Head Injury, from Man to Model

ISBN 90 5166 778 7

Cover Design: W.A. van den Brink i. s. m. Idefix vormgeving

Digitized CT scan of an injured human brain, transparantely masked by a digitized Hematoxylin-Eosin stained slice of an injured rodent brain. The vagueness of the image of the human brain, as if presented through the expirimentalists microscope, is obtained by "Gaussian blurring" using image editing software.

Eburon Publishers

PO Box 2867

2601 CW DELFT

info@eburon.nl

www.eburon.nl

Head Injury, from Man to Model

(Hersenletsel, van Patient tot Experiment)

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. dr P.W.C. Akkermans M.A.
en volgens het besluit van het college voor promoties

De openbare verdediging zal plaatsvinden op
woensdag 14 juni 2000 om 9.45 uur

door

Willem Aart van den Brink
geboren te Schiedam

Promotores: Prof. dr C.J.J. Avezaat
Prof. dr A. Marmarou

Overige leden: Prof. dr K.G. Go
Dr A.I.R. Maas (tevens co-promotor)
Prof. dr F.G.A. van der Meché
Prof. dr A.B. van Vugt

Het onderzoek dat in dit proefschrift wordt beschreven, is uitgevoerd binnen de Richard Roland Reynolds Neurosurgical Research Laboratories van de afdeling Neurochirurgie van het Medical College of Virginia te Richmond Virginia, in het Erasmus Dierexperimenteel Centrum te Rotterdam en in de Neuro Intensive Care Unit te Rotterdam.

Het onderzoek werd ondersteund door research grants NS-192235 en NS-12587 van het National Institute of Health en research grant H133B80029 van het National Institute of Disability and Rehabilitation Research.

Het verblijf van de promovendus in de Verenigde Staten werd financieel ondersteund door de Stichting Neurochirurgie en de Sophia Stichting Wetenschappelijk Onderzoek te Rotterdam.

De financiële ondersteuning voor publicatie van dit proefschrift door de Stichting Neurochirurgie, de Stichting Het Remmert Adriaan Laan Fonds, Promedics, Divisies Codman en DuPuy AcroMed van Johnson & Johnson Medical, Bayer, Carl Zeiss en Stöppler wordt zeer gewaardeerd.

Contents

Structure of the thesis

Introductory chapters, clinical relevance

Chapter 1 General introduction: Head injury, a silent epidemic

This chapter provides an epidemiological background of accidental injuries in general, and head injuries in particular. The data were provided by official institutions in the USA (CDC) and in the Netherlands (CBS). It places the magnitude of the problem of head injury in perspective. The burden to the patients, their relatives, and society is outlined.

Chapter 2 Pathophysiology of traumatic brain injury

In the second chapter the most important known pathophysiological sequelae of traumatic brain injury are summarized. The knowledge gathered over the last years, which has survived the 'experimental' stages, and which has found its place in current consensus, or which remain under serious debate, is summarized.

Chapter 3 Animal models of traumatic brain injury

This last introductory chapter precedes the experimental work, which will be the body of this thesis. It provides a historical perspective on head injury models. As head injury is diverse by nature several animal models, each covering a certain part of the spectrum of human head injury, are discussed.

Characterization of an experimental Closed Head Injury model

Chapter 4 A new model of diffuse brain injury in rats

This chapter is the basic article that introduces the Closed Head Injury model, its mechanical properties, and the principle pathophysiological characterization, to the scientific literature. As diffuse brain injury is present in most, and predominant in many head injured patients, the importance of a model causing such diffuse injury is stressed.

Chapter 5 Closed Head Injury: standardization and acceleration

As standardization is paramount in head injury modelling, and especially difficult in acceleration models in small animals, the CHI model is approached in a physical way. The mechanics responsible for acceleration of the brain, are approached sequentially to provide insight in the contribution of the determinators of skull-acceleration.

Chapter 6 Cortical dysfunction with preservation of brain stem function

Because models of Fluid Percussion Injury are hampered by predominant brain stem damage, even at moderate levels of injury, brain stem and cortical function after CHI were investigated electro physiologically by means of auditory brain stem and somatosensory evoked potentials.

Chapter 7 Blood brain barrier dysfunction and edema formation

Increased brain water content is one of the contributors to intra cranial hypertension. Increments of brain edema, and blood brain barrier dysfunction were studied in this model, which is not confounded by a post traumatic hypertensive surge, but is prominent in the Fluid Percussion model.

Experimental Closed Head Injury and oxygen

Chapter 8 Hypoxia augments behavioral deficits

Traumatic brain injury is often followed by secondary insults, which are deleterious with respect to patient outcome. The effects of secondary hypoxia on lower and higher order neuro behavioral function were studied.

Chapter 9 The brain parenchyma-$PbrO_2$ catheter interface

Monitoring brain tissue oxygen is a promising means of evaluation of the course of cerebral oxygenation after head injury. The micro catheter used measures partial pressure of oxygen in the brain tissue at the very proximity of the catheter tip. This 'probe tissue interface' was studied with histopathological and morphometric methods. Effects of local disturbances on measured $PbrO_2$ values are discussed.

Chapter 10 Brain oxygenation marginally impaired in Closed Head Injury

The main purpose of trauma care in head injury is the maintenance of cerebral blood flow high enough to assure metabolic demands. The effects of experimental CHI, systemic parameters, and global hypoxia on $PbrO_2$ values, reflecting local equilibrium of oxygen supply and demand, are evaluated.

Back from model to man: human head injury and oxygen

Chapter 11 Brain oxygen tension in human injury

Brain tissue oxygen monitoring can be considered 'state of the art' for the neuro intensive care. Before possibly targeting treatment using this technique, the occurrence, depth, and duration of cerebral hypoxia, and its relationship with outcome needs to be established. This final chapter reports our experience with this novel monitoring technique in severely head injured patients.

Abbreviations

ABCT Auditory Brain-stem Conduction Time

A / D Analogous / Digital (convertor)

ANOVA Analysis of Variance

ATLS Advanced Trauma Life Support

BAEP Brain-stem Auditory Evokend Potential

BBB Blood Brain Barrier

BWC Brain Water Content

CBS Centraal Bureau voor de Statistiek

CBF Cerebral Blood Flow

CBV Cerebral Blood Volume

CDC Centre of Disease Control

CHI Closed Head Injury

CPP Cerebral Perfusion Pressure

CSF Cerebrospinal Fluid

CT Computerized Tomography

DAI Diffuse Axonal Injury

H&E Hematoxylin & Eosin

ICD International Classification of Diseases

ICP Intracranial Pressure

EBIC European Brain Injury Consortium

EEG Electro- Encephalogram

FiO_2 Fraction of Inspired Oxygen

FPI Fluid Percussion Injury

SSEP Somato Sensory Evoked Potential

GOS Glasgow Outcome Scale

GCS Glasgow Coma Scale

IA Intra Arterial

IM Intra Muscular

ISO International Standards Organization

IV Intra Venous

LED Light Emitting Diode

LMR Landelijke Medische Registratie

MAP Mean Arterial Pressure

MRI Magnetic Resonance Imaging

MWM Morris Water Maze

nHL normal Hearing Level

NICU Neuro Intensive Care Unit

NO Nitric Oxide

N_2 Nitrogen

N_2O Nitrous Oxide

$PaCO_2$ Partial Pressure of arterial Carbondioxide

PaO_2 Partial Pressure of arterial Oxygen

$PbrO_2$ Partial Pressure of brain tissue Oxygen

PHTLS Prehospital Trauma Life Support

PI Permeability Index (of BBB)

P-value Probability Value

PVI Pressure Volume Index

RTA Road Traffic Accident

SAH Subarachnoid Hemorrhage

SCCT Somato Sensory Central Conduction Time

sd Standard Deviation

SOF Small Open Field

TBI Traumatic Brain Injury

TCDB Trauma Coma Data Bank

TNCC Trauma Nursing Core Course

Chapter 1
General introduction

Investigating the problem, I sometimes feel that we are in the position of the blind man describing the elephant. We are all aware of the phenomenon; our descriptions vary with the direction of approach.
Arthur E. Hirsch, 1966

1.1 Head injury, a silent epidemic

In well developed countries, injury is the leading cause of death and disability among young adults. In less developed countries the incidence of injury is high and rapidly increasing, but the relative mortality due to injuries is overshadowed by other causes, such as infections and malnutrition[15]. In the United States of America each year approximately 1.000.000 people are treated and released from hospital emergency departments because of head injury. About 80% of patients receiving medical attention can be categorised as mild (Glasgow Coma Score = GCS 14-15), 10% as moderate (GCS 9-13), and 10% as severe (GCS 3-8) [3]. According to the Centre of Disease Control[1], in 1996 95 patients per 100.000 inhabitants required hospitalisation, or died because of head injury. Leading causes of traumatic brain injury (TBI) in the USA are violence (self inflicted and assault, 44% fire arms), vehicle crashes (34%), falls (9%), and other miscellaneous causes (14%). In Europe the majority of external causes of traumatic brain injury is related to road traffic accidents and accidental falls.

Injury in the Netherlands

In the Netherlands, injury related mortality during the last decades showed a peak in the early seventies, with gradual decline thereafter for both road traffic accidents (RTA) and accidental falls [figure 1]. According to data from the "Centraal Bureau voor de Statistiek (CBS), from 1979 through 1995, injury in the Netherlands still accounted for an average annual number of deaths of more than 4000 (query from CBS mortality database). In the population younger than 40 years, injury related mortality outnumbers mortality caused by cancer and cardiovascular diseases. Injury in these patients is mainly caused by road traffic accidents (RTA) [table 1]. Head injury is considered the cause of instantaneous death in 60% of RTA-victims. These figures correspond to the early necropsy study of the 'Road Injuries Research Group' at the Birmingham accident hospital; claiming that 70-80% of the patients dying early after a RTA-accident sustain major brain damage[8]. A global classification scheme provides insight in the Dutch distribution of some primary causes of injury [figure 2].

figure 1 mortality due to injuries and its main subclasses in the Netherlands per 100.000 persons-year. After 1970 a decrease in mortality due to both road traffic accidents, as well as accidental falls is observed. Enforcement of safety regulations, and implementation of preventive measures started in this episode. The peak in 1953 is cause by the springtide in Zeeland, causing many casualties. Modified from van Beeck 1998[18].

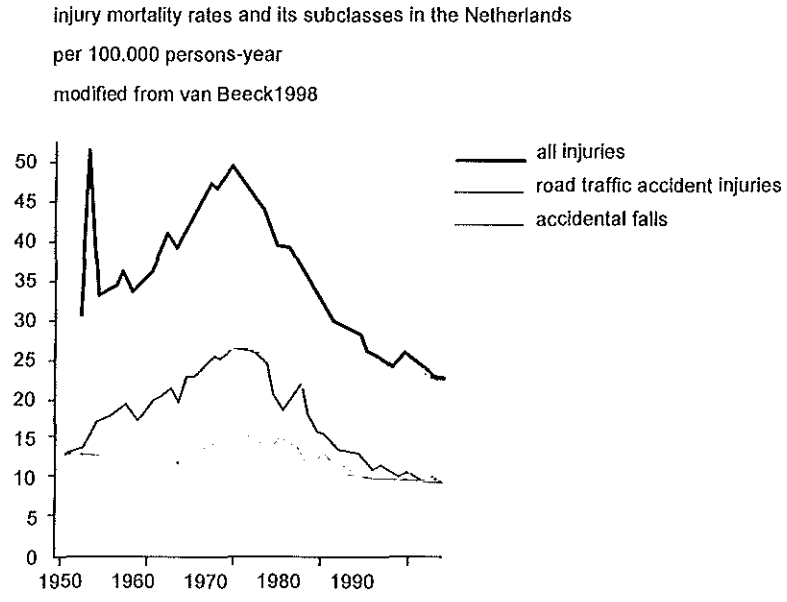
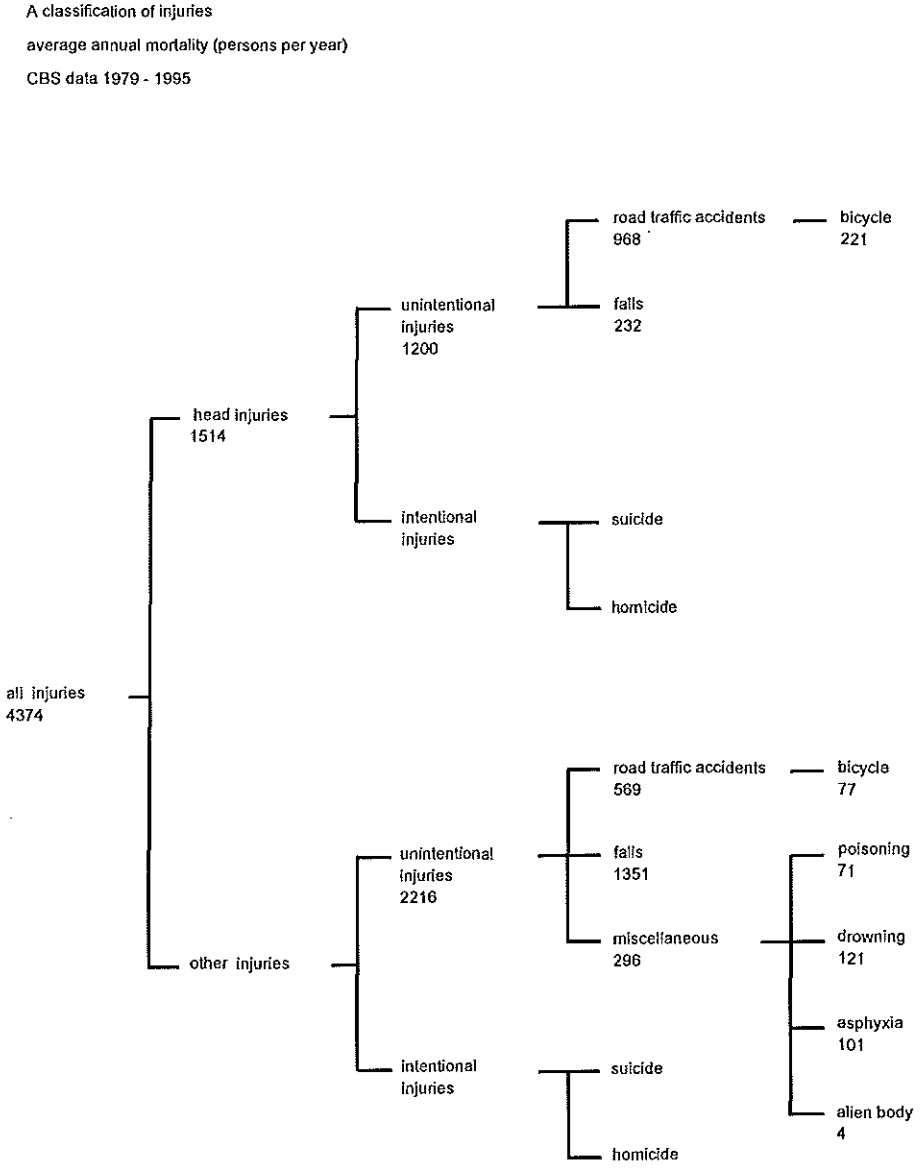


table 1 Mortality due to injuries in 1996 was higher than mortality from cancer or circulatory diseases during the first four decades of life. In these age groups by far the most injuries are cause by road traffic accidents. In the elderly population accidental falls account for the majority of injury related mortality. Data from Centraal Bureau Statistiek: Vademecum Gezondheidsstatistiek 1998.

Numbers of death due to injuries in 1996

	age groups				
	<15	15-39	40-64	>64	total number of deaths
accidents	140	782	534	1521	2977
rta	68	557	268	240	1133
fall	5	36	116	1155	1312
assault	18	108	64	17	207
cancer	114	773	9634	21565	32086
circulatory	44	402	6295	34562	41303

figure 2 Injuries can be classified according to several schemes. In the Netherlands injuries are largely due to road traffic accidents and accidental falls. A bicycle is involved in a relative large number of road traffic accidents. In case of head injuries, the number of intentional injuries, homicide and suicide, are relatively low. Data from Centraal Bureau Statistiek: query of injury related mortality database 1979 - 1995.



Injuries, predominant in the population of young adult males, cause a disproportional loss of productive years, and places a high burden on society due to its socio-economic costs. The financial burden of direct medical costs due to injuries in the Netherlands was calculated to be $952 million annually. Compared to $1746 million for cardiovascular diseases, and $ 915 million for cancer[18]. Using the human capital approach, indirect costs to society due to injuries are estimated to add up to $ 3.3 *10^9. Family grief, with relatives dying from (or becoming disabled after) head injury is immense and can not be expressed in financial units.

Head injury in the Netherlands

Van Beeck, in a joined study of the Erasmus University Rotterdam and 'Stichting Consument en Veiligheid' [19], modelled that each year 48.800 head injured patients are assessed at a hospital emergency room. 12.300 of them will be admitted with an average admission time of 7.3 days. The Dutch "Landelijke Medische Registratie" (LMR), a national institute collecting data on patient admissions to all hospitals in the Netherlands, provided information on admission diagnoses based on ICD-9 classification related to head injury. During the years 1995 - 1998 an annual average of 6852 patients were admitted because of a mild head injury (which was termed 'commotio cerebri'), 4696 were admitted for a more severe head injury, and 598 patients sustained some kind of traumatic intracranial hemorrhage. The annual number of admissions and mortality rates are displayed in table 2

table 2 Some insight in the mortality figures in the Netherlands can be presented when contusions and skull fractures are aggregated as 'complicated head injury' and 'commotio' as 'mild head injury'. Intracranial haematomas cause the highest mortality. Data from LMR.

admissions for traumatic brain injury in the Netherlands

	mild head injuries		complicated head injuries		Intracranial hematomas	
	n	mortality (%)	n	mortality (%)	n	mortality (%)
1995	6677	0	5052	9	602	28
1996	7076	0	4804	10	562	26
1997	7252	0	4702	11	621	27
1998	6403	0	4225	11	607	29

Statistics of mortality due to external causes, covering the years 1979 through 1995 was made available from the CBS. An abstract from the mortality database was based on an "external cause of death" query. Detailed information, though provided by both LMR and CBS, should be regarded with great care which is beyond the scope of this thesis. At a higher aggregation level, however, these data are accurate. Patients age, sex, location of decease, and cause of death codes according to ICD 9 and ICD 10 classifications were analyzed. The age distribution of head injury shows a peak at young adult age, with male predominance [figure 3].

Over the years a gradual decline in the total number of deaths can be observed. This is mainly caused by a decrease in lethal road traffic accidents [figure 4]. In this episode ample preventive measures were implemented. Fifty-two percent of head injured patients not surviving a road traffic accident, die on the scene of the accident. This might be caused by the very nature of severe head injury, though it is not unlikely that a certain amount of these patients could be salvaged by ultra early on site resuscitation.

figure 3 Number of deaths due to head injury. Young adult males form the largest group of victims of head injuries. At older age the male predominance decreases. Data from Centraal Bureau voor de Statistiek: mortality database, based on query "external cause of death" 1979 - 1995.

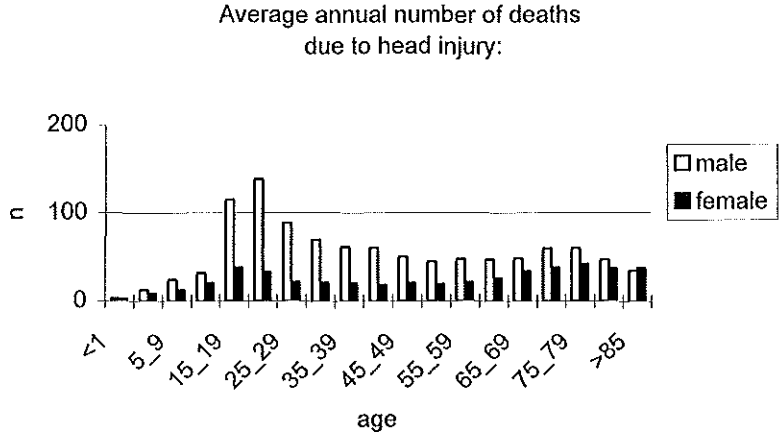
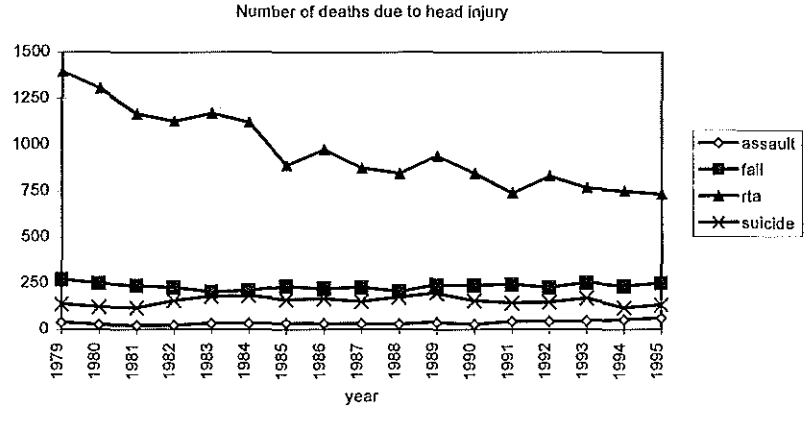


figure 4 Over the years the number of deaths due to head injury gradually decreases. This is mainly on account of a decrease in road traffic accidents. Data from Centraal Bureau voor de Statistiek: mortality database, based on query "external cause of death" 1979 - 1995.



Head Injury in the Rotterdam University Hospital

Patients from a large area around Rotterdam covering some 10-15% of the Dutch population, which comprises about 1.8 million regional inhabitants, are referred to the Academic Hospital for neurotrauma care. The hospital administration records of the Rotterdam University Hospital were reviewed for the period 1993 - 1998. An annual average of 251 patients with a diagnosis of head injury were admitted. 130 (52%) of these patients were admitted to an Intensive Care Unit [figure 5A]. Duration of hospital admission was more than one week in 100 patients (40%) [figure 5B]. Overall mortality rate was 15%, and 16% could not be discharged to their home environment [figure 5C].

Severe head injury is a major cause of death, predominantly among young male adults. Survivors often remain disabled, causing immense family grief and economic burden on our society. Physicians taking care for these patients must prepare their facilities for a considerable patient load.

1.2 Outcome in severe head injury

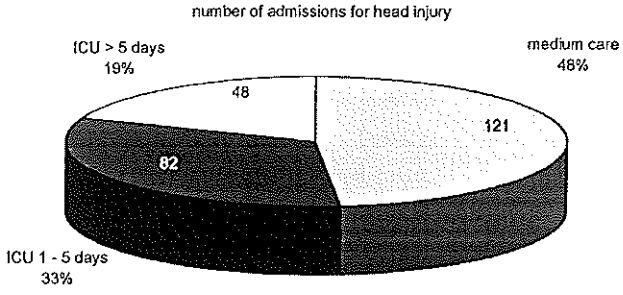
Variability of head injury mechanisms and pathophysiological responses of the brain result in heterogeneous patient populations. It is thus not surprising that reports concerning prognosis of head injury indicate wide variation in final outcome. Until the introduction of the Glasgow Coma Scale [table 3] and the Glasgow Outcome Scale [table 4] in the mid 1970-ties comparison of clinical severity (and of final outcome) between centres was hardly possible. The GCS is a scale which semi-quantifies the consciousness level of the head injured patient based on the ability to open the eyes, the reaction of the arms, and speech; either spontaneously or after external stimulation. Coma was defined as inability to open the eyes, to follow simple commands, or to utter comprehensible words[17]. Furthermore head injury can be divided in mild (GCS 14-15), moderate (GCS 9-13), or severe (GCS 3-8). For outcome prediction purposes the GOS is usually dichotomized in: favorable and unfavorable. In several large observational series of severely head injured patients, and in the placebo treated patient groups of trials of neuroprotective agents, the outcome distribution, using this dichotomy, is about fifty-fifty [table 5]. Individual patient outcome prediction is most reliably based on clinical parameters, the most important ones being: age, depth of coma, GCS motor score, pupillary reactivity, and the course of recovery of consciousness during the first days[5-7, 16, 20].

The prognosis after severe head injury is grave, and predictable only to a certain extent. Every effort possible should be undertaken to improve patient outcome.

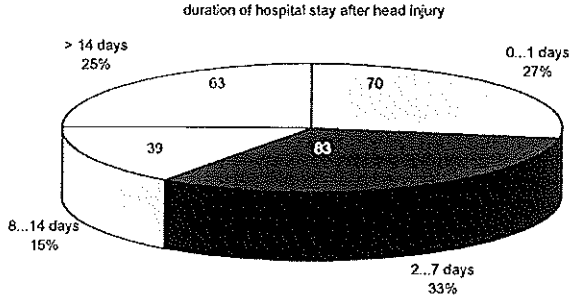
figure 5 [A admissions] [B duration] [C discharge] In the Rotterdam University hospital over the years 1993-1998 an annual average number of 251 patients were admitted for head injury. Approximately 50% of the patients were admitted to an ICU for dedicated neurotrauma care [a]. 40% were admitted in the hospital for more than one week [b]. Only 69 % could be discharged to their home environments [c].

Data from the AZR Hospital Administration

A



B



C

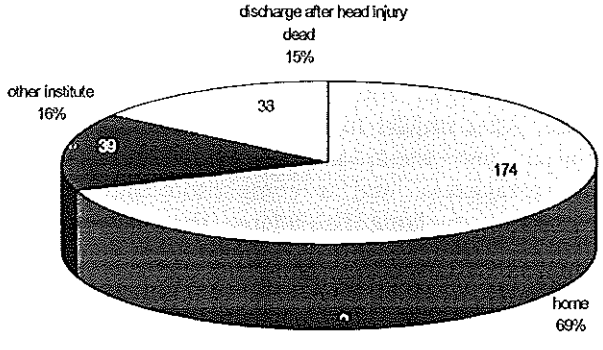


table 3 Glasgow Coma Scale as originally described. Comatose patients have a sum score lower than or equal to 8 and are usually considered "severely head injured".

Eye opening	spontaneously	4
	to verbal command	3
	to pain	2
	no response	1
Motor response	obeys commands	6
	localizing	5
	flexion-withdrawal	4
	abnormal flexion	3
	extension	2
	no response	1
Verbal response	oriented	5
	disoriented	4
	inappropriate words	3
	incomprehensible words	2
	no response	1

table 4 Glasgow Outcome Scale as measuring device of patient outcome. For practical purposes the scale is dichotomised: favorable (4&5) and unfavorable (1, 2&3).

Glasgow Outcome Scale		
dead	1	unfavorable
vegetative	2	
severely disabled	3	
moderately disabled	4	favorable
good recovery	5	

1.3 Treatment of head injured patients

Despite the complexity of clinical presentations of head injured patients, traumatic brain injury can be viewed as being caused by "primary injury", at the scene of the accident, and "secondary injury", evolving during the following episode. After impact to the head, damage to certain areas of the brain may be in part permanent and beyond cure. Other, less damaged areas, are potentially salvageable, and a comparison may be made with the penumbra, surrounding the core of an infarct. These surrounding areas, as well as undamaged areas of the brain, are susceptible to secondary damage which may be the result of a "secondary insult", for instance adverse systemic or intracranial pathophysiological events, requiring early recognition and treatment. Secondary brain damage may also occur as the result of secondary

'intrinsic damage'. Putative mechanisms on sub-cellular level superimpose their damaging consequences upon the already perturbed brain.

Early recognition and treatment of secondary injuries: Systemic secondary insults

Brain tissue is extremely dependent on continuous supply of metabolites. After traumatic brain injury, even if clinically moderate, neurons are considered to be more vulnerable to hypoxia and hypotension[10]. In the head injured patient all treatment strategies should thus be aimed at prevention, early recognition, and aggressive treatment of such systemic insults, which may lead to secondary deterioration[4, 11]. As such all patients presented at the first aid department should be treated according to management protocols in which there is no debate among treating physicians which organ system has priority. The Advanced Trauma Life Support (ATLS)[3] course stresses the ABC's (Airway, Breathing, Circulation) of trauma patient care using the adage 'treat first what kills first'. These elements, important for any traumatized patient, are even more valuable for the head injured patients. These basic treatment priorities are readily recognised and adopted in training programs for ambulance (Prehospital Trauma Life Support-PHTLS[14]) and first aid nurses (Trauma Nursing Core Course-TNCC [9]).

table 5 Several large observational series, and the placebo treated patients of clinical trials, show that severely head injured patients have a chance of 47% of an unfavorable outcome. Average mortality rate of these series is 27%.

Maas et al: Neurosurgery 1999	coma prognosis study: Jennett 1977	TCDB: Vollmer 1991	Westmead Survey: Fearnside 1993	EBIC Survey: Murray 1999	Triam-cinolone: Grumme 1995	HIT-I: Bailey 1991	HIT-II: ESG on Nimodipine 1994	HIT-III: Hardes 1996	PEGSOD: Young 1996	Tirilazad International: Marshall 1998	Tirilazad US: Straw 1995	Selfotel Morri submitte	
number of patients	700	661	315	796	209	175	414	61	162	459	557	427	4936
outcome dead	51	38.1	31	31	22	29	24	26	25	28	0	23	27
vegetative	2	5.1	4	3	5	1	5	10	6	4	36	3	7
severely disabled	9	15.7	10	16	18	21	12	10	15	13	0	17	13
moderately disabled	16	16	19	20	12	19	24	5	21	17	19	20	17
good recovery	22	25	36	31	41	30	36	49	33	38	45	36	35
unfavorable (%)	62	58.9	45	50	45	51	41	46	46	45	36	43	47
favorable (%)	38	41	55	51	53	49	60	54	54	55	64	56	53

Intracranial secondary insults

Concerning the occurrence of intracranial complications, the suspicion, early recognition, and adequate treatment of intracranial hematomas is paramount for physicians confronted with these patients. For early referral to a centre with neurosurgical expertise and capacity for emergency surgery regional agreements on referral conditions are mandatory. It is a political responsibility to offer enough financial support for the initiation and maintenance of dedicated and geographical evenly distributed trauma centres with up to date transportation facilities. After admission to the Neuro trauma centre, protocolar treatment of the head injured patient is the responsibility of the physicians taking care for these patients. Guidelines for the treatment of these patients, based on literature research are available[2]. Based on a more pragmatic approach, common understanding of pathophysiology, and consensus among experts, European Guidelines with more or less the same advises have been published[12].

Intrinsic secondary injuries

After traumatic brain injury has been inflicted, several cascades of secondary injuries, on a sub-cellular basis, have been brought up by researchers in this field. Ischemia and calcium influx seem to play a pivotal role in the final pathway leading to cell death. Theoretically these pathways should be amenable to pharmacological intervention, ameliorating its effects, and improving patient outcome. However clinical trials have not succeeded in proving beneficial effects of promising pharmaceutical agents in the overall population of patients with severe head injury[13]. The question has been raised whether trials should attempt to prove efficacy in the overall heterogeneous population of head injury or rather that trials of neuroprotective agents should be targeted to a sub population in whom the specific pathophysiological mechanism is likely to be active. Uncertainty exists concerning the time window within which pharmacological intervention may be worthwhile.

Secondary insults, resulting in neuronal ischemia, should by any means be avoided and treated aggressively. Methods of optimal advanced monitoring and treatment of cerebral homeostasis are still under debate. In this regard intensive collaboration between clinical scientists (confronted with a heterogeneous patient population) and experimental research units (utilising models of standardized head injury) is necessary to meet the challenge of providing efficient targeted treatment regimens.

References

1. Epidemiology of traumatic brain injury in the United States, in Centers for Disease Control and Prevention (ed): **Traumatic brain (head) injury (TBI).** http://www. cdc. gov/ncipc/dacrrd/tbi. htm, National Center for Injury Prevention and Control: 1999

2. American Association of Neurological Surgeons, Brain Trauma Foundation: **Guidelines for the management of severe head injury.** The Brain Trauma Foudation Inc.: 1-166, 1995

3. American College of Surgeons Committee on Trauma: **Advanced Trauma Life Support ®, Program for Physicians.** United States, First Impression: 1993

4. Becker DP, Gudeman SK: **Textbook of head injury.** Philadelphia, W.B. Saunders: 1989

5. Braakman R, Gelpke GJ: Probability tables. To be used as an aid in the prediction of outcome for comatose head injury patients. Rotterdam, Protocol Neurochirurgie: 7, 1984(UnPub)

6. Choi SC, Muizelaar JP, Barnes TY, et al: Prediction tree for severely head injured patients. **J Neurosurg 75**: 251-255, 1991

7. Choi SC, Narayan RK, Anderson RL, et al: Enhanced specificity of prognosis in severe head injury. **J Neurosurg 69**: 381-385, 1988

8. Gissane W: The nature and causation of road injuries. **Lancet 2**: 695-698, 1963

9. Jacobs BB, Baker P: **TNCC Trauma Nursing Core Course.** ENA: 1999

10. Jenkins LW, Moszynski K, Lyeth BG, et al: Increased vulnerability of the mildly traumatized rat brain to cerebral ischemia: the use of controlled secondary ischemia as a research tool to identify common or different mechanisms contributing to mechanical and ischemic brain injury. **Brain Res 477**: 211-224, 1989

11. Jones PA, Andrews PJ, Midgley S, et al: Measuring the burden of secondary insults in head injured patients during intensive care. **J Neurosurg Anest 6**: 4-14, 1994

12. Maas AIR, Dearden NM, Teasdale E, et al: EBIC-Guidelines for management of severe head injury. **Acta Neurochir 139**: 286-294, 1997

13. Maas AIR, Steyerberg EW, Murray G, et al: Why have recent trials of neuroprotective agents in head injury failed to show convincing efficacy? A pragmatic analysis and theoretical considerations. **Neurosurgery 44**: 1286-1298, 1999

14. McSwain NE, Frame S, Paturas JL: **PHTLS basic and advanced Prehospital Trauma Life Support.** St. Louis, Mosby: 1999

15. Murray CJL, Lopez AD: Estimating the causes of death: new methods and global and regional applications for 1990, in Murray CJL, Lopez AD (eds): **The global burden of disease.** Harvard, Harvard University Press: 118-201, 1990

16. Narayan RK, Greenberg RP, Miller JD, et al: Improved confidence of outcome prediction in severe head injury. A comparative analysis of the clinical examination, multimodality evoked potentials, CT scanning, and intracranial pressure. **J Neurosurg** 54: 751-762, 1981

17. Teasdale E, Jennett B: Assessment of coma and impaired consciousness. A practical scale. **Lancet 2**: 81, 1974

18. van Beeck EF: **Injuries: a continuous chalenge for public health**. Rotterdam, Thesis Erasmus University: 1998

19. van Beeck EF: Tussenrapportage ontwikkeling model voor de continue monitoring van de kosten van letsel. Amsterdam, Stichting Consument en Veiligheid: 18-19, 1998

20. van den Brink WA, Zwienenberg M, Zandee SM, et al: The prognostic importance of traumatic epidural and subdural hematomas revisited. **Acta Neurochir** 141: 509-514, 1999

Chapter 2

Pathophysiology of traumatic brain injury

It is imperative to realize that a severe head injury is not a simple or routine problem that will respond to a standard recipe. The clinical problems faced by a head-injured patient are a composite of the injury that the brain has sustained, associated injuries, and subsequent insults that occur when there are serious derangements. ***John D. Ward, Craniospinal Trauma, 1990***

2.1 Violation of the skull:

Heterogeneity of mechanisms and types of head injury.

The causes for severe head injury are various as there are: road traffic accidents, falls, assault, attempts of suicide e. g. Each mechanism inflicting the head can cause several primary injuries [table 1]. These are classically divided in localized brain injuries, and diffuse brain injuries. Localized contusions can be caused by local skull deformation, or deformation of the brain tissue against bony ridges of the inner table. Both result in brain tissue strains exceeding pial vascular and cortical tissue tolerances. Localized contusions may produce focal neurological deficits, and are generally easily visualized on CT scans. Penetrating head injuries cause focal lesions. High velocity missile injuries may cause generalized derangement due to a shock wave propulsed through the brain as well. Diffuse injuries are produced when rapid acceleration of the head occurs. Acceleration forces result in shearing lesions due to strain forces, caused by differential movement of centripetal layers of the brain. Its consequence is fundamentally different from localized injuries, as it results in widespread interruption of brain function. Loss of consciousness may be short-lived, as in concussion. Prolonged coma results from more severe diffuse axonal injury (DAI). The location of functional derangement resulting in prolonged coma, be it the brain stem or hemispheres, remains under debate. Often a admixture of focal and diffuse pathology is present in one patient.

Mechanisms of head injury, clinical presentation, and CT scan findings are heterogeneous.

