analysis and has past experience with this peptide.

We have included costings for CT perfusion studies in patients before and after they receive the CGRP. The study is not powered for efficacy but having these data will demonstrate a change in vessel diameter and make the project more attractive to a downstream funder/industrial partner.

Employee justification: PA: PI and clinical lead. DJW: expert clinical pharmacology input. Louise Sinclair: trial manager. LF: ethics and MHRA applications, co-authoring protocol, report writing. JD and IF: clinical support and study design. Cat Graham: statistical analysis and study design for analysis. Karen Briggs: identification of eligible patients (NHS support cost). R Buchanan (NHS Lothian Pharmacy): drug preparation. We will advertise for a clinical research fellow post. The clinical research fellow will be responsible for peptide administration, patient monitoring and transfer to and from the CT scanner, supervised by PA and LF.

6.13 Describe how the scientific or clinical environment(s) in which the research will be undertaken will increase the chances of success. Including, where relevant, how the research will benefit from facilities provided by the host institution or established clinical infrastructure such as Biomedical Research Units/Centres, Clinical Research Facilities or patient cohorts. (max 150 words)

The Western General Hospital is a regional referral centre for the treatment of aSAH and benefits from having neurointensive care on site. Edinburgh has a trial team which has significant experience in undertaking multi-centre randomised controlled trials in neurosurgical patients. We have support from neurosurgery and interventional neuroradiology, both of which are based on site with a CT scanner available for perfusion studies. As such, there is established clinical infrastructure to facilitate the research in this patient cohort. The University of Edinburgh has laboratory facilities close to the Western General Hospital with ELISA experts and previous experience in CGRP analysis. The Wellcome Trust Clinical Research Facility is available on site for sample storage and patient follow up.

6.14 For clinical studies, please outline the recruitment strategy and target recruitment rate. Include evidence of feasibility where appropriate (max 200 words)

A neurovascular clinical nurse specialist with expertise in aSAH and in contact with all aSAH patients at the Western General Hospital will identify potential patients. The nurse specialist will provide information to the patient/ their family and alert the trial team if they are willing to be involved. PA or the research fellow will then discuss the study further and obtain consent if appropriate.

The target recruitment rate is 1 to 2 patients per month. This is based upon recruiting a minimum of 21 and maximum of 42 patients within 18 months. Feasibility studies were carried out in 2011 and 2015. These showed that on average three patients per month were eligible of whom two would have consented to be in the study. This finding was consistent between patients and relatives giving consent in the case that patients lacked capacity. Assuming a recruitment rate of 2 per month and allowing a week between each dose level for analysis and trial meetings, we anticipate that the dose escalation can be completed within 18 months.

Section 7: Downstream Project Support

7.1 What are the major downstream hurdles that will need to be overcome if the project is to meet its ultimate aims? (max 100 words)

If this study demonstrates tolerability, the next stage will be securing funding for a Phase II trial. If this current trial is successful we do not anticipate ethical or MHRA approval will be difficult to obtain because we will have demonstrated safety and a potential benefit to patients. Access to finance will be achieved by partnering with a biotechnology company and joint application for phase II trial funding. Funding may also be sought from the MRC's EME programme. Finding a downstream biotechnology company is a potential downstream hurdle which is mitigated by Sunergos' assistance.

7.2 Will any new technologies, processes, etc - such as a new manufacturing process - be required to overcome these hurdles? If so, please describe them and what gives you confidence that they will be available when required? (max 100 words)

No new technologies or processes will be required. There is the potential to develop patentable methods of delivery of the CGRP but first a demonstration of safety and efficacy is required. In addition to this, it may be that a biotechnology partner could manufacture the peptide, thus saving costs. Ultimately, progression to Phase II relies upon successful completion of the currently proposed dose escalation study.

7.3 Following the end of the grant award, how will the project be sustained to enable it to meet its ultimate aims (i.e. what is your exit strategy)? What sources of subsequent funding/potential partners are available to you? What criteria will need to be met in order to access these funds/partnerships and how will the planned programme of work help to meet these criteria? (max 400 words)

Sunergos Innovations Ltd (SI), is responsible for commercialisation of medical translational research at the University of Edinburgh. SI will provide ongoing support throughout the programme of work and have been involved in this application. The team at SI will closely liaise with applicants throughout the duration of the study and play a key role in sustaining the technology after completion of the programme of work. This will be initiated throughout the work by

1. Sourcing further grant funding;
2. Licensing to a pharmaceutical partner or;
3. Spinning-out the technology.

SI has extensive links to potential partners in the pharmaceutical and biotech industry through existing and previous drug development programmes. These include collaborations with GSK, Eli Lilly, Galapagos, Biogen Idec and Genzyme.

Furthermore, SI (previously known as Edinburgh BioQuarter) has a proven track record of securing private investment and establishing companies in the medical translational area. Potential investment partners, with sufficient capital available to fund the next stage of clinical development include Index Ventures, Epidarex Capital, Syncona Partners and Novo Seeds. Other potential funding sources include other translational funders, such as the Wellcome Trust and Innovate UK. Over the last five years SI has assisted in securing £25m in translational grants and industry funding, formed 13 companies and raised £8m in private equity funding. This includes the majority of the top 20 companies based on orphan drug sales, which includes Novartis, Bristol-Myers Squibb, J &J (2). The team have close links with these companies and recently met with Pfizer's Rare Disease Unit.

We will explore the best way forward with potential partners as the study progresses. The criteria required to support onward investment is evidence of acceptable tolerability, and any data showing efficacy. The pharmacokinetic and pharmacodynamic data are important in enabling discussions. However, a reversal of cerebral vessel narrowing at safe doses of CGRP will significantly increase the likelihood of success.

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7.4 Have you made initial contacts with potential downstream funders/partners and, if so, who are they and what is the status of your discussions? If not, by which specific timepoint do such links need to be in place and with whom? (max 100 words)
SI has established contacts with potentially suitable companies (see 7.3). We have not contacted specific downstream funders/partners about this project at this stage as we are too early in the development of the intervention. Once we have data demonstrating an effect of CGRP on vessel diameter without SAEs we will proceed with contacting potential partners. We anticipate seeing an effect on vessel diameter by the fourth dose of CGRP and once we see an effect at a safe, tolerable dose of CGRP we would begin contacting potential partners with an aim to having a partner/further funding in place prior to the full completion of this work.

7.5 Have you sought an industrial partner for this project? If not, why did you feel that an industrial partnership was not appropriate (stage of project, type of project, nature of the commercial opportunity, etc)? If you have not managed to find a partner, why (stage of project, type of project, nature of the commercial opportunity, etc)? *Note that inclusion of an industrial partner is not mandatory and we recognise that this will not be appropriate for all projects*. (max 300 words)
As the project is currently in its infancy we need to generate data before seeking support from an industrial partner. The MRC funding is essential to provide proof of principle data which, alongside existing animal data, will provide a compelling case for industry partnership. However, due diligence carried out by the SI team has confirmed that there is a significant market opportunity.

7.6 Are there other potential non-academic beneficiaries, in addition to your identified end-users, who might benefit from your intervention or from advances made in its development? What are your plans for engaging with these other potential beneficiaries? (max 300 words)
CGRP has pleiotropic actions and has previously been investigated for its involvement in hypertension, heart failure, ischaemia and vessel remodelling, migraines, neurogenic inflammation and arthritis. There is also some pre-clinical evidence that CGRP may be beneficial in the treatment of pulmonary hypertension. In addition to this, studies have demonstrated improved wound healing by increased blood flow and flap survival following intravenous administration of CGRP (2). Data regarding the concentration of CGRP in CSF of patients after administration and the presence or absence of headache may be beneficial to those investigating CGRP antagonists in the treatment of migraine and could be useful in future studies of ischaemic stroke. There is a theoretical possibility that the administration of CGRP could be beneficial in patients who have suffered an ischaemic stroke. This is based on pre-clinical data suggesting that CGRP is involved in vessel remodelling, neurogenic inflammation and ischaemia.

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Section 8: Intellectual Property (IP)

It is expected that this section should be completed in partnership with your Institution's Technology Transfer Office (TTO), or equivalent, and failure to do so may prejudice your application. Please refer to the Guidance for Full Stage Applicantions. *Note that the generation of protectable intellectual property is not an essential requirement for this scheme; projects that will not generate patentable materials but that have the potential to provide health benefits are accepted on an equal basis.*

Core Technologies

8.1 Please list all existing technologies (together with patent application/patent numbers where applicable) that will be further developed as part of your proposed project ("Core Technologies"). (max 100 words)
The existing technology is the application of calcitonin gene-related peptide as an intervention in delayed cerebral ischaemia after subarachnoid haemorrhage. There is currently no patent regarding the use of CGRP in the treatment of SAH. The aim of this study is to develop knowledge around the dose and deliverability of CGRP into the CSF of patients with SAH.

8.2 Please provide a summary of the most relevant documents in relation to Core Technologies identified through a prior art search. (max 200 words)
An initial freedom to operate search suggests that there are no impediments to operate. We have identified one expired patent (WO8903686) with the title of "CGRP for Cerebral Blood Supply Improvement" that was filed by Celltech. There are no patents found in the Espacenet database relating to CGRP and delayed cerebral ischaemia. A search on clinicaltrials.gov has identified ongoing trials using CGRP investigating migraine and osteoporosis. There are no current clinical trials registered investigating CGRP and aSAH and the prevention of DCI. There are numerous patents around the medical use of CGRP however these date back to the 1980s and have expired e.g. WO 1989003686. Patents remaining in force related to sequence modified CGRP (e.g. US 20110150980; WO 2011051312). We will not be using a modified form of CGRP so our freedom to operate will not be impaired. Similarly, we will not be using modified release formulations of CGRP such as US 7976847 or US 20090023644.

8.3 Do the applicants and collaborator(s), if such exist, have rights to work on the Core Technologies? If yes, please specify how such rights have been acquired. If not, how does the applicant intend to secure such rights? (max 200 words)
Assuming ethical approval and MHRA approval is granted, there are no restrictions on the use of the peptide in a clinical setting. The technologies that will be used in this study are published and used routinely within a clinical setting, improvements to these delivery methods may be made in relation to delivery of CGRP, but there are no impediments to use. Should rights to any of the Core Technologies become required we would intend to secure such rights from the patent holder through commensurate and appropriate in-licensing of technology.

8.4 Do any of the academic applicants have a direct or indirect interest (consultancy, shareholding, options, etc) in the commercial owners of Core Technologies? If so, what is the nature of their interest and how are conflicts of interest being managed? (max 100 words)
None of the applicants have a direct or indirect interest in the commercial owners of core technologies.

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8.5 Please list the most important tools (materials, methods and data) that will be used in the project but will not form a part of the project end result and for which you need rights to ("Delivery Technologies"). (max 200 words)
There are no tools (as defined above) that require rights to use in this project.

8.6 Do the applicants and collaborator(s), if such exist, have rights to use the Delivery Technologies? If yes, please specify how such rights have been acquired. If not, how does the applicant intend to secure such rights. (max 200 words)
There are no delivery technologies which require rights for use in this study.

8.7 Do any of the academic applicants have a direct or indirect interest (consultancy, shareholding, options, etc) in the commercial owners of Delivery Technologies? If so, what is the nature of their interest and how are conflicts of interest being managed? (max 100 words)
None of the applicants have a direct or indirect interest in the commercial owners of delivery technologies (consultancies, shareholdings or options etc).

Management and Exploitation of Knowledge

8.8 Please describe your strategy for protecting the Knowledge. (max 200 words)
We are confident that we can secure the necessary protection to secure onward investments. Following discussions with Marks and Clerk patent attorneys, intellectual property around dosing regime is possible. Selecting the correct dose for a new drug is a crucial step in the development of drugs. The pharmacokinetic data will inform the optimised dose regimens and the determination of therapeutic ranges. Under UK patent law, in 2007, EPC 2000 went into force containing Article 54(5) which expressly declares that a newly found, specific further use of a substance or composition already known for use as a medicament in a method for therapeutic treatment, can be patentable under EPC 2000 Article 53(c). To protect our commercial position, we intend to apply for orphan designation in the European Union (EU) and US following a successful outcome from the trial. Aneurysmal subarachnoid haemorrhage affects approximately 1 in 10,000 people in the EU equivalent to a total of around 51,000 people. This is below the ceiling for orphan designation in both the EU and US and will allow us to apply for orphan drug protection under the orphan drugs act of 1983 (USA), 1993 (Japan) and EU (2000). Orphan designation will afford 10 and 7 years of market exclusivity in the EU and US respectively.

8.9 Please describe your plans and strategies regarding further development and exploitation of the Knowledge. (max 200 words)
Arising IP generated will be proactively managed by the Sunergos Innovations commercialisation team, collaboration opportunities will be sought with industry through licensing. There is potential to create an orphan drug company around the use of CGRP. Orphan drugs represent the biggest growth area for pharmaceutical companies. In 2015 revenue was $178bn, with a growth rate of 12%. By 2020 orphan drugs are set to account for 20% of the prescription market. This is in contrast to traditional pharmaceuticals, which have half the market size and a quarter of the growth rate. Pharmaceutical companies have invested heavily in this area and have experienced this as their biggest growth zone. The enduring appeal can be attributed to lower research and development costs, easily defined patient populations and the high sales prices of the drugs. The median cost per patient for an orphan drug in the USA is $66,000 (2). Even with a conservative price point of £10,000 per treatment the addressable market in the EU & USA is £6-10bn. Due to the potential market value of such interventions, investors and pharmaceutical companies are interested in these opportunities and, on positive completion of the study, we have a good prospect of securing further investment. Sunergos Innovations will lead on the commercialisation of the asset and progressing the orphan drug application.

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8.10 Will the academic applicants have the right to exploit the Knowledge developed by their activities at the end of the project? If there are any restrictions or limitations on exploitation, please describe them. (max 100 words)
Yes. There are no restrictions or limitations on exploitation of the knowledge. Previous patents on the use of CGRP in the treatment of SAH have expired and the potential use of CGRP in treating DCI after SAH is in the public domain. The University of Edinburgh and NHS Lothian have IP management agreements allowing commercialisation by SI.

8.11 Will the licensee or assignee of the Knowledge have freedom to operate it? If not, please list the relevant patents/patent applications including their owners and explain why you think they are relevant. (max 100 words)
We have not identified any major barriers to utilising any IP or innovation that arises from the proposed research plan. Patentable IP arising will be assessed against background IP and a strategy for patent claims identified. At this time no prohibitive blocking IP has been identified.