2.2 Pathology of traumatic brain injury

Brain contusion is the most predominantly occurring example of localized injury. Disruption of the blood brain barrier, resulting in vasogenic brain edema and areas of perivascular hemorrhage, result in mixed density lesions on the CT scan. If the pia mater is torn, cerebral lacerations occur, and further

damage to the arachnoid can result in burst lobes and acute subdural hematomas. Diffuse brain injury is hallmarked by axonal damage, microscopically visible as axonal swelling, axonal disruption, and the occurrence of retraction balls. Concomitant vascular injuries result in punctate hemorrhages in the same areas. High resolution CT scanners and MR Imaging can visualise these punctate lesions in the white matter, mainly corpus callosum, dorsal midbrain, and brain stem, which can serve as an indicator of the sustained axonal damage [1, 10, 14, 56]. Ischemic changes have been observed by Graham and Adams [16, 17], and remain the single most important secondary event after traumatic brain injury.

Head injuries require a radiological evaluation to estimate the histo-pathological changes occurring in the heterogeneous spectrum.

Table 1 Mechanisms of primary and secondary injuries. Primary injuries can be focal of diffuse, secondary insults can be systemic of intracranial. Prevention and early treatment of secondary insults is the pillar of head injury treatment.

primary focal injuries	diffuse injuries	secondary systemic	intracranial
contusions	concussion	hypoxia	hematomas
coup	mild	hypotension	epidural
contre-coup	classic	hypercarbia	subdural
intermediate	diffuse axonal injury	hypocarbia	intracerebral
	mild	hypovolaemia	increased ICP
	moderate	hypoglycaemia	vascular engorgement
	severe	hyperglycaemia	brain edema
		hyperthermia	vasogenic
			cytotoxic
			hydrocephalus
			meningitis

2.3 Pathophysiology of head injury

Intracranial hypertension

Intra-cranial pressure is the result of a dynamic interplay between the three main constituents of the skull and spinal canal; the brain tissue, blood and cerebrospinal fluid (CSF). The Monro-Kellie doctrine states that the volume in this container, the skull, is constant. An increase in either one of the volumes of its respective constituents must be balanced by an equal loss of volume of the others, or ICP will increase. In the compensated state, the volume of one component can increase without significant increase in ICP. In the uncompensated state, the compliance ($\Delta V/\Delta P$) will decrease and an increase in volume will result in an exponential increase in pressure. This relationship can be graphically depicted in the volume-pressure curve [figure 1]. The pressure

volume index (PVI = $\Delta V/\log_{10}(P_p/P_0)$ [37] is a measure of compliance, which could theoretically warn for impending uncontrollable ICP increases, and is related to outcome [38]. Increased intracranial pressure occurs in 33% of diffuse, and 50% of focal injuries. If uncontrollable, high ICP is related with poor patient outcome [table 2]. Following head injury several factors contribute to intracranial hypertension. Expanding haematomas and contusions elevate ICP by occupying intracranial space. Brain swelling either due to vascular engorgement (increase in Cerebral Blood Volume, CBV) [33] or brain edema (increase in Brain Water Content) [5,29] is the more important factor, as it is by no means treatable by straightforward surgical evacuation. Although increased ICP is related to poor patient outcome, it remains unknown whether or not high ICP is the cause of, rather than the result from, irreversible brain damage.

Figure 1 The pressure – volume relationship is exponential. The more the added volume amounts, the more the resultant increase of intracranial pressure will be. In the initial stages of volume expansion virtually no increase of pressure will occur.

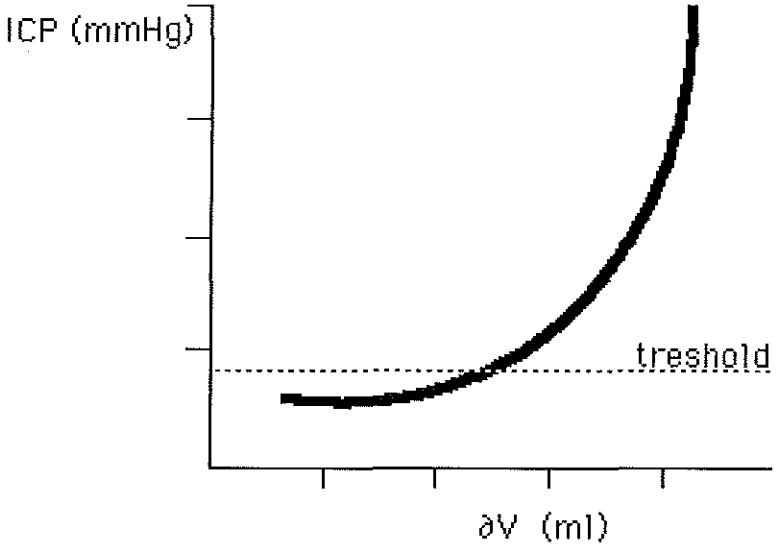


Table 2 Increased ICP relates to outcome. ICP's higher than 60 mmHg do not comply with survival.

relationship between ICP and mortality in severe head injured patients

highest		mass lesions		diffuse injury	
ICP level	mmHg	n	% mortality	n	% mortality
0-20		26	23	69	14
20-40		30	43	37	26
40-60		11	82	10	40
>60		16	100	2	100
	total	83	55	118	19

(miller et al. 1981)

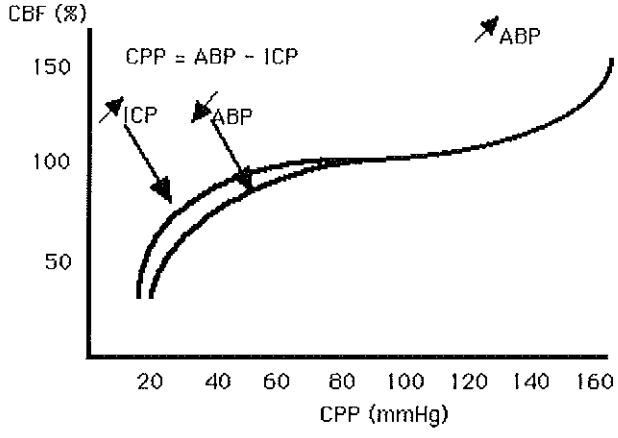
Decreased cerebral perfusion pressure

Because an energy storage in the brain does not exist, maintenance of blood flow to meet metabolic demand is pivotal. The driving force of CBF is cerebral perfusion pressure (CPP), defined as the difference between mean arterial blood pressure and ICP. Both a low arterial pressure and a high ICP compromise CPP after head injury. Rosner [2, 49] has advocated aggressive treatment of CPP, while the Lund group has hypothesised that, in the presence of a dysfunctional BBB, artificially raising blood pressure will increase the formation of vasogenic brain edema, and thus in the long run increase ICP [3, 18]. Experimental data did not support this hypothesis however: higher CPP causes smaller contusion volumes [32]. Additional standardized animal experiments are necessary to support one or the other hypothesis.

Cerebral blood flow, autoregulation, and ischaemia

After severe head injury both decreased cerebral blood flow, as well as area's of hyperaemia have been reported, the extremes of both have been related to poor patient outcome[6-8, 27, 34, 44, 46, 47]. The first mechanism is deleterious because of local tissue ischaemia, the latter because of vasodilation and increase of ICP, compromising nearby but relatively undamaged tissue. The intrinsic mechanism of autoregulation assures the brain of adequate CBF at variable levels of cerebral perfusion pressure. In the normal situation the limits of autoregulation are at approximately 50 to 150 mmHg [figure 2]. After head injury, either mild or severe, this mechanism is often impaired to a certain extent [13, 26, 46], rendering the brain vulnerable to spontaneous or iatrogenic fluctuations in arterial blood pressure. Cerebral hypoxia has been frequently observed after head injury, and is related to poor outcome [12, 28, 53-55]. Several mechanisms have been elucidated, but techniques to monitor these parameters on line are currently lacking and should be developed. Using animal models can serve this goal.

Figure 2 Autoregulation assures stable cerebral blood flow over a range of cerebral perfusion pressures. At the low end CBF will finally drop if perfusion pressure decreases below this threshold. In pathological states, the threshold may increase, resulting in higher blood pressures necessary to keep CBF physiological.



Biochemical derangement

Several putative mechanisms of cellular damage have been postulated, and shown to be active in both the secondary phase after head injury (intrinsic secondary damage) as well as after cerebral ischaemia. Without appearing to be complete several important mechanisms are mentioned below. Tissue acidosis is known to further damage traumatised brain tissue[41, 48, 58]. Excitatory amino acids play an important role in mediating secondary damage [9, 19, 45]. Free oxygen radicals accumulate during the reperfusion phase after ischaemia and damage cell membranes[24, 30]. Lipidperoxidation[20, 25, 43], NO synthetase up-regulation[11, 35, 50, 52], and cytokin surges[15, 21-23, 31, 42] have been demonstrated, and deserve more attention. Calcium accumulation in the cell is considered the final common pathway leading to cell death[4, 39, 40, 51, 57]. Intervention in these mechanisms, though promising in laboratory studies, have failed in clinical trials thus far[36]. It is fair to state that better insight in interrelation of such cellular mechanisms, and identification of the episode in which these are active is required, before the question whether these are potentially amendable to treatment can be raised.

Head injury induces a multitude of pathophysiological mechanisms. Relating cause, course, and consequences of these events to a certain type of head injury requires standardized models of head injury that cover part of the heterogeneous spectrum.

References

1. Adams JH, Graham DI, Murray LS, et al: Diffuse axonal injury due to non missile head injury in humans: an analysis of 45 cases. **Ann Neurol 12**: 1982

2. American Association of Neurological Surgeons, Brain Trauma Foundation: **Guidelines for the management of severe head injury**. The Brain Trauma Foudation Inc.: 1-166, 1995

3. Asgeirsson B, Grände PO, Nordström CH: A new therapy of post-trauma brain oedema based on haemodynamic principles for brain volume regulation. **Intensive Care Med 20**: 260-267, 1994

4. Baethmann A, Jansen M: Possible role of calcium entry blockers in brain protection. **Eur Neurol 25 Suppl 1**: 102-114, 1986

5. Barzó P, Marmarou A, Fatouros PP, et al: Contribution of vasogenic and cellular edema to traumatic brain swelling measured by diffusion weighted imaging. **J Neurosurg 87**: 900-907, 1997

6. Bouma GJ, Muizelaar JP, Bandoh K, et al: Blood pressure and intracranial pressure-volume dynamics in severe head injury: relationship with cerebral blood flow. **J Neurosurg 77**: 15-19, 1992

7. Bouma GJ, Muizelaar JP, Stringer WA, et al: Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. **J Neurosurg 77**: 360-368, 1992

8. Bruce DA, Langfitt TW, Miller JD: Regional cerebral blood flow, intracranial pressure and brain metabolism in comatose patients. **J Neurosurg 38**: 131-144, 1973

9. Bullock R, Zauner A, Woodward JJ, et al: Factors affecting excitatory amino acid release following severe human head injury. **J Neurosurg 89**: 507-518, 1998

10. Cordobes F, Lobato RD, Rivas JJ: Post-traumatic diffuse axonal brain injury. Analysis of 78 patients studied with computed tomography. **Acta Neurochir 81**: 27-35, 1986

11. de Rijck M: Protection of neurological function in stroke model and neuroprotective properties of Lubeluzole. **Cerebrovasc Dis 7**: 18-30, 1997

12. Dings J, Meixensberger J, Amschler J, et al: Brain tissue pO_2 in relation to cerebral perfusion pressure, TCD findings and TCD-CO_2-reactivity after severe head injury. **Acta Neurochir 138**: 425-434, 1996

13. Enevoldson EM, Jensen FT: Autoregulation and CO2 responses of cerebral blood flow in patients with acute severe head injury. **J Neurosurg 48**: 689-703, 1978

14. Firsching R, Woischneck D, Diedrich M, et al: Early magnetic resonance imaging of brainstem lesions after severe head injury. **J Neurosurg 89**: 707-712, 1998

15. Gourin CG, Shackford SR: Production of tumor necrosis factor-alpha and interleukin-1beta by human cerebral microvascular endothelium after percussive trauma. **J Trauma 42**: 1101-1107, 1997

16. Graham DI, Adams JH, Doyle D: Ischaemic brain damage in fatal non missile head injuries. J **Neurosci 39**: 213-234, 1987

17. Graham DI, Ford I, Adams JH, et al: Ischaemic brain damage is still common in fatal non missile head injury. J **Neurol Neurosurg Psychiatry 52**: 346-350, 1989

18. Grände PO, Asgeirsson B, Nordström CH: Aspects on the cerebral perfusion pressure during therapy of traumatic head injury. **Acta Anaesthesiol Scand 110 (Suppl)**: 36-40, 1997

19. Gurevich B, Artru AA, Lam AM, et al: Neuroprotective effects of NPS 846, a novel N-methyl-D aspartate receptor antagonist, after closed head trauma in rats. J **Neurosurg 88**: 1066-1074, 1998

20. Hall ED: Inhibition of lipid peroxidation in central nervous system trauma and ischemia. J **Neurol Sci 134 Suppl**: 79-83, 1995

21. Hans VNJ, Kossmann T, Lenzlinger PM, et al: Diffuse axonal injury triggers neuronal interleukin-6 mRNA synthesis and protein release into cerebrospinal fluid which promotes glial cell chemotaxis. 1998(UnPub)

22. Holmin S: **Inflammation in brain contusion, thesis**. Stockholm, Section of Neurosurgery, Karolinska Institute: 1997

23. Holmin S, Schalling M, Hojeberg B, et al: Delayed cytokine expression in rat brain following experimental contusion. J **Neurosurg 86**: 493-504, 1997

24. Ikeda Y, Long DM: The molecular basis of brain injury and brain edema: the role of oxygen free radicals. **Neurosurgery 27**: 1-11, 1990

25. Inci S, Ozcan OE, Kilinc K: Time-level relationship for lipid peroxidation and the protective effect of alpha-tocopherol in experimental mild and severe brain injury. **Neurosurgery 43**: 330-5; discussi, 1998

26. Junger EC, Newell DW, Grant GA, et al: Cerebral autoregulation following minor head injury. J **Neurosurg 86**: 425-432, 1997

27. Kelly DF, Kordestani RK, Martin NA, et al: Hyperemia following traumatic brain injury: relationship to intracranial hypertension and outcome. J **Neurosurg 85**: 762-771, 1996

28. Kiening KL, Unterberg AW, Bardt TF, et al: Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO2 versus jugular vein oxygen saturation. J **Neurosurg 85**: 751-757, 1996

29. Klatzo I: Neuropathological aspects of brain edema. J **Neuropathol Exp Neurol 26**: 1-14, 1967

Kontos HA, Wei EP: Superoxide production in experimental brain injury. J **Neurosurg 64**: 803-807, 1986

31. Kossmann T, Stahel PF, Lenzlinger PM, et al: Interleukin-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. J **Cereb Blood Flow Metab 17**: 280-289, 1997

32. Kroppenstedt SN, Kern M, Thomale UW, et al: Effect of cerebral perfusion pressure on contusion volume following impact injury. J **Neurosurg 90**: 520-526, 1999

33. Langfitt TW, Tannanbaum HM, Kassel NF: The etiology of acute brain swelling following experimental head injury. **J Neurosurg 24**: 47-56, 1966

34. Langfitt TW, Weinstein JD, Kassel NF: Cerebral vasomotor paralysis produced by intracranial hypertension. **Neurology 15**: 622-641, 1965

35. Lesage AS, Peeters L, Leysen JE: Lubeluzole, a novel long-term neuroprotectant, inhibits the glutamate-activated nitric oxide synthase pathway. **J Pharmacol Exp Ther 279**: 759-766, 1996

36. Maas AIR, Steyerberg EW, Murray G, et al: Why have recent trials of neuroprotective agents in head injury failed to show convincing efficacy? A pragmatic analysis and theoretical considerations. **Neurosurgery 44**: 1286-1298, 1999

37. Marmarou A, Shulman K, Rosende RM: A nonlinear analysis of the cerebrospinal fluid system and intracranial pressure dynamics. **J Neurosurg 48**: 332-344, 1978

38. Maset AL, Marmarou A, Ward JD, et al: Pressure-volume index in head injury. **J Neurosurg 67**: 832-840, 1987

39. McBurney RN, Daly D, Fischer JB, et al: New CNS-specific calcium antagonists. **J Neurotrauma 9 Suppl 2**: S531-S543, 1992

40. McIntosh TK, Juhler M, Wieloch T: Novel pharmacologic strategies in the treatment of experimental traumatic brain injury: 1998. **J Neurotrauma 15**: 731-769, 1998

41. Menzel M, Doppenberg EM, Zauner A, et al: Increased inspired oxygen concentration as a factor in improved brain tissue oxygenation and tissue lactate levels after severe human head injury. **J Neurosurg 91**: 1-10, 1999

42. Morganti-Kossmann MC, Kossmann T: The immunology of brain injury, in Rothwell NJ (ed): **Immune responses in the nervous system.** Oxford, Bios Scientific Publishers Ltd.: 159-187, 1995

43. Nishio S, Yunoki M, Noguchi Y, et al: Detection of lipid peroxidation and hydroxyl radicals in brain contusion of rats. **Acta Neurochir Suppl (Wien) 70**: 84-86, 1997

44. Obrist WD, Langfitt TW, Jaggi JL, et al: Cerebral blood flow and metabolism in comatose patients with acute head injury. Relationship to intracranial hypertension. **J Neurosurg 61**: 241-253, 1984

45. Okiyama K, Smith DH, Gennarelli TA, et al: The sodium channel blocker and glutamate release inhibitor BW1003C87 and magnesium attenuate regional cerebral edema following experimental brain injury in the rat. **J Neurochem 64**: 802-809, 1995

46. Overgaard J, Tweed W: Cerebral circulation after head injury. Part 1: Cerebral blood flow and its regulation after closed head injury with emphasis on clinical correlations. **J Neurosurg 41**: 531-541, 1974

47. Robertson CS, Narayan RK, Gokaslan ZL, et al: Cerebral arteriovenous oxygen difference as an estimate of cerebral blood flow in comatose patients. **J Neurosurg 70**: 222-230, 1989

48. Rosner MJ, Becker DP: Experimental brain injury: succesful therapy with the weak base, tromethamine. **J Neurosurg 60**: 961-971, 1984

49. Rosner MJ, Rosner SD, Johnson AH: Cerebral perfusion pressure: management protocol and clinical results. J **Neurosurg 83**: 949-962, 1995

50. Sakamoto KI, Fujisawa H, Koizumi H, et al: Effects of mild hypothermia on nitric oxide synthesis following contusion trauma in the rat. J **Neurotrauma** 14: 349-353, 1997

51. Siesjo BK, Siesjo P: Mechanisms of secondary brain injury. **Eur J Anaesthesiol** 13: 247-268, 1996

52. Sinz EH, Kochanek PM, Dixon CE, et al: Inducible nitric oxide synthase is an endogenous neuroprotectant after traumatic brain injury in rats and mice. J **Clin Invest** 104: 647-656, 1999

53. Valadka AB, Gopinath SP, Contant CF, et al: Relationship of brain tissue pO2 to outcome after severe head injury. **Crit Care Med 26(9)**: 1576-1581, 1998

54. van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Monitoring brain oxygen tension in severe head injury, early cerebral hypoxia is related with an unfavorable outcome. **Neurosurgery 46**: 868-878, 2000

55. van Santbrink H, Maas AIR, Avezaat CJJ: Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. **Neurosurgery 38**: 21-31, 1996

56. Willberger JE, Rothfus WE, Tabas J, et al: Acute tissue tear hemorrhages of the brain: Computed tomography and clinicopathological correlations. **Neurosurgery 27**: 208-213, 1990

Xiong Y, Peterson PL, Muizelaar JP, et al: Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. J **Neurotrauma** 14: 23-34, 1997

58. Yoshida K, Marmarou A: Effects of tromethamine and hyperventilation on brain injury in the cat. J **Neurosurg 74**: 87-96, 1991

Chapter 3

Animal models of traumatic brain injury

Biological models of head injury attempt to duplicate, in a reproducible manner, the loading conditions, neurological syndromes, and pathophysiology that occur in man. They serve, along with physical and analytical models, as one facet in developing a comprehensive understanding of the response of the brain and its coverings to mechanical trauma.

Thomas A. Gennarelli,
Central Nervous System Trauma Status Report, 1985

3.1 Introduction

Careful clinical observation and invasive monitoring of head injured patients in the ER, OR, and NICU provides direct information on the course of the disease. Head injuries are heterogeneous by nature, however. Mechanisms causing traumatic brain injury include localized damage to the cortex by, for instance, missile wounds, depressed skull fractures, and tissue lacerations. At the other end of the spectrum, diffuse brain injuries with or without brain swelling can be observed. To make this issue even more complex, combinations of localized and diffuse injury are not infrequently encountered. Observations in one group of patients can therefore not be generalized to the entire population. Standardized studies of traumatic brain injury, to elucidate mechanisms of (secondary) injury, to optimize monitoring modalities, and to develop therapeutic regimens, can only be performed in rigid bench test situations. These experiments exclude variability with respect to injury mechanism and pathophysiological response.

The development of one head injury model covering all pathophysiological entities encountered in the clinical situation is impossible, and not desirable. For example studies of penetrating head injury require different models than that of diffuse axonal injury. The objective of the investigator, and his scientific questions, will shape the development of the model used. Different objectives will thus result in different, but equally valid models, each appropriate to specific questions. Certain criteria are required for all models. The following hypothesis is implicit to brain injury modelling: "Part of the spectrum of human traumatic brain injury can be duplicated in non humans". This hypothesis allows for the assumption that inferences from one specific animal model can be made to the human condition. These inferences have their limitations. The main difficulties concern the difference in neuraxic structure, and the inability to test complex behavioral deficits. Furthermore, possible differences in receptors, neurotransmitters, and modes of signal transduction can not be neglected. Patho-anatomical, patho-physiological, neurobiological, and relatively simple behavioral observations can be performed in the laboratory setting.

Ideally, the mechanical load will resemble the biomechanics of human head injury. An important prerequisite for experimental modelling remains: "The induced response to trauma must be reproducible, and quantifiable covering a clinically relevant continuum". Of pivotal importance is the strive for minimal variability caused by animal characteristics, physiological parameters, anesthesia, and loading forces. Quantification of mechanical loading conditions, will facilitate the standardization of input parameters causing traumatic brain injury. The use of the laboratory rat has standardized animal characteristics. Inbred strains provide exact knowledge of genetic, physiological, and behavioral variables. Considering purchase price, housing, and anesthetic, isotope, and pharmacon use, these animals are economically efficient.

Utilising a head injury model provides the neuroscientist with the unique opportunity to study standardized traumatic brain injury; by these means pathophysiology can be elucidated, monitoring techniques can be optimised, and therapeutic regimens tested.

In vivo models of head injury.

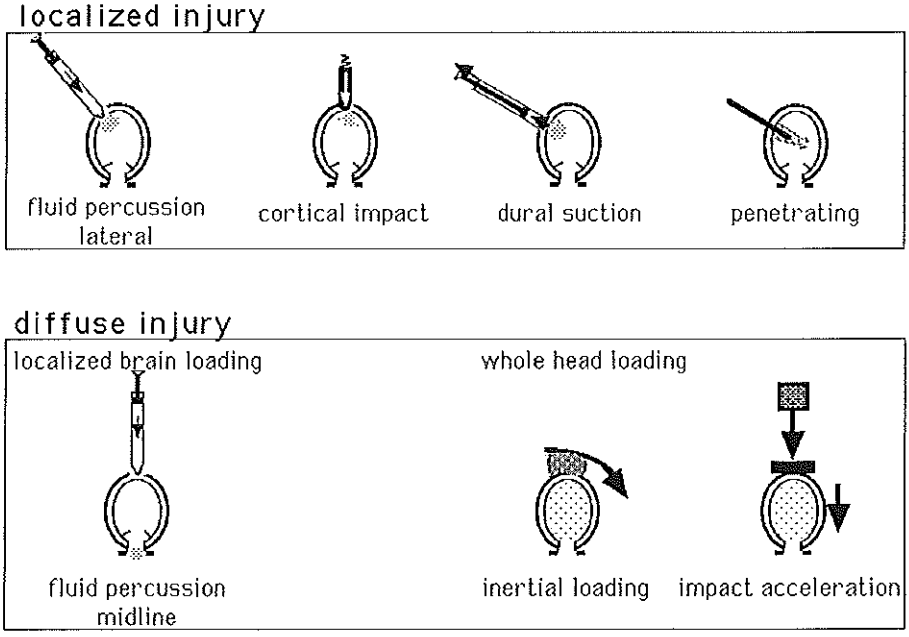
Animal models developed to mimic human brain injury can be divided into models that exert brain injury by means of mechanical loading of the brain, and models that utilise thermal or chemical stimuli. Only the former will be discussed. Since the early work of Denny Brown and Russell [6] experimental diffuse head injury can be submitted by means of percussion concussion and acceleration concussion. Current models can still be classified according to this scheme. Acceleration injury is induced by whole head loading, which can be exerted by means of impact or inertial forces. Localized brain loading, on the other hand, can result in diffuse and brain stem injury as in midline fluid percussion models, or in localized contusion as in lateral fluid percussion, cortical impact, and dural suction models. The resulting type of injury is often considered more important than the actual mechanism causing this injury [12], a classification of existing models is presented in figure 1. A historical overview and details of head injury models is presented in table 1. The utility of the most recent models to replicate part of the spectrum of human head injuries is presented in table 2. It is noteworthy to mention that small changes in imput parameters of most models will alter the type and grade of brain injury produced. These small changes can be used to shape a model to the scientific needs of the investigator.

A continuous development of head injury models will aid the investigator to make an optimal choice for the model most suited to answer his scientific questions.

3.2 Localized brain injury

Models of focal brain contusions are developed by exerting a standardized force directly to the exposed dura or brain. Cortical damage can be produced by lateral fluid percussion, weight drop, pneumatic indentation, or penetration models.

Figure 1 Classification of existing models of head injury. Both the type of injury (localized versus diffuse), and mechanism injury are displayed in the cartoons. The Closed Head Injury model described in this thesis is of the diffuse impact acceleration type.



Lateral Fluid Percussion Injury.

The fluid percussion injury (FPI) model, was first developed by Lindgren [20], and further adapted for use in cats and rats by Sullivan [30] and Dixon [8]. The model has gone through several stages of standardization and control of imput forces, though the principle remained the same. A rigid fluid filled cylinder craniectomy is connected to the intact dura through a craniectomy. The opposite side is sealed with a piston, which is struck by a falling pendulum. The pendulum causes a sudden increase in fluid pressure transmitted to the brain. Midline FPI models cause predominant brain stem damage, even at moderate levels of injury because of tissue strains amplified in the foramen magnum. McIntosh[23] developed the lateral FPI model which also causes cerebral contusions. Predictable neurological, physiological, and behavioral changes can be produced in a graded way with both models.

Cortical impact injury.

Direct rigid percussion of the cortical surface causes cerebral contusion, the size of which is dependent on the velocity, surface, and depth of the indentation[7, 11]. The extent of local damage can be titrated by careful changes of these parameters. Another means of inducing contusions in a mechanical way is by suction to the intact dura, Mathew[22] recently reported development of the contusions similar to those observed in the human situation.

Penetrating injury.

Penetrating brain injury is an entity in its own. Such models have been developed to study missile and non missile injury. Both local lacerations and general derangement of cerebral perfusion pressure, blood flow and metabolism have been reported[4,5].

3.3 Diffuse brain injury

Diffuse injury can be exerted by both fluid percussion and acceleration injury. Similar tissue strains can be expected in both models. Assuming that the type of injury produced is more important than the mechanism causing traumatic brain injury [12], has resulted in the development of several models of midline fluid percussion injury [8, 20, 30], to mimic diffuse injury. Brain stem damage is predominant in fluid percussion injury. From a mechanical point of view, a whole head loading model is preferable. Whether such loading results in acceleration, or compression, or penetration of the skull is dependent on the biodynamics of the collision, the so called loading conditions. In this aspect the mechanical input may be applied slowly (static loading, > 200 msec), or rapidly (dynamic loading, < 200 msec). Static loading, as can occur in crush injuries, by for instance a trash compactor, is rare. Models mimicking this type of injury have not been well developed. In exerting a dynamic load to the brain, both impact mediated, as well as inertial mediated skull acceleration have been applied. Contact phenomena, occurring after impact, are associated with compression, bending or even penetration of the skull, and with propagation of shock waves through brain tissue. Acceleration of the brain, occurring after both impact and inertial loading, results in tissue strains, either compressive, tensile, or shearing [figure 2]. Magnitude and location of these strains are dependent on direction of applied forces and dimensions of the skull.

Table 1 A comprehensive review of head injury models developed in the past. The models have been classified as localized, diffuse fluid percussion, and diffuse acceleration models. The name of the model, device used, species, first author, year of publication, and some specific characteristics are tabulated.

Historical overview of animal models of traumatic brain injury

models of localized brain injury								
model	*device*	*species*	*author*	*year*	*reference nr*	*penetrating*	*injury mechanism*	*pathological sequelae*
fluid percussion	lateral FPI	rat	McIntosh	1989	23	-	fpi	haemorrhage, cavitation, vascular disruption
concussion	air pressure	dog	Gurdjian	1954	16	-	air percussion	
cortical impact	weight drop	rat	Feeney	1981	11	-	mechanical percussion	contusion, cavitation
cortical impact	pneumatic impactor	ferret	Lighthall	1988		-	mechanical percussion	contusion
cortical impact	pneumatic impactor	rat	Dixon	1991	7	-	mechanical percussion	contusion
suction impact	dural suction	rat	Matthew	1996	22	-	vacuum suction	contusion, cellular swelling, CBF reduction
missile	lab gun	cat	Carey	1989	4	+	penetration	contusion
missile	air-rifle	monkey	Crockard	1977	5	+	penetration	contusion, brain swelling, brain stem effects
subdural haematoma	blood application	rat	Duhaime	1994	9	-	blood substrates	no injury, no ischaemia
subdural haematoma	blood injection	rat	Miller	1990	24	-	fluid volume & blood substrates	subdural haematoma, ischaemia, exitotoxic aminoacids

Historical overview of animal models of traumatic brain injury (continued)

models of diffuse brain injury (percussion-concussion)

model	*device*	*species*	*author*	*year*		*injury mechanism*	
fluid percussion	midline FPI	rabbit	Lindgren	1966	20	fpi	dai, brain stem
fluid percussion	midline FPI	cat	Sullivan	1976	30	fpi	SAH, brain stem, cerebellar tonsils
fluid percussion	midline FPI	rat	Dixon	1987	8	fpi	dai, brain stem
fluid percussion	midline FPI	micropig	Povlishock	1993		fpi	dai, brain stem

models of diffuse brain injury (acceleration-concussion)

model	*device*	*species*	*author*	*year*		*contact*	*head mobile*	*con-straint*	*impact*	*Accele-ration*	
Gehirn-shütterung	blow	frog	Witkowski	1877	33	+	+	-	+	+	concussion, pial vasoconstriction
cerebral concussion	pendulum	cat/dog/ monkey	Denny-Brown	1941	6	+	+	+	+	+	contusion, brainstem punctate hemorrhages
head trauma	piston	monkey	Gurdjian	1954	16	+	+	-	+	+	contusion
head injury	pendulum	cat	Langfitt	1966	19	+	+	+	+	-	contusion
	stunner	rat	Bean	1969	3	+	+	+	+	+	pulmonary pathology
experimental cerebral concussion	coil spring gun	rat	Ommaya	1971	27	+	+	-	+	+	concussion and learning deficits
whiplash	whiplash	monkey	Ommaya	1971	26	-	+	-	-	+	
brain concussion	bow and dowel	rat	Govons	1972	15	+	+	-	+	+	fractures
closed head injury	impact sled	monkey	Kobrine	1973	18	+	+	+	+	+	fractures, contusion, brain swelling

Historical overview of animal models of traumatic brain injury (continued)

models of diffuse brain injury (acceleration-concussion)

model	*device*	*species*	*author*	*year*		*contact*	*head mobile*	*con-straint*	*impact*	*Accele-ration-*	
	pendulum	rat	Bakay	1977	2	+	+	-	+	+	brain stem, mitochondrial swelling
impact acceleration	pneumatic	rat	Nilsson	1977	25	+	+	-	+	+	SAH, brain stem heamorrhages
concussive head injury	whole animal acceleration	rat	Huger	1979	17	+	+	-	+	+	SAH, brain stem, cathecholamine increases
head injury	occilation/vibration	cat	Nelson	1979		-	+	+	-	-	
angular acceleration	Penn II device	monkey	Gennarelli	1981	13	-	+	+	-	+	dai
closed head injury	stunner	cat	Tornheim	1983	32	+	+	-	+	+	contusion
experimental concussion	spring loaded knob	rat	Shaw	1985	29	+	+	-	+	+	no gross pathology, slight SAH
closed head injury	weight drop	rat	Shapira	1988	28	+	-	+	+	-	contusion
closed skull impact	pneumatic	rat	Dixon	1994		+	-	+	+	-	contusion
closed head injury	weight drop	rat	Marmarou	1994	21	+	+	+	+	+	dai, SAH, brain stem petechiae and edema
modified closed head injury	weight drop	rat	Engelborghs	1998	10	+	+	+	+	-	dai, SAH, frontobasal contusions

Table 2 Estimated value of replication of human head injury for the most important models developed. The CHI model described in this thesis is a model causing neuronal loss and diffuse axonal injury with some extent of subarachnoid hemorrhage. (modified from Genarelli, T.A.[12])

utility of models in replicating human traumatic brain injury										
					alterations in:					
model	*contusion*	*SAH*	*ASDH*	*ICH*	*neuronal loss*	*axonal injury*	*BBB*	*metabolic*	*CBF*	*vascular response*
fluid percussion midline	+	++	-	+	+	+	++	++	++	+
fluid percussion lateral	+	++	-	+	++	+	++	++	++	+
cortical impact	++	++	+	+	+	+	++	?	?	?
cortical suction	++	-	-	-	+	-	-	?	+	?
inertial acceleration	+++	+++	+++	+++	+++	+++	?	?	?	?
impact acceleration	±	++	-	+	++	++	++	++	++	?

± = inconsistent
? = no data exist
+ = duplicates to some degree
++ = duplicates with greater fidelity
+++ = duplicates with greatest fidelity

Inertial acceleration.

A model for constrained head movement without impact has been developed by Gennarelli and Thibault[13]. The so called 'Penn injury devices' induce highly controlled rotational acceleration of various magnitudes, resulting in exactly reproducible loading conditions. It was shown that widespread diffuse axonal damage results mainly of rotational acceleration in the coronal plane. Concussive states were attributed to widespread axonal damage. While translational accelerations did cause contusions, without loss of consciousness. High costs of both device and animal (primates), however, forestall widespread use.

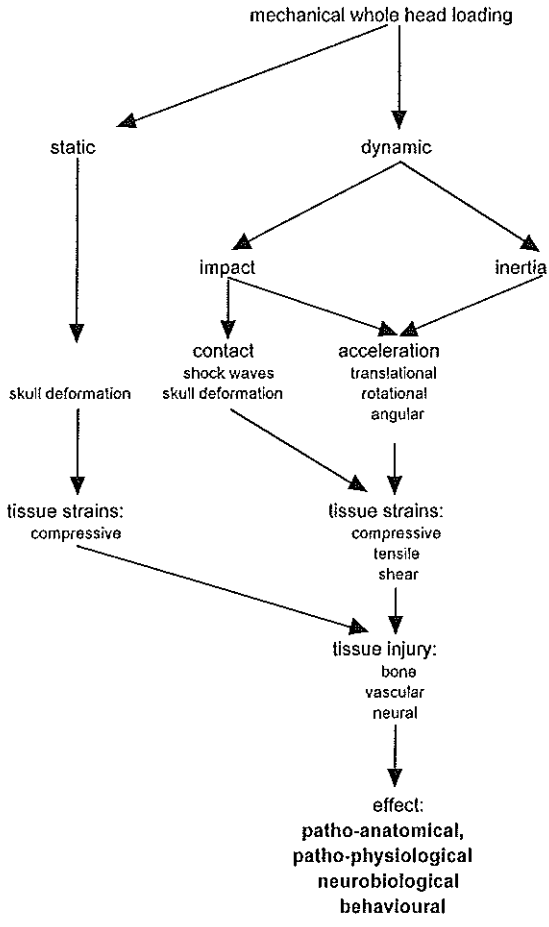
Impact acceleration.