8.12 If needed for exploitation of the Knowledge, will the applicant be able to pass rights to Core Technologies to the exploiting party (max 200 words)
Not applicable for the proposed study.

8.13 If needed for exploitation of the Knowledge, will the applicant be able to pass rights to Delivery Technologies to the exploiting party? (max 100 words)
Not applicable for the proposed study as all delivery technologies have been in routine use in the NHS for decades. However, it may be beneficial in future studies to develop delivery technologies (i.e. if less invasive routes of delivery were developed by the trial team). In which case, the applicants would be able to pass rights to the knowledge on to exploiting parties.

8.14 Any other points pertinent to protection or exploitation of knowledge not addressed in the sections above? (max 100 words)
Sunergos Innovations is a commercialisation catalyst that aims to transform innovative and disruptive research into real returns for entrepreneurs and our university partners. It is made up of experienced executives from industry, venture capital funds and contract research organisations. The Sunergos Innovations commercialisation team will work closely with the applicants to ensure that any arising IP is protected and maximal commercial benefit for the know-how/IP generated as part of this project will be achieved. The University has a defined process in collaboration with Edinburgh Research and Innovation to protect IP as developed.

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Section 9: Clinical Considerations

Please complete this section if your application includes clinical (human) research. Applicants who are not proposing human studies do not need to complete this section.

9.1 Are there any ethical issues which might complicate or prolong ethics approval? Please give particular consideration to any potential safety risks. (max 300 words)

Eligibility criteria include patients with subarachnoid haemorrhage who have a clinically indicated cerebral ventricular access device/drain. These patients are unlikely to have capacity due to their clinical condition. The trial team have experience of obtaining ethical approval for studies involving a similar group of neurosurgical patients who do not have capacity due to their clinical condition (Eurotherm3235) and we have taken advice from a member of the Scotland A Research Ethics Committee (REC). The REC will take into consideration the Adults with Incapacity (Scotland) Act 2000. This stipulates that research involving people unable to consent to take part can be done if it could not be carried out with people who can consent. In this study it could not. The research must be about the cause, diagnosis, care or treatment of the person's illness. It must be likely to produce benefit or to bring understanding that will help other people with the same condition. This study will bring understanding that will help other people in the same condition by furthering knowledge of this potential therapy and in some patients there may be benefit.

Our proposed study has potential safety risks associated with administration of CGRP, which are outlined by our adverse events (see section 6.3). We do not anticipate seeing any of these safety risks due to 1. the route of administration 2. the very low starting doses and 3. the absence of physiological changes seen from pre-clinical studies of intrathecal administration of CGRP. The potential benefits include amelioration of cerebral vessel narrowing and improved neurological outcomes.

9.2 Please provide details of the study sponsor and any relationship with a commercial partner. Please note that MRC is not the sponsor of university research. (max 100 words)

Edinburgh ACCORD, a partnership between NHS Lothian and the University of Edinburgh which supports around 1000 projects each year, have risk-assessed the protocol and agreed to sponsor the trial. ACCORD provides streamlined access to professional advice, expert regulatory support and world-class clinical research infrastructure. This partnership is underpinned by the first joint Research Framework Agreement in Scotland. Sunergos Innovations (previously Edinburgh Bioquarter), a partnership between the University of Edinburgh, NHS Lothian and Scottish Enterprise, aim to increase commercialisation and have demonstrable success in attracting partnership funding, including CIC, DCS and DPFS.

9.3 Please provide information on the status of required regulatory approvals. Please give particular consideration to any potential safety risks or ethical issues. (max 300 words)

The trial has been discussed with members of the MHRA at a "Regulatory Teleconference" meeting. The MHRA supported the study and minutes of the meeting are attached. We will apply to the MHRA for CTIMP authorisation once we have received feedback for this funding application.

In addition to the MHRA approval, we will seek ethical approval from Scotland A Research Ethics Committee as outlined in 9.1.

9.4 Please summarise the NHS support costs (note that NHS support costs should be listed in the "Additional Costs Proforma: NHS Support and Treatment costs" and attached separately to the application) (max 100 words)

There are NHS Service support costs of £71.76 per patient (max 42), for the processing of the patient record to identify NHS patients who may be suitable to approach to ask if they wish to participate in a research project. There is a cost of £82.29 per patient (max 42) for obtaining informed consent from patients because the study is a health research study

Annex I: Outsourcing

I.1 Who are you considering contracting to undertake the outsourced work? (max 100 words)
Dr Forbes Howie, based at the University of Edinburgh, will be contracted to carry out sample analysis. He has experience in ELISA and has previously studied CSF concentrations of CGRP in the CSF of humans. Bachem will be contracted to produce CGRP to GMP standards and deliver the peptide to the Western General Hospital.

I.2 What is each party contributing to the delivery of the project plan and what task(s) are they responsible for? Is the contribution unique or could a similar contribution be made by an alternate group/organisation? (max 150 words)
Dr Howie has previous experience of analysing CSF to determine CGRP concentration by ELISA. Bachem have the expertise and resources to supply CGRP at a grade suitable for clinical use. The company has GMP manufacturing methods for CGRP and can supply the peptide in a suitable solution ready for preparation by pharmacists at the Western General Hospital. To our knowledge Bachem are the only company with manufacturing capability for CGRP to GMP standards in Europe and a previous track record of manufacturing the peptide. We have investigated alternative sources and have been quoted similar prices from companies without prior experience of manufacturing this peptide. These companies also quoted longer lead times.

I.3 Please describe how the proposed outsourcing either enables the planned research to be undertaken or enables the planned research to be undertaken to the required quality or timescale. (max 150 words)
As per I.2. We would be unable to manufacture this peptide in-house due to cost constraints and the efforts required to generate suitable GMP manufacturing capabilities and have been unable to find a local, cheaper company to manufacture the peptide to the same standard as Bachem.

I.4 Please describe the agreement between the parties regarding management, ownership and rights to the project generated intellectual property. (max 100 words)
Bachem will be engaged as the manufacturer and release of finished dosage form of CGRP for use in the clinical study. Bachem will supply a quality assurance document which describes the responsibility for supply of the released CGRP. Bachem have no ownership or rights associated with the study and any Intellectual Property arising from the study will lie solely with applicants. Dr Howie is an employee of the University of Edinburgh. The study involves no other partners than the University of Edinburgh and NHS Lothian who have an agreement for IP sharing.

I.5 Do any of the participating academics have a direct or indirect interest (consultancy, shareholding, options, etc) in the industrial contractor(s)? If so, how are conflicts of interests between the parties being managed? (max 150 words)
Not applicable to this study.

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Annex II: References

II.1 References (max 700 words)

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8. Reilly C, Amidei C, Tolentino J, Jahromi BS, Macdonald RL. Clot volume and clearance rate as independent predictors of vasospasm after aneurysmal subarachnoid hemorrhage. J Neurosurg. 2004;101(2):255-61.
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Justification for Resources

Costs have been calculated based upon recruiting 42 patients and the trial running for 25 months. If no doses are repeated or the trial is stopped early (see full proposal, 6.3) costs will be reduced.

MHRA and MHRA amendments fees, ISCRCTN and clinical trials application fee £4,157: These are essential for MHRA approval and need to be in place prior to the trial starting.

Alpha-CGRP costs 348,000 CHF (£235,135): Please see exceptional costs letter. Converted to sterling by the University of Edinburgh finance department.

NHS pharmacy costs £4,595: CGRP is supplied to the Western General Hospital in 100 vials. NHS pharmacy will produce pre-filled syringes with the correct doses of CGRP to administer to the patient based upon body weight. The trial involves storage of the peptide at -20 degrees C, serial dilutions and dispensing costs. Pharmacy set up and administration fee: £500. Dose calculations/dispensing: £90 per dose (based on 1.5hrs of band 7 hourly rate).

CT perfusion scans £31,780: Evidence of efficacy from CT imaging will greatly improve our chances of receiving downstream funding or industrial partnership. CT angiograms £232.62; CTA with a regular CT head with IVCM £378.57. We have costed for 84 scans (42 pts pre- and post-alpha-CGRP).

Pregnancy tests and consumables for drug delivery and sample collection £928: Essential for drug delivery and sample collection. Prices are taken from a database used in intensive care when ordering replacements for routine consumables. Pregnancy tests are necessary to check eligibility criteria.

Sample analysis and storage £32,256: The quote is £32 per sample (maximum 1,008 samples) and includes sample storage for the life of the trial to -80 degrees Celsius. In addition, it includes ELISA kit costs and lab technician time.

Stationery and printing £1,100: Based on the known costs for a similar trial run by the same team (Eurotherm3235) and includes the use of a printer currently in use in the trial office we propose to use for this study.

Archiving costs £576: University of Edinburgh cost for archiving 4 boxes for fifteen years.

Equipment < £10,000: £1,450 for a Microsoft Surface Pro laptop with keyboard. A dedicated trial laptop is needed for bedside data entry to the online eCRF, it will also be used for other work on this trial. This device has the flexibility and capacity to be used in tablet form to facilitate bedside use and as a conventional laptop.

DMC and TSC costs £90: Teleconferencing – DMC x7, TSC x2. Two hours on average is allowed for each. Teleconferencing avoids travel costs and facilitates ease of meeting.

Staff training £160: Good clinical practice updates for the trial team (4 x half day updates).

Travel and subsistence £5,670: Milestone 1 - £2760, Milestone 2 - £1500, Milestone 3 - £1500 - 3 visits to London (MRC/MHRA) PA and LF (Milestone 1), 2 conferences PA and LF (1 each in Milestones 2 and 3).

Justification for Staff Employment:

Peter Andrews: Principal investigator. Considerable input on protocol development including background research, study design, drug dosing, statistical analysis and supporting information. Peter will supervise the clinical aspects of the trial in addition to fulfilling the requirements of Principal Investigator.

David Webb: Co-investigator. David has informed study design and has had input into the protocol design and dose levels. David has been a cardiovascular clinical investigator for over 30 years, from early phase 1 exploratory research to large phase 3 studies.

Liam Flynn: Co-investigator. Considerable input on protocol development including background research, study design, drug dosing, statistical analysis and supporting information. Liam is responsible for regulatory applications and write-up.

Cat Graham: Trial statistician. Involved with trial design and suggestion of 3+3 design. We have structured Cat's salary towards the end of the trial, as this is when we anticipate the statistical analysis of data to begin.

Ioannis Fouyas: Co-investigator. Consultant neurosurgeon with neurovascular interest. Ioannis will provide expert advice on neurosurgical aspects of the study and patients are admitted under neurosurgery.

Jonny Downer: Co-investigator. Consultant neuroradiologist. Jonny will provide expert advice on radiology as well as assessment of CT perfusion imaging.

Louise Sinclair: Trial manager with previous experience managing multi-centre RCT in critical care. Louise will be responsible for day-to-day management of the trial.

Clinical Research Fellow: The clinical research fellow (to be appointed) will assist with submitting MHRA and ethical applications in addition to monitoring the patient after CGRP administration and transfer to and from the CT scanner for perfusion imaging.

Name	Hours p/w	Period of Employment		Role
		From	To	
Professor Peter Andrews	8	16.01.17	15.02.19	PI
Professor David Webb	1	16.01.17	15.02.19	CO-I
Dr Liam Flynn	0.5	16.01.17	15.02.19	CO-I
Dr Jonny Downer	0.5	16.01.17	15.02.19	CO-I
Mr Ioannis Fouyas	0.5	16.01.17	15.02.19	CO-I
Louise Sinclair	50%	16.01.17	15.02.19	RA
Cat Graham	20%	16.01.17	15.02.19	RA
Clinical Research Fellow (to be appointed)	50%	16.01.17	15.02.19	RA

Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

CGRP Background

Calcitonin Gene-Related Peptide (CGRP), an endogenous 37-aminoacid peptide (3790 D), is normally synthesised in the body through alternative splicing of the Calcitonin gene. There are two known Calcitonin genes located on the short arm of chromosome 11 in humans. Alternative processing of the first Calcitonin gene results in synthesis of Calcitonin and CGRP. The transcription of the second Calcitonin gene does not seem to be differential, as only prepro-CGRP II mRNA and not preprocalcitonin II mRNA is detectable. This second Calcitonin gene also synthesises CGRP, which differs from that synthesised by the first Calcitonin gene by three aminoacids. These two CGRPs are called α- and β-CGRP.

Calcitonin gene-related peptide receptor activation is known to involve several crucial elements, in common with other G-protein-coupled receptors (GPCRs), such as the presence of a proline "kink" in transmembrane helix (TM)6, and a putative 'DRY' motif equivalent, similar to family A GPCRs. There is also evidence suggesting stabilisation of the calcitonin receptor-like receptor (CLR) interaction with G "alpha" s (Gas) by another 17kDa intracellular membrane protein, called receptor component protein (RCP).

Several mechanisms involved in CGRP-mediated vaso-relaxation have been identified. These mechanisms include either NO-dependent endothelium-dependent mechanisms or cAMP-mediated endothelium-independent pathways. Activation of the CGRP receptor is generally accepted to result in Gas-mediated activation of adenylate cyclase, with a subsequent increase in cAMP and activation of protein kinase A (PKA). In the absence of endothelium, CGRP is able to cause relaxation, suggesting it must act directly on the smooth muscle cells to stimulate adenylate cyclase. The resulting rise in cAMP then activates PKA, which phosphorylates and opens up ATP-sensitive K+ channels, thus leading to relaxation. Endothelium-independent relaxation to CGRP occurs in the majority of tissues examined to date.

CGRP is a vasodilator neuropeptide that is expressed in a subgroup of small neurons in the dorsal root ganglion, trigeminal and vagal ganglia. CGRP is released antidromically in the periphery, eliciting vasodilation as a component of neurogenic inflammation.

It is the potent vasodilating effect of α-CGRP on cerebral arterial vessel narrowing in patients after aneurysmal subarachnoid haemorrhage (aSAH) that is of interest. Importantly, this effect is independent of endothelial cells of blood vessels and their derived factors and deserves special consideration because endothelial cells are reported to be functionally and morphologically damaged in delayed cerebral ischaemia (DCI) after aSAH.

There are no data suggesting any off target toxicity with CGRP in any species studied, including non-human primates and humans.. Presumably this is because of receptor specificity. There are no human data on cerebral spinal fluid administration. However, lack of identifiable off-target effects in rodent and primate models when given in the CNS as well as lack of off-target effects when given intravenously in humans makes the likelihood of human off-target effects very low when given intrathecally. Alpha-CGRP exists within the CSF in humans naturally and has a high molecular weight with poor penetration through the Blood-Brain-Barrier. Therefore, we do not expect to see off-target effects in this study.

Kokkoris S, Andrews P, Webb DJ: **Role of calcitonin gene-related peptide in cerebral vasospasm, and as a therapeutic approach to subarachnoid hemorrhage**. *Frontiers in endocrinology* 2012, **3**:135.

Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

Alpha CGRP in subarachnoid haemorrhage: regulatory meeting by teleconference with MHRA - 28 May 2014 1400

Present: David Jones – Non-Clinical Assessor, MHRA Clinical Trials Unit; Martin O'Kane - Pharmaceutical Assessor (Acting Manager); Kirsty Wedderbuck – Clinical Assessor, MHRA Clinical Trials Unit and Peter Andrews (PA), Liam Flynn & Bridget Harris – Edinburgh

(Note of meeting: MHRA clarified this was a regulatory meeting and they would not be giving formal written advice).

PA gave a presentation on alpha CGRP in the context of subarachnoid haemorrhage – the data on administration into CSF is all in animals, the recommendation of the study in monkeys (Inoue et al Neurosurgery 1996;39:984-990) was that a human study was warranted, this has not yet been done.
MHRA members asked questions/commented:

1. **Martyn Ward** – asked about manufacture and quality issues regarding the peptide. PA explained that Bachem will manufacture GMP product that will be QP released. Martyn commented that in his view this was appropriate.

2. **David Jones** – non-clinical issues – commented that although GLP in the animal studies was probably not at a standard to support a clinical trial he did not at this stage see that as a stumbling block to the proposed study of intra-ventricular alpha CGRP in humans with SAH.

3. **Kirsty Wedderbuck** – clinical issues – noted it was harder to comment without having seen the protocol, PA explained that the intended population was 40 patients with a secured aneurysmal SAH who would be in critical care with an intraventricular drain and continuous cardiovascular monitoring as part of normal care, each would receive a single dose of alpha

CGRP into their ventricle, using the protocol for administering intrathecal antibiotics. The dose rationale is based on starting at 100th of the dose expected to have an effect, based on the animal data as the human data relates to IV administration only. CSF concentration would be monitored:- sampling might reduce the concentration – KW thought that this was

not such a problem in this study but might be in future studies. Advice from KW was that the existing human data needs to be discussed early in the IRB submission and protocol with a detailed discussion of all the safety monitoring and how the starting dose was determined (where the data determining it came from), and it is important to explain that alpha CGRP is present in CSF anyway and dosing is starting at a level less than that normally present. We need to justify the final dose (especially if it is significantly higher than any previous doses). The dosing schedule can be absolute doses or a % increases (which might allow a little leeway), there must be a detailed safety monitoring plan, strict stopping criteria for dose escalation and SAEs are needed, with very clear rules, especially regarding cardiovascular events (hypotension and tachycardia).

Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

Further points:

Edinburgh team to speak to MHRA again once the final protocol has been produced.

Meta-analysis data:

Two investigators independently extracted data relating to species of animal and weight; method of inducing SAH (single injection, double injection or clot placement); whether the basilar artery (BA), middle cerebral artery (MCA), anterior cerebral artery (ACA) or internal carotid artery (ICA) were measured; the method of measurement (angiography, *in vitro* measurement or direct *in vivo* visualisation); anaesthetic agent used; dose of αCGRP and time of administration from SAH; reporting and method of randomisation; reporting and method of blinding; animal welfare guideline statement; statement of sample size calculation; whether there was a statement of potential conflicts of interest and the use of animals with comorbidities. Study quality was assessed using the CAMARADES 10-item quality checklist.

21 publications investigating αCGRP and cerebral vessel narrowing were found after MEDLINE search. After examining the full papers of these abstracts and removing those with *in vitro* αCGRP administration (n=12) and those which lacked SAH models, nine eligible publications remained. The nine publications included in the review were published between 1989 and 2013 (median year 1996). From the 9 publications, 20 experiments were included in meta-analysis.

Characteristics of Studies

The total number of animals examined was 193 and the median number of animals used per experiment was 9 (interquartile range (IQR) 6 to 14). The median number of study quality checklist items scored was 4 (IQR 2 to 6). No studies used animals with comorbidities; reported a statement of potential conflicts of interest or stated an *a priori* sample size calculation. 40% of studies reported control of body temperature. 20% described a randomised treatment allocation and 45% reported allocation concealment. Half of the experiments used a blinded assessment of outcome and half used an anaesthetic agent other than ketamine. All studies were published in peer reviewed journals and 70% reported compliance with local animal welfare guidelines. In 16/20 (80%) of the experiments, SAH was induced by autologous blood injection by either single or double injection methods, the remainder were induced with blood clot placement.

Treatment Effect

Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

All 21 publications reporting *in-vivo* and *in-vitro* experiments demonstrated a dilation of cerebral arteries after αCGRP administration. Of the 20 eligible *in vivo* experiments included in meta-analysis, there was a 40.8 ± 8.2% (SE) increase in cerebral vessel diameter in the αCGRP group compared with controls ($p<0.0005$, 95% CI 23.7 to 57.9, I^2 96% Figure 2). There was also a significant dose-response to αCGRP in the 10 experiments which administered a single dose into the cerebroventricular system.

The effect size tended to be lower in studies that reported randomisation, blinded assessment of outcome, blinded induction of SAH and use of an anaesthetic agent without intrinsic neuroprotective properties. However, none of these observations reached statistical significance. There was also a trend towards lower effect size in studies reporting compliance with more quality checklist items. This ranged from 57.3 ± 10.7% ($p <0.05$) from experiments with a quality score of 1 to 28.1 ± 9.1% ($p<0.01$) from experiments with a quality score of 6.

Neurological outcome and adverse effects

Four studies reported an effect on neurological outcome after αCGRP administration. The standardised mean difference was 1.31 (95% CI -0.49 to 3.12, Q 40.5, n=65 animals) in favour of αCGRP. Tian et al. reported neurological outcome based on a comprehensive scoring system (0-48, 0 = best score, 48 = worst score) measured three times daily and based upon the assessment of four functions which has been used elsewhere (1). The mean neurological outcome on day 7 for the αCGRP group was significantly better than for the control group (10.67 ± 1.16 versus 22.33 ± 2.08 respectively, $p < 0.001$). Imaizumi et al. assessed neurological outcome based on food intake and a slope tolerance test on days 2, 3 and 4 post-SAH (2). No significant difference was found between the αCGRP and control groups for either assessment. Inoue et al. reported no significant difference in food intake, observable hemiparesis, consciousness disturbance or response to stimulation between the αCGRP and control groups (3). Ahmad et al. reported neurological outcome from grade I (normal) to grade III (unable to stand and presented abnormal posture) in addition to performing a slope tolerance test (4). Two rabbits in the control group were grade II (slow in response but able to walk) and III respectively, all other rabbits were normal and there was no statistical difference between the groups in the slope tolerance test. In all studies where it was measured, food intake was decreased after SAH but there was no significant difference between the αCGRP and control groups. Inoue et al. noted a significant decrease in weight in their αCGRP group compared with the control group at day 14, but note no other adverse effects and were unable to explain this change in weight (3).

Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

Eight publications reported physiological parameters which might be associated with adverse events. There was no significant difference in systemic arterial pressures or arterial blood gas results between the aCGRP or control groups. Imaizume et al. found that all animals tended to have an increased respiratory rate for approximately six hours after intrathecal injection of either αCGRP or vehicle and demonstrated a high blood pH and low pCO_2, but again no difference between the groups (5). Both Nozaki and Toshima et al. demonstrated a decrease in mean arterial blood pressure when αCGRP was administered intravenously, which was not seen by intrathecal administration (6, 7). The study by Tosima et al. demonstrates a marked decrease in mean arterial blood pressure following intravenous administration of αCGRP which is not seen with intracisternal administration (~70 mmHg versus ~40 mmHg at 30 minutes after αCGRP administration) (6).

1. Tian XH, Wang ZG, Meng H, Wang YH, Feng W, Wei F, et al. Tat peptide-decorated gelatin-siloxane nanoparticles for delivery of CGRP transgene in treatment of cerebral vasospasm. Int J Nanomedicine. 2013;8:865-76.
2. Imaizumi S, Shimizu H, Ahmad I, Kaminuma T, Tajima M, Yoshimoto T. Effect of calcitonin gene-related peptide on delayed cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. Surg Neurol. 1996;46(3):263-70; discussion 70-1.
3. Inoue T, Shimizu H, Kaminuma T, Tajima M, Watabe K, Yoshimoto T. Prevention of cerebral vasospasm by calcitonin gene-related peptide slow-release tablet after subarachnoid hemorrhage in monkeys. Neurosurgery. 1996;39(5):984-90.
4. Ahmad I, Imaizumi S, Shimizu H, Kaminuma T, Ochiai N, Tajima M, et al. Development of calcitonin gene-related peptide slow-release tablet implanted in CSF space for prevention of cerebral vasospasm after experimental subarachnoid haemorrhage. Acta Neurochir (Wien). 1996;138(10):1230-40.
5. Imaizumi S, Shimizu H, Ahmad I, Kaminuma T, Tajima M, Yoshimoto T. Effect of calcitonin gene-related peptide on delayed cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. Surg Neurol. 1996;46(3):263-71.
6. Nozaki K, Kikuchi H, Mizuno N. Changes of calcitonin gene-related peptide-like immunoreactivity in cerebrovascular nerve fibers in the dog after experimentally produced subarachnoid hemorrhage. Neurosci Lett. 1989;102(1):27-32.
7. Toshima M, Kassell NF, Tanaka Y, Dougherty DA. Effect of intracisternal and intravenous calcitonin gene-related peptide on experimental cerebral vasospasm in rabbits. Acta Neurochir (Wien). 1992;119(1-4):134-8.

DPFS: 4 MILESTONE FORM

Section 1: Project Summary

1.1 Title (max 150 characters) [same as Je-S Project Title]
Tolerability and dose-finding study of the effect of Calcitonin Gene-Related Peptide on cerebral perfusion after aneurysmal subarachnoid haemorrhage

1.2 Technical Summary (max 2000 characters) [same as Je-S Technical Summary]
Subarachnoid haemorrhage (SAH) accounts for 5% of strokes in the UK. However, because of its higher morbidity and mortality, its impact is said to equal that of ischaemic stroke, which accounts for 85% of all strokes. Of the 85-90% of patients who survive to reach hospital, 40% will die within one month of SAH. Aneurysmal SAH (aSAH) accounts for 85% of all non-traumatic SAH. Part of the severe associated morbidity and mortality can be attributed to delayed cerebral ischaemia (DCI) that occurs several days after the haemorrhage and is often fatal. DCI is substantially due to cerebral arterial vasoconstriction, occurs in 30-40% of patients and is the major cause of death from aSAH. Alpha calcitonin gene-related peptide (CGRP) is one of the most potent microvascular vasodilators known. There is a substantial treatment gap for DCI, and CGRP is a potential candidate agent. A number of studies have demonstrated that DCI following SAH is associated with a decrease in CGRP levels in nerves and an increase in CGRP levels in draining blood, suggesting that CGRP is released from nerves to oppose vasoconstriction. It is an almost immediate dilator of pre-constricted arteries and the arterial dilatory effect of CGRP is to greater than 90% of the pre-constriction levels. Tachyphylaxis or tolerance does not develop with CGRP treatment and the dilatory effect of CGRP on cerebral arteries is independent of endothelial cells, which are damaged after SAH. This evidence has led to the concept that exogenous CGRP may be beneficial in the treatment of DCI. Previous research has suggested that administration of CGRP into the cerebrospinal fluid (CSF) after aSAH will be more effective than intravenous administration. CGRP is a naturally occurring peptide within the CSF but we do not know how well tolerated this route of administration will be. We will administer CGRP into the CSF of patients following aSAH, assess the tolerability of ascending doses and measure cerebral perfusion.

1.3 Project Time and Cost	
Proposed duration of award (Months)	25
Full economic cost (£000s)	808,194.24

Section 2: Applicant Details

2.1 Principle Investigator [same as Je-S Principle Investigator]	
Name	Peter Andrews
Post Held	Consultant in Anaesthetics and Critical Care
Department	Centre for Clinical Brain Sciences
Institution	University of Edinburgh

DPFS: 4 MILESTONE FORM

Milestone 1 (M1)	
Time from Start to M1 (months)	6
Expenditure from Start to M1 (£000s)	359681
Estimate of meeting M1 criteria (%)	90
M1 Objectives (max 250 words)	
Scotland A Research Ethics Committee (REC) approval and MHRA approval.	
M1 Success Criteria and Target Values (max 250 words)	
Success criteria is approval from both bodies. Go = approval from MHRA and Scotland A REC.	
M1 Justification for Criteria and Values (max 250 words)	
Our sponsor (ACCORD) has advised us to seek funding for the study prior to ethical approval and MHRA approval prior to ethics approval. We will submit our MHRA application after a successful funding application. Funding is required to pay for an IMP dossier which is necessary for the MHRA application. The MHRA state that the initial assessment is completed within 30 days of application. If initially unsuccessful, further applications are assessed within 60 days from the original application. We will submit our ethics application to Scotland A REC by March 2017, which allows time for any requested changes to be tackled within milestone 1. Having previously been successful with applications to Scotland A REC in a similar patient cohort the trial team feel confident that we will gain ethical approval for this trial. However, we have made allowance for possible changes based on REC recommendations. Should we be granted ethical and MHRA approval prior to the 6 months we have allocated we would aim to expedite the study start date.	