After the initial experiments of Witkowski[33], in which an uncontrolled blow was delivered to the frog head, Denny-Brown and Russell [6] pioneered concussive injury by means of controlled impact to the unrestrained head of non human primates. They observed that with free movement of the head concussion was more likely to occur than would be the case with fixation of the skull, emphasising the importance of acceleration. Gurdjian[16] made an effort to obtain impact force tresholds for concussion, coma, and contusion. With the application of a helmet larger forces, resulting in higher acceleration, could be applied without producing skull fractures. Free head movement results in virtually unlimited degrees of freedom for three dimensional head movement which are not well controllable. Constraining head movement to a single plane improved the gradation and reproducibility of impact models only to a limited extent.

The Closed Head Injury model in the rat.

Utilisation of laboratory rats provided the possibility to perform a larger number of experiments to compensate for part of the reproducibility problem. Several limitations inherent to the use of small animals in impact-acceleration experiments were observed. The most important one being the fracture rate when inducing accelerations severe enough to cause concussive brain injury [14, 31]. To overcome this problem Marmarou [21] developed a model in which the forces directed to the skull are evenly distributed over a larger surface by means of a cemented metal disc. Placing the rodent head on a foam support, provided acceleration and partial constraint. Another problem, more difficult to overcome, is the so called on-off phenomenon. Small animals tend to either succumb, or survive without notable deficits. This fine line can be crossed by subtle changes in experimental design, impairing the introduction of gradable injury. This thesis reports some patho-physiological, and neurobehavioral sequelae of this model focused on several aspects considered important in the development of a new model. Both relevance to the elucidation of basic mechanisms, utilisation of advanced monitoring techniques, and the influence of secondary insults to the brain were considered in the experimental paradigms.

Figure 2 Mechanisms of brain tissue damage after whole head loading. In the Closed Head Injury Model described in this thesis acceleration is the most important factor causing tissue strains, and injury.



Both localized and diffuse head injury can be studied with a variety of standardized models; the author of this thesis has studied several aspects of diffuse head injury utilising the Closed Head Injury model in the rat.

References

1. Adams JH, Gennarelli TA, Graham DI, et al: Diffuse axonal injury in non-missile head injury, in Villiani R (ed): **Advances in neurotraumatology**. Amsterdam, Excerpta Medica: 53-58, 1983

2. Bakay L, Lee JC, Lee GC, et al: Experimental cerebral concussion. Part I: an electron microscopic study. **J Neurosurg** 47: 525-531, 1977

3. Bean JW, Beckman DL: Centrogenic pulmonary pathology in mechanical head injury. **J Appl Physiol** 27: 807-812, 1969

4. Carey ME, Gurcharan SS, Farrell JB: Brain edema following an experimental missile wound to the brain. **J Neurotrauma** 7: 13-20, 1990

5. Crockard HA, Brown FD, Calica AB, et al: Physiological consequences of experimental cerebral missile injury and use of data analysis to predict survival. **J Neurosurg** 46: 784-794, 1977

6. Denny Brown D, Russell WR: Experimental cerebral concussion. **Brain** 64: 2-3, 1941

7. Dixon CE, Clifton GL, Lighthall JW, et al: A controlled cortical impact model of traumatic brain injury in the rat. **J Neurosci Meth** 39: 253-262, 1991

8. Dixon CE, Lyeth BG, Povlishock JT, et al: A fluid percussion model of experimental brain injury in the rat. **J Neurosurg** 67: 110-119, 1987

9. Duhaime AC, Gennarelli LM, Yachnis A: Acute subdural hematoma: Is the blood itself toxic? **J Neurotrauma** 11: 669-678, 1994

10. Engelborghs K, Verlooy J, van Reempts J, et al: Temporal changes in intracranial pressure in a modified experimental model of closed head injury. **J Neurosurg** 89: 796-806, 1998

11. Feeney DM, Boyeson MG, Linn RT, et al: Responses to cortical injury, I: Methodology and local effects of contusions in the rat. **Brain Res** 211: 67-77, 1981

12. Gennarelli TA: Animate models of human head injury. **J Neurotrauma** 4: 357-368, 1994

13. Gennarelli TA, Thibault LE: Experimental production of prolonged traumatic coma in the primate, in Villiani R (ed): **Advances in Neurotraumatology**. Amsterdam, Excerpta Medica: 31-33, 1982

14. Gennarelli TA, Thibault LE: Biological models of head injury, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report**. Bethesda, MD, NINCDS: 391-404, 1985

15. Govons SR, Govons RB, Van Huss WD, et al: Brain concussion in the rat. **Exp Neurol** 34: 121-128, 1972

16. Gurdjian ES, Lissner HR, Webster JE, et al: Studies on experimental concussion, relation of physiologic effect to time duration of intracranial pressure increase at impact. **Neurology** 4: 674-681, 1954

17. Huger F, Patrick G: Effects of concussive head injury on central cathecholamine levels and synthesis rates in rat brain regions. **J Neurochem 33**: 89-95, 1979

18. Kobrine AI, Kempe LG: Studies in head injury. Part I: An experimental model of closed head injury. **Surg Neurol** 1: 34-37, 1973

19. Langfitt TW, Tannanbaum HM, Kassel NF: The etiology of acute brain swelling following experimental head injury. **J Neurosurg 24**: 47-56, 1966

20. Lindgren S, Rinder L: Experimental studies in head injury. II. Pressure propagation in "percussion concussion". **Biophysik 3**: 174-180, 1966

21. Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. **J Neurosurg 80**: 291-300, 1994

22. Mathew P, Bullock R, Graham DI, et al: A new experimental model of contusion in the rat. **J Neurosurg 85**: 860-870, 1996

23. McIntosh TK, Vink R, Noble LJ, et al: Traumatic brain injury in the rat: Characterization of a lateral fluid percussion model. **Neuroscience 28**: 233-244, 1989

24. Miller JD, Bullock R, Graham DI, et al: Ischaemic brain damage in a model of acute subdural hematoma. **Neurosurgery 27**: 433-439, 1990

25. Nilsson B, Ponten U, Voigt G: Experimental head injury in the rat. Part 1: Mechanics, pathophysiology, and morphology in an impact acceleration trauma model. **J Neurosurg 47**: 241-251, 1977

26. Ommaya AK: Experimental head injury in the monkey, in Caveness WF, Walker AE (eds): **Head injury conference proceedings**. Philadelphia, J. B. Lippincott Co.: 260-275, 1966

27. Ommaya AK, Geller A, Parsons LC: The effect of experimental head injury on one-trial learning in rats. **Int J Neurosci 1**: 371-378, 1971

28. Shapira Y, Shohami E, Sidi A, et al: Experimental closed head injury in rats: Mechanical, pathophysiological, and neurological properties. **Crit Care Med 16**: 258-265, 1988

29. Shaw NA: A simple device for experimental concussing the rat. **Physiol Behav 35**: 637-639, 1985

30. Sullivan HG, Martinez J, Becker DP: Fluid percussion model of mechanical brain injury in the cat. **J Neurosurg 45**: 520-534, 1976

31. Thibault LE, Gennarelli TA: Biomechanics and craniocerebral trauma, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report**. Bethesda, MD, NINCDS: 379-389, 1985

32. Tornheim PA, Prioleau GR, McLaurin RL: Acute responses to experimental blunt head trauma. **J Neurosurg 60**: 473-480, 1984

33. Witkowski L: Ueber Gehirnschütterung. **Virchows Arch A Pathol Anat Histopath 69**: 498-516, 1877

Aims of this thesis

The main purpose of the work summarized in this thesis is the development of a new model of diffuse head injury, the 'Closed Head Injury model'. As many models are either hampered by general disadvantages of larger animals, by pressure from animal rights committees, or by major pathophysiological limitations, a model devoid of these drawbacks is required. In the Closed Head Injury model mechanical similarities with the most important cause of traumatic brain injury, acceleration of the brain by sudden impact of the head, are obtained by weight drop. This method causes high levels of skull acceleration, the resulting traumatic brain injury thereof requires evaluation.

To this end, the model was characterized with respect to parameters considered most important in the clinical situation. A comparison with another well established model of diffuse brain injury, the Fluid Percussion model, was made for studies of several parameters.

Secondary global hypoxia was introduced in the experimental design to investigate its influence on cerebral oxygenation, and on animal outcome. The technique and pitfalls, of intra parenchymal $PbrO_2$-monitoring were evaluated in the experimental situation.

The clinical importance of early cerebral hypoxia was evaluated in severely head injured patients.

;

Chapter 4

A new model of diffuse brain injury in rats pathophysiology and biomechanics

The art of progress is to preserve order amid change
and to preserve change amid order. ***Alfred North Whitehead***

Abstract

This report describes the development of an experimental head injury model capable of producing diffuse brain injury in the rodent. A total of 161 anesthetized adult rats were injured utilizing a simple weight drop device consisting of a segmented brass weight free-falling through a plexiglass guide tube. Skull fracture was preved by cementing a small stainless-steel disc on the calvaria. Two groups of rats were tested: Group I, consisting of 54 rats, to establish fracture treshold; and group 2, consisting of 107 animals, to determine the primary cause of death at severe injury levels. Data from group 1 animals showed that a 450-gm weight falling from a 2-m height (0.9 kg-m) resulted in a mortality rate of 44% with low incidence (12.5%) of skull fracture. Impact was followed by apnea, convulsions, and moderate hypertension. The surviving rats developed decortication flexion deformity of the forelimbs, with behavioral depression and loss of muscle tone. Data from group 2 animals suggested that the cause of death was due to central respiratory depression; the mortality rate decreased markedly in animals mechanically ventilated during the impact. Analysis of mathematical models showed that this mass-height combination resulted in a brain acceleration of 900 G and a brain compression gradient of 0.28 mm. It is concluded that this simple model is capable of producing a graded brain injury in the rodent without a massive hypertensive surge or excessive brain-stem damage.

Introduction

Diffuse brain injury is associated with high mortality and morbidity rates, and recent studies by the Traumatic Coma Data Bank study group[11] show that 55% of patients comatose on admission suffer from diffuse brain injury, with 12.6% presenting with a normal computerized tomography scan. This type of injury has been difficult to study in the laboratory as present models, such as fluid-percussion or cortical impact, produce a focal brain contusion[5, 15, 26] and are associated with relatively minimal supratentorial axonal injury. At higher trauma levels, the fluid percussion model produces a significant brain-stem

injury and mechanical studies have shown that the region of maximum tissue strain is focused in the brain stem[25]. This occurs as a result of the mechanical force imposed upon the exposed dura and the resulting fluid volume introduced into the cranial vault[5]. We reasoned that a higher degree of injury could be produced if direct dural impact could be avoided and the mechanical insult delivered to the intact cranium.

This report describes the development of a new rodent closed head injury model in which the skull is protected to prevent fracture. This allows higher impact-acceleration levels to be achieved, which our companion paper has shown to result in a pronounced diffuse brain injury[1]. The first objective of this study was to identify the trauma levels that would induce mild head injury (with no mortality) and severe head injury (about 50% mortality rate) with a low incidence of skull fracture. The second objective was to begin isolating the cause of death in nonsurvivors in the group of severely head-injured rats. The third objctive was to develop a mathematical model to determine the level of acceleration achieved by the two degrees of impact producing the mild and severe head injuries.

Materials and Methods

Based on the analysis of our mathematical models, it was determined that one approach to obtaining high acceleration upon impact would be to lightly support the head in order to permit displacement immediately following impcat. Thus, the first series of rats was injured with a simple weight-drop device, and studied to determine the weight-height combination that resulted in a mortality rate of approximately 50% in nonventilated animals (Group 1). Having established this level, it was noted that nonsurvivors experienced prolonged apnea. Thus, a second series of rats (Group 2) was studied to establish whether this respiratory failure was related to a peripheral or central process.

Trauma Device

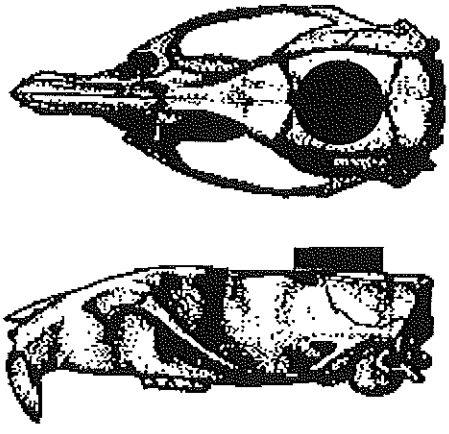
The trauma device consists of a column of brass weights falling freely by gravity onto a metallic helmet fixed to the skull vertex of the rat by dental acrylic. The brass weights, each 50 gm, were threaded so that they could be connected to produce a falling weight ranging from 50 to 500 gm. From a designated height, the weight falls through a 2-m vertical section of a transparent Plexiglas tube held in place with a ring stand. The helmet is a stainless-steel disc 10 mm in diameter and 3 mm thick. The contact side of the disc is grooved concentrically to accept acrylic and firm the contact.

Induction of Head Trauma

The scalp of the anesthetized animal was shaved, a midline incision was performed, and the periosteum covering the vertex was reflected. A stream of air was used to keep the area dry. The metallic disc was fixed to the central

portion of the skull vault of the rat between the coronal and the lambdoid sutures [Figure 1]. The animal was placed in a prone position on a foam bed of known spring constant contained within a Plexiglas frame [Figure 2] and secured in place with two belts. The lower end of the Plexiglas tube was then positioned directly above the helmet. The injury was delivered by dropping the weight from a predetermined height. Rebound impact was prevented simply by sliding the Plexiglas box (foam bed) containing the animal away from the tube immediately following the initial impact. All animal protocols were reviewed and approved by an internal animal review board and were in compliance with guidelines set forth by the National Institutes of Health.

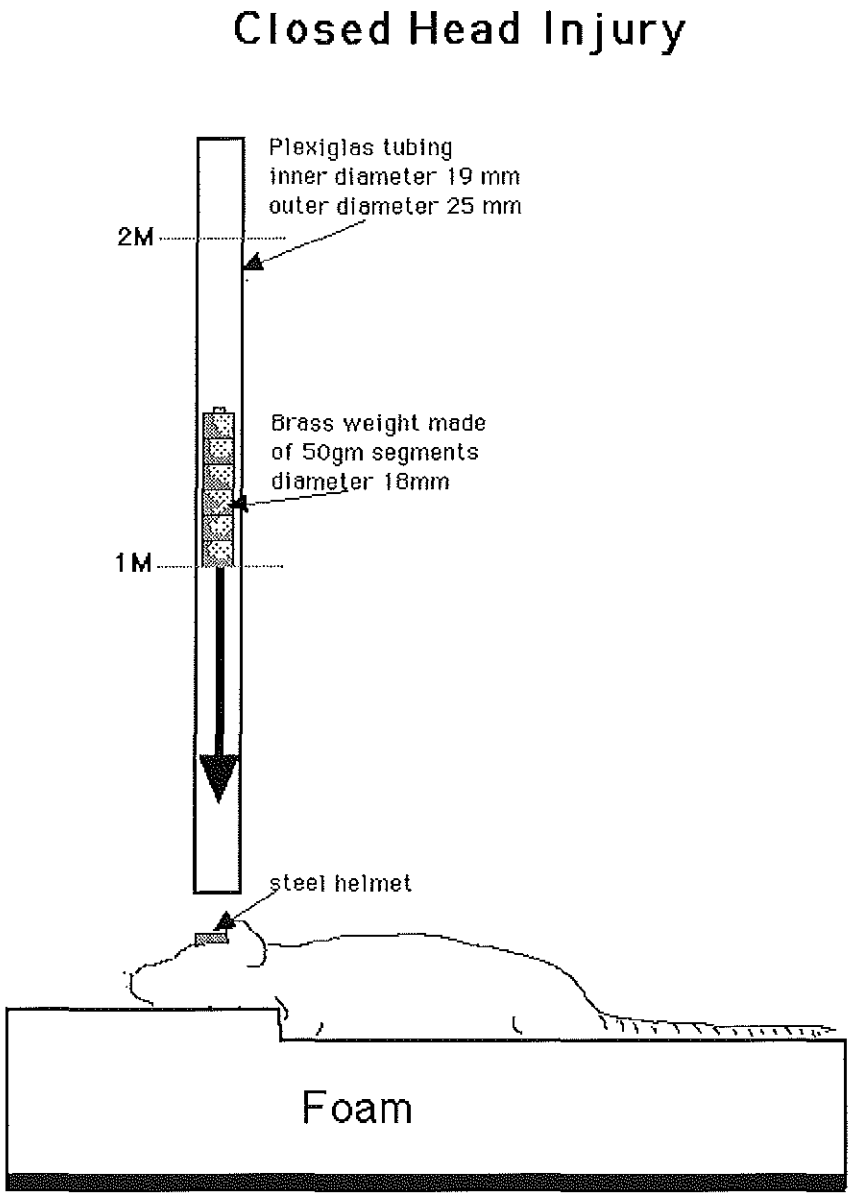
figure 1 Diagram of the rodent skull illustrating the positioning of the protective stainless-steel disc.



Group 1 Study

In Group 1, 54 adult Sprague-Dawley rats were anesthetized using methoxyflurane followed by intraperitoneal injections of alpha-chloralose (initial dose 90 mg/kg, maintenance dose 45 mg/kg). The animals were allowed to breathe spontaneously without tracheal intubation. The area of the inner thigh was shaved and the meforal artery was connulated for blood pressure monitoring. Rectal body temperature was maintained at 37 ° ± 0.5 ° C using a heat lamp. Respiration and heart rate were also monitored and recorded on a strip chart. Following the procedure described before, two different impacts (450 gm and 500 gm from a height of 2 m) were used to determine the upper limit that would produce a mortality rate of approximately 50% with a low incidence of skull fracture. Having established this severe head injury level, the height was reduced to 1 m, decreasing the energy delivery by 50% in order to induce a moderate head injury.

figure 2 Diagram of the head injury device. The upper weight is attached to a string and the segmented brass weights elevated to the desired height. The bottom opening of the Plexiglass cylinder is positioned in close proximity to the head of the rat and centered for the mass to strike directly upon the helmet. The helmet consists of a stainless-steel disc, 10 mm in diameter and 3 mm thick, cemented to the calvaria with a thin layer of acrylic. The foam (of known spring constant) is cut to fit in the Plexiglas frame without being compressed. After release of the weight and contact, the Plexiglas frame is removed rapidly to prevent a second impact



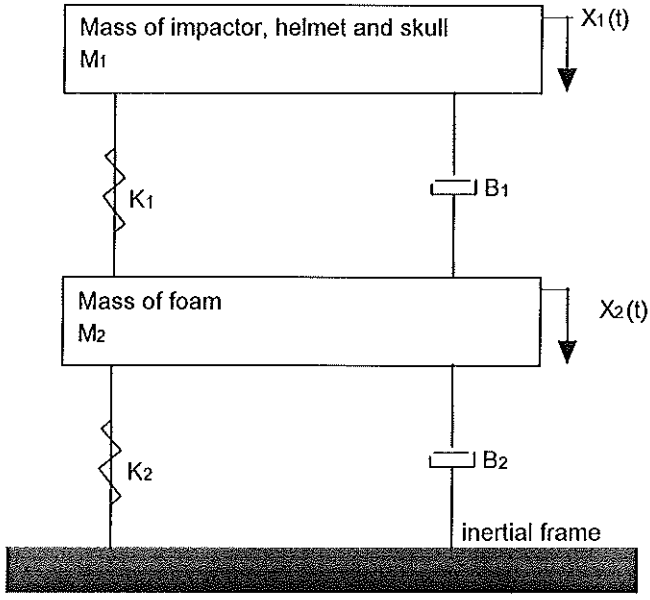
Group 2 Study

The 107 animals in Group 2 were anesthetized using isoflurane (1% to 2%) in a 33% oxygen - 66% nitrous oxide mixture. The rats were surgically prepared for trauma as previously described for the Group 1 animals. Severe head injury was induced using the 450 gm-2m weight-height impact. The animals in this group were subdivided into three subgroups: Group 2A, anesthetized via a mask without tracheal intubation and allowed to breathe spontaneously; Group 2B, intubated and allowed to breathe spontaneously; and Group 2C, intubated and mechanically ventilated during and after trauma. In addition, another two groups of animals were anesthetized using a mixture of halothane (1% to 2%) in a 2: 1 mixture of nitrous oxide and oxygen in order to study the changes in blood gas levels after this type of head injury. In the first group (Group 2D) 25 animals were anesthetized via a mask; six were control rats, five underwent a 450gm-1m weight-height impact, and 14 suffered a 450gm-2m impact. The 13 rats in the second group (Group 2E) were intubated and mechanically ventilated during the entire procedure; sic were control animals and seven underwent a 450gm-2m impact. The levels of pH, PaO_2, $PaCO_2$, and HCO_3^- in the blood were determined before impact and over a 2-hour period after trauma.

Biomechanical Analysis

A mathematical analysis of this simple weight-drop model as performed in order to estimate the acceleration, displacement, and compression gradient of the skull for various weight-height combinations. For this purpose, we elected to first model the impact-acceleration dynamics using a lumped parameter (spring-mass-dash pot) method. The basic configuration of the initial lumped parameter model [Figure 3] incorporated two Kelvin solids (with mass components) in a series, representing the material properties of the foam and the rat's skull with the helmet. Although a more thorough mathematical analysis will be presented in a separate report, a brief description of the methods and results are presented here in the interest of completeness. For simplification, six physical parameters were considered: mass of head and foam, stiffness of head and foam, and the mechanical impedance (viscous component) of the head and foam. The final equations relating displacement, velocity, and acceleration were derived and a computer provided graphic solutions for the weight-height combinations used in the studies described above.

figure 3 Diagram showing the basic configuration of the lumped parameter model. The model incorporates two Kelvin solids (with mass components) in a series, representing the material properties of the foam and the rat's head with the helmet. There are six parameters in the model: the mass of the impactor, helmet and skull (M1) and of the foam (M2); the stiffness of the rat's head (K1: 696, 200 N/m) and of the foam (K2: 2500 N/m); and the mechanical impedance (viscous component) of then head (B1: 20. 40 kg/sec) and of the foam (B2: 1.0 kg/sec).



Results

Group 1 Study

Mortality, Skull Fractures, and Trauma Level

In pilot experiments, fracture was observed in the unprotected skull supported on foam at a mass of 100 mg and a drop height of 50 cm. With the steel helmet in place, a weight-height trauma level of 450gm-2m produced a mortality rate of 44% (seven of 16 rats). Of the 16 animals impacted at this injury level, skull fracture was observed in two nonsurvivors (12.5%). At the 500gm-2m impact level, 11 (69%) of the 16 rats died and five (31%) survived; skull fracture was observed in five (31%) of the nonsurvivors. Due to the high mortality and skull fracture rates associated with the 500gm-2m impact, the 450gm-2m impact was considered the upper limit for producing severe head trauma, and this weight-height combination was selected for subsequent studies. The 450gm-1m impact (50% of the upper limit for producing severe head trauma) caused no mortality and no skull fracture in the 22 animals tested. Table 1 summarizes the mortality rates and incidence of skull fractures related to the severity of impact.

table 1 Mortality rate and incidence of skull fracture as related to the impact and type of respirations employed

* IP inj = intraperitoneal injection of alpha-chloralose; inhalation of isoflurane

Impact	anesthesia	respiration	no. of rats	mortality rate	skull fracture rate
500 gm-2 m	IP inj	non-intubated spontaneous	16	69.0%	31.0%
450 gm-2 m	IP inj	non-intubated spontaneous	16	44.0%	12.5%
450 gm-1 m	IP inj	non-intubated spontaneous	22	0.0%	0.0%
450 gm-2 m	inhalation	non-intubated spontaneous	58	58.6%	8.6%
450 gm-2 m	inhalation	intubated spontaneous	26	50.0%	3.8%
450 gm-2 m	inhalation	intubated mechanically assisted	23	8.7%	0.0%

Respiratory Distress

The nine animals surviving the 450gm-2m impact experienced apnea immediately after injury, with a reduction in respiratory rate of 20% for up to 30 minutes postinjury. Following this period, respiration in these animals gradually recovered and was not significantly different from that in the control rats by 2 hours postinjury. The seven animals impacted a the 450gm-2m level that did not survive experienced apnea lasting for up to 20 seconds immediately following impact, with a gradual slowing of respiration until death. Death occurred in five rats at a mean (± standard deviation) time of 4.2 ± 2. 2 minutes postinjury: the other two rats died at 2 and 3 hours postinjury. The animals impacted at the 450gm-1m level also experienced a brief (5 to 10 second) apneic period but rapidly recovered to control respiratory rates.

Response of Blood Pressure

In survivors of the severe head injury (450gm-2m impact), the blood pressure increased from a mean preinjury level of 102 ± 16 mm Hg to a peak of 123 ± 37 mm Hg measured at 15 seconds after impact. This was immediately followed by a period of hypotension and gradual return toward normal by 30 minutes postimpact [figures 4 and 5]. In nonsurvivors, the initial blood pressure profile was identical to that of survivors: however, more severe hypotension ensued without recovery.

The mild surge of blood pressure seen at severe head-injury levels was absent in mildly injured animals. The blood pressure of the animals subjected to the 450gm-1m impact decreased abruptly following trauma from a preinjury level of 104 ± 12 to 72 ± 18 mm Hg at 15 seconds after injury, and rapidly returned to control values within 2 minutes [Figure 5].

figure 4 Tracings of heart rate (*upper*), respiration (*center*), and systemic arterial blood pressure (SABP, *lower*) immediately following 450gm-2m impact in a spontaneously breathing animal anesthetized with alpha chloralose. A mild increase in blood pressure is observed followed by a hypotensive period prior to baseline recovery. Respiration is irregular and the respiratory rate gradually recovers to baseline within a 2-minute interval in survivors.

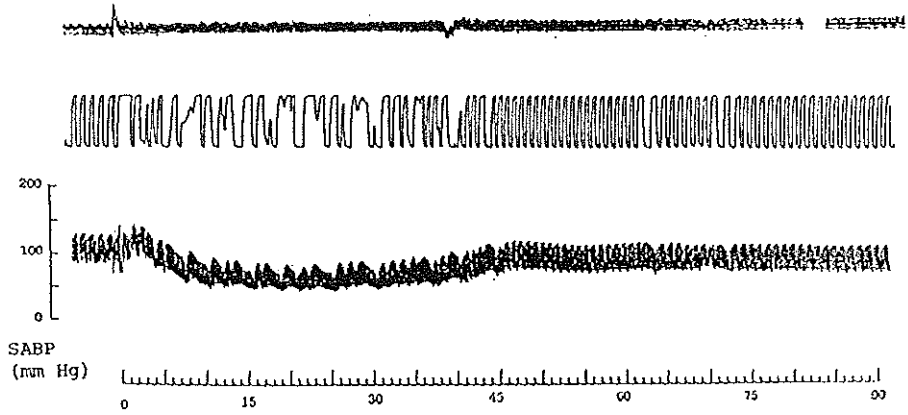
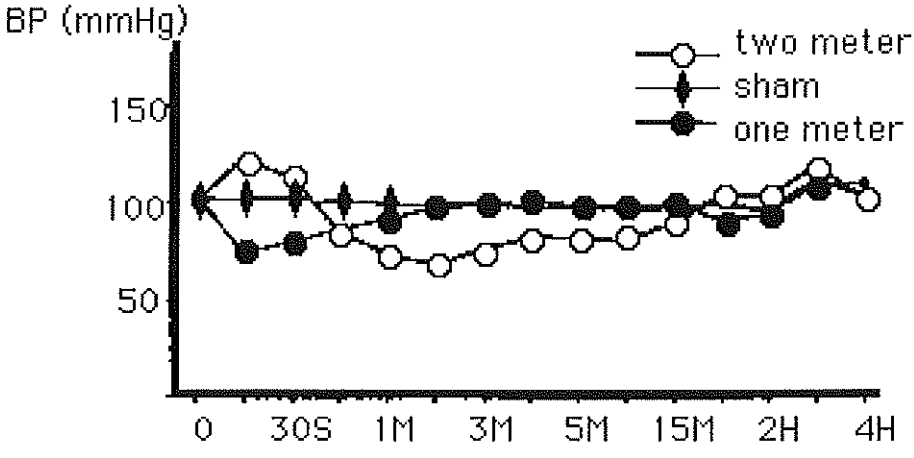


figure 5 Graph showing blood pressure (BP) response following mild (450gm-1m) and severe (450gm-2m) head injury. In the severely head-injured animals (*open circles*), a moderate transient elevation in blood pressure is followed by a hypotensive period prior to baseline return. The hypertensive surge is not seen in animals with mild head injury (*closed circles*) or in control rats (*diamonds*).



Response of Heart Rate

In survivors of the severe head injury (450gm-2m impact) the heart rate decreased from a preinjury level of 321 ± 36 to 241 ± 61 beats/min by 1 minute postinjury. Thereafter, heart rate gradually returned toward the control level and was not significantly different from baseline by 10 minutes postinjury. In

the five nonsurvivors of the 450gm-2m impact that died within the first few minutes of impact, marked bradycardia was observed and reached 50% of the control value by 2 minutes postinjury. This was followed by a gradual decrease in heart rate until death. In the two animals that died within a few hours, there was an increase in heart rate from a preinjury level of 323 ± 47 to 450 to 480 beats/min, and this tachycardia was sustained until death. In animals subjected to the 450gm-1m trauma, there was no significant change in heart rate from preinjury baseline levels.

Response of Blood Gases

In Group 2D (spontaneously breathing) rats, all control and mildly head-injured (450gm-1m impact) animals survived, while only six of 14 severely head-injured (450gm-2m impact) animals survived the trauma. Changes in blood gas levels after mild head trauma were similar to those in control animals [Table 2]. However, a progressive increase in $PaCO_2$ levels was observed in the survivors of severe head injury ($p<0.05$). In the four animals that died within 7 to 10 minutes after severe trauma (450gm-2m impact), $PaCO_2$ dramatically increased and PaO_2 decreased 5 minutes after impact [Table 3]. In contrast, all severely head-injured animals in the mechanically ventilated group (Group 2E) survived the trauma, and the blood gas changes in this group were similar to those in the control rats [Table 4].

table 2 Blood gas changes in spontaneously breathing animals after CHI

variable	group	pretrauma	posttrauma				
			5 Min	15 Min	30 Min	60 Min	120 Min
pH	control	7,35±0,05	7,37±0,03	7,38±0,05	7,36±0,04	7,37±0,02	7,35±0,02
	mild injury	7,35±0,03	7,35±0,03	7,35±0,02	7,34±0,01	7,33±0,03	7,33±0,02
	severe injury	7,40±0,01	7,34±0,04	7,36±0,04	7,36±0,04	7,33±0,03	7,33±0,04
PO_2	control	161,8±12,7	152,8±7,5	151,3±5,7	151,8±7,4	144,8±11,5	145,2±6,5
	mild injury	170,4±10,0	156,2±7,0	149,4±13,1	145,4±13,6	147,0±12,1	136,0±15,4
	severe injury	169,4±12,7	147,4±23,9	150,6±18,7	146,0±18,4	143,0±20,8	140,0±29,2
PCO_2	control	48,9±4,3	47,8±4,4	50,3±6,5	51,1±5,8	45,9±4,5	51,9±1,7
	mild injury	51,2±6,0	49,3±6,7	49,2±5,0	50,6±6,0	50,3±5,0	47,9±4,0
	severe injury	47,7±3,3	53,0±4,0	53,3±3,9	54,5±5,4	57,5±6,0 *	59,1±6,0
HCO_3-	control	32,3±2,0	28,9±4,7	32,5±1,2	32,6±2,0	29,9±2,9	32,9±0,7
	mild injury	28,0±3,7	27,0±2,9	27,2±2,9	27,4±2,7	26,7±2,5	25,5±2,3
	severe injury	29,0±1,8	28,5±1,7	29,9±1,7	30,0±3,3	29,8±2,1	31,0±1,9

* Mild injury = 450gm-1m impact; severe injury = 450gm-2m impact. Values are mean ± SD. Statistical significance of difference: * = p = 0.007, ** = p = 0.044

table 3 Blood gas changes in spontaneously breathing animals that died 7 to 10 minutes after severe head trauma

variable	pretrauma	5 Min posttrauma
no. of rats	4	4
pH	7,41±0,022	7,11±0,062
PO_2	182,5±17,99	34,03±12,93
PCO_2	44,78±2,89	90,98±10,14
HCO_3-	28,2±0,86	28,8±2,41

* severe injury = 450gm-2m impact. Values are mean ± SD.

table 4 Changes in blood gas levels in the mechanically ventilated animals following severe head trauma

variable	group	pretrauma	posttrauma	
			15 Min	120 Min
pH	control	7,45±0,007	7,43±0,008	7,44±0,005
	severe injury	7,46±0,012	7,43±0,025	7,45±0,006
PO_2	control	133,5±2,96	127,7±3,9	130,5±4,4
	severe injury	134,5±4,8	114,2±9,3	134,2±5,9
PCO_2	control	35,7±1,2	36,5±1,5	35,8±0,6
	severe injury	34,6±1,4	37,7±3,2	35,7±0,7

* severe injury = 450gm-2m impact. Values are mean ± SD.

Neurological Response

In addition to the apnea experienced immediately after injury, the animals exposed to the 450gm-2m impact developed severe generalized convulsions lasting 15 to 30 seconds; those seizures were confined to the immediate postinjury period. Survivors in this group as well as the mildly head-injured animals developed decortication flexion deformity of the forelimbs. Seizures in the animals subjected to the 450gm-1m injury were less frequent, less severe, and lasted for only several seconds.

Group 2 Study

Mortality and Respiratory Support

Table 1 summarizes the changes in the mortality rate and the incidence of skull fracture related to the severity of impact and the type of respiration employed during and after the impact. In the 58 non-intubated spontaneously breathing animals (Group 2A) an impact of 450gm-2m resulted in death in 34 animals (58.6%). Five nonsurvivors (8.6%) had evidence of skull fracture. Thirtyone animals died within a few minutes following impact and three died after prolonged behavioral suppression lasting for up to 3 hours. All animals that

suffered a 450gm-2m impact had a 10 to 20 second period of apnea associated with severe generalized convulsions for 15 to 30 seconds. In Group 2B (intubated nonventilated animals), 26 rats were impacted at the 450gm-2m level. A total of 13 (50%) survived and 13 (50%) died, with only one (3.8%) of the non-survivors found to have a skull fracture. In the 23 Group 2C (intubated and mechanically ventilated) rats, all animals survived the 450gm-2m impact except for two animals with skull fracture (8, 7%).

Electroencephalographic (EEG) studies in the animals that survived the 450gm-2m impact showed the development of cortical paroxysmal discharges for 40 to 60 seconds after impact, followed by a depression in the electrical activity of the brain for 10 minutes. The EEG recordings gradually returned to the preimpact control level by 30 minutes postimpact.

Biomechanical Analysis

Skull Acceleration

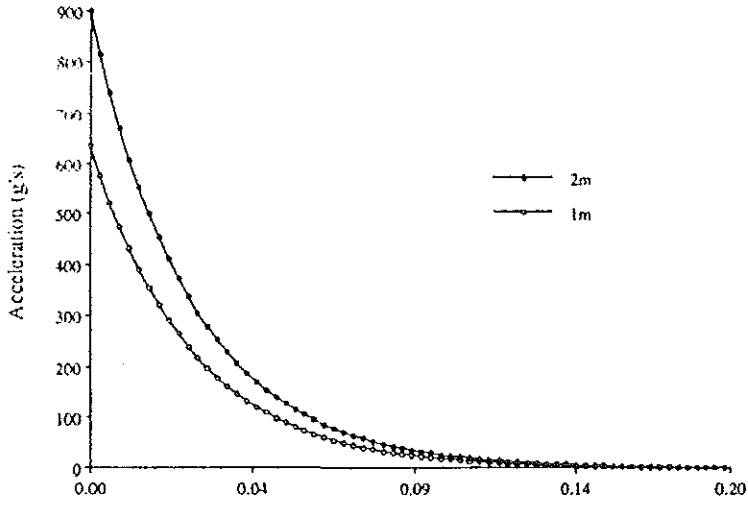
Since the force from the spring acts on both masses in proportion to their relative displacement and the damper yields a force on each mass proportional to its relative velocity, the following differential equations are found:

$$M_1X''_1=B_1 (X'_1-X'_2) +K_1 (X_1-X_2) = 0(1)$$

$$M_2X''_2 +B_2X'_2+B_1 (X'_2-X'_1)+K_2X_2 +K_1 (X_2-X_1) = 0(2)$$

Where M_1 = mass of impactor, helmet, and skull, M_2 = mass of the foam, X_1 = position of top surface of the rat's head, X_2 = position of bottom surface of the rats head, K_1 = stiffness of the rat's head (696, 200 N/m), and K_2 = stiffness of the foam (2500 N/m); prime and double prime symbols represent first and second derivative, respectively. The temporal course of acceleration corresponding to 450gm-2m and 450-gm-1m injuries is shown in figure 6. The results indicate that a peak acceleration of 900 g (1g = 980 radians/sec/sec) was formed by the 2m injury at the instant of impact, then rapidly dissipating within 0.2 msec. The 1m injury produced a 630 g acceleration, with a similar pattern of decline.