Milestone 2 (M2)	
Time from M1 to M2 (months)	18
Time from Start to M2 (months)	24
Expenditure from M1 to M2 (£000s)	425633
Expenditure from Start to M2 (£000s)	785314
Estimate of meeting M2 criteria, if M1 achieved (%)	90
M2 Objectives (max 250 words)	
Dose escalation progression and patient recruitment. The objectives for M2 are recruitment of sufficient patients to administer 7 doses of CGRP and assessment at each dose level to progress to the 7th dose.	
M2 Success Criteria and Target Values (max 250 words)	
Success criteria for M2 will be assessed at six month intervals: we require the recruitment of 1 to 3 patients per month depending upon the rate of dose escalation/incidence of adverse events. Go criteria at 6 months from the start of M2 = recruitment of 12 patients OR progression to dose level 3 (equivalent to minimum 6 patients, maximum 12 patients). Go criteria at 12 months from the start of M2 = recruitment of 24 patients OR progression to dose 6 (equivalent to minimum 12 patients, maximum 24 patients). Stop criteria at any time = the study will be stopped at any time should cardiorespiratory arrest, intracranial haemorrhage or death occur.	
M2 Justification for Criteria and Values (max 250 words)	
M2 can be measured by either patient recruitment numbers or the rate of dose escalation. Because this is a 3+3 design, the occurrence of serious adverse events will require the same dose to be repeated in a further 3 patients, thus increasing patient numbers. If a dose level is completed without any adverse events, it will not be necessary to recruit 3 patients each month. However, if each dose level is required to be repeated due to adverse events occurring, 3 patients will need to be recruited each month. Therefore, the stop/go criteria are based on either recruitment OR dose escalation. The minimum number of patients we would need to recruit per month if we repeated no dose levels is 1.2. The maximum number of patients we would need to recruit per month if we need to repeat every dose level is 2.3.	

Milestone 3 (M3) [if applicable]	
Time from M2 to M3 (months)	1
Time from Start to M3 (months)	25
Expenditure from M2 to M3 (£000s)	22881
Expenditure from Start to M3 (£000s)	808195
Estimate of meeting M3 criteria, if M2 achieved (%)	100
M3 Objectives (max 250 words)	
Complete analysis and write up of results.	
M3 Success Criteria and Target Values (max 250 words)	
Success criteria is completion of statistical analysis of CGRP concentrations based on ELISA and assessment of adverse events at each dose level. On completion of the study report the trial will have finished. Quantifiable evidence of M3 completion is a written report with formal analysis of the obtained data within month 24.	
M3 Justification for Criteria and Values (max 250 words)	
ELISA will be ongoing throughout the trial at the end of each dose level so results will be readily available by month 24. Therefore there should be no difficulty in meeting the M3 criteria.	

DPFS: 4 MILESTONE FORM

Milestone 4 (M4) [if applicable]	
Time from M3 to M4 (months)	0
Time from Start to M4 (months)	25
Expenditure from M3 to M4 (£000s)	0
Expenditure from Start to M4 (£000s)	858614
Estimate of meeting M4 criteria, if M3 achieved (%)	0
M4 Objectives (max 250 words)	
M4 Success Criteria and Target Values (max 250 words)	
M4 Justification for Criteria and Values (max 250 words)	

Appendix 4: First In Human Application

Study no: αCGRP Protocol Version:

EDINBURGH CLINICAL RESEARCH FACILITY
PHASE I / FIH STUDY REVIEW COMMITTEE
CLINICAL RISK ASSESSMENT REPORT

Study Title:	Toxicity response to alpha-Calcitonin Gene-Related Peptide administered into lumbar CSF of surgical patients		
Principal Investigator:	Professor David Webb		
Researcher conducting study:	Peter Andrews, David Webb & Liam Flynn		
CRF Study ref no.:		**CRF Site:**	Western General Hospital
Protocol version:	Dec 2013	**Risk Assessment version :**	**Version 02**
Study proposed start date:	February 1, 2013	**Date of Committee review:**	
Name of reviewers			
Documents submitted in support of application	CVs for all researchers & copies of GCP certification An updated study protocol A completed risk assessment form Product overview Unigene Laboratories, Inc. Clinical trial overview NCT00687947;FHM-CGRP-MA-2008 International patent application PCT/GB88/00877 Detailed review by applicant in Frontiers in Endocrinology McLatchie LM, et al., NATURE , Vol 393, 28 May 1998, 333-339. Kraenzlin Regulatory Peptides, 10 (1985), *189-197* Lancet 1990&1992 papers		

Clinical Risk Assessment of study submitted for approval to take place in the Edinburgh CRF

Refer to guidance in 'Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal Products' EMEA 2007

1.Study team

1. 1 Principal Investigator (Peter Andrews)

The *cv* of the PI must clearly identify the qualifications, experience and key publications in support of the role as PI for this study. Provide only concise, relevant information.
If the PI has no pharmacology qualification AND no experience, the role must be with supervision if at all. An appropriate supervisor must be identified.

1.1.1 Clinical Pharmacology qualifications and experience of PI:

Clinical pharmacology qualification:	CCT in clinical pharmacology & therapeutics	No, Year
	CCT in pharmaceutical medicine	No, Year
Clinical pharmacology experience:	PhD in clinical pharmacology	No, Year
	Industry experience	No, Year
	Academic experience	, Year

1.1.2 Research experience of PI

Experience in Phase I trials: Yes one, Trial steering committee

Experience in FIH trials: Yes one, Data monitoring committee

Experience in studies involving dose escalation: Yes

Please provide details below of this Phase I/FIH/dose escalation experience, indicating the publications that have resulted from this work:

NAP protocol – "Exploratory clinical study of microdosing NAP for optical molecular imaging in human lungs"

Trial Steering Committee. ISRCTN 23625128

The BRAIN TRIAL: a randomised, placebo controlled trial of a Bradykinin B2 receptor antagonist (Anatibant) in patients with traumatic brain injury. Trials 2009, 10:109doi:10.1186/1745-6215-10-109 - Published: 3 December 2009.

1.1.3 Role and competence of the PI

What clinical functions will the PI perform in this study (eg administration of drug, mentoring, clinical input etc)?:

Supervision, mentoring, study design, administration of drugs and clinical decision making relating to patients in WTCRF.

1.1.4 PI supervision (if required for role of PI)

What supervision does the PI require? *Prof David Webb will provide supervision on drug dose calculations for the initial starting doses and appropriate dose escalation and will provide the study the benefit of his 30 years of experience in clinical pharmacology.*

Name of proposed individual supervising the PI (if required): N/A

1.1.5 Training status of PI

GCP trained Yes Date of training: April 10, 2013 Training provider: WTCRF

ALS trained Yes Date of training: 1995, 2 yearly updates Training provider: NHS Lothian

Please provide details of other training/ skills essential for this study: Consultant Anaesthetist working in critical care.

1.2 Researcher(s) conducting the study

Please provide information for each researcher (other than PI)

1.2.1 Qualifications of researcher: David Webb

State relevant qualifications and training (including pharmacology).

CCT in Clinical Pharmacology and Therapeutics, MD, DSc

1.2.2 Research experience of researcher:

State FIH/ Phase I or relevant experience:

Experience in Phase I trials: Yes No. of trials and role: > 50 trials as Principal Investigator

Experience in FIH trials: Yes No. of trials and role: >20 trials as Principal Investigator

Experience in studies involving dose escalation: Yes

Please provide details below of this Phase I/FIH/dose escalation experience, indicating the publications that have resulted from this work:

Webb DJ, Cumming AMM, Leckie BJ, Lever AF, Morton JJ, Robertson JIS, Szelke M, Donovan B. Reduction of blood pressure in man with H-142, a potent new renin inhibitor. Lancet 1983;ii:1486-7.

Webb DJ, Benjamin N, Vallance P. The potassium channel opening drug cromakalim produces arterioselective vasodilation in the upper limbs of healthy volunteers. Br J Clin Pharmacol 1989;27:757-61.

Haynes WG, Noon JP, Walker BR, Webb DJ. L-NMMA increases blood pressure in man. Lancet 1993;342:931-2.

Dhaun N, Macintyre IM, Melville V, Lilitkarntakul P, Johnston NR, Goddard J, Webb DJ. Blood pressure-independent reduction in proteinuria and arterial stiffness after acute endothelin-A receptor antagonism in chronic kidney disease. Hypertension 2009;54:113-9.

Oliver JJ, Dear JW, Webb DJ. Clinical potential of combined organic nitrate and phosphodiesterase type 5 inhibitor in treatment-resistant hypertension. Hypertension 2010;56:62-7.

Dhaun N, Macintyre IM, Kerr D, Melville V, Johnston NR, Haughie S, Goddard J, Webb DJ. Selective endothelin-A receptor antagonism reduces proteinuria, blood pressure, and arterial stiffness in chronic proteinuric kidney disease. Hypertension 2011;57:772-9.

Ras RT, Zock PL, Zebregs YEMP, Johnston NR, Webb DJ Draijer R. Consumption of grape seed extract does not lower daytime ambulatory blood pressure in subjects with above optimal blood pressure levels. Am J Clin Nutr 2013 in press

1.2.3 Supervision of researcher

Name of supervisor for the researcher(s) if not the PI: Peter Andrews

What supervision will be provided?: *Will work as part of the team on this study and will be supervised by senior team members.*

1.2.4Training status of researcher

GCP trained Yes Date of training: November 14, 2012 Training provider: Pfiser

ALS trained Yes Date of training: Training provider:

Please provide details of other training/ skills essential for this study: Over 30 years experience in running clinical trials in academia and in collaboration with the pharmaceutical industry, and has directed the University's Clinical Research Centre for over 20 years. The University's representative to the Lothian Research Ethics Committee for 1-years and a member of the University Phase I Study Scientific Review Committee for 2 years as well as directing the clinical research training programme at Edinburgh's Wellcome Trust Clinical Research Facility for over 10 years. Trained many laboratory and clinical investigators, including over 10 successfully completed MDs and over 30 PhDs.

Please provide information for each researcher (other than PI)

1.2.1 Qualifications of researcher: *Liam Flynn*

State relevant qualifications and training (including pharmacology).

MB ChB

1.2.2 Research experience of researcher:

State FIH/ Phase I or relevant experience:

Experience in Phase I trials: No No. of trials and role:

Experience in FIH trials: No No. of trials and role:

Experience in studies involving dose escalation: No

Please provide details below of this Phase I/FIH/dose escalation experience, indicating the publications that have resulted from this work:

1.2.3 Supervision of researcher

Name of supervisor for the researcher(s) if not the PI: Peter Andrews

What supervision will be provided?: *Will work as part of the team on this study and will be supervised by senior team members.*

1.2.4Training status of researcher

GCP trained	Yes	Date of training: GCP 20.08.13	Training provider: WTCRF
ALS trained	Yes	Date of training: 02.05.13	Training provider: Aberdeen Royal Infirmary

Please provide details of other training/ skills essential for this study: ALS instructor. ATLS provider. Previous anaesthetic and ITU experience.

Section 1 - Committee use only:

1. Is the PI competent to conduct early phase trial?: Yes/ No / With supervision
2. Is the proposed supervisor of the PI acceptable (if required)?: Yes/ No/ NA
3. Is the named supervisor of the researcher(s) acceptable (if not the PI)? Yes/ No/ NA
4. Is the degree of supervision of the researcher(s) conducting the study acceptable? Yes/ No/ NA
5. Training needs identified for PI and researcher appropriately? Yes/ No

Comments/ Recommendations:

2. Pre-clinical development data (please complete for each IMP)

Provide key papers that detail the pre-clinical data of the study IMP(s)

Role of calcitonin gene-related peptide in cerebral vasospasm, and as a therapeutic approach to subarachnoid hemorrhage. Kokkoris S, Andrews P, Webb DJ. Front Endocrinol (Lausanne). 2012;3:135. doi: 10.3389/fendo.2012.00135.

Product overview Unigene Laboratories, Inc.

Clinical trial overview NCT00687947;FHM-CGRP-MA-2008

International patent application PCT/GB88/00877

2.1 Is there evidence of previous exposure of humans to compounds with related modes of action?: yes
Provide details:

LANCET 1990. Intravenous Administration. In 15patients (ll women, 4 men; mean age 48 years [range 22-70]), 0.035 microg/min CGRP was administered intravenously and the infusion rate was doubled every 10 min until either a clinical response was obtained or a max dose of 1.15 (stated in paper but is probably 1.12) micrograms/min was reached at 1 hour.

Systolic blood pressure decreased by 35.7 mm Hg during CGRP infusion (p < 0.05 compared with placebo; 95% CI

9.2, 75.8 mm Hg), and mean / diastolic blood pressure decreased by 21.3 mm Hg (p < 0.01 compared with placebo; 95% CI 9.8, 46.6 mmHg).

5 patients did not improve on either treatment, 1 improved on both, 8 improved on CGRP but not on placebo, and 1 improved on placebo but not on CGRP-thus of the 9 patients who showed a treatment preference, 8 (88.9%) favoured CGRP (p < 0.05; 95% CI 51.8, 99.9%).

LANCET 1992. Intravenous Administration. 62 patients were randomly assigned an infusion of 0.6microg/min CGRP for 4 h, then up to a maximum of 10 days, and 55 patients standard best management (controls). 41 patients discontinued treatment because of adverse events (19 patients), lack of improvement at 4 h (17) or later (4), or patient's request (1 patient). Hypotension was a common side-effect of the CGRP intravenous infusion. Facial flushing or raised ICP were not recorded.

TABLE III—OUTCOME AT 3 MO

—	No (%) of patients	
	CGRP	Control
Good outcome	41 *(66%)*	33 *(60%)*
Good recovery	27 *(43%)*	26 *(47%)*
Moderately disabled	14 *(23%)*	7 *(13%)*
Poor outcome	21 *(34%)*	22 *(40%)*
Severely disabled	9 *(14%)*	11 *(20%)*
Vegetative	1 *(2%)*	0
Dead	11 *(18%)*	11 *(20%)*

2.2 Is there evidence from animal models for potential risk of serious pharmacologically mediated toxicity?: no
Provide details: Relevant Animal Model data

Neurosurgery Vol 39(5), November 1996, pp 984-990. Cerebral Ventricular Administration. Microspheres containing CGRP (human [alpha]-CGRP, molecular weight 3790; Bachem, Feinchemikalien, Bubendorf, Switzerland) were prepared by using an in water drying method, through a water/oil/water emulsion.

Ten male cynomolgus monkeys (Macaca fascicularis), each weighing between 4 and 8 kg. The animals were divided into one of three groups, including CGRP (n = 5) and placebo (n = 3) groups, which were treated with the CGRP and placebo tablets, respectively, after SAH. The remaining two animals underwent SAH but received no treatment with tablets (SAH group). Each CGRP tablet used in this experiment contained 400 micrograms of CGRP.

Pharmacodynamic & Pharmacokinetic Data

In Hartmann's solution, there was no CGRP release from the CGRP tablet for the initial 6 days. During the next 4 days, there was a significant release of CGRP; approximately 4 micrograms of CGRP were released each day.