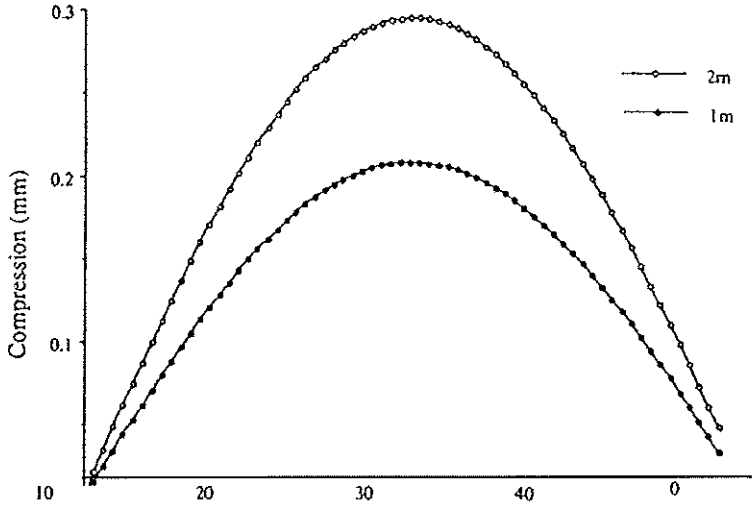
figure 6 Graph demonstrating the temporal course of acceleration for mild, 1-m (*open circles*) and severe, 2-m (*closed circles*) head injury levels based on the mathematical model described in the text. The acceleration is brief, lasting for approximately 0.20 msec., and approaches peak magnitudes of 900 and 630 g for severe and mild injury levels, respectively. Acceleration profiles remain identical for varying mass and increase only with increased height. The limiting factor for further energy transfer at impact is the fracture treshold of the rodent skull.



Estimated Magnitude of Skull Compression

The computer results indicated that the skull is depressed upon impact and, since the foam has negligible resistance to acceleration, the base of the skull continues to descend without being compressed significantly. According to the model, virtually all compression is the result of displacement of the vertex. Both the impactor and the skull descend in unison for a period equal to the time of contact between the impactor and the skull. The contact duration was determined by establishing a small electrical potential between electrodes connected to the impactor and the skull and measuring the electrical current on an oscilloscope; the time of contact was found to be 40 msec. On inserting these data into the model, the impactor continues to descend with the skull for approximately one-half the contact duration. At the 20-msec time point (one-half the contact duration) compression is at a maximum [Figure 7]. Following this cycle, the impactor and the skull move upward together, returning to zero displacement. When the impactor and the skull change direction and ascend, the acceleration is in the opposite direction and the skull base moves faster that the vertex, thereby exposing the brain surface opposite the impact site to a high accelleration gradient. Experimentally, multiple contact was eliminated by simply moving the rat from under the cylinder while the impactor was ascending. This simplified the model considerably. Based on this analysis, the skull undergoes a maximum compression of 0.28 mm for the 450gm-2m injury and 0. 20 mm for the 450gm-1m injury.

figure 7 Graph demonstrating the temporal course of skull compression for mild, 1-m (*open circles*) and severe, 2-m (*closed circles*) head injury levels based on the mathematical model described in the text. Skull and thus brain compression approaches a maximum of 0.3 mm, peaking at a time point equivalent to one-half the contact duration. As in acceleration the compression curves are identical for varying mass but vary directly with increases in height.



Discussion

This report describes the development of a simple, reproducible, and practical model of rodent closed head injury that has certain advantages over other models. First, a lethal level of closed head injury can be achieved without predominant brain-stem damage as seen with direct dural impact.[25] Second, at lethal levels, the transient rise in blood pressure seen in closed head injury immediately postimpact is mild and does not reach levels consistent with breakthrough of cerebral blood flow autoregulation or blood-brain barrier compromise. Thus, the effect of trauma in the absence of posttraumatic hypertension can be isolated. Third, data from our companion paper[1] show that the model produces a pronounced diffuse axonal injury consistent with the features of human diffuse axonal injury described by Adams, et al[2]. Finally, posttraumatic ventriculomegaly is observed in survivors of severe closed head injury at 4 to 6 weeks postinjury (unplished data), which also mirrors the experience in human head injury[10, 12, 28]

Closed Head Injury in the Rat

Over the past 20 years, numerous investigators have studied the response of the rat to experimental closed head injury. In a study of head injury by

Beckman and Bean,[4] the heads of hand-held, nonanesthetized rats cushioned with a sponge rubber were impacted with a bolt. In those animals that did not survive the impact injury, the heart rate was found to decrease significantly, cases of pulmonary edema were noticeably more severe, lung weight: body weight ratios were significantly higher, and contusions, subarachnoid hemorrhage (SAH), and subdural hemorrhage were frequently found in the brain. Hand-held rats were impacted with padded darts from a pistol in a study by West, et al.,[29], investigating concussion and EEG response. One-third of the rats displayed severe concussion with impairment of consciousness and apnea, associated with depressed EEG amplitudes lasting until recovery approximately 2 hours after impact. Huger and Patrick [11] injured rats using a hinge-drop impact mechanism. Hyperventilation, convulsions, and SAH were noted effects of concussion. Althought tyrosine and dopamine levels and synthesis rates were increased in the traumatized rats, there was no significant difference between the effects in traumatized rats and in those undergoing a sham drop, suggesting that catecholamine level changes were due to stress in the non-anesthetized animals.

A similar study on the effects of closed head injury in which mice were impacted with a sliding bolt striking the immobilized head was conducted by Nelson, et al.[18]

However, unlike the previous three studies mentioned in which constant amounts of energy were imparted to the rats' heads, the experiment by Nelson and coworkers included an adjustable impactor for the purpose of producing graded trauma. Shapira, et al[24] introduced another model for closed head injury in rats using a weight-drop impact to one side of the unprotected skull. This model produced an ipsilateral focal brain contusion and a blood pressure rise for more than 10 minutes posttrauma, and thus would be suitable for studying the focal but not the diffuse forms of brain injury.

In our study, the cortical paroxysmal discharges observed in the EEG recordings of severely head-injured animals during the first minute after impact correlate with the severe generalized convulsion observed in those animals immediately after trauma. On the other hand, the subsequent depression in EEG amplitude would explain the delayed recovery from anesthesia in these animals, [1] which was prolonged compared to the recovery in control rats.

Fracture Threshold of the Rodent Skull

Studies by Nilsson and colleagues [19-21] were directed toward an investigation into the physiological response of closed head injury in the rat. A piston accelerated by compressed gas was used to impact the supine rat in the region of the occipital protuberance. The velocity of the piston at impact was adjustable, thereby eliciting concussion (defined as the loss of reaction to pain stimuli) of variable severity; at 6m/sec, no concussion resulted; at 9 m/sec, the rats were comatose for long periods. The mortality rate was similarly related to impact velocity, ranging from 10% (two of 20 rats) at 7m/sec to 67% (four of six rats) at 11 m/sec. Gross pathological examination revealed SAH in the occipital

cistern at the velocity corresponding to concussion threshold (7m/sec), with more extensive brainstem hemorrhage at higher velocities. However, these studies as well as those by Bakay, et al., [3] in which a pendulum device was used to cause concussion, found thin linear fractures at moderate concussion levels (9m/sec) and shattering fractures at impactor velocities causing greater than 50% mortality. The rodent skull at the vertex is extremely thin and almost transparent. Thus, the ability to produce concussive closed head injury without fracture was the first objective of this study. Our results show that the protection offered by the stainless-steel helmet was sufficient to prevent fracture and achieve high kinetic energy levels at impact delivery. The impact and resultant high transient acceleration was sufficient to produce a severe brain injury without extensive brain-stem damage. Of interest was the fact that the acceleration in this model was confined generally to the sagittal plane. A distinction between rotational and translational acceleration has been made in at least one biological model[7, 8, 23] in which it was found that visible brain lesions resulted from injuries caused by both translation and rotational accelleration, with a greater frequency and severity after rotation. The development of a rodent acceleration model, similar to that used by the Gennarelli group[6], is extremely difficult because of the relatively small brain mass of the rodent (2 gm). Levels of acceleration necessary to produce diffuse axonal injury by accelleration alone would be excessively high. From studies of diffuse axonal injury produced by our model, it appears that this is overcome with the combination impact.

Effect of Impact

A considerable effort was made in the preliminary stages of development of this model to select the optimum support for the rodent skull. We selected a foam of known spring constant based on the following consideration: dynamic loading of the head can be divided into impact and inertial effects, the former associated with the generation of transient stress waves in the tissue and skull and the latter with differential acceleration of tissue (regional gradients) and skull. Thus, the degree to which head motion is restricted is of chief importance in determining the relative contribution of impact and inertial components in head trauma[6] That is, when an impactor strikes the head, the compression effects and associated 'contact phenomena' are most damaging whereas, if the head is free to move following impact, the shear forces between tissues are predominant. The size of the impactor relative to the skull is also important in determining the relative importance of contact and acceleration effects. A small high-velocity object causes the head to move very little and thus its kinetic energy is dissipated primarily through contact phenomena, while a large, blunt object primarily acts to accelerate the head with minimum contact effects[6]. For this reason, we focused our experiment in the 400- to 500 gm weight range, and selected foam to provide reasonable support while allowing the head to accelerate.

Absence of Posttraumatic Hypertension in Rodent CHI

Of importance is the response of blood pressure immediately following impact (Fig. 5). The fluid-percussion model and others involving direct dural impact result in a significant hypertension produced immediately following injury. With fluid percussion, blood pressure exceeds 180 mm Hg and does not recover for several minutes[30]. This results in pressure breakthrough, loss of autoregulation, and a dramatic increase in cerebral blood flow[13]. In contrast, closed head injury in the rat results in only a small elevation of blood pressure with hypotension developing within 1 minute of impact. Thus, as hypertension is not a feature of rodent closed head injury, it allows the investigator to isolate more clearly the effects of trauma upon barrier function and autoregulation.

Respiratory Depression in Closed Head Injury

We attribute the major cause of death due to impact to respiratory depression followed by an ensuing hypotension. Posttraumatic apnea and respiratory depression are observations consistently seen in both clinical and experimental head injury[9, 16, 17, 19, 22]. In our experimental studies of spontaneously breathing animals, impact was immediately followed by apnea lasting for up to 20 seconds and a gradual slowing of respiration. In these animals, death occurred within minutes of impact as a result of severe hypoxia and hypercapnia. Tracheal intubation alone did not improve the mortality rate in these animals (Group 2B). When animals were intubated and mechanically ventilated during and for a few minutes after impact, the mortality rate decreased from more than 50% to less than 10%. These observations and those reported by others[9], suggest a central rather than a peripheral mechanism accounting for this respiratory depression. The transient apnea may be explained by the transient changes in brain-stem auditory evoked potentials (BAEP's) demonstrated by van den Brink, et al[27]. In these studies, althought BAEP's remained intact, wave IV was less consistent in the severely injured rats than in the mildly injured or the control animals. Thus, we suspect that the respiratory depression seen in animals without respiratory support is caused by a transient brain-stem physiological dysfunction that can be overcome with mechanical ventilation. These results emphasize the need for early respiratory support in severely head-injured patients.

Mathematical Analysis of the Weight-Drop Model

One advantage of mathematical models is the ability to predict the kinetic energy transfer and acceleration profiles for different weight-height combinations, as demonstrated in this report. Previous models of impact acceleration injury have considered various methods through which the kinetic energy of the impacting device may be lost or transformed into injury-causing forces. One such model characterizes the uniaxial impact of a material with mechanical properties described in terms of ideal elastic, viscous, and inertial elements, that is, the spring, dash pot, and mass, respectively. This type of model is expressed mathematically in terms of a set of linear ordinary

differential equations, thus resulting in solutions that are relatively simple to implement. However, the disadvantage of the lumped parameter model is that it cannot reveal the time history of stresses and strains occurring within the material. Models that describe the energy transformation more specifically (as a combination of deformation, stress, and pressure wave propagations, and contact phenomena throughout the head) are, for certain idealized geometries, able to predict the distribution of strains within the system.

As a starting point in our analysis, we elected to model the impact-acceleration system using the lumped parameter (spring-mass-dash pot) method. Our objective was to utilize this model to discribe 'whole head' motion. Based upon several simple measurements of the impact dynamics of our model and the relationship between loading duration and the comparative extent to which impact and impulse effects contribute to a given head injury, we believe that the inherent limitation of the spring-dash pot model may be mitigated in the context of our model. As described by Ommaya and Gennarelli[23], the impact component of dynamic loading is associated with skull bending or fracture and the propagation of shock waves through skull and tissue. The impulse component of dynamic loading, on the other hand, consists of "whole head" motion in either translational or ratational directions. Although the impact and impulse components of head injury are both ultimately manifested as tissue strains (shear, tensile, or compressive), it is known that, as the impact duration increases, the relative amount of tissue strain due to impact effects decreases and the tissue strain due to impulse effects increases. More extensive studies of contact duration are necessary to resolve the degree to which impact effects predominate.

Conclusions

A new practical and simple model of head injury in the rat has been developed with several features similar to the experience in the clinical setting. It is hoped that with continued investigation this model will contribute to our understanding of human head injury.

References

Paper:
Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. **J Neurosurg** 80:291-300, 1994

Presentations, abstracts:
van den Brink WA, Avezaat CJJ, Marmarou A: Een nieuw model van gesloten-schedel hersen letsel in de rat. **Wetenschappelijke Vergadering Nederlandse Vereniging van Neurochirurgen Nov.**: 1991 (Presentation)

van den Brink WA, Marmarou A, Avezaat CJJ: Current methods of inducing head injury. **Topics in Intensive Care** 70, 1991 (Abstract)

1. Abd-Elfattah Foda MA, Marmarou A: A new model of diffuse brain injury in rats. Part II: Morphological characterization. **J Neurosurg 80**: 301-313, 1994

2. Adams JH, Doyle D, Ford I, et al: Diffuse axonal injury in head injury: definition, diagnosis and grading. **Histopathology 15**: 49-59, 1989

3. Bakay L, Lee JC, Lee GC, et al: Experimental cerebral concussion. Part I: an electron microscopic study. **J Neurosurg 47**: 525-531, 1977

4. Beckman DL, Bean JW: Pulmonary damage and head injury. **Proc Soc Exp Biol Med 130**: 5-9, 1969

5. Dixon CE, Lyeth BG, Povlishock JT, et al: A fluid percussion model of experimental brain injury in the rat. **J Neurosurg 67**: 110-119, 1987

6. Gennarelli TA, Thibault LE: Biological models of head injury, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report**. Bethesda, MD, NINCDS: 391-404, 1985

7. Gennarelli TA, Thibault LE, Adams JH, et al: Diffuse axonal injury and traumatic coma in the primate. **Ann Neurol 12**: 564-574, 1982

8. Gennarelli TA, Thibault LE, Ommaya AK: Pathophysiologic responses to rotational and translational accelerations of the head, in Society of automobile engineers (ed): **Proceedings of the 16th Strapp Car Crash Conference**. New York, 295-308, 1972

9. Grubb RLJr, Naumann RA, Ommaya AK: Respiration and the cerebrospinal fluid in experimental cerebral concussion. **J Neurosurg 32**: 320-329, 1970

10. Hawkins TD, Lloyd AD, Fletscher GIC, et al: Ventricular size following head injury: a clinicoradiological study. **Clin Radiol 27**: 279-289, 1976

11. Huger F, Patrick G: Effects of concussive head injury on central cathecholamine levels and synthesis rates in rat brain regions. **J Neurochem 33**: 89-95, 1979

12. Levin HS, Meyers CA, Grossman RG, et al: Ventricular enlargement after closed head injury. **Arch Neurol 38**: 623-629, 1981

13. Lewelt W, Jenkins LW, Miller JD: Autoregulation of cerebral blood flow after experimental fluid percussion injury of the brain. **J Neurosurg 53**: 500-511, 1990

14. Marshall LF, Bowers Marshall S, Klauber MR, et al: A new classification of head injury based on computerized tomography. **J Neurosurg 75 (suppl)**: S14-S20, 1991

15. McIntosh TK, Vink R, Noble LJ, et al: Traumatic brain injury in the rat: Characterization of a lateral fluid percussion model. **Neuroscience 28**: 233-244, 1989

16. Meyer JS, Kondo A, Nomura F, et al: Cerebral hemodynamics and metabolism following experimental head injury. **J Neurosurg 32**: 304-319, 1970

17. Millen JE, Glauser FL, Zimmerman M: Physiological effects of controlled concussive brain trauma. **J Appl Physiol 49**: 856-862, 1980

18. Nelson SR, Lowry OH, Passonneau JV: Changes in energy reserves in mouse brain associated with compressive head injury, in Caveness WF, Walker AE (eds): **Head injury conference proceedings**. Philadelphia, J. B. Lippincott Co.: 444-447, 1966

19. Nilsson B, Nordström CH: Rate of cerebral energy consumption in concussive head injury in the rat. J **Neurosurg** 47: 274-281, 1977

20. Nilsson B, Nordström CH: Experimental head injury in the rat. Part 3: Cerebral blood flow and oxygen consumption after concussive impact acceleration. J **Neurosurg** 47: 262-273, 1977

21. Nilsson B, Ponten U: Experimental head injury in the rat. Part 2: Regional brain energy metabolism in concussive trauma. J **Neurosurg** 47: 252-261, 1977

22. Nilsson B, Ponten U, Voigt G: Experimental head injury in the rat. Part 1: Mechanics, pathophysiology, and morphology in an impact acceleration trauma model. J **Neurosurg** 47: 241-251, 1977

23. Ommaya AK, Gennarelli TA: Cerebral concussion and traumatic unconsciousness correlation of experimental and clinical observations on blunt head injuries. **Brain** 97: 633-654, 1974

24. Shapira Y, Shohami E, Sidi A, et al: Experimental closed head injury in rats: Mechanical, pathophysiological, and neurological properties. **Crit Care Med** 16: 258-265, 1988

25. Shima K, Marmarou A: Evaluation of brain stem dysfunction following severe fluid percussion head injury to the cat. J **Neurosurg** 74: 270-277, 1991

26. Sullivan HG, Martinez J, Becker DP: Fluid percussion model of mechanical brain injury in the cat. J **Neurosurg** 45: 520-534, 1976

27. van den Brink WA, Slomka W, Marmarou A, et al: Neurophysiological evidence of preservation of brain stem function in experimental closed head injury in the rat, in Avezaat CJJ, van Eijndhoven JHM, Maas AIR, et al (eds): **Intracranial Pressure VIII**. Berlin, Heidelberg, New York, Springer-Verlag: 221-226, 1993

28. van Dongen KJ, Braakman R: Late computed tomography in survivors of severe head injury. **Neurosurgery** 7: 14-22, 1980

29. West M, Parkinson D, Havlicek V: Spectral analysis of the electroencephalographic response to experimental concussion in the rat. **Electroencephalogr Clin Neurophysiol** 53: 192-200, 1982

30. Yuan XQ, Wade CE, Clifford CB: Immediate hypertensive response to fluid percussion brain injury may be related to intracerebral hemorrhage and hypothalamic damage. J **Neurotrauma** 8: 219-228, 1991

Chapter 5

Standardization of the weight drop model for experimental Closed Head Injury: influence of foam characteristics on acceleration forces and influence on animal outcome.

At impact the skull moves away and the brain lags behind. The brain then swirls to follow the head, appearing to rotate on an axis passing through the center of gravity of the head.

Ayub K. Ommaya, Head Injury Conference Proceedings, 1966

Summary

Introduction: In the weight drop model of Closed Head Injury acceleration of the head is caused by impact of a weight dropped from a certain height onto the rodent skull. Displacement of the rat's skull is limited by a foam head support. Although the mechanics for producing injuries are strictly standardized, the resulting clinical severity of the injury produced is variable. It has been suggested that this may be caused by variability of foam properties and static compression tests have been developed and used for investigation of foam properties. These static tests however may not adequately reflect foam properties important to fast dynamic processes, such as occur during acceleration induced by this model of Closed Head Injury. A dynamic calibration procedure would appear to be more appropriate.

Object: To investigate dynamic and static properties of foam used to restrain the rodent skull during the delivery of head injury inflicted with the weight drop model.

Methods: A dynamic foam tester based on an ISO standard was developed. Three foams with different firmness were tested in vitro. Subsequently, the three different types of foam were utilized in the experimental model in which 48 rats were subjected to closed head injury.

Results: Dynamic compressibility of used foam specimen did not correlate with results obtained from static stress/strain compression tests. In animal experiments no influence of foam properties on mortality, brain edema, blood glucose, lactate and gases could be demonstrated.

Conclusion: Foam, as used in the Closed Head Injury model, is a visco-elastic material. If meticulous standardization of fast dynamic experiments is warranted, both the viscous as well as the elastic properties should be tested in a dynamic procedure. Pragmatically however, foam characteristics appear to be an over-criticized issue, without demonstrable influence on outcome parameters.

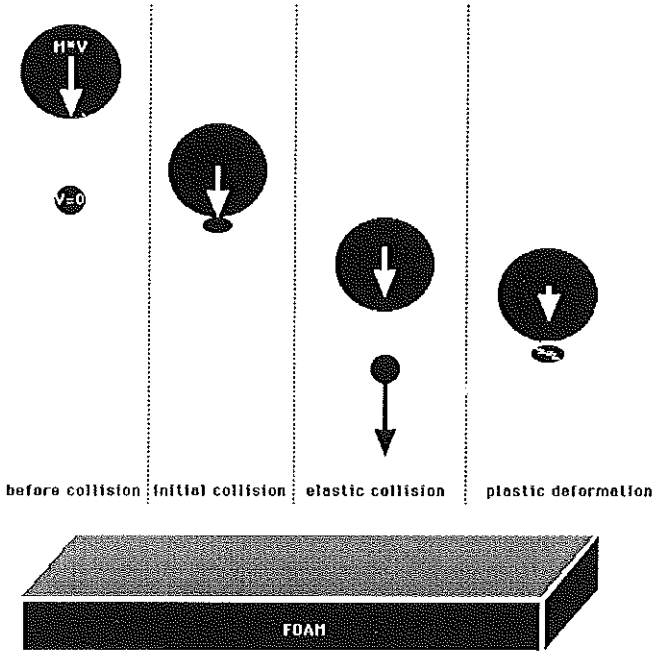
Introduction

To study head injury, and the pathophysiologic consequences thereof in the experimental situation, a variety of models, each focusing on part of the spectrum of human traumatic brain injury has been proposed. Standardization of models, and of resulting injury, is a prerequisite in head injury research. The reproducibility of brain injury is dependent on characteristics of the device used and of the animal chosen. The weight drop model of closed head injury (CHI) in the rat, developed by Marmarou [8] is widely used as a model of diffuse traumatic brain injury [1-3, 6, 10-12]. Despite the standardized mechanics of the model it has however been our experience, as well as that of other groups, that severity of injury resulting from this model is variable. This is evidenced by variability in primary outcome measures, such as mortality. The question is whether this variability results from specific aspects of the device used, or this variability is primarily related to different (unknown) animal characteristics.

In the CHI model the head is accelerated as a result of an impulse onto the intact skull, a highly dynamic process, occurring within milliseconds, and subsequently decelerated by the supporting block of foam under the rat's head. Direct measurement of acceleration of the skull during each of the animal experiments, though straightforward, is difficult to realize and impracticable. Moreover, such measurements would only indicate a possible variability in the delivery of CHI, without any indication how to prevent the variability. Following a deductionistic approach, the model may be subdivided in standardizable parts:

The formation of the momentum of the impactor, its impulse to and acceleration of the skull, followed by the deceleration and rebound by the foam. These aspects, amenable to pragmatic insight of subsequent events and mathematical modeling, might give a better insight in the physical course of events, offer an opportunity to react specifically, and thus improve possibilities for standardization of the model. The momentum ($p = m \times v$) with which the skull is struck, can be graded by changing either mass (m) or velocity (v) of the falling impactor. Velocity is determined by the height from which the weight is dropped and resistance experienced during the fall. If impactor mass and height are kept constant, impactor velocity, as may be measured by a photogate just before collision, should stay constant. Resulting acceleration of the head is also influenced by elasticity of the collision between impactor and the rat's skull[7]. At impact the skull will be compressed. During recapture of its shape elastic forces between impactor and skull provide an additional acceleration of the skull, causing it, in case of a completely elastic collision, to move away from the falling weight at increased speed. In case of an inelastic collision, plastic deformation, fractures etc., occur and the impactor and skull will move together at slightly reduced velocity [figure 1]. After acceleration the head decelerates in the foam support due to 'viscous' and 'elastic' reactive forces in the foam. 'Rigidity' or firmness of the foam has been an issue of

figure 1 Phases of collision. During the initial phase of collision both bodies move together with deformation of the skull, velocity of both bodies is lower than of velocity of the impactor just before initial contact. In the second stage the skull undergoes a new acceleration due to the recapturing of its shape. If not prevented by the supporting foam, the skull could even loose its contact with the impactor if the elasticity of the collision is high enough. Persistent plastic deformation can occur in case of a fracture, in which the original shape of the skull is not recaptured.



discussion with respect to the reproducibility of the injury. The springiness of a foam in general depends on the nature and packing of its cells, whereas the mechanical properties of the cushion as a whole also depend on its relative dimensions, the shape factor S, and the structural restraint of its mounting. To overcome problems due to foam characteristics Piper [9] advised 'architectural' adaptation of the foam bed based on static measurements. Static measurements however may not be appropriate to characterize foam properties in response to fast dynamic processes, such as occur in the weight drop model. Static measurements are particularly relevant to slow elastic processes, for instance when testing the comfort of mattresses. In a fast dynamic process viscous damping forces contribute to total reactive forces at compression of the foam besides the elastic forces. Damping forces, among others, depend on the ease with which naturally entrapped air can escape from a compressed foam (open or closed cells and the openness of the frame supporting the foam). To investigate dynamic properties of the foam a representative dynamic foam tester, based on ISO standards (ISO 4651, ISO 2439 B, ISO 3386), was

developed taking into account elastic and transient viscous forces. The purpose of the study was twofold: First to develop a routine for determining foam characteristics, which could produce uniform output parameters, and to quantify differences between aging and fresh pieces of foam. Secondly to investigate whether differences in rigidity would influence principle pathophysiologic outcome parameters in rats, submitted to standardized severe CHI.

Materials and methods

Closed Head Injury model

The weight drop model for experimental CHI was developed as model of diffuse head injury in rodents. Mechanical details of the trauma apparatus and pathophysiologic characteristics of the model have been described previously [8]. Briefly, the CHI model produces trauma by weight drop on the intact skull, supported on a foam bed. A metal disc, cemented onto the vertex of the rat skull, provides an even distribution of the impact-load over the skull. The mounted disc is positioned precisely under the center and perpendicular to the opening of the guidance tube through which the weight is dropped. In the original model a hoisting rope pulled the weight with a mass of 450 grams to a height of 2 or 1 meters in a perspex tube. At release of the rope, free fall of the weight results in collision with the skull. Displacement and deceleration are controlled by the foam supporting the head of the rat. The hoisting rope caused variability in impactor velocity when released [9]. To exclude this source of variation, in our CHI apparatus the weight was released by means of an electrically driven magnet, vertical position of the tube was checked by means of a plummet, to minimize frictional losses. A LED photogate, measuring the velocity of the impactor, was mounted at the near end of the guidance tube. To prevent a second hit after rebound of the impactor, the foam bed with the rat was quickly removed after the first collision.

Study design

Three types of foam were subjected to a series of static and dynamic tests: a fresh piece and an earlier used bed of the original 'Richmond Standard' foam (courtesy of A. Marmarou, Richmond, Virginia), and a soft and hard foam, 20-S and 20-H, manufactured by UXEM-Holland. CHI experiments were performed to investigate the relevance of rigidity of various foams on several important outcome parameters.

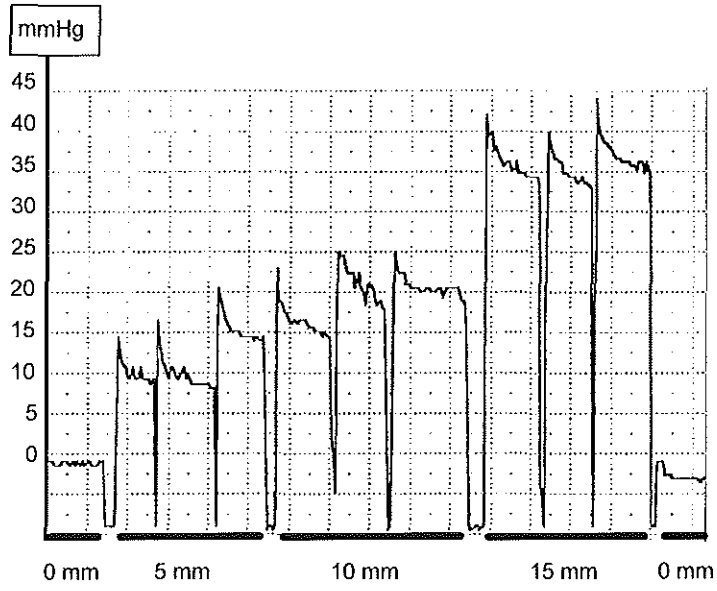
In vitro foam testing

Static foam tests

A test device measuring static stress at predefined indentation similar to the report of Piper[9]. was used. Briefly, a strain gauge pressure transducer with a sensor area of approximately 1 cm^2 was pressed into 2.5 cm thick unrestrained

pieces of foam with a sample size of approximately 12 by 12 cm to three predefined depths (5, 10, and 15 mm). Stable pressure-values, measured after an initial viscous peak [figure 2], were transformed to static stress to strain curves. Each sample was tested three times and values were averaged.

figure 2 Recording of pressure levels during slow static impression at three predetermined depths. Note the viscous pressure peak preceding the plateau from which values for calculation of the modulus of elasticity were taken.

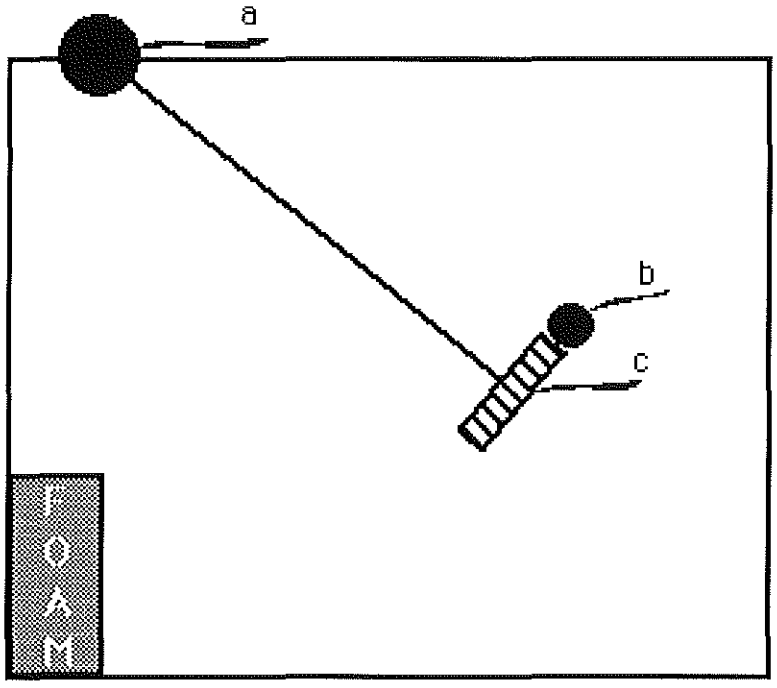


Dynamic foam tests

The in-house developed dynamic foam testing device consists of a 100 cm pendulum with a mass of 550 grams, lifted to 45 degrees above unrestrained geometrical identical blocks of foam, loosely positioned against the anvil of the device [figure 3]. Prior to release of the impactor a 'zero-calibration' procedure is performed. Velocity, just prior to contact with the foam block, is calculated from the time path of the impactor in a photogate. An accelerometer mounted on the falling impactor records deceleration at the moment of foam penetration. The rebound of the impactor was recorded by a potentiometer mounted at the center of rotation of the pendulum.

Electric signals were measured using a Macintosh II CI computer (Apple Computer Inc., Cupertino, USA), using a conventional A/D converter. A virtual instrument utilizing Superscope software (Superscope IIe, 1.44, 1994, GW Instruments, Somerville, Massachusetts USA) was developed to measure and store momentaneous values of indentation, deceleration, and rebound. Each foam specimen was tested five times and values were averaged.

figure 3 Drawing of the dynamic foam tester with three output ports to the ad-converter of the computer. a = output of joint potentiometer indicating rebound distance. b = output of accelerometer. c = output of bar code odometer indicating both velocity and foam indentation depth.



In vivo testing

Experimental protocols

A total of 48 male wagrij rats, weighing 283 ± 61 grams, were studied, seven served as sham controls, 41 were subjected to CHI. Thirteen rats were supported by 'Richmond Standard' foam, 16 by UXEM 20-H foam, and 12 by UXEM 20-S foam. Two impactor masses of 550 and 600 grams were applied respectively, height of fall was two meters. Table 1 provides details of animal groups.

Animal preparation

Anesthesia was ether-induced using the chamber technique with fresh air flow. When a surgical level of anesthesia was achieved, the non-recovery anesthetic urethane was intraperitoneally injected (1.39 mg/kg). The skull was prepared and CHI was delivered as described[8]. After CHI the animal was kept in a warmed cage to maintain body temperature at 37.5[0] Celsius. Heart rate, respiratory rate, rectal temperature, were monitored for 30 minutes. Blood gases, glucose and lactate analyses were performed at 15, 30 and 240 minutes through orbital punctures. After termination of the experiment brains were analyzed for water content by means of the wet weight - dry weight method[4].

table 1 Experimental groups and number of animals in each foam - impactor mass combination. The lower mortality in the Richmond foam group (23%) was not significantly different from the mortality using a foam of either a higher or lower hardness (37% and 41% respectively) (Chi-square test: p = 0.6)

injury/sham	foam	Number	mortality
sham	-	7	0
CHI	Uxem 20-H	16	6
CHI	'Richmond'	13	3
CHI	Uxem 20-S	12	5

Calculations and statistical analysis

Values are plotted in diagrams and presented as means (± standard deviation). The slope of the momentaneous stress/strain diagram represents the dynamic compressibility of the foam. Differences between continuous data were tested by means of ANOVA and Student's T-tests for unpaired observations. Contingency tables were constructed and tested for significance, using the Chi-square statistic for associations. P-values < 0.05 were considered significant.

Results

Foam characterization

Static foam test

In the original recordings, made during compression of the foam, a distinctive peak was observed. Shortly thereafter, at the same penetration depth, pressure stabilized [figure 2]. The values of the plateau phase are presented in figure 4. The diagrams enable the calculation of a 'stress to strain ratio' of a particular type of foam. These values are presented in the upper part of table 2. Measured this way, UXEM 20-H appears to be the hardest type of foam.

Dynamic foam tests

Derived parameters, calculated from the momentaneous measured values, obtained from the dynamic foam test apparatus, are presented in the lower part of table 2. Dynamically measured, 'Richmond Standard' foam appeared to posses the lowest compressibility, whereas UXEM 20-H, having the largest static stress to strain ratio, had a lower dynamic compressibility than the 'Richmond Standard' foam. Quite expectedly, there was no relationship between the dynamic and static properties of the foams. In the foam block that had been used in previous animal experiments, the dynamic compressibility had decreased, indicating mechanical wear.

table 2 The upper part of the table displays the statically obtained stress to strain ratio. Harder foam, as subjectively perceived by manual compression, had a higher value. Dynamic characterization parameters, as displayed in the lower part, do not correlate with the static modulus of elasticity.