The RIA analysis of the CSF for CGRP concentration was performed before SAH and on Days 7 and 14 for each animal. The non-CGRP group showed no detectable CGRP in the CSF at any time point. In the CGRP group, 6.5 ± 5.4 nmol/L CGRP (mean ± standard deviation, n = 5) was detected in the CSF on Day 7. It was otherwise undetectable.

	Changes in Diameter (%)[a]							
	Day 7				Day 14			
	IC[b]	MC	AC	Total[c]	IC	MC	AC	Total
SAH group (n = 3)	63.7 ± 3.5	53.8 ± 7.1	46.7 ± 10.6	55.8 ± 9.2	87.4 ± 3.6	91.3 ± 16.2	81.4 ± 20.4	87.3 ± 12.5
Placebo group (n = 2)	41.6 ± 19.7	74.9 ± 11.6	56.0 ± 20.0	57.5 ± 20.2	74.9 ± 27.1	78.1 ± 28.9	99.7 ± 17.5	84.2 ± 22.8
Non-CGRP group[d] (n = 5)	54.8 ± 15.8	62.3 ± 13.8	51.3 ± 14.0	56.5 ± 20.2	82.4 ± 15.4	86.0 ± 19.8	90.5 ± 18.8	86.0 ± 16.9
CGRP group (n = 5)	56.6 ± 14.7	81.7 ± 11.3[e]	81.1 ± 17.4[f]	73.1 ± 18.2[g]	88.7 ± 23.5	90.6 ± 13.5	84.0 ± 6.6	87.8 ± 15.2

[a] Values are mean ± standard deviation, expressed as percentage of vessel diameter on Day 0.
[b] IC, internal carotid artery; MC, middle cerebral artery; AC, anterior cerebral arery; CGRP, calcitonin gene-related peptide.
[c] Total represents the mean of IC, MC, and AC values.
[d] SAH and placebo groups are pooled into the non-CGRP group.
[e] $P < 0.05$ versus non-CGRP group.
[f] $P < 0.03$ versus non-CGRP group.
[g] $P < 0.02$ versus non-CGRP group.

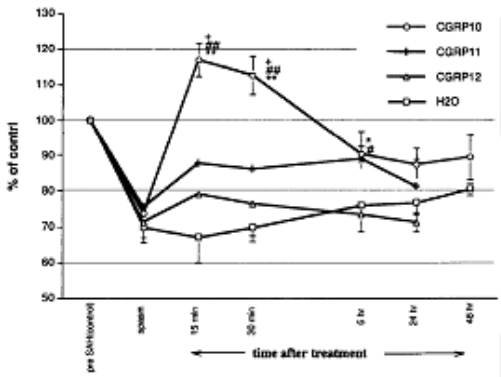
Safety Pharmacology

No difference was found between the non-CGRP and CGRP groups in arterial blood gas parameters or in arterial blood pressures. The only significant factor was a difference in body weight on Day 14.
In the CGRP group, vasospasm was significantly ameliorated at the middle cerebral artery; at the anterior cerebral artery, and on average (81.7, 81.1, and 75.7%, $P < 0.05$, 0.03, and 0.02, respectively).

Surgical Neurology, 1996, 46; 263-71. **CSF Administration.** 40 Japanese white rabbits weighing 2.5-3.5 kg. Day 4 after SAH and following confirmation of basilar artery constriction, CGRP or sterile water was injected into the cisterna magna. 10^{-10}, 10^{-11} and 10^{-12} mol/kg CGRP solution.
Spontaneous movements of the animals were less after SAH, but neither consciousness disturbance nor motor deficit was observed. One day after drug or water injection, normal gait was observed on stimulation, while spontaneous movement almost disappeared in all groups.
Toxiciology. All animals tended to hyperventilate for up to 4 hours after injection of CGRP and placebo (sterile water). The blood pH was high and pCO_2 was low within 4 hours after drug injection. No difference, however, was seen among the groups at each time point. Arterial blood pressure was stable at measured time points after injection of each drug and sterile water.

Pharmacodynamic Data

Fifteen minutes after 10^{-10} mol/kg CGRP injection, the basilar artery dilated from 73.6% +/- 2.1% to 117.1% +/- 4.8% ($P< 0.001$). At 6 hours after 10^{-10} mol/kg CGRP injection, the basilar artery was still dilated to 90.3% +/- 2.1% ($P < 0.05$). In the 10^{-11} mol/kg CGRP group, the basilar artery was dilated to 87.7% 2 8.2% ($p < 0.05$) 15 minutes after the injection.



2.3 What animal species have been studied? (see also 3.4) Rodents, rabbits and primates.

2.4 Provide evidence of a risk analysis of the pre-clinical data for the IMP(s) including:

Alpha CGRP has been given intravenously to humans in phase 2 & 3 studies. The doses required to cause vasodilation caused low blood pressure. Alpha CGRP has been given into cerebral spinal fluid around the brain of primates, rabbits and rats. Primate model data are thought to best reflect human biology & pathophysiology; including the blood clot around subarachnoid blood vessels.

2.4.1 Identification of the on-target and off-target areas of the IMP(s).

Hypotension (mean blood pressure<80 mmHg), facial flushing, hyperventilation ($PaCO_2$ measurement) or pain. Assessed by objective physiological measurement, self reporting and clinical observation (by research and clinical team).

There are no data suggesting any off target toxicity in any species studied, including humans. Presumably because of receptor specificity. There are no human data on cerebral spinal fluid administration. However, lack of identifiable off-target effects in rodent and primate models when given in the CNS as well as lack of off-target effects when given intravenously in humans makes the likelihood of human off-target effects very low when given intrathecally. Alpha-CGRP exists within the CSF in humans naturally and has a high molecular weight with poor penetration through the Blood-Brain-Barrier. We do not expect to see off-target effects in this study.

Linda M. McLatchie†, Neil J. Fraser*, Martin J. Main*, Alan Wise*, Jason Brown*, Nicola Thompson*, Roberto Solari*, Melanie G. Lee* & Steven M. Foord*. Nature , (393) 28 May 1998, 333-339.*

2.4.2 The adverse events associated with the on-target and off-target areas.

The additional tests to be undertaken to monitor for possible effects on other target areas: *In the Wellcome Trust Clinical Research Facility the patient will have continuous monitoring of physiology (blood pressure, heart rate, temperature, urine output, $PaCO_2$, SpO_2) and will have the continuous presence of a trained ICU doctor and nurse.*

Section 2 – Committee use only

1. Is the risk analysis of the pre-clinical development data for the IMP(s) acceptable in terms of previous human exposure and animal models? Yes/No

2. Have on-target and off-target areas been accurately identified and the possible associated adverse events? Yes/No

Comments/Recommendations

3. Investigational Medicinal Product (please complete for *each IMP*)

Provide the full information available about the nature of the agent to be tested. *(extract below is taken from the review by Kokkoris, Andrews & Webb. Reference numbering is as the review)*

Calcitonin Gene-Related Peptide (CGRP), an endogenous 37-aminoacid peptide (3790 D) is normally synthesised in the body through alternative splicing of the Calcitonin gene. There are two known Calcitonin genes located in the short arm of chromosome 11 in humans. Alternative processing of the first Calcitonin gene results in synthesis of Calcitonin and CGRP. The transcription of the second Calcitonin gene does not seem to be differential, as only prepro-CGRP II mRNA and not preprocalcitonin II mRNA is detectable. This second Calcitonin gene also synthesises CGRP, which differs from that synthesised by the first Calcitonin gene by three aminoacids. These two CGRPs are called α- and β-CGRP (9).

CGRP is a vasodilator neuropeptide that is expressed in a subgroup of small neurons in the dorsal root ganglion, trigeminal and vagal ganglia. CGRP is released antidromically in the periphery, eliciting vasodilation as a component of neurogenic inflammation. CGRP-containing pathways from the parabrachial nuclear complex and posterior thalamus convey nociceptive and visceral sensation to the amygdala and the insular cortex(10).

It is the potent vasodilating effect of α-$CGRP_{h(human)}$ on cerebral arterial vessel narrowing in patients after aSAH that is of interest in this study. Importantly, this effect is independent of endothelial cells of blood vessels and

their derived factors (11) and deserves special consideration because endothelial cells are reported to be functionally and morphologically damaged in DCI after aSAH (12).

Results of any pilot studies

Previous studies have shown the importance of α-CGRP in cerebral arterial vessel narrowing after aSAH. Investigations in experimental models in which CGRP was administered intrathecally, as well as in vitro and in vivo studies, have all proven the potent dilatory effects of α-CGRP in cerebral vessel narrowing after aSAH (13,14). Given intrathecally in rabbits, 10^{-10} mol/kg resulted in 6hours of >110% vessel dilation in a model of aSAH cerebral arterial vessel narrowing (14,15).

Clinical trials of intravenous administration of α-CGRP (n=62 patients) with cerebral arterial vessel narrowing after aSAH showed a potential role of α-CGRP in dilating spastic cerebral vessels compared with best standard care (n=55). Outcome, measured on the Glasgow outcome scale, at 3 months was good in 66% of those treated with CGRP and 60% in the controls; the relative risk of a poor outcome in α-CGRP-treated patients was 0.88 (95% confidence interval 0.60 to 1.28). 66% of the α-CGRP group did not complete treatment because of adverse events (19 patients), lack of improvement at 4h (17 patients) or later (4 patients), or patient's request (1 patient). The trials were stopped early because of systemic side effects such as hypotension (13%), tachycardia and facial flushing (16,17).

The European CGRP investigators concluded "Subarachnoid instillation may be more rational, since the peptide is normally found on the adventitial rather than the endothelial side of the cerebral vessel wall. The action of α-CGRP on the vessel wall is direct, by activation of adenylate cyclase, and does not require intact endothelial cells or the release of endothelium-derived relaxing factor, which is inhibited after aSAH"(16).

3.1 Mode of action:

Novel molecular structure: Yes (see below) Biological cascade/ cytokine release: No

Multiple signalling pathways No

Calcitonin gene-related peptide receptor activation is known to involve several crucial elements, in common with other GPCRs, such as the presence of a proline "kink" in transmembrane helix (TM)6 (Conner et al., 2005), and a putative'DRY" motif equivalent (Conner et al., 2007), similar to family A GPCRs. There is also evidence suggesting stabilization of the CLR interaction with G "alpha" s (Gas) by another 17kDa intracellular membrane protein, called RCP (Evans et al., 2000).

The existence of two receptors, CGRP1 and CGRP2, was originally proposed in the late 1980s, with the CGRP1 receptor being the predominant mediator of cardiovascular effects. This receptor classification was developed as a consequence of pharmacological studies carried out with different agonists and antagonists in a range of tissue preparations, especially the positive inotropic effect in the guinea pig or rat atrium for determination of CGRP1 receptor activity, and the inhibition of electrically evoked twitch responses in the rat vas deferens for determination of CGRP2 receptor activity (Dennis et al., 1989, 1990; Dumont et al., 1997). In general, receptors that can be antagonized by the 30-amino acid fragment of CGRP, CGRP8−37, with an approximate pA2 value of 7.0 are designated as CGRP1 receptors,while those that CGRP8−37 block with a pA2 of 6.0 or less are classified as CGRP2 receptors (Quirion et al., 1992; Poyner, 1995). However, it is questionable whether the CGRP2 receptor is a single receptor type or whether it is, in fact, explained by multiple molecular entities (Hay, 2007). In contrast, CGRP1 is a well-defined receptor type consisting of CLR and RAMP1.

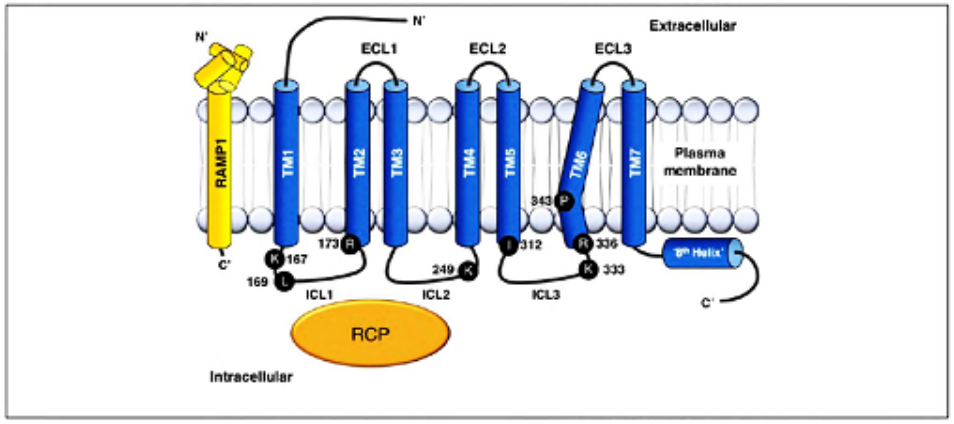


FIGURE 2 | Structure of CGRP receptor. CGRP receptor components and important residues for receptor signaling and internalization. The CGRP receptor is formed by CLR (blue), RAMP1 (yellow), and RCP (orange). Functionally important residues are shown as single letter abbreviations. CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; RAMP, receptor activity-modifying protein; RCP, receptor component protein; C', C-terminal; EC, extracellular loop; ICL, intracellular loop; N', N-terminal; TM, transmembrane. From Walker et al. (2010), with permission.

From Kokkoris, Andrews, Webb, 2012.

3.2 Nature of target:

Provide available knowledge on the nature of the target and variation between individuals

SIGNAL TRANSDUCTION OF CGRP RECEPTOR

Several mechanisms involved in CGRP-mediated vasorelaxation have been identified. These mechanisms include either NO-dependent endothelium-dependent mechanisms or cAMP-mediated endothelium-independent pathways. The most common pathway is NO- and endothelium-independent. Activation of the CGRP receptor is generally accepted to result in Gas-mediated activation of adenylate cyclase, with a subsequent increase in cAMP and activation of protein kinase A (PKA). In the absence of endothelium, CGRP is able to cause relaxation, suggesting it must act directly on the smooth muscle cells to stimulate adenylate cyclase (Edvinsson et al., 1985, 1998; Crossman et al., 1990). The resulting rise in cAMP then activates PKA, which phosphorylates and opens up ATP-sensitive K+ channels, thus leading to relaxation (Figure 3A; Nelson et al., 1990). Endothelium-independent relaxation to CGRP occurs in the majority of tissues examined to date. Exceptions include the rat aorta, where the relaxation to CGRP occurs only in the presence of an intact endothelium and is attenuated by inhibitors of NO synthase, implying an NO-dependent mechanism (Brain et al., 1985; Gray and Marshall, 1992a,b). A significant increase in both cAMP and cGMP occurs and is also dependent on the presence of endothelium (Gray and Marshall, 1992a). This implicates the release of NO from the endothelium, which then relaxes the smooth muscle cells through activation of guanylate cyclase and accumulation of cGMP. Moreover, it has been shown that cAMP is able to stimulate eNOS activity, leading to increased synthesis and release of NO (Ferro et al., 1999; Queen et al., 2000). The activation of eNOS via cAMP is probably mediated via PKA, as a study demonstrated that various protein kinases can phosphorylate and activate eNOS (Butt et al., 2000). It is a possibility that CGRP causes an increase in cAMP in endothelial cells, which leads to PKA activation. PKA, in turn, activates eNOS, which results in NO release, and thus relaxation of smooth muscle (Figure 3A). There is some evidence for Gai/o signaling by the CGRP receptor, which is traditionally identified by sensitivity to pertussis toxin (PTX; Figure 3B). The CGRP-mediated stimulation of Ca2+ transients in rat nodose neurons and the activation of c-Jun N-terminal kinase (JNK) in SK-N-MC cells (which express endogenous CGRP receptors) both displayed PTX sensitivity (Wiley et al., 1992; Disa et al., 2000).

The CGRP receptor may also be able to stimulate intracellular activity through a different G protein. Aiyar et al.

(1999) reported that CGRP was able to activate phospholipase C (PLC) in HEK293 cells, leading to an increase in intracellular Ca2+ via inositol trisphosphate (IP3) activity. This increase in Ca2+ occurred concurrently with the stimulation of adenylyl cyclase and accumulation of cAMP. Activation of PLC is considered to occur through $G_{q/11\alpha}$, rather than through $G_{\alpha s}$, suggesting that the activated CGRP receptor is able to interact with both types of G protein. If this mechanism is present in endothelial cells, it provides an alternative explanation for CGRP activation of eNOS (which is traditionally considered to be dependent on Ca2+/calmodulin for activation), independently of cAMP accumulation. The possibility that CGRP receptors may be coupled to phosphatidylinositol turnover is supported by another study that found this secondary messenger pathway in skeletal muscle (Laufer and Changeux,1989; Figure 3C). Recently, Meens et al. (2012) reported that activated CGRP receptors induce cyclic nucleotide-independent relaxation of vascular smooth muscle cells in mesenteric resistance arteries and terminate arterial effects of ET-1 via $G_{\beta\gamma}$. More specifically, CGRP receptor activation causes cAMP production but the relaxation of rat mesenteric resistance arteries induced by activation of this receptor involves $G_{\beta\gamma}$ and is not dependent on cAMP (Figure 3D). Another study by Meens et al. (2010) discovered that CGRP released from peri-arterial sensory motor nerves terminates longlasting vasoconstrictor effects of ET-1 by promoting dissociation of ET-1/ETA-receptor complexes.

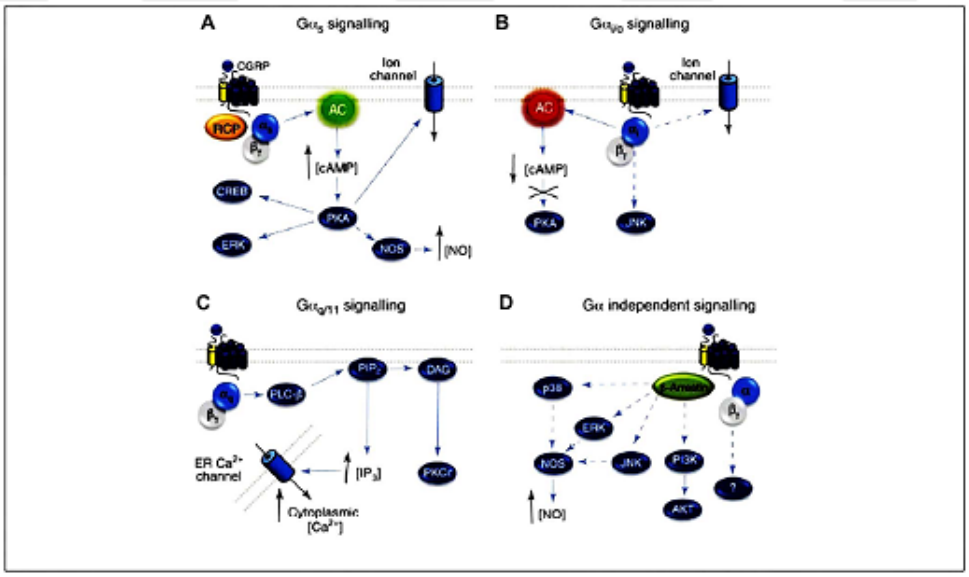


FIGURE 3 | CGRP receptor-mediated intracellular signaling. (A) $G\alpha_s$ signaling increases AC (green) activity, elevating intracellular cAMP, activating PKA and subsequently many potential downstream effectors. **(B)** The CGRP receptor might also couple to $G\alpha_{i/o}$, reducing AC (red) activity, decreasing intracellular cAMP and reducing PKA activity. **(C)** CGRP signaling via $G\alpha_q$ activates PLC-b, which cleaves PIP_2 into IP_3 and DAG, resulting in elevated intracellular Ca^{2+} and PKC activation. **(D)** The CGRP receptor might also utilize Ga-independent signaling, and $G_{\beta\gamma}$- or b-arrestin-mediated signaling pathways. Arrows represent reported pathways; broken arrows represent potential or inferred pathways. CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; Ga, α subunit of the G protein; NO, nitric oxide; NOS, nitric oxide synthase; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; RCP, receptor component protein; AC, adenylate cyclase; ER, endoplasmic reticulum; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol. From Walker et al. (2010), with permission.

The CGRP receptor can also potentially activate other downstream signaling molecules, such as PKC and mitogen-activated protein kinase (MAPK) cascades, such as p38, JNK, and extracellular receptor activated kinase 1/2 (ERK ½; Walker et al., 2010). CGRP receptor signaling is regulated by desensitization, internalization, and trafficking, which, as with other GPCRs, involves GPCR kinases (GRK), β-arrestin, and clathrin- and dynamin dependent endocytosis (Walker et al., 2010). Padilla et al. (2007) proposed a mechanism by which endosomal endothelin converting enzyme-1 (ECE-1) degrades CGRP in endosomes to disrupt the peptide/receptor/β-arrestin complex, freeing internalized receptors from β–arrestins and promoting recycling and resensitization, resulting in long-lasting

vascular relaxing response to CGRP.

3.3 Relevance of animal species and models:
Justify the use of the model used, ensuring evidence of comparison of available animal species to humans (in support of 2.3)

The best data come from work in monkeys, where αCGRP was administered into the Cistern Magna. Neurosurgery 39(5), November 1996, pp 984-990.

In this study a slow release tablet of 400micrograms was placed in the Cisterna Magna, releasing 4micrograms per day. This achieved maximum CSF concentrations of 7.7-10.5nanomoles/ litre measured by aspiration of CSF from the same site. 0.4 micrograms is roughly equal to 0.1nM of human recombinant alpha CGRP. This is not ICV administration but is close to the fourth ventricle. Blood clot was placed around the middle and anterior cerebral arteries in this study, requiring CSF circulation for efficacy. A reduction in vessel narrowing was shown.

The volume of CSF in the monkey (4-8kg) is about 10ml compared with 150ml in humans (25mls in each ventricle). The maximum concentration at the time of bolus administration of the starting dose will be 0.01nm (10^{-11}mol) into about 25 mls of ventricle CSF. This will give a peak concentration of 0.4 nanomoles/ litre (20 times less than the laboratory study above).

3.4 CT(EAG) review:

Does this study have risk factors requiring review by the Expert Advisory Group (EAG) to the Committee on Human Medicines (CHM)? No

Section 3 Committee use only

1. Is there sufficient information provided on the nature of the agent(s) including mode of action, target and species model and justification for use in humans? Yes/ No

2. Does the Committee assess this study as requiring review by the CT(EAG)?: Yes/ No

Comments/ Recommendations

4. Administration of doses (provide information for each IMP)

Ensure that the trial design and protocol are clearly described and flow diagrams accompany the submission.

4.1 What is the route of administration? *Intrathecal (i.e. into lumbar CSF) and intravenous infusion.*

4.2 What is the rate of administration?

Intrathecal dosing will follow the NHS Lothian intrathecal protocol. Briefly; using an aseptic technique and full barrier precautions, 2mls of dead space CSF will be aspirated and discarded, 1ml of CSF will be aspirated and stored for pharmacokinetic analysis. 2mls of vehicle and alpha-CGRP will be injected over 30 seconds. Thereafter, 2ml of sterile 0.9% sodium chloride will be injected.

Intravenous infusion will be continuous; 0.035 micrograms/min CGRP (17.5 nanograms/ml concentration) will be administered intravenously and the infusion rate doubled every 10 minutes until a maximum dose of 1.12 micrograms/min is reached at 1 hour. The infusion at this rate will be continued for a further 20 minutes.

4.3 What is the estimation of first dose? Calculation of Intrathecal Administration dosing schedule
The molecular weight is 3790. Therefore 0.4micrograms of alpha-CGRPh ~ 0.1 nanomoles.
Macaca fascicularis have CSF volume 3ml/kg and weigh 4-8kg; CSF volume and body weight are about 10% of human physiology.
Study dosing was about 4 micrograms/ day (1 nanomole) to give very low levels, measurable at day 7 of 6+/-5 nanomoles/litre
Previous studies have suggested that a minimum CSF level of 23 nanomoles/ litre is the minimum effect level for aCGRP in CSF to cause vasodilation.
To achieve the minimum CSF concentration of 23 nanomoles/litre[20], predicted to have a biological effect, requires 3.45 nanomoles, assuming complete CSF distribution.
10^{-13} mol /kg and 10^{-12} mol /kg and below the expected "no effect" dosing level of 10^{-12} mol /kg aCGRP.

4.4 What method was used to calculate the dose? *EMEA CHMP guidelines, 2007 were followed.*

The MABEL method was used. There is no data on intrathecal or CNS alpha-CGRP dose administration in man and so we have followed EMEA CHMP guidelines and used the MABEL method to determine the expected 'no effect' dose in humans. We will measure the CSF concentration of CGRP in the intravenous group to inform us of the first below 'no effect' dose in the intrathecal group. We will not proceed to intrathecal administration until we have the results of CSF concentrations of alpha-CGRP following IV administration.

4.5 Who will be responsible for checking the calculations at dosing time? *The PI, research pharmacist and the researcher*

4.6 What is the expected total exposure of the associated drug and the anticipated plasma concentrations? The anticipated plasma concentrations in the intrathecal arm of the study are expected to be undetectable. The intravenous infusion arm will receive 10^3 lower dosage than the previous studies that measured plasma levels.

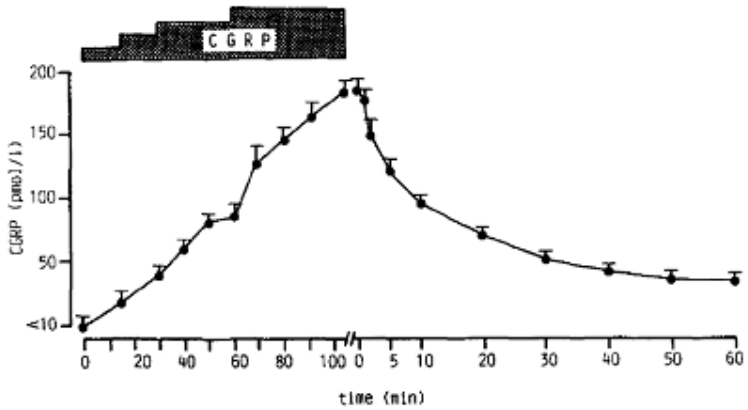


Fig. 3. Plasma levels of calcitonin gene-related peptide in 6 healthy subjects during i.v. infusion of CGRP at a rate of 0.32, 0.64, 1.28 and 2.56 pmol/kg per min. Data points represent means and S.E.M. of incremental post-infusion values.

The lowest and highest calculated rate of infusion given i.v. was 0.32 pmol/kg per rain and 2.56 pmol/kg per rain, respectively. Analysis of the infusate showed that the radioimmunoassay-measured rates of infusion were 0.24 + 0.2 pmol/kg per min and 1.93 +/- 0.14 pmol/kg per min, respectively. The achieved plasma concentration and disappearance of immunoreactive CGRP from plasma is shown in Fig. 3. A steady state concentration was not reached but the partial infusion data estimation of steady state concentration yielded a value of 227 4- 13 pmol/1 compared to the measured 184 +/- 9 pmol/l just before stopping the infusion. There was a two-phase or bi-exponential decay curve. - Regulatory Peptides, 10 (1985) 189-197.

4.7 How do these values compare against the exposure and achieved concentrations in the animal models previously studied? These values will be significantly lower; the starting dose is 1000th that given to primate models and escalates to a dose that may achieve a therapeutic level if animal data are translated to clinical trials. There are no data regarding the difference between human and animal pharmacokinetics regarding CSF/CNS administration of alpha-CGRP as the peptide has only been given intravenously in humans previously.

4.8 What is the dose escalation strategy? A modified Fibonacci sequence - successive cohorts of patients are treated with increasing doses of the drug.

Intravenous Dosing (arm for pharmacokinetic data)

This will follow the same dosing schedule used in the Lancet 1990 study[24].

0.035 micrograms/min CGRP will be administered intravenously and the infusion rate doubled every 10 minutes until a max dose of 1.12 micrograms/min is reached at 1 hour.

Intrathecal

Baseline surgical data including respiratory rate, blood pressure, heart rate, temperature and SpO_2 will be measured and used in the absence of a formal control. These measurements will be made again following initial administration of spinal anaesthesia.