	'Richmond' standard	UXEM 20-H	UXEM 20-S	
static test modulus of elasticity s/e	7,8	9,2	5,0	
45 ° Angle	'Richmond' standard	UXEM 20h	UXEM 20z	'Richmond' used
measured:				
Speed at Impact m/s	1,50 (0,00)	1,50 (0,00)	1,50 (0,00)	1,50 (0,00)
Max Indentation cm	47,60 (0,89)	48,40 (0,89)	54,80 (1,10)	54,00 (0,00)
Max Deceleration m/s^2	14,33 (0,88)	13,93 (1,10)	12,01 (0,61)	13,42 (0,88)
Deceleration time sec.	0,03 (0,00)	0,03 (0,00)	0,03 (0,00)	0,03 (0,00)
Rebounce cm	-0,38 (0,00)	-0,44 (0,01)	-0,47 (0,01)	-0,35 (0,00)
Dynamic test				
Work N	0,27 (0,00)	0,27 (0,00)	0,27 (0,00)	0,27 (0,00)
Foam Coëfficient $\Delta s/\Delta e$	0,56 (0,02)	0,51 (0,02)	0,42 (0,01)	0,47 (0,02)

figure 4 Stress to strain curves from static tests of three different foams show a clear difference among the three foams. For fast dynamic acceleration experiments however, these should not be considered 'fingerprints'[9] of the foam used.

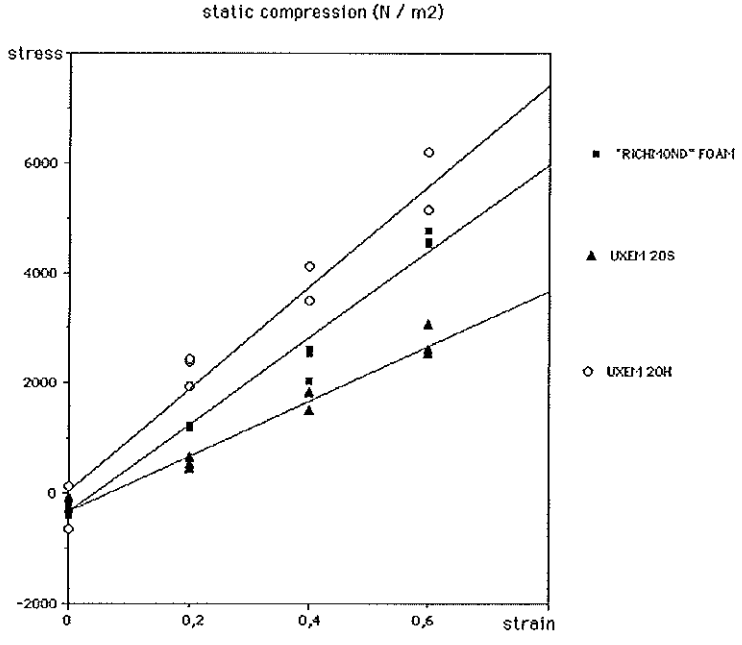
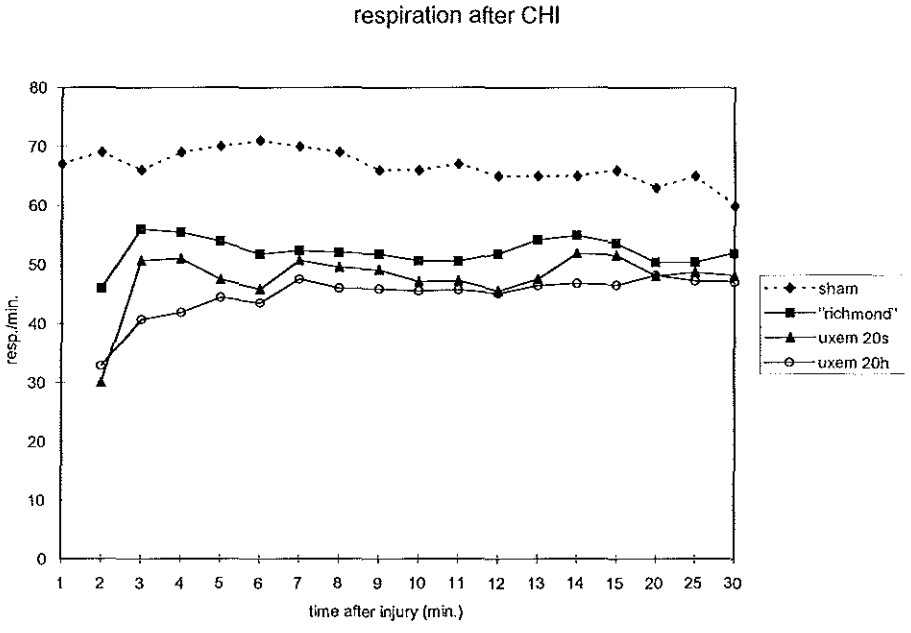


figure 5 Respiratory frequency of animals subjected to CHI with the heads supported by different foams. Only traumatized rats displayed an initial respiratory depression during the first few minutes. This respiratory depression gradually stabilizes to values slightly lower than sham controls. No difference of this pattern was observed when comparing the effects of injury utilizing either of the three foam types.



Animal experiments

Velocity of impactor

The magnetic weight release, annihilating hoisting rope resistance during free fall of the impactor, resulted in a constant impactor velocity. Separate velocity measurements indicated that angulation of the guidance tube from an exact vertical position, as checked with the plummet, is of no importance with respect to impactor velocity at impact. Even at an obliqueness of six degrees, which is obvious to any observer, and easy to correct for, a decrease of velocity was not measured. Airflow obstruction, which did decrease impactor speed, was prevented by keeping a proper distance between tube exit and skull.

Skull fractures and mortality

In these experiments skull fractures were not observed. Overall mortality rate was 34%. Mortality rates for each type of foam and impactor weight are presented in table 1, the observed differences were not statistically significantly different among foam types ($p = 0.58$) or impactor weights ($p = 0.57$).

Physiological parameters

Respiratory Rate

All traumatized animals had initial depression of respiratory rate, followed by irregular breathing. In surviving animals a gradual recovery to respiratory rates slightly lower than those of sham controls occurred [figure 5]. $PaCO_2$ values indicated hypoventilation during the first 15 and 30 minutes after CHI [table 3]. There was no difference within the experimental groups, with respect to the type of supporting foam.

table 3 Values of blood gas analysis, glucose and lactate. Injured rats have decreased ventilation and oxygenation as compared to sham controls. No difference can be observed when comparing the results of injury utilizing either of the three foam types.

foam	sham		CHI 'Richmond'		CHI UXEM 20H		CHI UXEM 20S	
	mean	sd	mean	sd	mean	sd	mean	sd
pH								
15'	7,38	(0,03)	7,35	(0,04)	7,34	(0,04)	7,36	(0,02)
30'	7,39	(0,02)	7,34	(0,05)	7,36	(0,06)	7,37	(0,02)
240'	7,35	(0,03)	7,30	(0,08)	7,29	(0,08)	7,35	(0,04)
pCO_2								
15'	52,9	(3,3)	63,0	(8,5)	60,7	(9,7)	58,8	(3,5)
30'	51,7	(5,9)	64,9	(9,4)	61,5	(8,7)	59,2	(3,9)
240'	55,9	(1,9)	58,1	(9,0)	57,6	(10,3)	54,5	(4,1)
pO_2								
15'	57,7	(9,9)	59,8	(10,1)	61,8	(8,4)	60,0	(7,7)
30'	61,6	(10,4)	53,7	(6,8)	62,7	(9,7)	56,9	(5,2)
240'	44,3	(1,3)	40,9	(9,2)	37,5	(8,9)	45,4	(6,1)
gluc								
15'	12,4	(1,3)	13,5	(1,8)	14,4	(1,9)	13,3	(1,7)
30'	12,3	(0,8)	14,0	(2,3)	14,4	(3,2)	13,0	(1,0)
240'	12,9	(0,8)	12,8	(2,1)	11,3	(3,1)	12,7	(1,9)
lact								
15'	2,2	(0,7)	2,4	(0,6)	2,0	(0,6)	2,5	(0,6)
30'	1,9	(0,2)	2,4	(0,6)	2,3	(0,9)	2,2	(0,5)
240'	1,3	(0,2)	1,8	(0,5)	2,2	(1,5)	1,4	(0,3)

Blood gases, glucose, lactate

All samples were taken from orbital punctures and thus of mixed capillary origin. There was a substantial difference of blood gas values at four hours, possibly related to this technique, sampling from a previously punctured orbit. As presented in table 3, pH was becoming slightly lower over time in all groups of animals. Traumatized rats were more acidotic than sham controls. PO_2 was

not different after trauma as compared to sham controls. Glucose and lactate values were higher in traumatized animals as compared to sham controls. Concerning these samples, differences with respect to supporting types of foam were not observed.

Brain edema

Brain water content after injury was 77.52 ± 0.73%, as compared to 77.13 ± 0.56% in the sham rats. This 0.39% increase in brain water was however not significant. With respect to supporting foam [table 4] a difference in accumulation of brain water content, during the four-hour experiment, was not observed.

table 4 Brain water contents as determined with the wet weight/dry weight method. All values of traumatized animals were higher than sham controls. No difference was observed when comparing the effects of injury utilizing either of the three foam types.

	sham	CHI	CHI	CHI
foam		'Richmond'	UXEM 20H	UXEM 20S
	mean sd	mean sd	mean sd	mean sd
edema				
hemisphere	78,23 (0,36)	78,44 (0,50)	78,57 (0,32)	78,47 (0,66)
brainstem	75,18 (1,41)	76,20 (1,44)	76,01 (0,54)	75,76 (0,88)
total	77,13 (0,56)	77,56 (0,35)	77,56 (0,87)	77,43 (0,92)

Discussion

In a model of head injury, each experiment should be completely reproducible with respect to mechanical loading of the brain, and preferentially also with respect to pathophysiological sequelae. Models of whole head mechanical loading can be divided in models with static loading and dynamic loading (energy transfer within 200 msec)[5]. As acceleration takes place within a few milliseconds, the CHI model can be classified as a model with fast dynamic head loading properties[8]. In the CHI model, it is highly impractical to directly monitor acceleration of the skull, the most important traumatizing source. Thus, we used a deductionistic approach, subdividing the model in standardizable phases in the collision process. These phases can be characterized by values at the beginning and the end of each phase, applying the basic formulas for the conservation of momentum and/or energy. The CHI model is mathematically and physically subdivided in the formation of the momentum of the impactor (1), its impulse to and acceleration of the skull (2), followed by its deceleration by the foam (3) and the subsequent rebound. It was hypothesized that with standardized episodes the model as a whole can be kept within acceptable limits of variation. The effect of the mechanical resistance of the hoist rope on velocity in the original injury apparatus [9], was eliminated by

the use of an electromagnet. Impactor velocity was not altered by obliqueness of the guiding tube, even when this was substantial. Air-outflow obstruction however does impair impactor velocity, but is prevented easily by keeping a proper distance between the outlet of the guidance tube and the head of the animal. Velocity of the impactor is mainly dependent on the height from which the impactor is released and thus easily controlled for. During the early phase of collision the skull sustains acceleration induced by the impactor. It has been shown that the duration of contact between impactor and helmet, comprehending the subsequent episodes of skull acceleration - deceleration - and rebound, lasted approximately 40 msec[8]. In our dynamic foam testing apparatus deceleration of the falling indentor occurred only much later. With respect to the magnitude of acceleration, the foam characteristics are much less prominent.

Static foam testing

Static tests of the used types of foams, obviously different in hardness as can subjectively be observed by manual compression of selected blocks of foam, resulted in different static 'stress to strain ratios'. Even during slow compression of the foam a distinctive counteractive 'viscous peak' always preceded the plateau phase, as has been shown in figure 2. The duration of this peak exceeds the whole time span of the collision in the CHI model. The parameters obtained using a static test disregard these viscous forces occurring during decelleration in the foam. The static 'stress to strain ratio' describes elastic properties of foam. However these elastic forces are much smaller than the viscous counterforces in acceleration experiments, and should not be considered an important 'fingerprint' of the foam used in CHI experiments.

Dynamic foam testing

A foam-testing pendulum, based on a device described in ISO standards 4651, 2439 B, and 3386, was custom developed in our laboratories. The pendulum was adapted to approximate impulse ranges used in CHI experiments. It was observed that with angulations larger than 45 degrees the foam was compressed more than 50%. Since larger compression ratios than 50% confound appropriate foam testing, we chose to adhere to the 45-degree angle, noting that impactor velocities would be lower than those observed in the CHI trauma apparatus. The normalized stress to strain ratio, provided by static tests, was not related to the dynamic compressibility in our used types of foam. Quite understandable, dynamic properties cannot be estimated from measured static stress to strain ratios. This lack of relationship emphasizes the importance of accurate dynamic testing, if repetitive foam quality control is deemed necessary. Used 'Richmond Standard' foam showed lower dynamic compressibility than unused foam, indicating mechanical wear during use in previous CHI experiments.

Animal experiments

Despite of the different mechanical properties of the tested foams, no significant differences in several important outcome parameters could be observed. The variability in mortality might suggest that a foam with a lower dynamic foam coefficient results in higher mortality, this variation was however far from statistically significant. This observation confirms our hypothesis that foam characteristics might play a theoretical role in influencing deceleration and rebound after collision. With respect to animal outcome parameters however, the contribution of foam properties is negligible. In the CHI model, acceleration is the most important physical factor inducing (shearing) injury. Velocity of impactor is the major determinant in skull acceleration. In the first report on this model[8] mortality was reduced to 0% if the weight was dropped from 1 meter height, obviously due to much lower impactor velocities. The magnitude of deceleration (change of velocity in the direction opposite of velocity of the skull, caused by resistance forces of both elastic as well as viscous foam properties) is much lower than the acceleration caused by the impactor during the initial phases of the collision. In the CHI model, the deceleration process does not play a role in the induction of traumatic brain injury.

Conclusion

In the CHI model, acceleration is first and foremost dependent on impactor velocity. Partial restraint of the skull by a supporting foam does not affect acceleration, but does result in narrower limits of head motion, and allows for controlled deceleration. Characteristics of the foam can be standardized to maintain absolute reproducibility utilizing a dynamic foam testing apparatus only. Pragmatically, these foam characteristics play a minor role with respect to reproducibility of mortality, or other parameters related to the severity of head injury.

References

Paper:
van den Brink WA, de Jong DA, van den Heuvel A, et al: Standardization of factors involved in acceleration in the closed head injury model; influence on animal outcome parameters **J Neurosci Meth** 2000 (Submitted)

Presentation, abstract:
van den Brink WA, de Jong DA, van den Heuvel A, et al: The closed head injury model, standardization of acceleration mechanisms. **Intracranial Pressure X.** Williamsburg, 1998 (Abstract)

1. Abd-Elfattah Foda MA, Marmarou A: A new model of diffuse brain injury in rats. Part II: Morphological characterization. J **Neurosurg 80**: 301-313, 1994

2. Barzó P, Marmarou A, Fatouros PP, et al: Magnetic resonance imaging-monitored acute blood-brain-barrier changes in experimental traumatic brain injury. J **Neurosurg 85**: 1113-1121, 1996

3. Barzó P, Marmarou A, Fatouros PP, et al: Contribution of vasogenic and cellular edema to traumatic brain swelling measured by diffusion weighted imaging. J **Neurosurg 87**: 900-907, 1997

4. Elliott KAC, Jasper H: Measurement of experimentally induced brain swelling and shrinkage. **Am J Physiol 157**: 122-129, 1949

5. Gennarelli TA, Thibault LE: Biological models of head injury, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report**. Bethesda, MD, NINCDS: 391-404, 1985

6. Ito J, Marmarou A, Barzó P, et al: Characterization of edema by diffusion-weighted imaging in experimental traumatic brain injury. J **Neurosurg 84**: 97-103, 1996

7. Ludolph GL, Legger RJ: Botsing, in **Theoretische mechanica**. Groningen, J. B. Wolters: 367-204, 1963

8. Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J **Neurosurg 80**: 291-300, 1994

9. Piper IR, Thomson D, Miller JD: Monitoring of weight drop velocity and foam stiffness as an aid to quality control of a rodent model of impact acceleration neurotrauma. J **Neurosci Meth 69**: 171-174, 1996

10. van den Brink WA, de Jong DA, van den Heuvel A, et al: The closed head injury model, standardization of acceleration mechanisms. **Intracranial Pressure X** 1998 (Abstract)

11. van den Brink WA, Marmarou A, Avezaat CJJ: Brain oedema in experimental closed head injury in the rat. **Acta Neurochir Suppl. 51**: 261-262, 1990

12. van den Brink WA, Santos BO, Marmarou A, et al: Temporary blood brain barrier damage and continued edema formation in experimental closed head injury in the rat, in Avezaat CJJ, van Eijndhoven JHM, Maas AIR, et al (eds): **Intracranial Pressure VIII**. Berlin, Heidelberg, New York, Springer-Verlag: 144-147, 1993

Chapter 6

Cortical Dysfunction with Preservation of Brain-Stem Function after Experimental Closed Head Injury

In our earlier studies we questioned whether focal damage could occur in the brain stem as a result of head injury as an isolated event and we suggested at the time that the structural basis of the clinical syndrome of 'primary brain stem injury' was the type of brain damage now referred to as diffuse axonal injury... ***J. Hume Adams, Histopathology, 1989***

Abstract

Object: To examine the extent of brain-stem and cortical dysfunction associated with experimental Closed Head Injury (CHI).

Methods: Male Sprague Dawley rats were submitted to severe or moderate CHI, induced by the weight drop model. In this model of CHI impact is directed to the intact skull, protected against fractures with a steel helmet. During impact the head is held on a foam head support, allowing for acceleration forces inducing diffuse injury. Brain-stem Auditory Evoked Potentials (BAEP) and Somato Sensory Evoked Potentials (SSEP) measuring brain-stem and cortical function, were acquired at regular intervals in survivors up to 4 (n=26) or 24 (n=6) hours after trauma. Sham groups, undergoing all manipulations except for the actual injury, served as control. Result obtained in this model were compared to results obtained in severe Fluid Percussion Injury (FPI) (n=6)

Conclusions: In contrast to traumatic brain injury induced by FPI, primary brain-stem dysfunction was not observed in survivors of CHI. By 24 hours, there were indications for mild brain-stem impairment, probably due to secondary mechanisms such as brain-stem compression or ischemia. At moderate levels of CHI, cortical function showed mild initial impairment with rapid recovery to baseline. At high level CHI, cortical function was severely impaired and did not completely recover within 4 hours.

Introduction

Animal models of head injury have contributed to the understanding of the pathophysiology, and underlying mechanisms of traumatic brain injury. The Fluid Percussion Injury (FPI) model, employed in both cats and rats, has become a standard in modeling diffuse traumatic brain injury, as it represents a reproducible model producing gradational injury[10, 24]. However, at high pressure levels the model produces a predominant brain-stem component[10, 37].

The impact model by Nilson also causes profound brain-stem and cervical cord damage, resulting from the more than 90 degree dorsiflexion of the neck[30]. Referring to the human situation, this is important as severe injury is usually not associated with isolated brain-stem damage[2]. A rodent model of Closed Head Injury (CHI), which consists of both a mechanical impact and acceleration force to the intact skull, was developed by Marmarou[23]. At severe levels this weight drop model produces 50% mortality in the absence of skull fractures, and levels of unconsciousness which are similar to those obtained in FPI. The objective of this study was to study the integrity of the brain-stem, and cortical function after CHI. This was performed utilizing both Brain-stem Auditory Evoked Potentials (BAEP) and Somato Sensory Evoked Potentials (SSEP), as they provide neuro-physiological information of the structures of interest.

Materials and methods

Experimental Protocol:

Studies were performed in male Sprague Dawley rats weighing 342 ± 40 grams (mean ± sd). Anesthesia was induced with methofane using the chamber technique and maintained with either α-chloralose (90 mg/kg, intraperitoneal) or halothane through an endotracheal tube (1 to 2%, in a nitrous oxide: oxygen mixture 66%: 33%). Heart rate and respiration were monitored by means of subcutaneous needle electrodes. Core body temperature, was monitored by a rectal probe and maintained at 38^0 C.

Ten rats were subjected to severe (> 3.0 atm) trauma injury with the midline FPI model as described by Dixon[10]. BAEP's were measured in six survivors. Twenty two rats were subjected to severe CHI induced by the weight drop model[23]. BAEP's were measured in four, SSEP's in eight surviving animals. Fourteen rats were subjected to mild CHI. All rats survived this level of injury; BAEP's were measured in eight rats, SSEP's in six. Duration of these studies was four hours. Control values were always obtained just before trauma. BAEP's were acquired at 5, 15, 30 minutes and every hour after trauma. Near field SSEP's were recorded for the first 15 minutes, at 30 minutes and every hour after trauma, additional far field SSEP's were recorded every hour after 30 minutes. Additionally BAEP's and SSEP's were studied after 24 hours in six rats surviving severe CHI. Twelve sham experiments, in which all procedures except for the actual trauma were performed, served as controls (six for BAEP's and six for SSEP's). Details of the different injury protocol groups (group 0-V) are shown in table 1.

The protocols were approved by the Institutional Animal Care and Use Committee, Medical College of Virginia, Richmond, Virginia.

table 1 Diagram of the five different protocol groups. Mild injury does not cause mortality. Mortality of the FPI injured rats at the > 3 atm. levels used in this study is comparable to mortality after severe CHI. Interesting is the mortality change in the animals that were intubated. Emphasizing the importance of an open airway early after injury.

	trauma (level)	number (survivors)	intubated	anaesthetic	evoked potential	duration
group 0	FPI (severe)	10 (6)	yes	halothane	BAEP	4 hrs
group I	CHI (severe)	11 (4)	no	chloralose	BAEP	4 hrs
group II	CHI (mild)	6 (6)	no	chloralose	BAEP	4 hrs
group III	CHI (severe)	11 (8)	yes	halothane	SSEP	4 hrs
group IV	CHI (mild)	8 (8)	yes	halothane	SSEP	4 hrs
group V	CHI (severe)	10 (6)	yes	halothane	BAEP & SSEP	24 hrs

Trauma Model

The mechanics of the new CHI model employed in group I - V, have been reported in chapter 4. Briefly the rat is submitted to traumatic brain injury by weight drop on the intact skull. Limited movement, impact, compression, and acceleration are provided by supporting the skull with foam. A stainless steel disk is cemented on the vertex to prevent skull fractures. The 450 gram weight was dropped onto the disc from a height of either 1 or 2 meter. These levels will be referred to as 'mild' and 'severe' injury. Rebounding is prevented by quickly removing the foam bed with the rat sideways after the first contact.

Neuro-Physiological Measurements

Brain-stem Auditory Evoked Potentials

Square wave click stimuli with a duration of 100 msec and an intensity of 85 dB nHL (normal Hearing Level) were generated by a Nicolet Compact Four signal averager (Nicolet Biomedical Instruments, Madison, Wisconsin) at a 11.4 Hz rate.

Recordings in the CHI model were made with carefully secured needle electrodes at the left and right mastoid (A1, A2), over the frontal sinus (Fpz), in the occipito-cervical muscles (Cz), and over the right sensory cortex (C4). In the FPI experiments Cz was represented with an epidural vertex screw, which was also used for luer lock fixation of the trauma device. The Fpz electrode was grounded. A2 (ipsilateral mastoid) was used as the reference. A1 and Cz were active electrodes. Impedances of electrodes referred to the ground electrode were below 7 kΩ. The responses were filtered (30-3000 Hz), amplified (250 mV/full scale) and averaged 500 times for an epoch length of 10 msec. To confirm consistency two averages per condition were acquired before and at frequent intervals after trauma. The data were stored on floppy disks, and printed out with a M8510 + dot matrix printer, active electrode positive

displayed upwards. According to Jewett and Romano the positive peaks were referred to sequentially with roman numerals[19] The often obtained small cochlear microphonic potential about 0,51 msec. before wave I was labeled C.M. Peak latencies were measured utilizing the averager system software, referring to the beginning of the stimulation artifact. Auditory Brain-stem Conduction Time (ABCT) is defined as the latency difference between the IVth and Ist wave[36]. This ABCT is considered the main indicator of adequate brain-stem function. Amplitudes were measured from positive peak to following trough. Somato Sensory Evoked Potentials

Square wave current stimuli with a duration of 100 msec and intensity of 5 mA, just enough to show a clearly definable paw twitch, were generated by the Nicolet Compact Four at a 4.2 Hz rate and delivered by two needle electrodes placed in each forepaw with the cathode placed proximal.

The active epidural screw electrode was located over the sensory cortex, just anterior to the coronal suture and 2 mm lateral from the sagittal suture (C4). Fpz was used as reference, the Cz electrode was grounded. Impedances of the electrodes referred to the ground electrode were below 7 kΩ.

The SSEP responses were filtered (5-3000 Hz) and 'amplified (250 mV/full scale), averaged 150 times with an epoch length of 20 msec. To be able to clearly examine the far field somatosensory components, that are often no more than small deflections of the curve leading to the first positive potential, we changed the band pass, number of averages and epoch length to 1-3000 Hz, 500, 10 msec. respectively. Separate far field potentials were acquired from 30 min. post trauma to the end of the experiment. SSEP's were stored and printed as mentioned, the active electrode positive was displayed downward. The rat near field SSEP consists of 2 major positive and 2 major negative peaks, labeled P1, N1, P2, N2 in succession[34]. The far field potentials that visualized just before P1 were labeled I, II, III[41]. Latencies were analyzed from the stimulation artifact to peak. Somato Sensory Central Conduction Time (SCCT) is defined as the latency difference between the P1 wave and the component II. Near field peak amplitudes were measured from peak to following peak. SSEP activity can be quantified by means of amplitude summation, as this method results in values that are amenable to statistical analysis.

Statistics

Comparisons of each time point versus control, and versus results of sham animals were made. Analysis was performed using the student T-test, P-values < 0.05 were considered significant.

Results

Mortality and skull fractures

Two rats in the entire series of CHI injured rats (n=45) had a skull fracture, both were injured at the severe 450 gram 2 meter level. One rat had a dura tear due to screw placement. Data from these three experiments were not used for analysis. Mortality in mild CHI (groups II and IV) was zero, in severe CHI (groups I, II, and V) 37 %. Intubated animals had a considerably lower mortality (25%) than non-intubated animals (60%). In severe FPI (group 0) mortality was 40%, comparable to severe CHI.

Electrode configuration, anesthesia, and reference values

BAEP responses from the Cz-A2 and the A1-A2 needle electrode configuration, as used in CHI experiments, were acquired simultaneously. These were similar with respect to latencies, but different with respect to amplitudes, an observation previously described by Plantz et al.[32]. Individual waves obtained by the A1-A2 configuration were more clearly distinguishable. Baseline recordings of all CHI experiments were grouped to obtain our reference data set, BAEP latencies and amplitudes are shown in table 2. For CHI, the results of the A1-A2 configuration will be reported. When utilizing the Cz epidural screw, only possible in FPI experiments, a more pronounced peak IV was obtained. For FPI, results of the Cz-A2 configuration will be reported. BAEP's remained robust and did not differ among different anesthetic agents, or intubation protocols. Baseline SSEP latencies and amplitudes are shown in table 3. In both BAEP and SSEP recordings variation of amplitudes was more substantial than latency variability. Fourth wave amplitude is too small for statistical analysis.

table 2 Reference data set of rat BAEP latencies and amplitudes (mean ± sd). Values of both electrode configurations (CZ-A2, and A1-A2) are shown. The differences are caused by near field effects due to the small volume of the head. Amplitudes have larger standard deviations than latencies.

latencies	cm	I	II	III	IV	I - IV
Cz-A2	0, 67 (0, 06)	1, 21 (0, 08)	2, 28 (0, 26)	2, 94 (0, 21)	4, 02 (0, 33)	2, 81 (0, 05)
A1-A2	0, 67 (0, 05)	1, 14 (0, 07)	2, 08 (0, 13)	2, 77 (0, 19)	3, 89 (0, 30)	2, 75 (0, 27)
amplitudes	cm	I	II	III	IV	
Cz-A2	0, 17 (0, 12)	1, 72 (0, 76)	0, 35 (0, 40)	1, 10 (0, 50)	0, 58 (0, 54)	
A1-A2	0, 14 (0, 10)	0, 86 (0, 39)	1, 67 (0, 55)	1, 56 (0, 50)	0, 40 (0, 82)	

table 3 Reference data set of rat SSEP latencies and amplitudes (mean ± sd).

C4-FPz	component I	component II	component III	P1	N1	P2	N2	II-P1
latencies	1.60 (0.02)	3.42 (0.04)	5.11 (0.06)	6.89 (0.08)	8.49 (0.07)	10.66 (0.10)	14.11 (1.16)	3.47 (0.09)
								ampl. summ.
amplitudes				6.78 (0.34)	2.86 (0.26)	14.30 (1.31)	5.87 (0.65)	32.91 (15.97)

Brain-stem Auditory Evoked Potentials

Fluid Percussion Injury

After FPI the BAEP- third and fourth wave, were abolished. Figure 1A shows a typical example of BAEP's after FPI with partial recovery only after 120 minutes. Until four hours the latency of the fourth wave was prolonged, and its amplitude remained depressed. Figure 2B shows the mean ABCT over time. It was not always possible to define the fourth wave after FPI. Peaks that were visible had prolonged latencies, and ABCT's were significant during the four hours of the experiment. Peak abolishment and prolongation of ABCT are indicative of prolonged brain-stem dysfunction.

mild Closed Head Injury

After mild trauma there was no difference in the BAEP's as compared with the BAEP's obtained at baseline. As in sham animals, wave form morphology remained intact, no peaks disappeared. Figure 1B displays an example of the BAEP's during the 4 hour experiment. Prolongation of latencies, or ABCT [figure 2C] as a indicators of brain-stem integrity, were not observed.

severe Closed Head Injury

In surviving animals, severe trauma did not cause alteration of the BAEP's. Wave form morphology remained intact, no peaks disappeared [figure 1C]. Prolongation of latencies, or ABCT [figure 2D] as a indicators of brain-stem integrity, were not observed, during the first 4 hours. BAEP's obtained 24 hours after injury (group 5), did not show a significant prolongation of ABCT, as compared to baseline values. Although in sham rats [figure 2A] ABCT was significantly shorter after 24 hours than ABCT 24 hours after severe CHI.

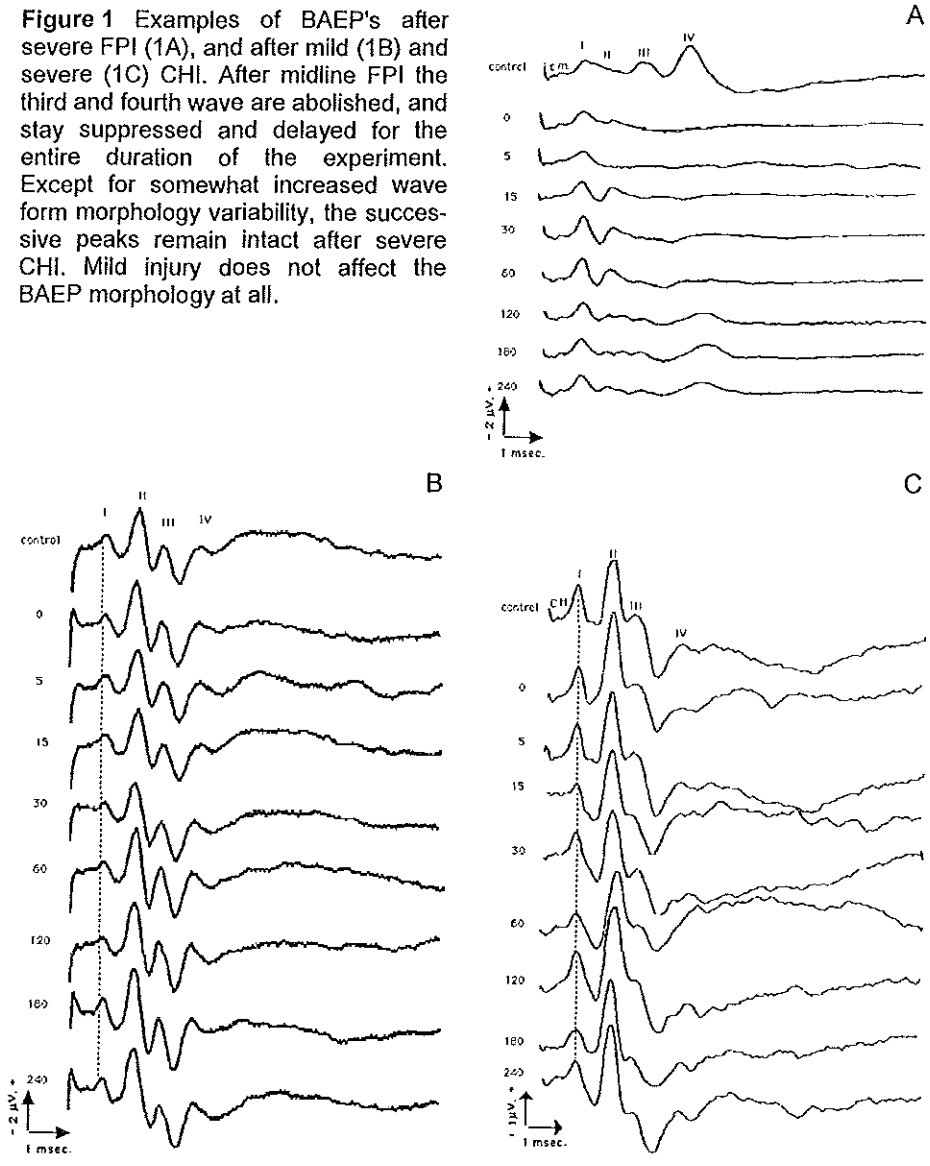
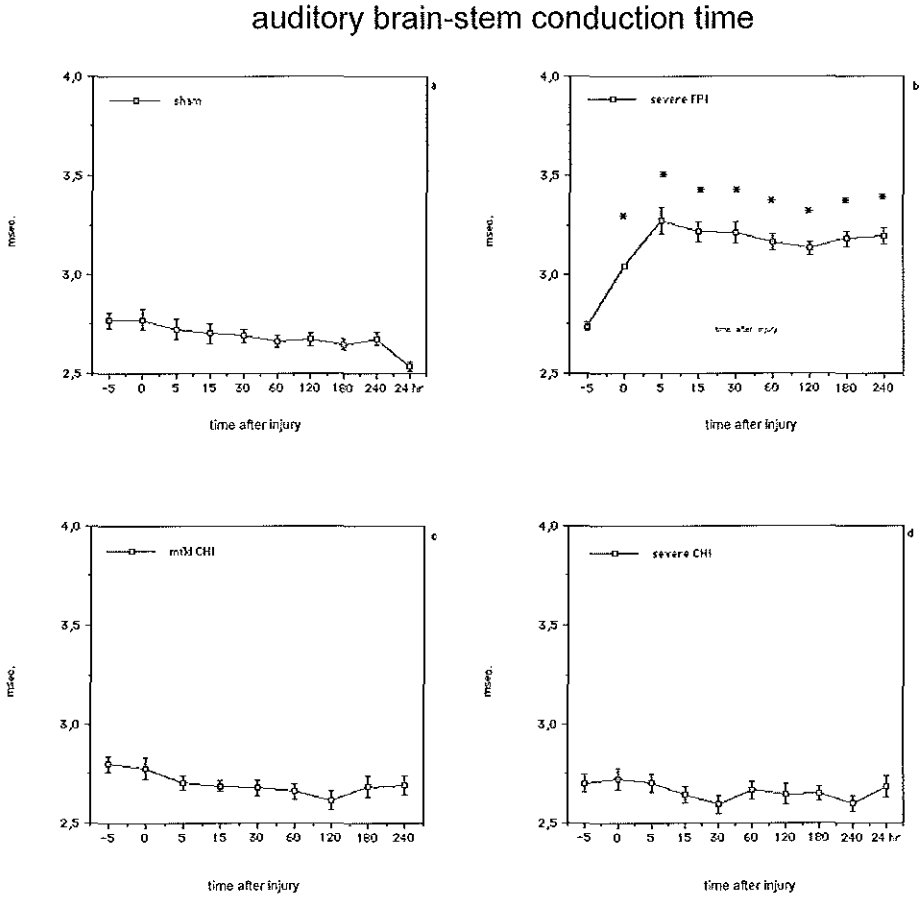


Figure 1 Examples of BAEP's after severe FPI (1A), and after mild (1B) and severe (1C) CHI. After midline FPI the third and fourth wave are abolished, and stay suppressed and delayed for the entire duration of the experiment. Except for somewhat increased wave form morphology variability, the successive peaks remain intact after severe CHI. Mild injury does not affect the BAEP morphology at all.

Figure 2 BAEP-ABCT (mean ± sem) after injury. There is no prolongation of ABCT in sham animals (A) or after CHI (C&D). ABCT is significantly prolonged after FPI (B).