A. Below expected "no effect" dosing level: 10^{-12} mol /kg aCGRP (n=6)

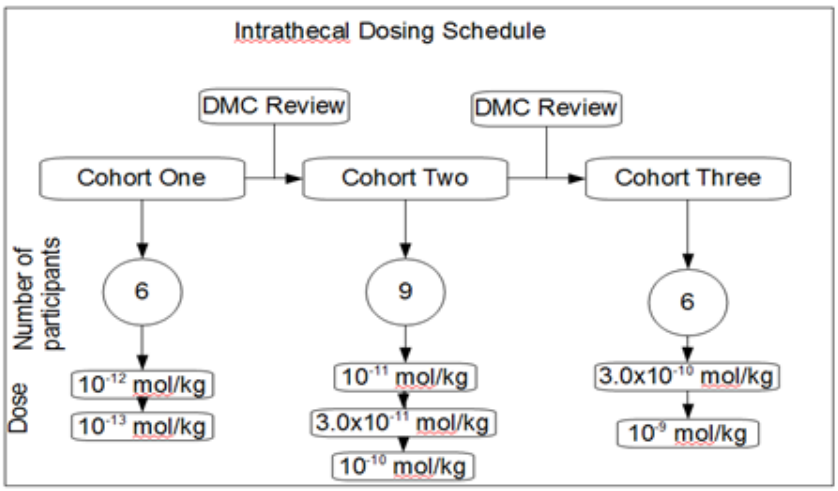
Three participants will receive one active dose of 10^{-13} mol/kg of αCGRP. Three separate participants will receive one active dose of 10^{-12} mol/kg of αCGRP.

B. At expected "no effect" dosing level 10^{-12} mol /kg aCGRP (n=9)

Nine participants will receive one active dose of either; 10^{-11} mol /kg, $3.0x10^{-11}$ mol /kg or 10^{-10} mol /kg of the intervention. Three of the nine participants will be assigned to each dose.

C. Above expected "no effect" dosing level 10^{-12} mol/kg aCGRP (n=6)

Three participants will receive one active dose of $3.0x10^{-10}$ mol /kg αCGRP and three separate participants will receive one dose of 10^{-9} mol /kg of the intervention.



Toxicity monitoring

Key adverse events that will be monitored:

Serious Adverse events (SAEs):

1. Haemodynamic instability including: systolic blood pressure decrease >25 mmHg; a mean blood pressure decrease >20 mmHg; heart rate increase to >145 bpm.
   A blood pressure SAE will be recorded after 3 confirming non-invasive blood pressure measurements with change assessed from baseline (time zero) Or any symptomatic hypotension (a reduction in blood pressure associated with symptoms including lightheadness). A heart rate SAE will be a heart rate greater than 145 bpm, sustained for more than 5 minutes, Or any symptomatic tachycardia (an increase in heart rate associated with symptoms including chest tightness).
2. Reduction in Glasgow Coma Scale >2 points or 1 motor point; change in pupil response or size
3. Clinical evidence of a seizure
4. Death or serious adverse event - the timing in relation to peptide delivery and a root cause analysis will be performed (not expected as the compound has been given at similar doses intravenously)

Adverse Events (AEs):

1. Spontaneous hyperventilation; $PaCO_2$ < 4.0 kPa, monitored using a TOSCA device (Radiometer Medical, Åkandevej 21, DK-2700 Brønshøj, Denmark). This is possible after cerebral ventricular system injection[21], but unlikely after lumbar CSF injection but will be assessed for completeness.
2. Facial flushing. If observed, an assessment of the involvement 1-4 facial quadrants will be made and an assessment of intensity of rubor made (mild, moderate, intense). Volunteers will be asked about tolerability of the flushing, on a simple yes/ no scale.
3. Any other adverse event (self-reported or otherwise) will also be recorded such as headache. For headache a standard visual analogue score will be used.

Any AE that is considered intolerable will be treated as an SAE.

Stopping Rules and Decision Making

The study will be stopped if 3 participants develop cardiovascular SAEs.

Recruitment will be suspended after any SAE and further dosing in that volunteer stopped. A DMC review will be undertaken to decide if further doses can be given to subsequent volunteers. Escalation to a higher dosing level will only occur after a DMC review.

The study will be stopped if *cardiovascular* SAEs are observed in three research subjects and associated with intrathecal administration. The study will not be stopped following a single cardiovascular SAE after intrathecal administration as this may be unrelated to the peptide. However, the DMC will review every SAE and the study will only continue to recruit once the DMC are satisfied. The study will be stopped following one cardiorespiratory arrest, intracranial haemorrhage or death.

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Precautions to apply between doses within cohorts

A doctor (Flynn or Andrews) will be available in the WTCRF throughout the study. Critical care staff will be informed of the study and the possible SAE/AEs associated with the peptide. The on call Intensive Care Consultant will be made aware on the day of the study. A WTCRF nurse will also monitor the patient throughout. Full critical care organ support, neuroradiology and neurosurgery advice and intervention will be available if required. Rapid access to the treatment allocation codes will be available if needed.

Precautions to apply between cohorts

Pre-specified SAE and AE criteria will be used to identify and mitigate the risk of progressing to a subsequent cohort. Administration in the next cohort will not occur before participants in the previous cohort have been treated and data/results from those participants are reviewed in accordance with the protocol and signed off by the Data Monitoring Committee (DMC). The DMC (1 statistician and 2 clinicians) will decide how many patients should be studied before changing dosing level. Decisions will be based upon data from 3 or more patient cohorts.

4.9 What is the justification for the strategy adopted? CGRP receptors of the perivascular nerve endings are located on the abluminal side (adventitia-media) of the arterial walls. The abluminal sides of the cerebral arteries lie in the subarachnoid space and are accessible through the cerebral ventricle system (CVS) or lumbar CSF (intrathecal) administration. Moreover, CGRP has a high molecular weight with poor penetration through the Blood-Brain-Barrier (BBB). Systemic administration of alpha-CGRPh to treat cerebral vasospasm possibly did not result in adequate therapeutic concentrations in the cerebral subarachnoid space, but CSF concentration was not measured. And as higher dosing was required, untoward systemic side effects occurred. Administration in CSF has theoretical pharmacokinetic benefits such as a reduction in the volume of distribution (Vd), avoidance of distribution to other organs/ tissues and circumvents the first pass effect, which taken together minimises the effective dose.

Intrathecal administration of drugs is safer than CVS, but results in greater variation of drug concentration in the cerebral subarachnoid space. Peak concentrations are achieved within minutes but there are few data suggesting that rarely this may occur as late as three to four hours after administration and requiring a higher volume of study agent.

With intravenous administration, AEs and SAEs are expected to be evident in minutes up to one hour and will be easily assessable by the DMC. After intrathecal administration AEs and SAEs are expected to be evident in minutes up to one hour, but may in take as long as 3-4 hours. Research subjects will be observed and continuously monitored for 6 hours, or longer if an AE or SAE is reported. If an AE or SAE develops at the end of the protocol, admission to a critical care bed will requested so that further monitoring can continue. Administration directly into CSF was associated with an effect on vessel diameter within 15 minutes (Neurosurgery Vol 39(5), November 1996, pp 984-990).

4.10 Who will administer the first dose? (eg PI/ researcher) The CI will administer the first dose and will be present during and after administration.

4.11 Which members of the study team will be present at the administration of first dose? Andrews & Flynn. Andrews will be present during the initial administration of αCGRP. Dr Flynn will be present at all times to take samples and observations from the subjects. Prof. Andrews will be in the Western General Hospital throughout the study but not in the same department as the research subjects.

4.12 What is the period of observation of first subject prior to subsequent doses? (Justify this decision) The trial design is a one dose strategy and patients will be monitored for six hours in the WTCRF following the first dose and for 24hrs on the ward after the study is complete. We do not expect any toxicity at the dosage tested in the first subjects. Subsequent participants will be recruited 24hrs after the first dose.

4.13 What is the period of observation between administration of IMP to subsequent subjects within the cohort? (Justify this decision). Only one subject will studied per day. The period of observation in the WTCRF will be 6 hours after administration of the final dosage increment and the subject will be followed up for 24 hours on the ward after the study is complete. Thereafter the next subject will be tested.

4.14 What medical cover will be in place on dosing days?
Include information on the process for the first subject and subsequent subjects.
Provide information on who will be present, the duration of cover and the physical locality of the researcher for first dose and subsequent doses.
Flynn will be present during and after administration, Andrews will be immediately available during and after dosing for up to 6 hours after the dose. The research subject will be in the WTCRF which is located in close proximity to the Intensive Care Unit at the Western General Hospital. After 6 hours have elapsed, following the administration of CGRP, the patient will be transferred to their ward.

Section 4 Committee Use only

1. Trial design acceptable? Yes/ No

2. Route and rate of administration acceptable (each IMP)? Yes/ No

3. Estimation of first dose and method of calculation used acceptable? Yes/No

4. Dosing calculation confirmed and provided by:

5. Expected exposure and plasma concentrations acceptable? Yes/ No

6. Comparison with exposure and achieved concentrations in animal model acceptable? Yes/ No

7. Dose escalation strategy acceptable? Yes/ No

8. Period of observation of first subject prior to subsequent doses acceptable? Yes/No

9. Interval between initial and subsequent subjects acceptable? Yes/ No

10. Level of subject supervision acceptable for first and subsequent doses? Yes/ No

Comments/ Recommendations

5. Statistics

Note: Please ensure that appropriate statistical advice has been sought. The Committee will review the statistical aspects of the study from the protocol and ethics form submitted. Provide any additional, relevant information below.
Analysis
Dose response curves will be produced at the conclusion of each cohort showing the levels of CGRP found in both blood and CSF for all subjects. Descriptive analysis will be presented including any AE, SAE or withdrawals where available this AE/ SAE/ withdrawal information will be presented in relation to the timing of doses.

Section 5 Committee Use only

1. Is the statistical analysis appropriate for the trial design? Yes/ No

2. Is the sample size appropriate? Yes/ No

Comments/ Recommendations

6. Adverse reactions (account for each IMP):

61 Describe the measures in place to monitor key on-target and off-target areas as identified in 2.4.1, including the additional tests to be undertaken to monitor for possible off-target effects (eg bp, glucose, liver toxicity etc).
Continuous monitoring of physiology during and after administration of the peptide. Hospital admission thereafter and 24 hour study team follow up. All AEs and SAEs will be recorded from the time a participant attends the WTCRF to take part in the study until 24 hours later (including telephone follow up after hospital discharge). A medically qualified member of the research team will ask about the occurrence of AEs/ SAEs during the evening follow-up visit the same day and on a surveillance call the next day. Open-ended and non-leading verbal questioning of the participant will be used to enquire about AE/SAE occurrence.
AEs and SAEs may also be identified by support departments e.g. laboratories. The laboratory may fax the results directly to ACCORD. Details of the fax number will be placed on the laboratory request form as well as

the mobile phone number of the chief investigator.

6.2 Describe the strategy to manage likely adverse events or adverse reactions as identified in 2.4.2.

Symptomatic relief will be given for headache (paracetamol/co-codamol), and similar will be available for back or surgery/ catheter related post-operative pain. Intravenous fluids will be administered for low blood pressure. Full critical care organ support will be available if necessary.

6.3 Probability of event (categories) low

6.4 Seriousness of possible event: Low blood pressure is the most likely event. This will be treated as "serious".

6.5 Anticipated responses: As above. Initially, intravenous fluids and then vasopressors if necessary.

6.6 Potential long-term consequences: None.

6.7 Will an antidote be immediately available on site? no

If no, justify and detail contingency plan. There is no known antidote for clinical usage, but volume resuscitation and vasopressor therapy are expected to be sufficient. The PI is an expert at managing such circumstances.

If yes, provide details of the antidote and plan for subject management:

6.8 Detail AE reporting system to be used: *Key adverse events that will be monitored*:

Serious Adverse events (SAEs):

1. Haemodynamic instability including: systolic blood pressure decrease >25 mmHg; a mean blood pressure decrease >20 mmHg; heart rate increase to >145 bpm.
   A blood pressure SAE will be recorded after 3 confirming non-invasive blood pressure measurements with change assessed from baseline (time zero) Or any symptomatic hypotension (a reduction in blood pressure associated with symptoms including lightheadedness). A heart rate SAE will be a heart rate greater than 145 bpm, sustained for more than 5 minutes, Or any symptomatic tachycardia (an increase in heart rate associated with symptoms including chest tightness).
2. Reduction in Glasgow Coma Scale >2 points or 1 motor point; change in pupil response or size
3. Clinical evidence of a seizure
4. Death or serious adverse event - the timing in relation to peptide delivery and a root cause analysis will be performed (not expected as the compound has been given at similar doses intravenously)

Adverse Events (AEs):

1. Spontaneous hyperventilation; $PaCO_2$ < 4.0 kPa, monitored using a TOSCA device (Radiometer Medical, Åkandevej 21, DK-2700 Brønshøj, Denmark). This is possible after cerebral ventricular system injection[21], but unlikely after lumbar CSF injection but will be assessed for completeness.
2. Facial flushing. If observed, an assessment of the involvement 1-4 facial quadrants will be made and an assessment of intensity of rubor made (mild, moderate, intense). Volunteers will be asked about tolerability of the flushing, on a simple yes/ no scale.
3. Any other adverse event (self-reported or otherwise) will also be recorded such as headache. For headache a standard visual analogue score will be used.

Any AE that is considered intolerable will be treated as an SAE.

The investigators (Andrews & Flynn) will not be blind.

Section 6 Committee use only

1. Is the strategy to monitor and manage adverse events acceptable? Yes/ No
2. Is the antidote or contingency plan acceptable? Yes/ No
3. Is the AE reporting system acceptable? Yes/ No
4. Procedure for emergency unblinding of study drugs acceptable? Yes/ No

Comments/ Recommendations

7. Suitability of subjects

Note: It is a requirement that confirmation of subjects' past medical history (PMH) for these trials is received via the subjects' GP or other medic such as a hospital consultant for patient studies, to provide assurance that inclusion and exclusion criteria are met.

7.1 Justify choice of subjects for this study: These subjects will have spinal anaesthesia for a minor urological procedure and a spinal catheter left in situ thereafter for administration of the peptide and CSF sampling. These patients would normally have spinal anaesthesia for this procedure. There is no current evidence on the effect of intrathecal administration of anaesthesia to plasma or CSF concentrations of CGRP. Two studies have investigated the effect of administration of desflurane and propofol anaesthesia on plasma concentrations of CGRP (Wang T et al. 2004 and Luo F et al. 2009 respectively). Plasma concentrations of CGRP decreased during propofol anaesthesia and there was no statistically significant difference in plasma CGRP concentrations following desflurane anaesthesia. This is perhaps due to the decreased peripheral resistance associated with propofol and the well-documented vasodilatory properties of CGRP. The researchers do not believe prior spinal anaesthesia will have an effect on the CSF concentrations of CGRP. Spinal anaesthesia for these procedures is usually with bupivacaine, which has a half-life of 3.5 hours. Patients will have fully recovered from their spinal anaesthesia and be deemed to be haemodynamically stable and fit for transfer by the anaesthetic team prior to arriving at the WTCRF. Patients are likely to be normovolaemic following surgery as they are likely to receive intravenous fluids during anaesthesia/their operation. Being normo- or hypervolaemic means patients are less likely to show toxicity to CGRP than hypovolaemic patients as any vasodilatory effect will be less apparent. However, participants will not be excluded/included in the study based on whether they received IV fluids. They will only be included if they have sufficiently recovered from surgery and spinal anaesthesia, which involves assessment of their haemodynamic status by the clinical anaesthetist.