Somato Sensory Evoked Potentials

Mild Closed Head Injury

A typical example of the course of the SSEP's after mild trauma is displayed in figure 3A. Some of the late near field SSEP peaks are abolished during the first minutes. This is followed by a rapid recovery. The P1 and N1 waves with the short latencies tend to recover earlier than the P2 and N2 waves. The three far field potentials I, II, and III, did not show latency prolongation after trauma. SCCT was prolonged significantly up to 30 minutes. SCCT values in relation to baseline are shown in figure 4. SSEP measurements, quantified by summing peak to peak amplitudes resulted in significant decreases of activity during the first 120 minutes. Relative decreases of amplitude summation in relation to baseline observations are shown in figure 5.

Severe Closed Head Injury

A typical example of the course of the SSEP's of a surviving rat after trauma is displayed in figure 3B. All near field SSEP's are abolished directly after severe injury. This is followed by a gradual recovery. Again waves with the short latencies tend to recover earlier. The three far field potentials I, II, and III, did not show latency prolongation after trauma. SCCT was prolonged slightly but significantly up to 60 minutes, and again after 24 hours. SCCT values in relation to baseline are shown in figure 4. SSEP amplitude summations resulted in significant decreases of activity during the first 60 minutes, and again after 24 hours. Relative decreases in relation to baseline observations are shown in figure 5.

Figure 3 Examples of SSEP's after mild (3A) and severe (3B) CHI. The SSEP is abolished directly after trauma. After severe injury all the waves are affected longer and more pronounced than after mild injury.

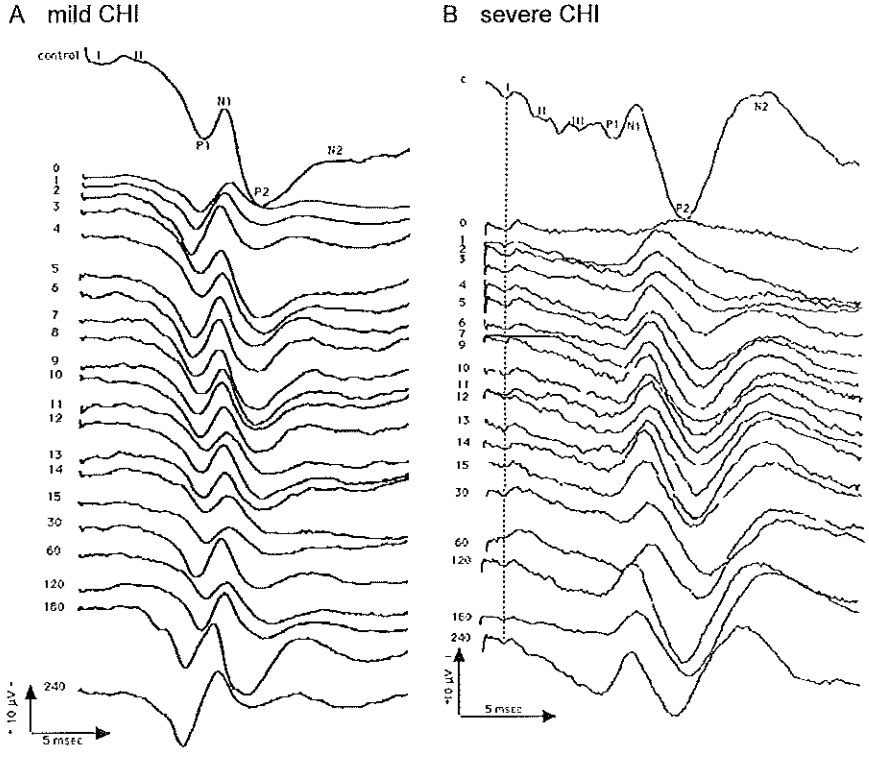


Figure 4 Somato Sensory Central Conduction time, expressed in percentage of each baseline value. CCT's are significantly prolonged for the first 60 minutes after trauma. During the first 15 minutes far field SSEP's were not acquired. It is likely that during the first period the CCT was prolonged even more.

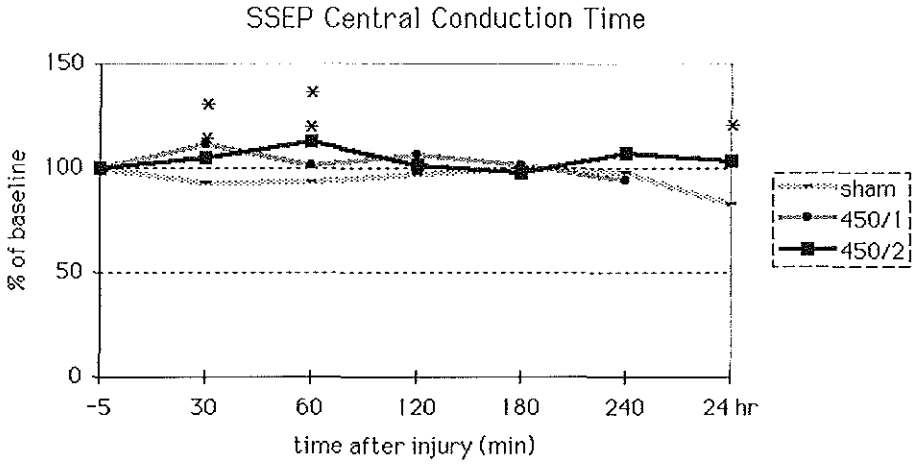
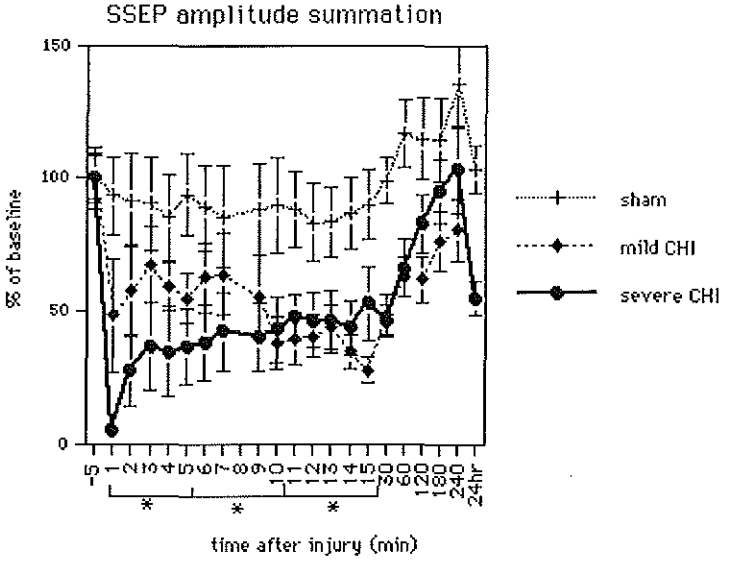


Figure 5 SSEP near field amplitude summation (mean) expressed in percentage of each baseline value. Data from the first three 5-minute blocks of observations were grouped and tested for significance. Values obtained after the first 15 minutes were tested individually. Amplitudes are significantly reduced for the first 60-120 minutes after trauma, and gradually recovering. After 24 hours the amplitude summation of severely injured animals decreases again.



Discussion

Our study on rats injured with the FPI device confirms the observations of the cat experiments as reported by Shima et al.[37]: FPI causes significant and sustained brain-stem dysfunction. This dysfunction is evident by changes of the fourth wave characteristics, the rodent equivalent of the human fifth wave, thought to be generated in the rostral brain-stem[26, 36]. ABCT prolongation does not have a straight forward relationship with the severity of brain-stem damage, especially not when recorded very early after injury, when electrical dysfunction might be temporary. ABCT holds however a prognostic value in head injured patients, and complete and persistent absence of peak V does relate to very poor outcome[21]. Based on morpho-pathological findings in their major characterization studies Dixon et al.[10] and McIntosh et al.[21] independently suggested that in the rat the FPI model causes predominantly lower brain-stem damage. The question can be raised whether the observed brain-stem dysfunction in the FPI model is due to characteristics specific to this model, and not related to the diffuse injury itself. If the explanation for the damage to the stem lies in the mechanical distortion of the contents of the cranial vault at foraminal level, occurring with the sudden increase of volume in the closed cranium, extrapolation of results from the FPI model to the clinical situation is in many settings, debatable. A model without this feature is a necessity to study pathophysiological mechanisms in severe experimental closed head injury[10, 13, 29, 37].

BAEP studies

Direct brain-stem compression is not a feature of the weight drop model, with a completely different mechanical induction of acceleration trauma. The authors hypothesized that the characteristic brain-stem damage observed after FPI, may be absent in this different model of severe diffuse CHI. The results described provide evidence for this hypothesis: Directly after severe injury, BAEP's remained unaffected in all animals surviving CHI. The ABCT, indicator of brain-stem function, was not prolonged. Obviously brain-stem function remains preserved immediately after CHI. Twenty-four hours after trauma however BAEP parameters were significantly different from the sham control group. This suggests a delayed mechanism, possibly caused by ongoing secondary insults, resulting in brain-stem compression. Edema studies in this model as discussed in chapter 7 support this hypothesis[19]. Rats not surviving CHI died early, before an adequate evoked potential study could be performed. Brain-stem injury in non-survivors can therefore not be excluded.

SSEP studies

Impact acceleration models are often associated with persistent severe damage to the cervical cord[30]. In this model however the three far field components, generated in the posterior column (I), nucleus cuneatus (II), and ventral posterior thalamus and cerebellar pathways (III), remain intact during the experiment. The rapid return of the early near field potential P1, which is generated in the thalamus or its projections[4, 11], also indicates that CHI does not

induce permanent damage to the cervical cord. Although SCCT was prolonged early after injury, this prolongation was relatively small.

Evidence for supratentorial damage is found in the effect of injury on cortical generated SSEP waves. These waves can be quantified by means of amplitude summation, as the obtained values have a good correlation to clinical outcomes and to other methods of SSEP grading [5]. Moreover this method results in values that are amenable to statistical analysis. Mild injury caused abolishment of the P2 and N2 peaks for up to 16 minutes in some cases. Recovery was rapid and complete in most instances. After severe injury there is an initial abolishment of all peaks. Partial recovery takes place after 15 minutes. 24 hours after severe injury, prolonged SCCT and lower amplitudes, indicate a decrease in cortical activity again. This delayed event indicates either a secondary injury mechanism or an ongoing neurological deterioration[18].

Relation to the clinical situation

Electro-neurophysiology is an accurate means for quantitatively investigating neurological function of the brain and brain-stem in vivo, particularly when pharmacological sedation forestalls clinical assessment. The practical value of evoked potential monitoring in the evaluation of head injury patients has been reported by several groups[5, 7, 12, 15, 16, 21, 27, 28]. Although it has been shown that brain-stem injury is predictive of poor outcome[22, 33], the mentioned studies show that mainly SSEP's, and not BAEP's have a predictive value. Among other studies [22, 39] the report by Lindsay et al [21] did however show a correlation of ABCT with outcome. Moreover, they noted that none of the patients with an absent fifth wave have even a moderate outcome. Isolated or predominant injury to the brain-stem is a rare entity however [3, 25]. This clinical experience is consistent with the experimental findings of Ommaya and Gennarelli[31], who observed that the distribution of damaging strains as induced by inertial loading would decrease in magnitude from the surface to the center of the brain. In the field of animal research the use of BAEP's and SSEP's and the alteration of the different wave characteristics due to physiological alterations[6, 14, 38] and pharmacological influences[8, 9, 17, 35] have been thoroughly investigated. Letcher et al.[20], in a monkey study of experimental acceleration injury, observed that the duration of depressed consciousness is very comparable to the duration of SSEP depression, concluding that abnormalities of evoked potentials, rather than of EEG accompany 'concussion'. In our studies of post-traumatic behavior we found that the duration of depressed consciousness is in the same order of magnitude as the duration of SSEP depression (unpublished data). In a less standardized model of acceleration injury in the awake rat, Shaw [34] has utilized SSEP's and found similar abnormalities as we did. He concluded that temporal synaptic dysfunction is the main reason for the clinical state of cerebral 'concussion', although the level therefrom remained unclear. Our study supports the cortical origin of cerebral 'concussion', as brain-stem parameters remained intact during the abnormalities of SSEP's. One might argue however that the BAEP is too robust to indicate subtle dysfunction of the nearby located reticular formation.

Conclusions

In this study neuro-physiological parameters were used to evaluate the function of neural pathways in the cervical cord, brain-stem and cortical areas after mild and severe CHI. In contrast to the findings after FPI, with the known and in this study confirmed disadvantage of severe brain-stem damage, are the results of our findings in the CHI in the rat. While survivors of FPI clearly suffer damage to the brain-stem, the neuro-physiological cervical cord and brain-stem parameters after CHI remain unaffected. The SSEP's suggest dysfunction at cortical level, which is of considerable duration. Both the mechanics of the model, and the brain-stem preservation, bare close resemblance with human head injury.

References

Presentations, abstracts:
van den Brink WA, Slomka W, de Jong DA, et al: Neurophysiologische karakterisatie van gesloten schedel hersen letsel; een experimentele studie in de rat. **Symp Exp Ond Heelk Spec** 5:11-28, 1992 (Abstract)

van den Brink WA, Slomka W, Marmarou A, et al: Neurophysiological evidence of preservation of brain stem function in experimental closed head injury in the rat, in Avezaat CJJ, van Eijndhoven JHM, Maas AIR, et al (eds): **Intracranial Pressure VIII**. Rotterdam, Springer-Verlag:221-226, 1993 (Abstract)

1. Abd-Elfattah Foda MA, Marmarou A: A new model of diffuse brain injury in rats. Part II: Morphological characterization. J **Neurosurg 80**: 301-313, 1994

2. Adams JH, Graham DI, Murray LS, et al: Diffuse axonal injury due to non missile head injury in humans: an analysis of 45 cases. **Ann Neurol 12**: 1982

3. Adams JH, Mitchell DE, Graham DI, et al: Diffuse brain damage of immediate impact type. Its relationship to 'primary brain-stem damage' in head injury. **Brain 100**: 489-502, 1977

4. Allison T, Hume AL: A comparative analysis of short latency somatosensory evoked potentials in man, monkey, cat and rat. **Exp Neurol 72**: 592-611, 1981

5. Brown JIM, Moulton RJ, Konasiewicz SJ, et al: Cerebral oxidative metabolism and evoked potential deterioration after severe brain injury: New evidence of early posttraumatic ischemia. **Neurosurgery 42**: 1057-1064, 1998

6. Budnick B, McKeown KL, Wiederholt WC: Hypothermia induced changes in rat short latency somato sensory evoked potentials. **Electroencephalogr Clin Neurophysiol 51**: 19-31, 1981

7. Cant BR, Hume AL, Judson JA, et al: The assessment of severe head injury by short-latency somatosensory and brain-stem auditory evoked potentials. **Electroencephalogr Clin Neurophysiol 65**: 188-195, 1986

8. Church MW, Gritzke R: Effects of ketamine anaesthesia on the rat brain-stem auditory evoked potential as a function of dose and stimulus intensity. **Electroencephalogr Clin Neurophysiol 67**: 570-583, 1987

9. Church MW, Gritzke R: Dose dependent effect of atropine sulfate on the brain-stem and cortical evoked potentials in the rat. **Brain Res 456**: 224-234, 1988

10. Dixon CE, Lyeth BG, Povlishock JT, et al: A fluid percussion model of experimental brain injury in the rat. J **Neurosurg 67**: 110-119, 1987

11. Dong WK, Harkins SW, Ashleman BT: Origins of cat somatosensory far field and early near field evoked potentials. **Electroencephalogr Clin Neurophysiol 53**: 143-165, 1982

12. Firsching R, Frowein RA: Multimodality evoked potentials and early prognosis in comatose patients. **Neurosurg Rev 13**: 141-146, 1990

13. Gennarelli TA, Thibault LE: Biological models of head injury, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report**. Bethesda, MD, NINCDS: 391-404, 1985

14. Gold S, Cahani M, Sohmer H, et al: Effects of body temperature elevation on auditory nerve brain-stem evoked potentials and EEG's in rats. **Electroencephalogr Clin Neurophysiol 60**: 146-153, 1985

15. Greenberg RP, Becker DP, Miller JD, et al: Evaluation of brain function in severe human head trauma with multi modality evoked potentials. Part 2: Localization of brain dysfunction and correlation with posttraumatic neurological conditions. J **Neurosurg 47**: 163-177, 1977

16. Greenberg RP, Mayer DJ, Becker DP, et al: Evaluation of brain function in severe human head trauma with multi modality evoked potentials. Part 1: Evoked brain injury potentials, methods, and analysis. J **Neurosurg 47**: 150-162, 1977

17. Hirose G, Kitagawa Y, Chujo T, et al: Acute effects of phenytoin on brain-stem auditory evoked potentials: Clinical and experimental study. **Neurology 36**: 1521-1542, 1986

18. Holmin S, Schalling M, Hojeberg B, et al: Delayed cytokine expression in rat brain following experimental contusion. **J Neurosurg 86**: 493-504, 1997

19. Jewett DL, Romano MN: Neonatal development of auditory system potentials averaged from the scalp of rat and cat. **Brain Res 36**: 101-115, 1972

20. Letcher FS, Corrao PG, Ommaya AK: Head injury in the chimpanzee Part 2: Spontaneous and evoked epidural potentials as indices of injury severity. **J Neurosurg 39**: 167-177, 1973

21. Lindsay KW, Pasaoglu A, Hirst D, et al: Somatosensory and auditory brain-stem conduction after head injury: a comparison with clinical features in prediction of outcome. **Neurosurgery 26**: 278-285, 1990

22. Lütschg J, Pfenninger J, Ludin HP, et al: Brain-stem auditory evoked potentials and early somatosensory evoked potentials in neurointensively treated comatose children. **Am J Dis Child 137**: 421-426, 1983

23. Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. **J Neurosurg 80**: 291-300, 1994

24. McIntosh TK, Noble LJ, Nadrews B, et al: Traumatic brain injury in the rat: characterization of a midline fluid percussion model. **Cent Nerv Syst Trauma 4**: 119-134, 1987

25. Mitchell DE, Adams JH: Primary focal impact damage to the brain-stem in blunt head injuries - does it exist? **Lancet 2**: 215-218, 1973

26. Moller AR, Burgess J: Neuronal generators of the brain-stem auditory evoked potentials (BAEP's) in the rhesus monkey. **Electroencephalogr Clin Neurophysiol 53**: 361-372, 1986

27. Moulton RJ, Shedden PM, Tucker WS, et al: Somatosensory evoked potential monitoring following severe closed head injury. **Clin Invest Med 17: 3**: 187-195, 1993

28. Narayan RK, Greenberg RP, Miller JD, et al: Improved confidence of outcome prediction in severe head injury. A comparative analysis of the clinical examination, multimodality evoked potentials, CT scanning, and intracranial pressure. **J Neurosurg 54**: 751-762, 1981

29. Nilsson B, Ponten U: Metabolism and neurophysiological function following head injury, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report**. Bethesda, MD, NINCDS: 1985

30. Nilsson B, Ponten U, Voigt G: Experimental head injury in the rat. Part 1: Mechanics, pathophysiology, and morphology in an impact acceleration trauma model. **J Neurosurg 47**: 241-251, 1977

31. Ommaya AK, Gennarelli TA: Cerebral concussion and traumatic unconsciousness correlation of experimental and clinical observations on blunt head injuries. **Brain 97**: 633-654, 1974

32. Plantz RG, Williston JS, Jewett DL: Spatio-temporal distribution of auditory-evoked far field potentials in rat and cat. **Brain Res 68**: 55-71, 1974

33. Seelig JM, Greenberg RP, Becker DP, et al: Reversible brain-stem dysfunction following acute subdural hematoma. **J Neurosurg 55**: 516-523, 1981

34. Shaw NA: Somatosensory evoked potentials after experimental head injury in the awake rat. **J Neurol Sci 74**: 257-270, 1986

35. Shaw NA: The effect of pentobarbital on the auditory evoked response in the brain-stem of the rat. **Neuropharmacology 25**: 63-69, 1986

36. Shaw NA: The auditory evoked potential in the rat--a review. **Prog Neurobiol 31**: 19-45, 1988

37. Shima K, Marmarou A: Evaluation of brain-stem dysfunction following severe fluid percussion head injury to the cat. **J Neurosurg 74**: 270-277, 1991

38. Simpson GV, Knight RT, Brailowsky S, et al: Altered peripheral and brain-stem auditory function in aged rats. **Brain Res 348**: 28-35, 1985

39. Tsubokawa T, Nishimoto H, Yamamoto T, et al: Assessment of brainstem damage by the auditory brainstem response in acute severe head injury. **J Neurol Neurosurg Psychiatry 43**: 1005-1011, 1980

40. van den Brink WA, Marmarou A, Avezaat CJJ: Brain oedema in experimental closed head injury in the rat. **Acta Neurochir Suppl. 51**: 261-262, 1990

41. Wiederholt WC, Iragui Madoz VJ: Far field somato sensory potentials in the rat. **Electroencephalogr Clin Neurophysiol 42**: 456-465, 1977

Chapter 7
Blood Brain Barrier Dysfunction and Edema in Experimental Diffuse Head Injury.

It must be remembered, however, that the sudden removal of pressure from the brain when the blood-pressure has been forced to considerable heights may be followed by a paralysis (of the cerebral vessels).... The occasion of this can be readily brought out by post mortem examinations....

Harvey Cushing.
The American Journal of the Medical Sciences, 1902

Abstract

The integrity of the Blood Brain Barrier and temporal course of edema formation were studied in the weight drop model of closed head injury in the rat. In this model diffuse brain injury is induced by impact and acceleration of the protected skull. In contrast to fluid percussion injury this model does not produce a post-traumatic hypertensive surge. Using an albumin-bound radioactive tracer it was shown that blood brain barrier disruption is of short duration and occurs early after head injury. A subsequent rise in arterial pressure further increases the magnitude of this dysfunction. Microgravimetrical techniques were used to study brain water content (BWC) after trauma. Brain edema gradually increases during the first 24 hours after injury. These studies indicate that vascular damage might play a role in the initiation of cerebral edema, subsequent events of a different origin however, must be held responsible for the continuation of edema formation. With respect to the importance of the well-documented post-traumatic cerebral ischemia, cellular edema is most likely an important contributor to post-traumatic brain swelling in diffuse traumatic brain injury.

Introduction

Raised intracranial pressure (ICP), due to brain swelling, is one of the main factors causing secondary deterioration of the clinical condition and worsening of the neurological outcome in severe head injury[15, 22, 50]. Swelling of the brain has been attributed to both vascular engorgement and brain edema[15, 23, 24, 26, 27, 32, 51]. Elucidation of the relative importance of these factors and the time course of their development can help towards targeting treatment of raised ICP. Two main types of edema have been described in brain injury: cytotoxic, and vasogenic[18]. Cytotoxic brain edema is caused by swelling of the astroglia, due to cellular ischaemia or hypoxia. This type of edema is most often modeled by means of vascular occlusion studies[18, 21, 35]. Vasogenic edema has been implicated as most important in head injury, primarily based on experimental studies utilizing the cryogenic injury model of focal cerebral contusion [18, 20, 45]. Studies

with the fluid percussion injury (FPI) model, more closely allied to diffuse traumatic brain injury, supported these findings[6, 29, 34]. In this model, however, a dramatic surge of blood pressure is observed immediately upon impact, which may exacerbate fluid flow over the damaged barrier and promote further edema formation[7, 24, 32, 33].

The objective of this research was to study blood brain barrier integrity and development of edema in a model of diffuse Closed Head Injury (CHI), in which the surge of arterial pressure immediately following trauma is considerably less than in models of direct dural impact.

Materials and methods

Trauma model:

Experimental diffuse head injury was induced with the weight drop model. In this model impact/acceleration trauma is induced by dropping a weight of 450 grams from a height of 2.00 meters on the intact skull, supported by a bed of foam. The occurrence of fractures is prevented by protecting the exposed skull with a stainless steel disk. Rebound was prevented by quickly removing the foam bed with the rat sideways after the first contact. In this model, the severity of induced injury depends mainly on acceleration and duration of impact. Magnitude of acceleration depends on the height from which the weight is dropped, but also on characteristics of the foam bedding. Impact, and duration thereof, is dependent on the weight dropped on the skull. Biomechanics and pathophysiology of this model have been described in detail[28]. An additional seven rats were traumatized with the midline FPI model at 3.3 atm level. This injury severity level is comparable to 450 gram/2 meters CHI.

Animal preparation

CHI studies were performed in male Sprague Dawley rats weighing 353 ± 41 gram. Anesthesia was induced in a glass chamber saturated with Methophane. When appropriate levels of anesthesia were achieved, as assessed by the absence of corneal and hind paw withdrawal reflexes, the animals were intubated. Anesthesia was maintained using Isoflurane in a 1 to 2% concentration in an oxygen: nitrous oxide mixture (1: 2). In 10 animals the anesthetic regimen with Ketamine/Xylazine (87 mg/kg; 13 mg/kg I. M.), of one of the cornerstone articles concerning FPI[7], was used. Thirty-six surviving rats were allocated to studies on blood brain barrier integrity (18 injured, 18 sham) and 24 to studies of edema formation (12 injured, 12 sham)

Blood brain barrier studies

In studies of the BBB, 30 µCi I^{125} Radio labeled Bovine Serum Albumin (RISA) in 0.8 ml phosphate buffered Saline (PBS) was injected in the left femoral vein. The RISA was allowed to circulate and distribute evenly over the vascular compartment for 30 minutes before experimental trauma was inflicted. Fifteen

rats served as sham controls; 25 rats were subjected to experimental CHI; 10 of these died within 30 minutes of trauma. Seven rats were subjected to midline FPI, four of these died within 30 minutes after trauma. The surviving 36 rats (15 CHI, 15 sham; 3 FPI, 3 sham) were divided into 8 groups as specified in table 1A.

Table 1 Overview of the number of animals, anaesthetic agents, and time of termination. Table 1A shows the groups used for BBB studies, table 1B the groups for edema studies.

Table 1A

	survivors of injury	sham controls	time of termination	anesthesia
group 1	CHI = 5	-	4 hours	Isoflurane
group 2	-	n = 5	4 hours	Isoflurane
group 3	CHI = 5	-	15 hours	Isoflurane
group 4	-	n = 5	15 hours	Isoflurane
group 5	CHI = 5	-	4 hours	Ket/Xyl
group 6	-	n = 5	4 hours	Ket/Xyl
group 7	FPI = 3	-	15 hours	Isoflurane
group 8	-	n = 3	15 hours	Isoflurane

Rats were killed at 4, 10, and 15 hours after injury by intraperitoneal injection with Pentobarbital (60 mg/kg). Intra-vascular blood was removed by rapid trans-cardial saline perfusion at 80 mmHg until the return of fluid was clear. Hereafter the skull was inspected for fractures, opened, and the brain with the first two segments of spinal cord removed. The arachnoid membrane was carefully dissected from the convexity and basal area to remove any traumatic subarachnoid hemorrhage. The specimen was subsequently frosted for no longer than three hours to facilitate standardized cutting in five supratentorial and two infratentorial segments. Blood samples and brain segments were weighed with a.001 gram accuracy (Mettler Scale, Scientific Products, Evanston Illinois). The radioactivity of each segment was counted in a gamma counter (Beckmann Gamma 4000, Beckmann Instruments Inc., Silverspring Maryland) for two minutes. The radioactivity in counts per minute (CPM) of each sample was corrected for background activity. Subsequently the CPM was divided by the weight of the sample to obtain the concentration of radioactivity in the sample (CPM/gm). Permeability index (PI) in each brain section was calculated by dividing the calculated CPM/gm by the blood CPM/gm and multiplying this number by 1000 [8].

Table 1B, brain edema studies

	survivors of injury	sham controls	time of termination	anesthesia
group 9	CHI = 4	-	4 hours	Isoflurane
group 10	-	n = 4	4 hours	Isoflurane
group 11	CHI = 4	-	15 hours	Isoflurane
group 12	-	n = 4	15 hours	Isoflurane
group 13	CHI = 4	-	24 hours	Isoflurane
group 14	-	n = 4	24 hours	Isoflurane

Edema studies:

Thirty animals were used for edema studies. Eighteen rats were subjected to experimental CHI, 12 served as sham controls. 6 rats died immediately following injury. The remaining 24 rats (12 CHI, 12 sham) were studied in six groups of four animals each as specified in table 1B.

Animals used for edema studies were killed with an intra-cardial injection of 2 mm KCl at 4, 15 and 24 hours. For determination of water content the slices of the frozen brains that have not been perfused were defrosted on ice for 30 minutes. Micrograviметrical techniques were used to determine the BWC of five supratentorial slices[30]. The 100 ml gravimetric gradient of the mixture of benzene bromobenzene was prepared one day before and calibrated immediately before use. If the linear regression coefficient of the calibration with five potassiumsulfate drops of known specific gravity was less than 0.998 the gradient was rejected. A 14 gauge needle was used to obtain brain samples of equal weight, which were gently dropped in the gradient. The depth of the sample after two minutes of settling was measured and plotted on the regression curve to obtain the specific gravity and water content. Water content data were expressed in percentage as gram water/gram tissue x 100.

Statistical analysis

Values are displayed as means (± standard error of the means). Differences between contiguous data, of traumatized versus sham operated animals, were tested by means of student T-tests for unpaired observations. Ordinal data are displayed in contingency tables and tested using the Chi square statistic for of associations (DataDesc®, Description Inc. Ithaca, NY, USA). P-values < 0.05 were considered significant.

Results

Skull fractures and mortality

Three rats in the total of 43 animals, submitted to CHI, suffered a skull fracture and died shortly after injury. Overall mortality after CHI, without a skull fracture, in this series was 33%. This is indicative of severe trauma. Data from non surviving animals were not acquired. Mortality and fracture rates correspond to the initial pathophysiology and biomechanics report[28]. Table 2 displays mortality rates among groups. Despite the fact that the same level of injury was inflicted, all animals under the anesthetic regimen with ketamine/xylazine survived CHI.

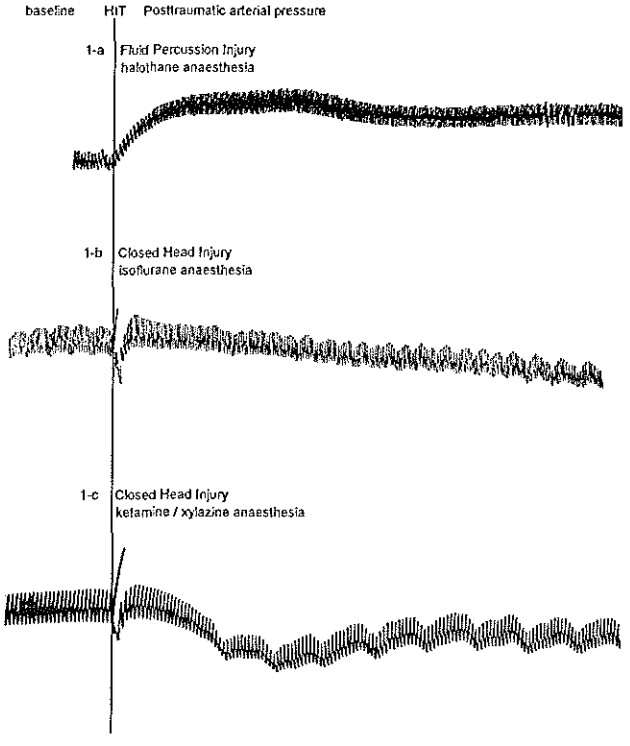
Table 2 Mortality after CHI in relation to anaesthesia protocol used. Overall mortality was 33%. All animals under ketamine/xylazine anaesthesia survived trauma, in the isoflurane group mortality was 37%. This difference is not statistically significant (CHI^{-2} 2.751, $p>0.05$)

	isoflurane	ketamine/xylazine
Survivor	22	5
non-survivor	13	0
Total	35	5

Arterial blood pressure

Examples of early blood pressure responses of animals surviving closed head injury under different anesthetic protocols are given in figure 1. These arterial blood pressure data are derived from the experiments in which the focus was on the physiologic response to CHI, as reported earlier[28]. In the present study an arterial catheter was not used. FPI results in a hypertensive surge lasting several minutes [figure 1A]. In CHI under volatile anesthesia only a small transient of increased blood pressure with subsequent hypotension is observed [figure 1B]. This transient was completely abolished if the I. M. anesthetics are used, in this situation the post-traumatic hypotensive period was of immediate onset [figure 1C].

figure 1 Typical examples of arterial pressure response to traumatic brain injury.



A

following severe Fluid Percussion Injury, note the impressive pressure surge, similar to previous reports characterizing this model.

B

following severe Closed Head Injury, isoflurane anesthesia, note the small increase in systolic and pulse pressure with slowly decreasing pressure afterwards.

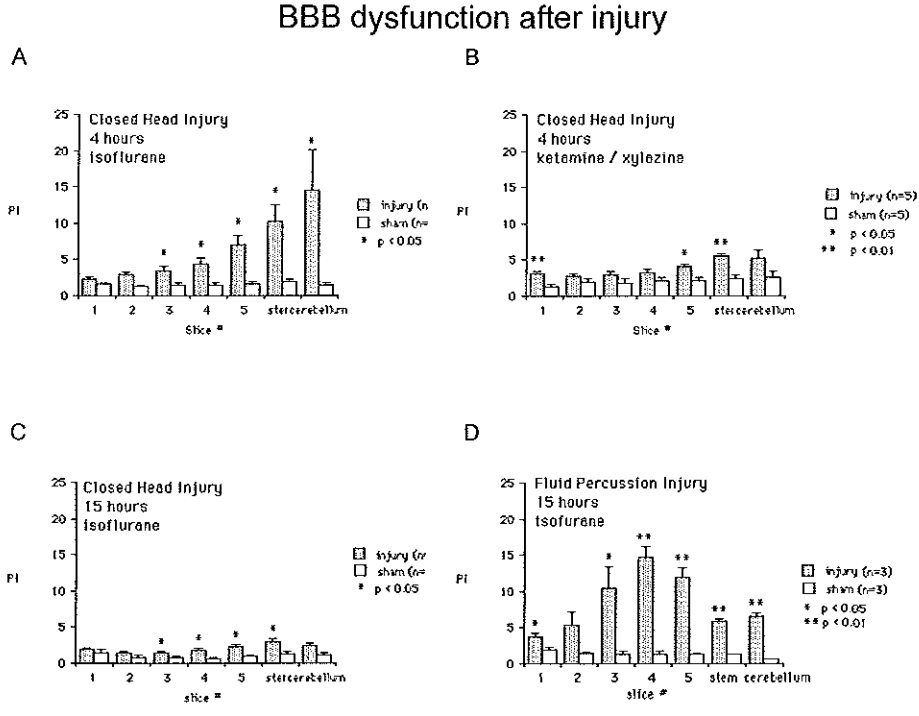
C

following severe Closed Head Injury, ketamine xylazine anesthesia, the pressure 'peak' is completely abolished, and a period of more pronounced hypotension follows.

Blood Brain Barrier dysfunction (I^{-125} albumin permeability)

The disruption of the BBB is quantitatively reflected in the rise in permeability index (PI) for I^{-125} RISA. Brain specimen from both injured and sham treated animals were always responsible for higher than background activity [figure 2]. In all slices the PI of injured animals was consistently higher than PI of sham animals. As is shown in figure 2A, permeability after 4 hours is significantly higher in the caudal tissue samples. Main increase in the supratentorial compartment is in the brain slices 4 and 5, just under the helmet, the area where most of the skull and brain deformation due to impact is to be expected. The ketamine/xylazine anesthetic regimen, completely abolishing the transient rise in blood pressure following injury, causes significant less barrier dysfunction after CHI [figure 2B]. After 15 hours there is a clear diminution of the BBB compromise as compared to 4 hours after injury [figure 2C]. The spatial distribution remains the same. After severe fluid percussion injury (3.3 atm) the BBB compromise is much higher than after CHI, and despite the smaller numbers of animals significant in all slices but slice 2.

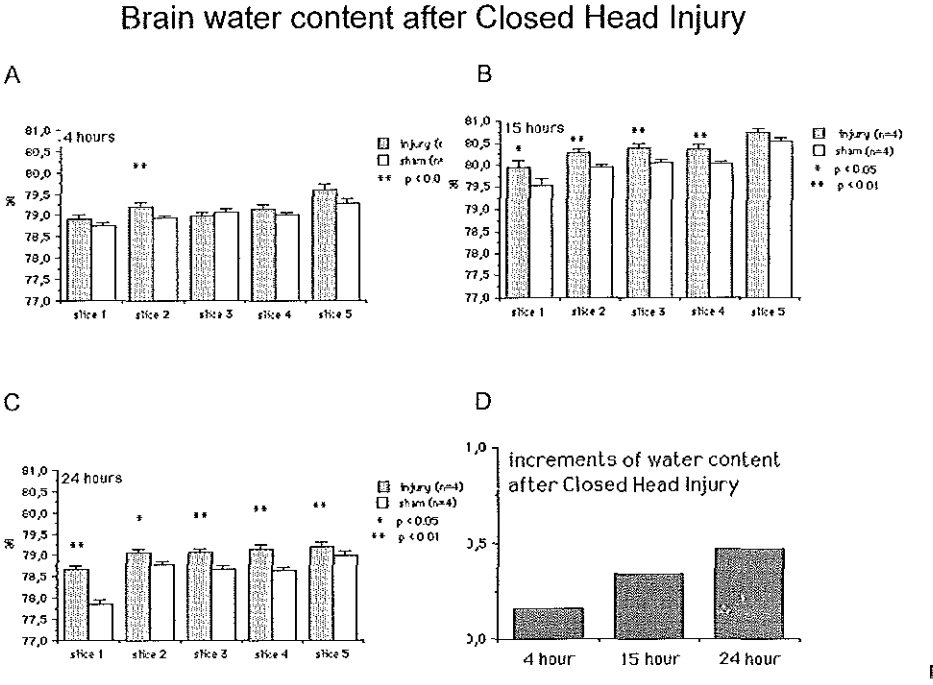
figure 2 Cumulative cerebrovascular permeability to I^{-125} bovine serum albumin following severe traumatic brain injury as expressed in Permeability Index (PI = brain count per minute per gram/blood count per minute per gram *1000). After injury the BBB is compromised in all animals, and in all slices of brain tissue.



Brain Edema (specific gravimetry)

Edema formation was studied in comparison with the same day sham operated animals. BWC was consistently higher in the (CHI) injured animals [figure 3]. Although these differences are only significant in one of five slices after four hours [figure 3A] a trend is evident. After fifteen hours survival this trend is more manifest with significant increases of water content in the majority of brain slices [figure 3B]. Twenty-four hours survival after CHI is the latest time point studied. Here all sections display significant edema formation [figure 3C]. The global increase in BWC, as measured at these different times, is shown in figure 3D. The edema measurements indicate that the process of edema formation after CHI is ongoing for at least the first 24 hours.

figure 3 BWC of the five supratentorial slices, as determined by standardized gravimetrical techniques. Differences between injured (Closed Head Injury) and sham animals are small, though over time an increase in water content in injured animals can be appreciated, and eventually becomes significant in all slices.



Discussion

Impact acceleration injury, induced with the CHI model, causes an initial vascular leak of the blood brain barrier with subsequent brain edema, in the absence of a post-traumatic hypertensive surge. Acute post-traumatic hypertension is one of the characteristic of the FPI model[7, 33]. Effects of acute arterial blood pressure increases on permeability of the barrier and vasogenic edema formation are well known, and have been studied extensively by Johansson et all.[17]. In our study, the relevance of even minor post-traumatic increases in arterial pressure, with respect to the study of barrier function, is reflected in lower permeability when the small rise of MAP was completely abolished. Another explanation for the lower PI, might be the protective effect of the weak glutamate receptor antagonist ketamine in this protocol. The absence of mortality in this small series supports this explanation. Because the focus of this research was not on the potentially protective effect of one agent over the other, this conclusion can not be definitively drawn, and warrants a dedicated study. Nilsson et al. found the cerebral vasculature to be pressure passive in the early stages following concussive impact acceleration injury in rats[37]. This loss of autoregulation renders the micro-vasculature of the brain more vulnerable to fluctuations of arterial blood pressure, as discussed earlier by Langfitt et al[24] The observation that PI for albumin bound Iodine tracer decreases over time, indicates that the dysfunctional barrier is, at least partially, restored within the first few hours after injury. In contrast to the BBB disruption, brain edema increases over time. Edema formation in these later periods must be of a different origin than pure 'vasogenic', as previously implicated by the cold injury models of focal brain injury[19, 21, 38, 45]. In perspective of the high incidence of cerebral ischemia following head injury, recently observed in clinical studies[5], cytotoxic ischemic brain edema is most likely the cause of ongoing increases in water content.

Study of blood brain barrier

The integrity and defects of the blood brain barrier can be studied by tracing and identification of substances, that do normally not cross the barrier, into the neuropil. Much like the work of Ellis et al.[8], the method used provides quantitative measures of BBB integrity. Increase in PI after CHI is consistent, and after 15 hours PI is considerably less than after 4 hours. This can be explained by early protein extravasation being cleared from the brain parenchyma, without replacement of new protein bound tracer. In more quantitative terms of barrier damage and interstitial fluid resolution, inflow of tracer becomes lower than outflow shortly after injury. Obviously the barrier closes again at some time point after trauma, suggesting that the temporary BBB dysfunction following CHI is functional, and not a mechanical laceration of the vessels. This finding is in accordance with earlier observations in FPI that the duration of post-traumatic BBB permeability lasts for a few hours or less[40, 42]. Our study showed a larger increase in PI 15 hours after FPI than after CHI,

indicating the importance of the arterial pressure surge after brain injury. Barzó et al.[4] performed an extensive study on early post-traumatic barrier dysfunction utilizing a gadolinium permeability and MRI detection technique. They concluded that restoration of barrier compromise is probably taking place within 15 minutes. In contrast to models of diffuse injury, models of focal contusion, as the cold injury model[45], and the contusion models of direct cerebral impression[13], result in much longer and biphasic BBB compromise.

Brain edema studies

Brain edema, in this study, was determined utilizing the technique of regional quantification of the BWC, as developed by Marmarou[30]. The rise in BWC following CHI is displayed in figure 4, showing that edema formation is a process that continues over time. In a FPI study, McIntosh et al.[31] showed the same phenomenon of continuously increasing regional edema reaching the maximum at 48 hours and persisting for 5 days. In both CHI and FPI models of diffuse experimental injury an increase in brain edema in the absence of an open barrier is observed. Foda et al.[1], in a microscopic characterization study of this CHI model, described two forms of edema: pericappilary and extracellular edema. In a sequential MRI study in which secondary insults were added to CHI, Ito et al.[16] concluded that post-traumatic intracranial hypertension is most likely due to cellular swelling. Barzó et al.[4] extended this research over time and found a biphasic response, with initial vasogenic, and later cellular brain edema. The hypothesis that in diffuse brain injury, cytotoxicity is the main cause of edema, is confirmed by these studies utilizing advanced imaging techniques. Observations of early ischemia after head injury[5] support this mechanism. In a model of traumatic impact brain contusion, Shapira et al.[48] found edema in the contused hemisphere reaching maximum values at 18 hours. And in a model of weight drop cerebral contusion, Holmin et al.[13, 14], found biphasic vasogenic brain edema, which was attributed to an inflammatory response. As in freezing lesions[19, 38], increase of brain water in models of focal contusion is of vasogenic origin. In these contused areas, with BBB damage, both hydrostatic and osmotic pressure gradients promote edema formation and brain swelling[20, 46].

Experimental traumatic brain injury and hypertension

In studies of BBB damage and brain edema, the FPI model has the shortcoming of documented severe post-traumatic increase in arterial pressure[7, 33]. Our FPI study, 15 hours after injury, did show an overall 7 fold BBB-permeability increase in the traumatized brains. In a FPI study, Qian et al.[41] described that the arterial pressure surge preceded a CBF increase, and speculate that the BBB damage may be due to the sudden increase of CBF. After head injury, autoregulation is inflicted. Cerebrovascular responsiveness to changes in arterial pressure is diminished, and the cerebral capillary bed is rendered more vulnerable to increases of systemic blood pressure, which might aggravate cerebral swelling[23-25, 32, 36, 39, 46]. Duration of barrier dysfunction is dependent on severity and duration of the hypertensive surge[9, 12, 17]. The

decrease in permeability after CHI without the small blood pressure transient, further supports the importance of minor elevations of blood pressure in the early period after injury. In cerebral contusions, arterial blood pressure is the main driving forces for plasma constituents to cross the leaky barrier into the extracellular compartment, to aggravate brain edema[18, 20]. Studies of the blood brain barrier and brain edema after head injury should be performed utilizing a trauma model devoid of contaminating systemic factors.

Relation to the clinical situation

In the clinical situation, heterogeneity of traumatic brain injury is well known. Marshall, on behalf of the trauma coma databank[31], described different types of injury based on CT scan characteristics, and divided them into three types of diffuse injury, and focal space occupying lesions. Obviously, both diffuse and focal injury can exist in one patient, in which one type of injury might prevail over the other. In diffuse traumatic brain injury, as produced by the CHI, and the FPI model, the period of BBB leak is short. In cerebral contusion it has been shown that the disruption of the BBB with concomitant vasogenic brain edema is more extensive and of longer duration[14, 47, 50]. This study and studies of focal injury indicate that the origin of brain edema varies according to the cause. Dependent on the type of injury prevailing in the individual patient (diffuse versus focal), one type of brain edema will prevail (cytotoxic versus vasogenic). Development of brain edema contributes to brain swelling. Brain swelling in the closed cranium is responsible for intracranial hypertension, one of the most worrisome consequences of head injury. Raised ICP impairs cerebral blood flow by decreasing cerebral perfusion pressure, and as such causes brain tissue ischemia, further increasing brain edema[36]. Our study has provided insight in the development of blood brain barrier damage and brain edema in a model of CHI. Because in diffuse injury, the BBB is opened only in the acute period following trauma, edema is most likely of cytotoxic nature. Clinicians treating patients with severe head injury need to bear in mind the main cause of raised ICP in the individual patient. Rosner advocated the active increasing of mean arterial pressure to ensure adequate CPP, indiscriminate of the presence of contusions [43]. The Lund group however presumes that cautious lowering of arterial blood pressure, might decrease vascular edema formation, by decreasing hydrostatic capillary pressure[2, 3, 10, 11]. In case of massive BBB dysfunction, as in large cerebral contusions, a more conservative approach with respect to iatrogenic hypertension and hypervolemia might be appropriate. In case of diffuse injury, we have shown that, at least in the experimental setting, the BBB opening is of short duration.

Conclusion

Experimental traumatic brain injury, in a model of diffuse closed head injury, is followed by a temporal dysfunction of the blood brain barrier, enabling indiscriminate passage of plasma constituents into the neuropil during a short period of time. In the early period following traumatic brain injury, vascular leak is aggravated by increases in arterial blood pressure. The Closed Head Injury model is characterized by a relative absence of pressure rise, as compared to the impressive surge observed in models of direct dural percussion. Even in this scenario the barrier is compromised. The duration of barrier opening after CHI has been shown to be very short-lived in MRI studies, and is likely to be dependent on severity of trauma and secondary insults. Unlike the barrier opening, being a temporal incident, edema formation following severe CHI is, at least for the first 24 hours, an ongoing phenomenon. Taking these observations in concert, it is concluded that the main contributor to brain edema following diffuse head injury, is vasogenic brain water in the ultra-early post-traumatic period, and cellular water in the late post-traumatic period. Other studies have shown that vasogenic edema is much more important in focal brain contusions. This emphasizes the heterogeneity in head injuries, and warrants well-considered decision making, and close observation, should hypertensive therapy be instituted. Aggressive hypertensive therapy in the early post-traumatic period might not be as beneficial as advocated, and should especially in cases of severe disruption of the BBB, as is the case in and around contusions, be used judicially, or not al all.

References

Presentations, abstracts:
van den Brink WA, Marmarou A, Avezaat CJJ: Brain oedema in experimental closed head injury in the rat. **Acta Neurochir Suppl.** 51: 261-262, 1990

van den Brink WA, Santos BO, Marmarou A, et al: Bloed-hersen-barriere dysfunctie in twee modellen van hersenletsel in de rat. **Symp Exp Ond Heelk Spec** 6:11-19, 1993 (Abstract)

van den Brink WA, Santos BO, Marmarou A, et al: Quantitative analysis of blood brain barrier damage in experimental head injury in the rat; the role of posttraumatic hypertension. **J Neurotrauma** 7-6, 1993

van den Brink WA, Santos BO, Marmarou A, et al: Temporary blood brain barrier damage and continued edema formation in experimental closed head injury in the rat, in Avezaat CJJ, van Eijndhoven JHM, Maas AIR, et al (eds): **Intracranial Pressure VIII.** Berlin, Heidelberg, New York, Springer-Verlag: 144-147, 1993 (Abstract)

van den Brink WA, Santos BO, Marmarou A, et al: Quantitative analysis of blood brain barrier damage in two models of experimental head injury in the rat. **Acta Neurochir Suppl** 60:456-458, 1994

1. Abd-Elfattah Foda MA, Marmarou A: A new model of diffuse brain injury in rats. Part II: Morphological characterization. J **Neurosurg 80**: 301-313, 1994

2. Asgeirsson B, Grände PO, Nordström CH: A new therapy of post-trauma brain oedema based on haemodynamic principles for brain vollume regulation. **Intensive Care Med 20**: 260-267, 1994

3. Asgeirsson B, Grände PO, Nordström CH, et al: Effects of hypotensive treatment with alpha 2-agonist and beta 1-antagonist on cerebral haemodynamics in severely head injured patients. **Acta Anaesthesiol Scand 39**: 347-351, 1995

4. Barzó P, Marmarou A, Fatouros PP, et al: Contribution of vasogenic and cellular edema to traumatic brain swelling measured by diffusion weighted imaging. J **Neurosurg 87**: 900-907, 1997

5. Bouma GJ, Muizelaar JP, Choi SC, et al: Cerebral circulation and metabolism after severe traumatic brain injury: The elusive role of ischemia. J **Neurosurg 75**: 685-693, 1991

6. Cortez SC, McIntosh TK, Noble LJ: Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. **Brain Res 482**: 271-282, 1989

7. Dixon CE, Lighthall JW, Anderson TE: Physiologic, histopathologic, and cineradiographic characterization of a new fluid percussion model of experimental brain injury in the rat. J **Neurotrauma 5**: 99-104, 1988

8. Ellis EF, Chao J, Heizer ML: Brain kininogen following experimental brain injury: evidence for a secondary event. J **Neurosurg 71**: 437-442, 1989

9. Ellison MD, Povlishock JT, Hayes RL: Examination of the blood-to-brain transfer of -aminoisobutyric acid and horseradish peroxidase: Regional alterations in blood-brain barrier function following acute hypertension. J **Cereb Blood Flow Metabol 6**: 471-480, 1986

10. Grände PO, Asgeirsson B, Nordström CH: Aspects on the cerebral perfusion pressure during therapy of traumatic head injury. **Acta Anaesthesiol Scand 110 (Suppl)**: 36-40, 1997

11. Grände PO, Asgeirsson B, Nordström CH: Physiologic principles for volume regulation of a tissue enclosed in a rigid shell with application to the injured brain. J **Trauma 42** (5 Suppl): S23-S31, 1997

12. Haggendal E, Johansson B: On the pathophysiology of the increased cerebrovascular permeability in acute arterial hypertension in cats. **Acta Neurol Scand 48**: 265-270, 1972

13. Holmin S, Mathiesen T: Biphasic edema development after experimental brain contusion in rat. **Neurosci Lett 194**: 97-100, 1995

14. Holmin S, Mathiesen T, Shetey J, et al: Intracerebral inflammatory response to experimental brain contusion. **Acta Neurochir 132**: 110-119, 1995

15. Ito H, Tomita H, Yamazaki Sh, et al: Brain swelling and brain edema in acute head injury. **Acta Neurochir 79**: 120-124, 1986

16. Ito J, Marmarou A, Barzó P, et al: Characterization of edema by diffusion-weighted imaging in experimantal traumatic brain injury. J **Neurosurg 84**: 97-103, 1996

17. Johansson B, Linder LE: Reversibility of the blood-brain barrier dysfunction induced by acute hypertension. **Acta Neurol Scand** 57: 345-348, 1978

18. Klatzo I: Neuropathological aspects of brain edema. J **Neuropathol Exp Neurol** 26: 1-14, 1967

19. Klatzo I, Piraun A, Laskowski EJ: The relationship between edema, blood brain barrier and tissue elements in a local brain injury. **J Neuropathol Exp Neurol** 17: 548-564, 1958

20. Kuroiwa T, Shibutani M, Tajima T, et al: Hydrostatic pressure versus osmotic pressure in the development of vasogenic brain edema induced by cold injury, in Long D (ed): **Advances in Neurology**. New York, Raven Press: 11-19, 1990

21. Kuroiwa T, Ueki M, Chen Q, et al: Biomechanical characteristics of brain edema: the difference between vasogenic-type and cytotoxic-type edema. **Acta Neurochir Suppl** 60: 158-161, 1994

22. Lang DA, Teasdale E, Macpherson P, et al: Diffuse brain swelling after head injury: more often malignant in adults than children? **J Neurosurg** 80: 675-680, 1994

23. Langfitt TW, Tannanbaum HM, Kassel NF: The etiology of acute brain swelling following experimental head injury. **J Neurosurg** 24: 47-56, 1966

24. Langfitt TW, Weinstein JD, Kassel NF, et al: Contributions of trauma, anoxia, and arterial hypertension to experimental acute brain swelling. **Trans Am Neurol Assoc** 92: 257-259, 1967

25. Lewelt W, Jenkins LW, Miller JD: Effects of experimental fluid-percussion injury of the brain on cerebrovascular reactivity to hypoxia and to hypercapnia. **J Neurosurg** 56: 332-338, 1982

26. Long DM: Traumatic brain edema, in The Congress of Neurological Surgeons (ed): **Clinical Neurosurgery vol. 29.** Baltimore, London, Williams & Wilkins: 174-202, 1982

27. Marmarou A: Traumatic brain edema: an overview. **Acta Neurochir Suppl** 60: 421-424, 1994

28. Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. **J Neurosurg** 80: 291-300, 1994

29. Marmarou A, Shima K: Comparative studies of edema produced by fluid percussion injury with lateral and central modes of injury in cats, in Long D (ed): **Advances in Neurology**. New York, Raven Press, Ltd.: 233-236, 1990

30. Marmarou A, Tanaka K, Shulman K: An improved gravimetric measure of cerebral edema. **J Neurosurg** 56: 246-253, 1982

31. Marshall LF, Bowers Marshall S, Klauber MR, et al: A new classification of head injury based on computerized tomography. **J Neurosurg 75 (suppl)**: S14-S20, 1991

32. Marshall WJS, Jackson JLF, Langfitt TW: Brain swelling caused by trauma and arterial hypertension. **Arch Neurol** 21: 545-553, 1969

33. McIntosh TK, Noble LJ, Nadrews B, et al: Traumatic brain injury in the rat: characterization of a midline fluid percussion model. **Cent Nerv Syst Trauma** 4: 119-134, 1987

34. McIntosh TK, Soares H, Thomas M, et al: Development of regional cerebral oedema after lateral fluid-percussion brain injury in the rat. **Acta Neurochir Suppl. 51**: 263-264, 1990

35. Menzies SA, Betz AL, Hoff JT: Contributions of ions and albumin to the formation and resolution of ischemic brain edema. J **Neurosurg 78**: 257-266, 1993

36. Milhorat TH, Johnson WD, Dow-Edwards DL: Relationship between oedema, blood pressure, and blood flow following local brain injury. **Neurol Res 11**: 29-32, 1989

37. Nilsson B, Nordström CH: Experimental head injury in the rat. Part 3: Cerebral blood flow and oxygen consumption after concussive impact acceleration. J **Neurosurg 47**: 262-273, 1977

38. Pash MP, Tweed WA: Tissue albumin and water content in the early stages of vasogenic brain edema formation. **Can J Neurol Sci 6**: 423-426, 1979

39. Pfenninger EG, Reith A, Breitig D, et al: Early changes of intracranial pressure, perfusion pressure, and blood flow after acute head injury. J **Neurosurg 70**: 774-779, 1989

40. Povlishock JT, Becker DP, Sullivan HG, et al: Vascular permeability alterations to horseradish peroxidase in experimental brain injury. **Brain Res 153**: 223-239, 1978

41. Qian L, Ohno K, Maehara T, et al: Changes in lCBF, morphology and related parameters by fluid percussion injury. **Acta Neurochir 138**: 90-98, 1996

42. Rinder L, Olsson Y: Studies on vascular permeability changes in experimental brain concussion 2. Duration of altered permeability. **Acta Neuropathol 11**: 201-209, 1968

43. Rosner MJ, Rosner SD, Johnson AH: Cerebral perfusion pressure: management protocol and clinical results. J **Neurosurg 83**: 949-962, 1995

44. Saito N, Chang C, Kawai K, et al: Role of neuroexcitation in development of blood-brain barrier and oedematous changes following cerebral ischaemia and traumatic brain injury. **Acta Neurochir 51 suppl.:** 186-188, 1990

45. Schneider GH, Hennig S, Lanksch WR, et al: Dynamics of posttraumatic brain swelling following a cryogenic injury in rats. **Acta Neurochir Suppl 60**: 437-439, 1994

46. Schutta HS, Kassel NF, Langfitt TW: Brain swelling produced by injury and aggravated by arterial hypertension. **Brain 91**: 281-294, 1986

47. Shapira Y, Setton D, Artru AA, et al: Blood-brain barrier permeability, cerebral edema, and neurologic function after closed head injury in rats. **Anesth Analg 77**: 141-148, 1993

48. Shapira Y, Shohami E, Sidi A, et al: Experimental closed head injury in rats: Mechanical, pathophysiological, and neurological properties. **Crit Care Med 16**: 258-265, 1988

49. Shima K, Marmarou A: Evaluation of brain stem dysfunction following severe fluid percussion head injury to the cat. J **Neurosurg 74**: 270-277, 1991

50. Todd NV, Graham DI: Blood-brain barrier damage in traumatic brain contusions. **Acta Neurochir Suppl 51**: 296-299, 1990

51. Tornheim PA, McLaurin RL: Acute changes in regional brain water content following experimental closed head injury. J **Neurosurg 55**: 407-413, 1981

Chapter 8
Hypoxia induces augmentation of behavioral deficits in experimental Closed Head Injury

Paralytic dilatation of the pupils is a measure of the degree of hypoxia incidental to the anesthesia; not a measure of the depth of anesthesia from the anesthetic agent per se, its value as a 'sign' of anesthesia is limited to such interpretation. ***Arthur E. Guedel, Inhalation Anesthesia, 1951***

Abstract

Object

Hypoxia is one of the most important secondary insults in head injury. Its occurrence is related to unfavorable outcome. In animal models of traumatic brain injury, deficits in behavioral outcome may be enhanced by addition of secondary insults. This is particularly relevant because in some experimental studies on neuroprotective agents in head injury hypoxic insults have been added to primary injury. We evaluated the effects of secondary hypoxia on spontaneous behavior, motor, and cognitive function in the model of Closed Head Injury (CHI) in the rat.

Methods

Rats were submitted to severe CHI using a weight drop model. Either CHI alone, or a combination of CHI and hypoxia ($FiO_2 = 14\%$ during 30 minutes) was delivered. Two sham groups, one without, and one with hypoxia were used as controls. In surviving animals, time of recurrence to normal reflexes, abolished by trauma, was recorded. The following week, tests of spontaneous behavior, and motor function were performed daily. During the second week after CHI, visuo-spatial memory and learning capability was evaluated using the Morris Water Maze.

Results and conclusions

Severe CHI caused some alterations of spontaneous behavior, and impaired motor and cognitive function. Secondary hypoxia, administered after CHI, resulted in a significantly larger impairment of both motor function and learning capabilities. This augmentation of deficits can be employed in the evaluation of new treatment regimens. Projected to the human situation, this study confirms the importance of hypoxia after head injury with respect to functional outcome.

Introduction

Many trauma patients suffer both systemic injury and head injury. Severe head injury remains the most important factor contributing to mortality following trauma[2, 7, 17]. The coexistence of moderate head injury in patients with predominant extracranial injury, doubles mortality predicted by Injury Severity Score (ISS)[17]. In patients with severe head injury, systemic hypoxia (SaO_2 < 90%) was observed by helicopter crew in 24% of 50 patients at the accident scene[21]. On arrival at a Trauma Coma Databank hospital emergency room 19% of 717 patients had a PaO_2 less than 60 mmHg[3]. Episodes of hypoxia were recorded in 40% of 124 head injured patients during the ICU stay in Edinburgh[12]. Secondary insults are related to unfavorable outcome.

End point parameters used in experimental models of traumatic brain injury have often been pathophysiological variables, expected to relate to functional outcome. Direct measurements of functional outcome in rodent models of diffuse traumatic brain injury requires much effort, as behavioral deficits are often subtle, and hard to quantify. The failure to show effects on neurobehavioral outcome might be caused by the relatively small deficits in animals surviving traumatic brain injury[5]. We expected that secondary insults would deteriorate functional outcome after diffuse experimental Closed Head Injury (CHI). The aim of the present study was to investigate the sensitivity of behavioral endpoint evaluation in the CHI model, as developed by Marmarou[16], and to evaluate the additional effects of secondary hypoxia on a battery of functional outcome parameters.

Materials and methods

Animal preparation

Studies were performed in 91 male Wagrij rats weighing 255 ± 36 grams (mean ± sd). During one week before the experiment, and during the behavioral tests, the rats were housed in a reversed day-night cycle, with free access to food and water. Anesthesia was ether-induced using the chamber technique, and maintained with isoflurane through an endotracheal tube (1 to 2 - 2.5 % isoflurane, in an oxygen: nitrogen mixture of 33%: 66%). Animals not submitted to secondary injury breathed spontaneously. While inducing hypoxia (14% FiO_2 for 30 min.), reflex hyperventilation occurred, requiring pharmacological paralysis (pavulon 0.1 mg IM), and artificial ventilation. The animals were killed by Euthasate (sodiumpentobarbital 300 mg) injection at specified time points after injury. The protocols were approved by the Institutional Animal Care and Use Committee, Erasmus University Rotterdam.

Trauma Model

The mechanics and pathophysiology of the CHI model employed, have been reported in detail elsewhere[16]. Briefly, diffuse closed head injury is induced by weight drop on the intact skull. A stainless steel disk is cemented onto the vertex to evenly distribute the impact forces of the falling weight, preventing skull fractures. During impact the head is partially restrained on a foam head support, allowing for standardized skull acceleration. To minimize and standardize anesthesia effects, isoflurane was withdrawn before inducing CHI, the weight was not released until a hindpaw withdrawal on squeezing was observed. After weight drop (550 gram, 210 centimeters), a second hit of the impactor is prevented by quickly removing the foam bed with the rat sideways after first collision. The skull was inspected for overt fractures, and the wound was quickly sutured. When the rats had regained full consciousness, they were returned into their cages with free access to food and water.

Experimental groups

Ninety-one experiments were performed in four separate groups [table 1], in which the effects of trauma, hypoxia, and a combination of the two were investigated. Thirty-nine rats were submitted to CHI (group I), twelve rats served as sham-controls, (group II) in which all procedures except for traumatization and hypoxia were performed. Twenty-eight animals were traumatized and submitted to 30 minutes hypoxia by decreasing FiO_2 to 14 % (group III), and 12 rats underwent only hypoxia (group IV). A number of experiments were terminated earlier than 14 days after injury for histological and immunological examinations. The return of acute reflexes of all experiments are reported. With respect to long term behavioral tests, results of rats surviving 7 or 14 days after surgery are presented.

table 1 Protocol groups, in which the effects of trauma, and secondary hypoxia on behavioral outcome were studied. One rat in the CHI & hypoxia group was not tested for its acute reflexes.

Group nr	trauma (level)	30 min. hypoxia	nr of experiments (non - survivors)	nr of acute reflex tests	nr of 14 days behavioral tests
I	CHI (severe)	no	39 (7)	32	9
II	sham	no	12 (0)	12	5
III	CHI (severe)	yes	28 (3)	24	6
IV	sham	yes	12 (2)	10	6

Neuro behavioral observations

acute reflexes

Severe CHI completely abolishes all normal neurological reflexes for a certain time. During the early recovery phase, the time to return of these reflexes was recorded. These somatomotor functions were grouped according to their complexity in table 2 [1]. Reflexes that could not be elicited were scored as 3600 seconds, as this was more than the largest observed delay time.

table 2 Reflexes which are lost immediately following experimental head injury. Classification according to complexity and postural characteristics[4].

I	simple	nonpostural	pinna reflex, corneal reflex
II	simple	postural	paw withdrawal on pinching, movement on tail pinching
III	more complex	nonpostural	whisker reflex, startle reflex on click
IV	more complex	postural	head support, spontaneous righting, escape response on pinching
V	spontaneous movement		time of 5 and 10 line crossings in the small open field

spontaneous behavior

All testing material was cleaned with alcohol before each test to remove scent marks of previously tested rats, and rats were weighed before each testing trial. Observations were made prior to, and daily after injury in a Small Open Field (SOF). The SOF consists of a fresh white paper-covered platform with a diameter of 40 cm, divided into four equal quadrants. A vertical transparant round tube, separating the rat from the environment makes inconspicuous observation possible. Rats were observed with respect to their spontaneous behavior for three minutes, numbers of line crossings, rearing, and grooming were scored.

vestibulomotor equilibrium

Gross vestibulomotor equilibrium function was tested on a beam balance rod as described by Dixon et al.[4] The training procedure for the beam tests was started three days before head injury. The wooden rod was 1.5 * 1.5 cm and 20 cm long and was suspended 75 cm above a soft surface. One end was covered with a vertical black board. The observation period was 90 seconds, during which the ease of balancing was semiquantified using a five point scale: 1 being falling of the rod, 2, 3, and 4 performing gradually better and 5 moving around comfortably with explorative behavior and righting. Observations after trauma were started at the second day, to allow for a recovery period.

motor function

Delicate motor function and coordination were scored using a beam walking test[1]. The walking beam consists of a 2 meters tall rod of 1.5 * 1.5 cm which was suspended 75 cm above a soft surface. Difficulty level was increased by 3 cm tall metal pegs placed every 20 cm. Strong light and loud pink noise served as negative-reinforcement paradigm. Termination of these adverse stimuli, at entry of a dark goal box at the end of the rod, served as reward. Rats were allowed to stay in this goal box for 60 sec. The times from placement on the beam untill start of movement (hesitation period), and entering the dark box (walking time) were scored. Three consecutive trials were performed and averaged every day. Time of hesitation and walking was limited at 90 seconds, for rats unwilling or unable to walk. Falling off the rod was scored as 100

seconds. Rats were trained prior to injury. Observations after trauma were started at the second day, to allow for a recovery period.

cognitive function

Cognitive function tests, using a Morris Water Maze (MWM)[19,20], were started 7 days after CHI, to avoid confounding effects of motor deficits. The rats were not trained in the MWM before CHI. A black pool, 180 cm in diameter, was filled with 50-cm water at 20 -22 ° C. A platform, 8 cm in diameter, with a fixed position 1 cm under water surface, was used as the hidden goal platform. The pool was located in a room with ample visual extra-maze cues (walls, cages, pipes), that remained constant during the testing period. Rats were placed in one of four positions (north, east, south, and west) in random order, with the snout facing the wall. These four consecutive trials were performed daily and escape latencies to swim to the hidden platform were averaged. A maximum swimming time of 90 seconds was allowed. If the platform was not found within this time the rats were guided to the platform, where they stayed for 15 sec. After the MWM tests the rats were placed in a warmed incubator. Performance of this task is impaired by hippocampal damage, and to a lesser extent by cortical damage[19,20]

Statistics

Single observations were summarized and tested for significance using the Mann Whitney-U test for nominal observations, and when a non-normal distribution was expected. Repeated observations after CHI were compared with data from sham animals, presented over time in representative graphs, and tested for statistical significance using time series analysis of variance (ANOVA) using SPSS software (version 7.5, SPSS Inc., Chicago, Ill, USA). P-values less than 0.05 were considered significant.

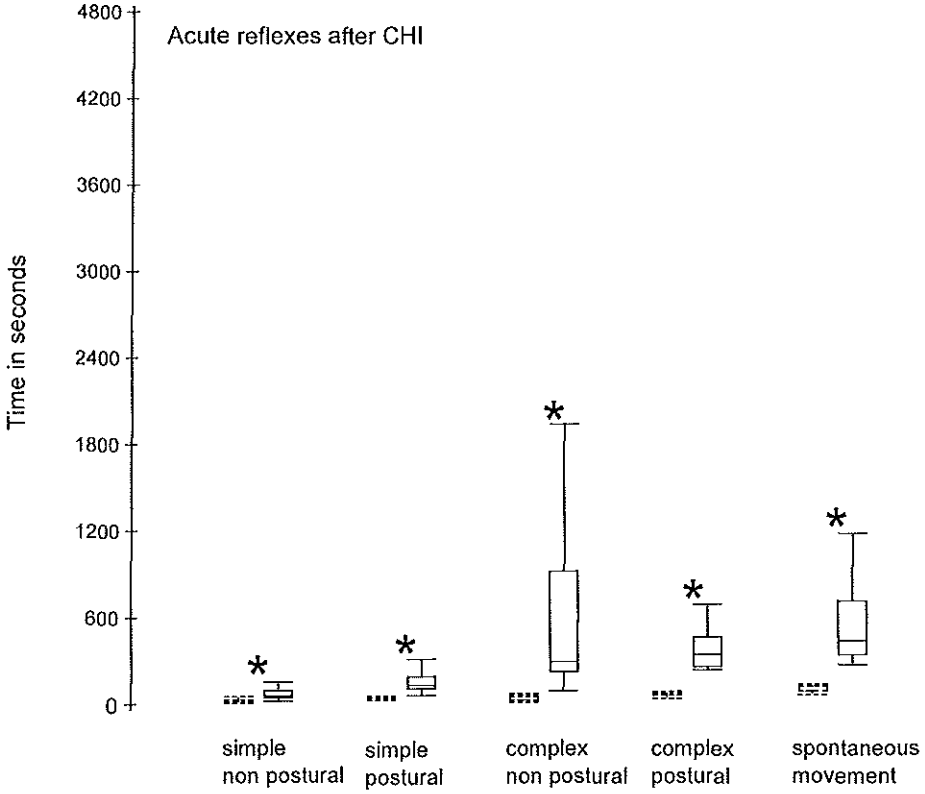
Results

Mortality and skull fractures

In this series of experiments a total number of 67 rats were submitted to CHI; three suffered a skull fracture. Of the 39 rats submitted to CHI only (group I), 7 died (mortality 18%). Of the 28 rats submitted to trauma and hypoxia (group III), 3 died (mortality 10%). The differences in mortality between groups I and III were not significant ($p = 0.32$, Fischer exact test). Two animals from control group IV did not survive hypoxia only. All sham treated animals (group II) survived experimentation.