7.2 Give clear guidance on what constitutes a valid subject.

Inclusion Criteria

- Males or females aged over 18 years
- Not pregnant
- Scheduled for transurethral bladder or prostate surgery considered 'small' and low risk by the surgeon

responsible
- ASA grade I or II
- Clinical decision to have a spinal anaesthetic
- Written informed consent to participate in the study must be obtained from the patient prior to initiation of any study-mandated procedures
- Sufficiently recovered from surgery and anaesthesia in the opinion of clinical anaesthetist and surgeon, who will document this prior to patient transfer to WTCRF. Sufficiently recovered implies haemodynamic stability and normovolaemia.

Exclusion Criteria
- Presence of hypertension, defined as blood pressure ≥ 140/90 taken at the time consent is given and confirmed on three separate measurements. Treated hypertension is not one of the exclusion criteria as long as the patient is normotensive on treatment.
- Any previous subarachnoid haemorrhage
- Migraine history
- Post-operative bleeding requiring >2 unit transfusion
- Any contra-indication to spinal anaesthesia
- Rosacea (skin condition causing reddening of facial skin)
- Any severe or unstable concomitant condition or disease (e.g., known significant neurological deficit, cancer, haematological, coronary disease, psychiatric disorder), which would affect assessment of the safety or efficacy of the intervention (investigator's opinion).

Screening, Enrolment and Consent

12 elective surgical patients, ASA I and II, will be recruited from urological surgery lists. They will be scheduled for either a transurethral resection of bladder tumour or prostate. Resections will be considered "small" and low risk by the surgeon responsible for the case.

Screening and risk assessment will be made by research staff including; presence of hypertension, any aSAH, migraine and contra-indications to spinal anaesthesia.

Participants will be consented for insertion of a spinal (intradural) anaesthetic for the procedure and a continuous intrathecal catheter for research purposes and for sampling of blood and CSF for pharmacokinetic assessment.

Identifying Participants

Potentially eligible participants will be identified by urological surgery consultants & clinic nurses who will give information to the patient.

Informing Potential Participants about the Trial

The clinical team responsible for patient care will identify potential participants. Potential participants will be provided with a brief information sheet and asked to make contact with the research team if they might be interested in participating. Interested individuals who contact the research team will be sent the full participant information sheet and invited to arrange a screening visit or to decline participation. If no reply is received after 2 weeks, the research team will send a reminder.

Consenting

Once eligibility has been confirmed, an authorised member of the research team will go through the study with the potential participant, ask if they are ready to decide whether or not to join the study and if willing give the participant the consent form. After the doctor has checked that the consent form is understood, they will invite the participant to sign the form, add their own name and countersign it. The original will be filed in the patient's notes along with the Information sheet. One copy will be given to the patient and one stored in the trial master file.

7.4 Do inclusion/ exclusion criteria take account of possible allergic response to planned antidotes or treatment of AEs? Yes

Section 7 Committee use only

1. Is subject choice valid? Yes/ No
2. Is PMH check acceptable? Yes/No
3. Are possible allergic responses to antidote or treatment of AEs adequately accounted for in inclusion/ exclusion criteria? Yes/ No

Comments/ Recommendations

8. Pharmacy information

8.1Source of IMP(s)

Where is IMP coming from? Bachem Holding AG, Hauptstrasse 144, 4416 Bubendorf, Switzerland, Tel +41 61 935 2333, Fax+41 61 935 2324. Email: ir@bachem.com

Is availability guaranteed for the duration of the study? Yes.

Provide QP certification for manufacture and further manipulation or processing.

The synthesis of Alpha CGRP is carried out using standard solid phase chemistry at a GLP compliant manufacturing site, BaChem, Switzerland. No materials at risk of transmitting transmissible spongiform encephalopathy (TSE) are used in the manufacturing process. Alpha CGRP manufacture and filling, under aseptic conditions, and QP release are performed by Pharmacy Production Unit, Western Infirmary, 100 University Place Glasgow, G12 8TA, and are carried out according to Good Manufacturing Practices. The quality of the product is controlled by ensuring compliance to internal specifications set with due regard to European guidance on the control of IMPs.

8.2 Stability of the IMP(s)

What is the shelf life of the IMP(s)? The peptide is to be stored frozen at -20°C (+/- 5oC). Stability studies are being conducted to confirm the stability of CGRP formulation over the proposed study duration.

What is the length of time of maintenance of IMP activity once prepared for administration? Greater than 7 days.

8.3 Storage of the IMP(s)

What are the storage requirements for the IMP(s) eg frozen?

The peptide is to be stored frozen at -20°C (+/- 5oC). Stability studies are being conducted to confirm the stability of CGRP formulation over the proposed study duration. CGRP will be stored in the pharmacy at the WGH.

8.4 Reliability of very small doses

Is there a need for demonstration that the intended formulation of the doses to be administered provides the intended dose?
The peptide will supplied by BaChem, Switzerland, prepared to GMP standard and QP released from Glasgow Western Infirmary and prepared for administration in this study using "aseptic worksheets" and multiple dilutions. No post-dilution measurement of the peptide will be performed.

Section 8 Committee use only

9. Contingency planning and safety considerations. To be completed by the study team.

Please provide details of the contingency plans for the study within the CRF, include information relevant to ICU.
To be included as a minimum:

1. The criteria and protocol for premature discontinuation of IMP in an individual? (This should include clear details on clinical parameters).

Discontinuation Procedures

A blood pressure SAE will be recorded after 3 confirming non-invasive blood pressure measurements with change assessed from baseline (time zero) Or any symptomatic hypotension (a reduction in blood pressure associated with symptoms including lightheadedness). A heart rate SAE will be a heart rate greater than 145 bpm, sustained for more than 5 minutes, Or any symptomatic tachycardia (an increase in heart rate associated with symptoms including chest tightness).

Any AE that is considered intolerable will be treated as an SAE.

Stopping Rules and Decision Making

Recruitment will be suspended after any SAE and further dosing in that volunteer stopped. A DMC review will be undertaken to decide if further doses can be given to subsequent volunteers. Escalation to a higher dosing level will only occur after a DMC review.

The study will be stopped if *cardiovascular* SAEs are observed in three research subjects and associated with intrathecal administration. The study will not be stopped following a single cardiovascular SAE after intrathecal administration as this may be unrelated to the peptide. However, recruitment will be stopped and the DMC will review every SAE and the study will only continue once the DMC are satisfied. The study will be stopped following one cardiorespiratory arrest, intracranial haemorrhage or death.

If a participant chooses to withdraw completely from the study and all study assessments this will be recorded in the CRF. Any concerns expressed by the participant will be addressed by the research team.
Clinical parameters for discontinuation of peptide.

2. Specific instructions for antidote (if applicable). N/A, however, resuscitation fluids, oxygen therapy and vasopressors will be immediately available.

3. Contingency plan for on- and off-target effects, including details of specific treatments that must be held on-site to manage expected adverse reactions. Symptomatic relief including paracetamol, cocodamol and intravenous fluids will be available and resuscitation equipment and drugs will be kept on site.

4. Is it essential that ICU is aware? Yes

5. Is it essential ICU have a bed available? No, but a ward bed will be kept for the surgical volunteer.

6. State if a relevant specialist is required to be available to support emergencies and adverse event management. Yes
   Please detail who is required and under what circumstances. The PI is a consultant in critical care.

7. Time period between first, second and subsequent participants. Between first and second. 24 hours and between cohorts at least 5 working days to allow DMC review of the data.

8. Plan for data and safety review following first dose and prior to subsequent dosing. Data will be sent to the DMC after each cohort or after an SAE (see protocol). There is a requirement for written agreement to continue to the next dosage increments.

9. Plan for data and safety review prior to subsequent subject recruitment. As above.

10. The criteria for stopping the trial. The study will be stopped after any 3 cardiovascular SAEs. Recruitment will be suspended after any SAE and a DMC review will be undertaken to decide if further doses can be given. Escalation to a higher dosing level will only occur after a DMC review. The justification for this is to balance the risks to the participants against the potential information gained by the research. Stopping the study due to one SAE potentially unrelated to the peptide would be imprudent. The study will be stopped following one cardiorespiratory arrest, intracranial haemorrhage or death. In addition, if the DMC deems that the SAE is serious enough to stop the study at any point, it will be stopped.

11. Medical staff level of cover on first dose. There will be a nurse, trainee anaesthetist and a consultant in Critical Care.

12. Medical staff level of cover for subsequent doses: As above.

13. Duration of medical cover post dose (hrs). 4 hours in WTCRF, the participant will then be transferred to a ward.

14. CRF Nursing staff level requested per subject. 1 nurse.

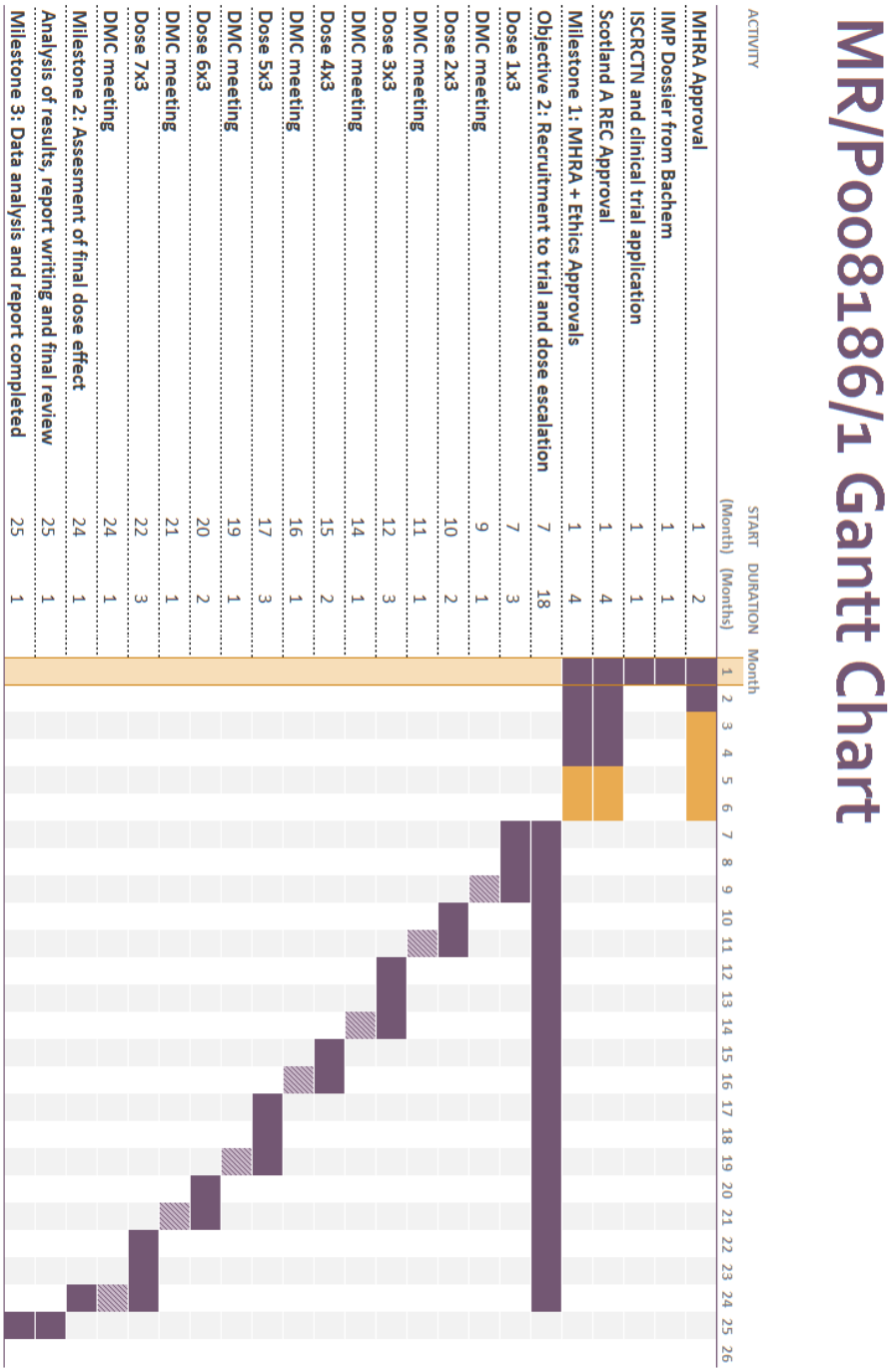
15. Identify proposed room allocation or specific requirements for participant observation for duration of CRF admission.

Yes. Double oxygen point, wall suction point, high flow oxygen equipment and intravenous fluids.

16. Maximum number of subjects on any given day (after review of initial dosing studies). One.

17. Need for overnight observation? All surgical subjects will be kept in hospital overnight and will have a 22:00 review by the trainee anaesthetist. In the event of an SAE, the patient will be admitted to a critical care bed, of appropriate level, for ongoing observation.

Additional comments:


MR/P0081861 Gantt Chart
ACTIVITY
START (Month)
DURATION (Months)
Month
MHRA Approval 1 2
IMP Dossier from Bachem 1 1
ISCRCTN and clinical trial application 1 1
Scotland A REC Approval 1 4
Milestone 1: MHRA + Ethics Approvals 1 4
Objective 2: Recruitment to trial and dose escalation 7 18
Dose 1x3 7 3
DMC meeting 9 1
Dose 2x3 10 2
DMC meeting 11 1
Dose 3x3 12 3
DMC meeting 14 1
Dose 4x3 15 2
DMC meeting 16 1
Dose 5x3 17 3
DMC meeting 19 1
Dose 6x3 20 2
DMC meeting 21 1
Dose 7x3 22 3
DMC meeting 24 1
Milestone 2: Assesment of final dose effect 24 1
Analysis of results, report writing and final review 25 1
Milestone 3: Data analysis and report completed 25 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26