Figure 1 Time before return to normal reflexes, after CHI (continuous line) and sham (dotted line) (fig 1a). All reflexes returned significantly later in CHI traumatized rats. In rat with hypoxia only and both CHI and hypoxia (fig 1b), the differences, though larger, were only significant in complex postural reflexes, due to larger variations. Data presented are medians and interquartile ranges. (* = $p<0.05$)

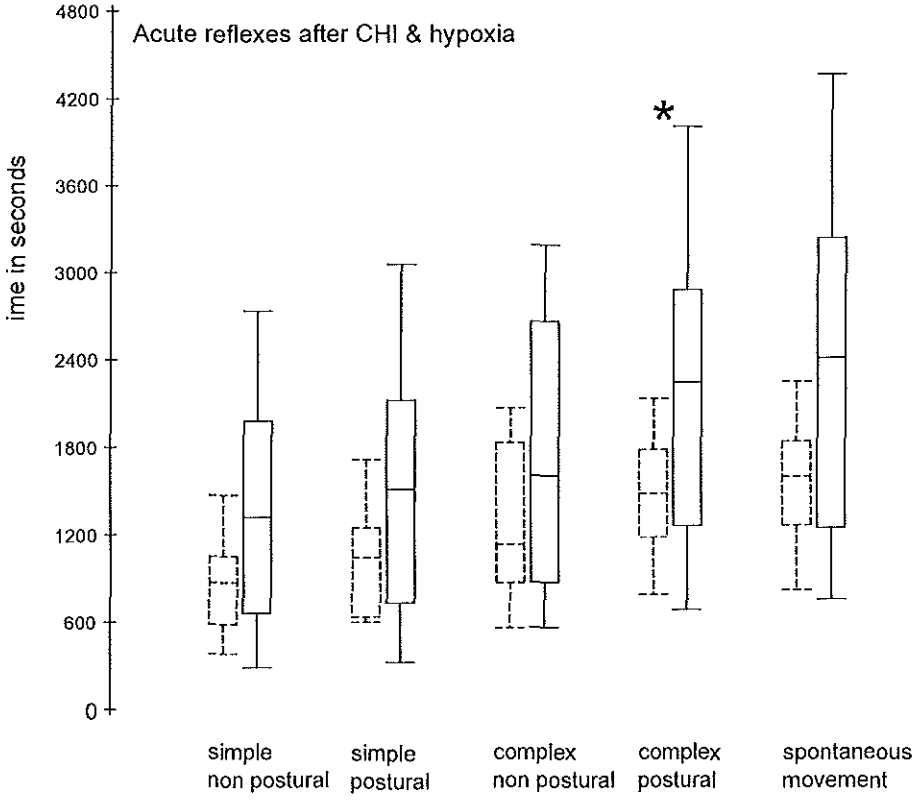
A



Acute transiently abolished reflexes

Since the pinna reflex, and head support were either difficult to elicit and caused much interobserver disagreement, these observations were not considered robust enough, and were discarded from further analysis. Only data of surviving rats are analyzed and presented. The times before reflexes could be elicited are presented in figure 1. In sham operated animals all reflexes had returned within a median interval of 80 seconds, while spontaneous movement was noted after a median time of 133 seconds. After CHI all rats were stunned for several minutes. The median recurrence of simple reflexes was within 150 seconds. Complex reflexes returned after a median time of 350 seconds, while spontaneous movement enough to cross 10 lines occurred after 450 seconds. Posttraumatic hypoxia required 30 minutes of artificial ventilation and paralysis, with subsequent weaning of the ventilator. The recurrence interval of acute reflexes is for a large extent determined by the prolonged anaesthesia, thus the differences between the two hypoxia groups III and IV were not significant.

figure 1B

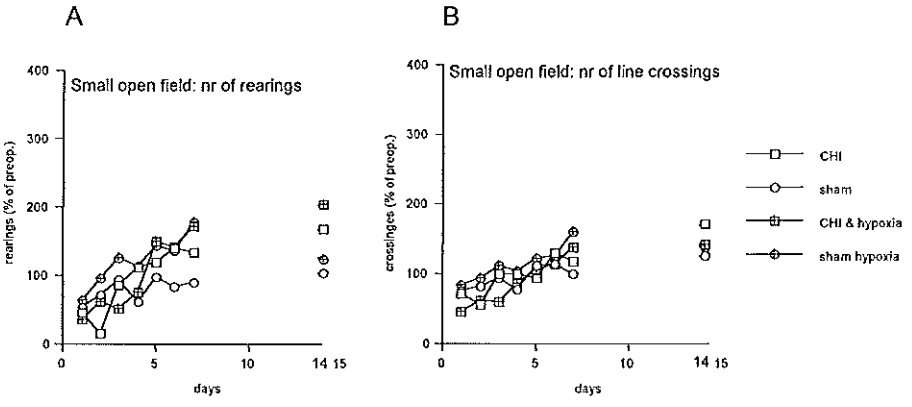


spontaneous behavior

Body weight decreased after experimentation. Both hypoxia ($F(1, 34) = 14.26$, $p < 0.005$) and CHI ($F(1, 34) = 29.31$, $p < 0.001$) decreased body weight, resulting in the largest loss in rats submitted to both CHI and hypoxia. Body weights increased over the postoperative period at the same rate for all groups as evidenced by a main effect of days ($F(6, 204) = 18.88$, $p < 0.001$).

The number of groomings, line crossings, and rearings were taken as measures of spontaneous activity in the Small Open Field (SOF) [figure 2]. Grooming occurred at a relatively low stable level throughout the experiment (0 - 2 in 3 minutes), whereas both other measures were reduced after surgery, and increased over the postoperative period. (crossing: $F(6, 204) = 12.25$ $p < 0.001$; rearing: $F(6, 204) = 12.58$ $p < 0.001$). Neither CHI nor hypoxia affected spontaneous activity.

Figure 2 Small open field scores of rearings (2a), and line crossings (2b) expressed in percentage of pre-injury baseline recording. Significant decreases in spontaneous movement and inquisitive behavior were observed in all animals undergoing experimentation. As differences between groups were not significant, the overlapping error bars are not presented.



Vestibulo motor equilibrium function

With respect to the vestibulomotor equilibrium function both CHI alone and hypoxia alone showed a moderate decrease in beam balancing performance of an average of 0.5 point on the five point grading scale. Both CHI ($F(1, 34) = 13.20$ $p < 0.005$) and hypoxia ($F(1, 34) = 10.70$ $p < 0.005$) disrupted vestibulomotor function as tested with the beam balance test [figure 3]. The largest deficit was observed in group III, in which the effects of CHI and hypoxia were additional, as evidenced by the absence of a significant interaction between treatments ($p=0.24$).

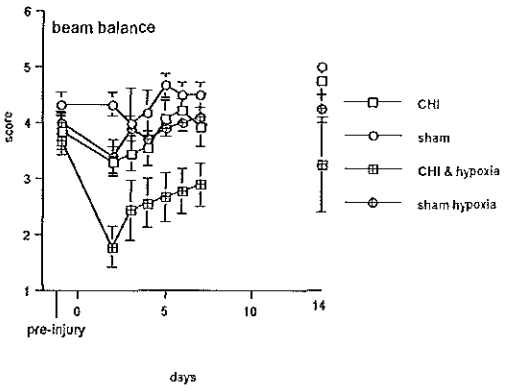
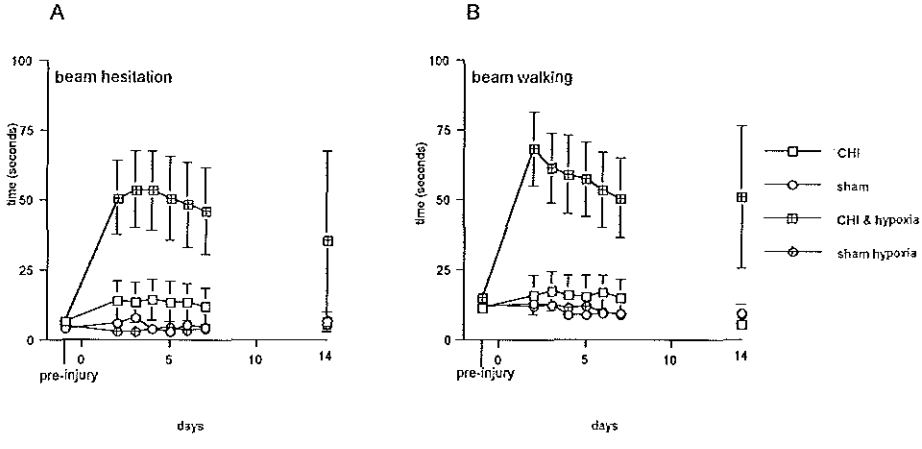


Figure 3 Beam balance vestibulomotor equilibrium scores (mean ± sem) before, and at days 1-7 and 14 after CHI. Both trauma and hypoxia caused significant impairment of gross vestibulomotor function ($p < 0.005$). Combination of the two did result in an expected decrease, which was not significantly different than the numerical sum of the two insults ($p = 0.24$)

motor function

Before the rats were startled enought by the mildly aversive sound and light stimulus to start moving, a period of hesitation was observed and scored seperately. Pre-injury beam hesitation and walking performance were equal for all groups. Hesitation was increased by CHI ($F(1, 34) = 10.37$ $p < 0.005$) and by hypoxia CHI ($F(1, 34) = 4.26$ $p < 0.05$). However a significant CHI * hypoxia interaction ($F(1, 34) = 4, 88$ $p < 0.05$) and additional analyses indicated that hypoxia by itself did not affect hesitation time, but that it amplified the effect of CHI on this measure [figure 4a]. Very similar results were obtained for beam walking, for which significant main effects of CHI ($F(1, 34) = 11.45$ $p < 0.005$) and hypoxia $F(1, 34) = 7.52$ $p < 0.02$), as well as a significant CHI * hypoxia interaction ($F(1, 34) = 7.06$ $p < 0.02$) were found. Again, hypoxia had no effect by itself, but augmented the effect of CHI on motor function as tested by beam walking [figure 4b].

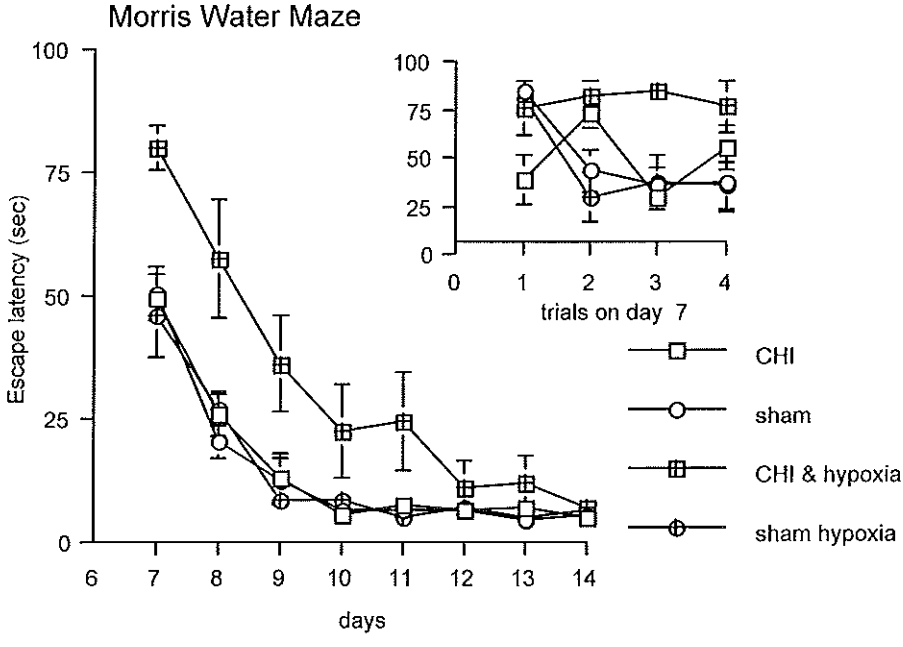
Figure 4 Beam hesitation (4a) and walking (4b) times (mean ± sem in seconds) before, and at days 1-7 and 14 after CHI. Trauma caused significant prolongations of both hesitation (4a) and walking (4b) times. Addition of secondary hypoxia significantly augmented these delay times ($p < 0.05$)



cognitive function

Escape latencies for all experimental groups obtained in the spatial learning paradigm in the Morris Water Maze are shown in figure 5. Escape latencies decreased as a function of days ($F(7, 154) = 92.03$, $p < 0.001$) as all rats learned to locate the hidden platform over the training period. However a days * CHI * hypoxia interaction ($F(7, 154) = 2.86$ $p < 0.01$) indicated that the rate of acquisition was differentially affected by the insults. Although both main effects of CHI ($F(1, 22) = 8.67$, $p < 0.01$) and hypoxia ($F(1, 22) = 7.04$, $p < 0.02$) were significant, a significant CHI * hypoxia interaction ($F(1, 22) = 6.77$, $p < 0.02$), and additional analyses, indicated that escape latencies were only affected in group III, in which the insults were combined. Subsequent analysis of escape latencies for the four trials at the first day of training [insert figure 5], indicated that all groups, except for group III decreased their escape latencies over subsequent trials on day 1.

Figure 5 MWM escape latencies (mean ± sem) on days 7 - 14 after surgery. Isolated CHI or hypoxia did not cause cognitive impairment. Only combination of the two insults did result in a significantly delayed escape latency curve during the first 5 days ($p < 0.02$). The inset graph presents the four trials at the first day of testing. Mean escape latencies were equal at the first trial, thereafter the double insult rats failed to improve, indicating learning deficits.



Discussion

Severe CHI caused an initial abolishment of non-postural and postural reflexes. A gradual return of these reflexes was observed in animals surviving injury. Observations in the SOF showed decreased spontaneous movement, and less inquisitive behavior during the first days after surgery in all four groups. The beam tests, representing lower order functions, such as vestibulomotor equilibrium- and motor- function, showed decreased performance of rats submitted to double insults. Higher order functions such as initiation of walking on the beam, and space navigational cognition, showed that the double insult resulted in impaired learning. It is unlikely that escape latencies are prolonged due to motor impairment at time of testing. Although swimming patterns were not recorded in the present study, several arguments are in favor of a deficit of spatial navigation process, rather than motor impairment. At first, escape latencies during the first trials were equal for all groups. Secondly, escape latencies of the CHI and hypoxia group ultimately reached the same levels as the ones observed in the other groups, indicating that the treatments resulted in a shift of the acquisition curve, i. e. a learning deficit[19]. The equal initial escape latencies during the first four trials of MWM testing, are another strong argument in favor of cognitive deficits, rather than motor impairment, in animals submitted to the double insult.

Even at this level of CHI, usually considered severe, and in this experiment resulting in 18 % mortality, the neuro-behavioral deficits after CHI only were not impressive. Neither did thirty minutes hypoxia by itself, of which we have shown that they result in PaO_2 levels of 43 ± 8 mmHg[26], cause significant impairment. The higher survival rate of animals submitted to the double insult can be explained by the thirty minutes artificial ventilation in these rats, even though the oxygen concentration was low. The addition of hypoxia to CHI, significantly augmented both lower and higher order behavioral deficits in surviving animals. In our previous study, with measurements of brain tissue oxygen ($PbrO_2$) with an equal secondary injury protocol[26], thirty minutes hypoxia cause very low levels of $PbrO_2$ during this episode only. After cessation of the induced hypoxia, brain oxygenation rapidly increased to slightly subnormal values. For the injured brain, thirty minutes impaired brain tissue oxygenation is obviously enough to induce significant neurological deficit. These observations support the findings in midline fluid percussion injury (FPI) experiments, in which augmented neuronal damage was observed, when secondary ischemia by means of carotid clamping was added[11]. In the latter study, injury was most prominent in the CA1 hippocampal region, while in the cortical impact model the CA3 region was more affected[23]. Hippocampal damage causes most severe spatio-visual navigation impairment in rats, but cortical lesions also influence this task[19, 20]. Ishige[9] showed global abnormalities of contralateral movement during the first 24 hours after temporal FPI, which were augmented by secondary hypoxia. In addition to these observations, our study shows that, both motor deficits and cognitive functional impairments,

induced by the combination of CHI and hypoxia, are lasting for weeks after injury. Jenkins et al.[11] hypothesized, based on their FPI & ischemia experiments, that mild traumatic brain injury decreases neuronal treshold for ischaemia. As autoregulation is impaired in the CHI model [21, 22], it is likely that the ability to accomodate cerebral vasculature to hypoxic conditions is also impaired[15]. This presumed loss of responsiveness to hypoxemia causes an increased vulnerability of the injured brain for secondary hypoxia. This relationship between systemic and traumatic brain injury might be bi-directional. It has been suggested that head injury does impair normal cardio-vascular compensation mechanisms in cases of hemorrhagic shock[17, 27].

In animal models of head injury, several parameters can be used in evaluation of new treatment strategies. Measurements of presumed pathophysiological target mechanisms of the new treatment strategy should serve as initial parameters in such studies. A logical continuation of 'target mechanism evaluation' would be the study of functional outcome, which is closely allied to what is considered important in the recovery of human head injury[13, 11]. We studied representative parameters in a standardized battery of tests[4, 19]. As severe CHI only did not produce significant deficits in this model, addition of a secondary insult will be required to induce measurable loss of neurological function, which could then be ameliorated with newly developed treatment strategies. Because in the human population secondary insults frequently occur after trauma[3, 12, 17, 24], the addition of such an insult in an animal model should not interfere with the human situation modelled. However, standardization of both the actual delivery of mechanical injury, as well as the depth and duration of the secondary insult should be defined, and kept constant, for all subjects[6, 8, 25]. From a modelling point of view, it remains important to realize that changes in anesthesia, and oxygenation, can significantly bias the results of a behavioral study.

Extrapolating our observations to the human situation, does again emphasize the importance of hypoxia after head injury. The clinical observation that secondary insults after head injury are of prognostic importance[3, 12, 24], is supported by this rodent study. Moreover, our study indicates that in cases of comparative severity of primary head injury, individuals with secondary insults sustain an additional deleterious effect on functional outcome, directly reducing individual chances for a good recovery. The guidelines, directed at the early recognition and aggressive treatment of such insults, are already widely adopted in teaching programs for professionals in this field[1, 10, 18].

Conclusion

In the model of Closed Head Injury in the rat, behavioral deficits are augmented by secondary hypoxia. This observation has implications for strict standardization of anesthesiologic management in animal studies with neurobehavioral endpoints. The augmentation of such behavioral deficits can be used in studies of efficacy of new treatment regiments. Extrapolating these findings to the human population of head injured patients indicates that the occurrence of hypoxia is not merely correlated to outcome. It moreover proves that secondary hypoxia, with the same severity of primary traumatic brain injury, evokes an obvious detrimental effect on neurological outcome. This report stresses once again the importance of adequate management of secondary insults.

References

Paper:
van den Brink WA, van Haagen OBHAM, van Rooijen A, et al: Secondary hypoxia augments behavioral deficits in experimental closed head injury. **J Neurotrauma** 2000 (Submitted)

Presentation, abstract:
van den Brink WA, Demetriadou E, Marmarou A, et al: Post traumatisch gedrag in een nieuw model van gesloten schedel-hersen-letsel in de rat. **Symp Exp Ond Heelk Spec** 11, 1994 (Abstract)

1. American College of Surgeons Committee on Trauma: **Advanced Trauma Life Support ®, Program for Physicians.** United States, First Impression: 1993

2. Centers for Disease Control and Prevention: Epidemiology of traumatic brain injury in the United States, in **Traumatic brain (head) injury (TBI).** http: //www. cdc. gov/ncipc/dacrrd/tbi. htm, National Center for Injury Prevention and Control: 1999

3. Chesnut RM, Marshall LF, Klauber MR, et al: The role of secondary brain damage in determining outcome from severe head injury. **J Trauma 34**: 216-222, 1993

4. Dixon CE, Lyeth BG, Povlishock JT, et al: A fluid percussion model of experimental brain injury in the rat. **J Neurosurg 67**: 110-119, 1987

5. Forbes ML, Clark RSB, Dixon CE, et al: Augmented neuronal death in CA 3 hippocampus following hyperventilation early after controlled cortical impact. **J Neurosurg 88**: 549-556, 1998

6. Gennarelli TA: Animate models of human head injury. **J Neurotrauma 4**: 357-368, 1994

7. Gennarelli TA, Champion HR, Copes WS, et al: Comparison of mortality, morbidity, and severity of 59. 713 head injured patients with 114. 447 patients with extracranial injuries. **J Trauma 37(6)**: 962-968, 1994

8. Gennarelli TA, Thibault LE: Biological models of head injury, in Povlishock JT, Becker DP (eds): **Central Nervous System Trauma Status Report.** Bethesda, MD, NINCDS: 391-404, 1985

9. Ishige N, Pitts LH, Hashimoto T, et al: Effect of hypoxia on traumatic brain injury in rats: Part 1. **Neurosurgery 20**: 848-853, 1987

10. Jacobs BB, Baker P: **TNCC Trauma Nursing Core Course.** ENA: 1999

11. Jenkins LW, Moszynski K, Lyeth BG, et al: Increased vulnerability of the mildly traumatized rat brain to cerebral ischemia: the use of controlled secondary ischemia as a research tool to identify common or different mechanisms contributing to mechanical and ischemic brain injury. **Brain Res 477**: 211-224, 1989

12. Jones PA, Andrews PJ, Midgley S, et al: Measuring the burden of secondary insults in head injured patients during intensive care. **J Neurosurg Anest 6**: 4-14, 1994

13. Levin HS: Head injury and its rehabilitation. **Curr Opin Neurol Neurosurg 5**: 673-676, 1992

14. Levin HS, Gary HE, Eisenberg HM, et al: Neurobehavioral outcome 1 year after severe head injury. Experience of the traumatic coma data bank. **J Neurosurg 73**: 699-709, 1990

15. Lewelt W, Jenkins LW, Miller JD: Effects of experimental fluid-percussion injury of the brain on cerebrovascular reactivity to hypoxia and to hypercapnia. **J Neurosurg 56**: 332-338, 1982

16. Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. **J Neurosurg 80**: 291-300, 1994

17. McMahon CG, Yates DW, Campbell FM, et al: Unexpected contribution of moderate traumatic brain injury to death after major trauma. **J Trauma** (In Press)

18. McSwain NE, Frame S, Paturas JL: **PHTLS basic and advanced Prehospital Trauma Life Support**. St. Louis, Mosby: 1999

19. Morris RGM, Garrud P, Rawlins JNP, et al: Place navigation impaired in rats with hippocampal lesions. **Nature 297**: 681-683, 1982

20. Morris RGM, Hagan JJ: Allocentric spatial learning by hippocampectomised rats: a further test of the 'spatial mapping' and 'working memory' theories of hippocampal function. **Q J Exp Psychol 38b**: 365-395, 1986

21. Nawashiro H, Shima K, Chigasaki H: Immediate cerebrovascular responses to closed head injury in the rat. **J Neurotrauma 12(2)**: 189-197, 1995

22. Prat R, Markiv V, Dujovny M, et al: Failure of cerebral autoregulation in an experimental diffuse brain injury model. **Acta Neurochir Suppl 71**: 123-126, 1998

23. Sinz EH, Kochanek PM, Dixon CE, et al: Inducible nitric oxide synthase is an endogenous neuroprotectant after traumatic brain injury in rats and mice. **J Clin Invest 104**: 647-656, 1999

24. Stocchetti N, Furlan A, Volta F: Hypoxemia and arterial hypotension at the accident scene in head injury. **J Trauma 40**: 764-767, 1996

25. van den Brink WA, de Jong DA, van den Heuvel A, et al: The closed head injury model, standardization of acceleration mechanisms. **Intracranial Pressure and Neuromonitoring in Brain Injury** 1998(Abstract)

26. van den Brink WA, ter Horst M, van den Heuvel A, et al: Brain oxygenation is impaired in experimental closed head injury. (submitted)

27. Yuan XQ, Wade CE: Traumatic brain injury attenuates the effectiveness of lactated Ringers solution resuscitation of haemorrhagic shock in rats. **Surg Gynaecol Obstet 174**: 305-312, 1992

Chapter 9
Brain parenchyma - $PbrO_2$ catheter interface, A histopathological study in the rat

The microneurosurgeon must be constantly analytical.
Each case must be analyzed before and after its completion.
Laligam N. Sekhar,
Cranial Microneurosurgery, 1999

Abstract

Local cerebral oxygenation can be monitored continuously using an intraparenchymal Clark-type pO_2 sensitive catheter. Measured values of brain tissue PO_2 ($PbrO_2$) not only depend on the clinically interesting balance between oxygen offer and demand, but also on catheter properties and characteristics of the probe tissue interface. Microdamage surrounding PO_2 sensitive needles, inserted into various tissues, has been reported; we evaluated histologic changes at the probe tissue interface after insertion of PO_2 probes, suitable for clinical use, in the rat brain. The effect of insertion of the probe itself (mechanical damage), the application of micropotential during the measurements and the effect of time was evaluated using digital image analysis of Haematoxyline-Eosine stained histological slices. Surrounding the probe tract a zone of edema with an average radius of 126.8 µm was seen; microhemorrhages with an average surface area of 56.2 x 103 μm^2 were observed in nearly all cases. The area of edema and the presence of microhemorrhages were not influenced by performed measurements, and do not increase over time. Intraventricular blood was observed in 10 of 19 rats studied. Measured low $PbrO_2$ values were related to the presence of a microhemorrhage in either probe tract or ventricles. Tissue damage due to the measurements is negligable and the amount of edema itself does not influence the accuracy or response time of the PO_2 probe. Low $PbrO_2$ readings, however, could be caused by local microhemorrhages, undetectable on CT or MRI.

Introduction

Continuous delivery of oxygen is necessary for adequate function of organs and cells. Different organs can survive variable periods of relative hypoxia. The brain is especially sensitive to periods of oxygen deprivation and irreversible damage already occurs after a few minutes of circulatory arrest. Ischemia is a common denominator in many cerebral disorders, not only confined to stroke or heart attack victims. In patients with aneurysmal subarachnoid hemorrhage early ischemia occurs during the 'tamponade phase' and delayed ischemia results from vaso-spasm. In patients with head injury, ischemia is the common

result of various pathophysiologic cascades, further potentiated by systemic insults and episodes of decreased cerebral perfusion pressure.

For clinicians treating patients with these disorders monitoring of brain oxygenation is indicated and can facilitate more targetted therapy. Jugular oximetry, measuring oxygen saturation in cerebral venous blood, as monitor of global cerebral oxygenation is currently widely used in patients with severe head injury [21, 22, 28]. However, results of this technique can potentially underestimate the incidence of ischemia when anemia exists or arteriovenous shunting occurs. Moreover technical problems are frequent and complicate measurements [7]. Alternatively we chose to investigate the clinical application of local measurements of brain tissue PO_2 ($PbrO_2$) as parameter for cerebral oxygenation after head injury [31,32]. Methods for measurement of tissue oxygen tensions have initially been reported by Clark [2] and Davies and Brink [6].

Brain tissue PO_2 has been the subject of a large number of studies in animals [3, 9, 15-17, 20, 26, 27]. Many efforts have been made to refine and improve the reliability and applicability for clinical use. In the human situation $PbrO_2$-studies have been confined to observations during intracranial procedures [4, 12, 19] and for long term monitoring in the ICU in which it was mainly confined to severely head injured patients [13, 17, 18, 30-33]. Measured values of brain tissue PO_2 ($PbrO_2$) depend not only on the clinically interesting balance between oxygen offer and demand, but also on catheter properties and characteristics of the probe tissue interface. In studies in muscle and liver tissue using PO_2 needle probes local tissue damage has been observed[1, 24]. Around the needle probe a small zone of tissue necrosis occurs with compression of blood vessels within a diameter of 70 µm. Surrounding this area, some local edema causing disturbance of the microcirculation is found. It is conceivable that the introduction of a flexible catheter into the brain and the measurements itself could cause changes in the structure of the surrounding tissue and its microvascular supply when used in brain tissue as well. The present study was undertaken to evaluate the probe tissue interface at a light microscopic histological level to investigate whether local aspects could influence accuracy and reliability of measurements in the clinical situation.

Materials and Methods

PO_2 probes

The probes used were flexible polarographic Clark-type PO_2 probes, identical to the ones used in our clinical program, with a polyethylene surface (diameter 0.5 mm) in combination with a Licox measuring computer (Licox PO_2 computer, GMS mbH, Kiel-Mielkendorf, Germany). A small DC potential (795 mV) is applied to the 5 mm tip of the probe. O_2 molecules are reduced at the surface and an oxygen tension dependent current (0-10 µA) is generated and measured. Premeasurement calibrations of the catheter were performed as follows: The actual barometric pressure was measured utilizing an electronic

barometer and this value, together with the catheter specific values provided by the manufacturer, were entered in the Licox-measuring device. The catheter was kept in its sterile vial in the aluminum temperature block connected to the temperature inlet wire, as such correcting for ambient room temperature. After obtaining stable values, which should not be out of range for more than 10%, the value of 154 mmHg was used as the upper calibration level. Subsequently a zero calibration was performed with freshly made zero solution, containing $Na_2S_2O_4$. After this calibration procedure the catheter was rinsed with sterile saline several times and stored in the water saturated vial until insertion in the brain parenchyma. During the measurement of the cerebral oxygenation the body temperature of the animal was monitored and kept within a close range of 37.5 °C. Individual temporary deviations from these values were manually corrected for.

Experimental protocols

Experiments were conducted in male Sprague Dawley rats. All experiments were performed according to the 'Guidelines for the Care and Use of Laboratory Animals', Erasmus University Rotterdam. In each animal two $PbrO_2$ probes were inserted, one on each side. Before use approximately 3 mm of the tip was cut off under microscopic vision while taking care not to open the seal, to ensure that the measuring part of the probe was positioned fully in the brain parenchyma for measurements. Catheters were introduced under microscopic control in both frontoparietal regions of the brain after opening the dura with a sharp hook. As such the measurement area of the tip of the catheter was placed 0.5 to 1.0 mm underneath the brain surface, prohibiting potential contamination by room air. Contamination by high oxygen values of the cerebrospinal fluid (CSF) could however not be excluded. The catheter in the right hemisphere was connected to the measuring computer and $PbrO_2$ monitored, the left catheter was not connected. In this way, possible damage occurring due to the measurement itself in addition to mechanical damage resulting from introduction of the catheter alone could be evaluated. The protocol focussed on analysis of histologic changes at the probe tissue interface, one hour and 24 hours after introduction of the catheters for $PbrO_2$ measurements. To this end two experimental groups were studied: $PbrO_2$ catheters were introduced in 12 male Sprague Dawley rats, measurements performed for one hour after which the animals were sacrificed and brains removed for histologic analysis. In seven rats measurements were performed for four hours, after which period the brain oxygen measurement was stopped and the animals sacrificed 20 hours later.

One-hour histology

Rats in this group were sedated with Diethylether, endotracheally intubated and allowed to breath spontaneously. Isoflurane (1.5%) in a mixture of 25% O_2, 25% N_2 and 50% N_2O was used to allow adequate oxygenation, sedation and analgesia, while minimally interfering with cerebral blood flow. The temperature was kept stable by using a heating pad and an infrared light

source. A polyethylene catheter was inserted in the left femoral artery for continuous measurement of arterial blood pressure, and blood samples at the start and the end of the measurements. The rats were fixated in a stereotactic device to minimize movement artefacts caused by breathing. $PbrO_2$ catheters were inserted into both hemispheres. Influence of body temperature on PO_2 measurement were compensated for by manually entering the temperature in the measuring computer. Rats were then monitored for one hour and values of mean arterial blood pressure, heart rate, respiratory rate, temperature and $PbrO_2$ recorded every 15 minutes. Blood samples were analysed on a blood gas analyser (model AVL 945, Graz-Austria). At the end of the measurement period animals were killed with a pentobarbital overdose. The rats were rapidly transported to the perfusion setup, perfused with saline at a perfusion pressure of 70 mmHg; thereafter a glutaraldehyde 1%/formaldehyde 4% mixture was perfused for five minutes, the catheters removed, the brain taken out of the skull and immersed in the same glutaraldehyde/formaldehyde mixture.

24-H histology

Experiments were performed in seven rats to evaluate the effect of the presence of indwelling catheters over a 24 hour period. The rats were sedated with Diethylether and subsequently an adequate dose of the non recovery anesthetic agent urethane was administered by intramuscular injection (1.39 mg urethane/gram body-weight). The rats were not intubated, nor was an arterial catheter inserted. $PbrO_2$ catheters were introduced in the same way as described in the one hour protocol. In this group $PbrO_2$ was monitored for four hours and all vital parameters noted every 15 minutes. At the end of the four hour period the Licox computer was disconnected, while the catheters remained in situ. The rats were given a subcutaneous fluid depot of 10 ml saline and remained in the stereotactic device for another 20 hours. During this period, EKG, respiratory, and temperature tracings were paper recorded using a polygraph. After this period the vital parameters were again noted, and the animals subsequently killed, perfused, and brains removed as in the first group.

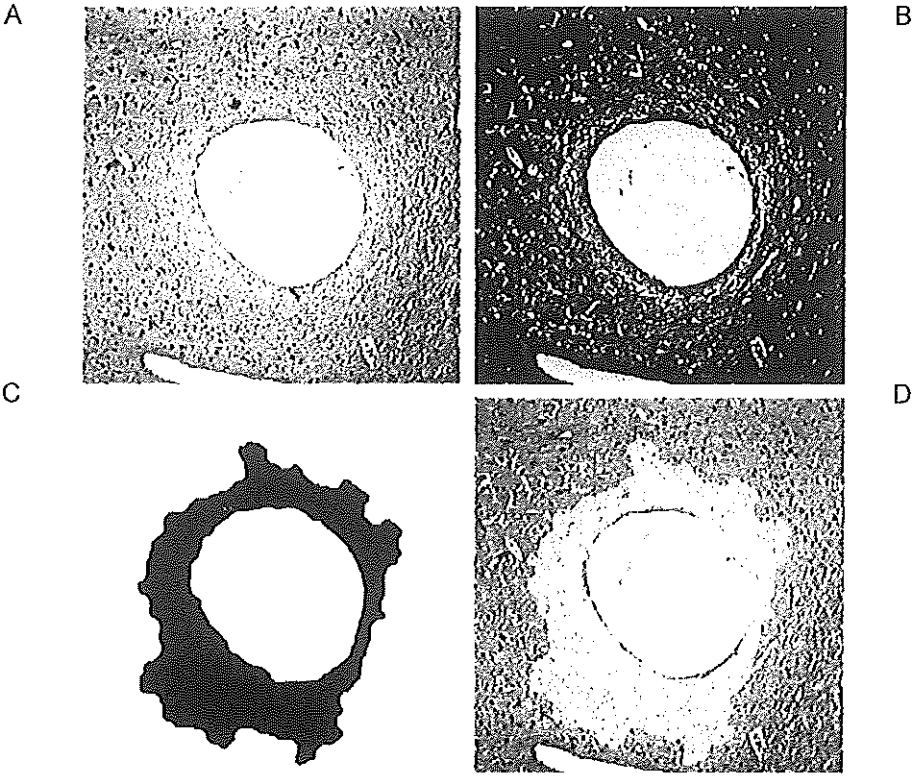
Histologic procedures

The brains were stored for 24 hours in the glutaraldehyde/formaldehyde mixture. The right side of the brain was marked with Indian ink, after which it was cut into five 3 mm thick coronal slices and placed into tissue cassettes used for routine histology. Subsequently they were dehydrated, cleared and infiltrated with paraffin in a tissue processor (model VIP, Tissue Tek, Bayer Diagnostics, Germany). Microtomized sections (4 µm thick) were made of sections two, three and four of the brain. The microtomized sections were stained with hematoxylin and eosin. Initially other stainings (Kluwer, Bodian, CD68 and Panleuko) were also employed, but as these did not yield any additional information these procedures were abandoned after the pilot studies.

Digital Image Analysis

Areas of the probe tract, edema and hemorrhage were measured using a digital image analysis system: a 3 chip CCD color camera (Sony DXC 930 P, Sony, Japan) mounted on a microscope (Zeiss, Axioplan, Germany) and a 66 MHz PC were used for collecting digital images of the microscopic slices. After shading correction and contrast enhancement [figure 1A and B] area measurements were performed with the use of KS400 digital image analysis software (Kontron Elektronik GmbH, Germany). With the aid of this program it is possible to identify areas by their colour characteristics. Areas of probe tract, edema, and hemorrhage were determined by interactive thresholding of the red and green channels of the color images [figure 1C and D] [23]. After visual inspection of the results the respective surface areas were automatically calculated and stored for further statistical analysis.

figure 1 - Digital image analysis in four steps. (A) Gray-scale image of a shading-corrected digital color image of a tissue slice stained with H&E and recorded with a 3-chip color CCD-camera. The outline of the less intense staining (edema) near the probe tract (white hole) is difficult to identify. (B) Image shown in A after contrast enhancement. The edematous area can now clearly be distinguished from the surrounding tissue. (C) Area selected by tresholding of the green and red channels of the color image. The surface area of the selected region was calculated and taken as a measure of catheter-induced edematous damage. (D) Parts A and C merged in one image.



Statistical Analysis

The observed values were compared with repeated measures ANOVA analysis for unbalanced data, and associations were tested with the Fischer exact test, using BMDP software package (BMDP statistical software, version 1990, Los Angeles, California, USA). P-values < 0.05 were considered significant.

Results

Physiological findings

Vital parameters remained within physiologic limits during the measurement period [table 1A]. An example of $PbrO_2$-readings during one hour after introduction is shown in figure 2. The mean trend over time after introduction is shown in figure 3 the value of 154 mmHg room air PO_2 drops quickly to tissue levels slightly lower than stabilized measurements after 60 min. As such, even without using an introducer, there is some time of equilibration needed before stable values are recorded. In six out of 19 experiments a slow increase of 8.6 +/- 2.3 mmHg during the first hour was seen. Stable values were obtained within one hour in five, and within two hours in all of these measurements. The arterial PaO_2 and $PaCO_2$ were not controlled for during the experiment and as such both these major determinators of tissue oxygen values showed a large interindividual variation, as did the $PbrO_2$-measurements [table 1 and 2].

table 1 Measured vital parameters and blood gas analysis of both experimentation groups for the 1-H group

	t = 0	t = 30 min	t = 60 min
arterial pressure, mm Hg	94.1 ± 8.4	91.9 ± 10.6	389.3 ± 12.7
heart rate,/min	397.5 ± 37	407.5 ± 37.7	407.5 ± 31.1
respiratory rate,/min	67.8 ± 12.4	65.3 ± 8.6	66.1 ± 16.9
temperature, ° C	37.3 ± 0.8	37.1 ± 0.6	37.1 ± 0.7
$PbrO_2$, mm Hg	25.4 ± 19.6	26.0 ± 18.8	31.7 ± 19.4
pH	7.40 ± 0.05	-	7.36 ± 0.09
PaO_2, mm Hg	124.8 ± 20	-	108.7 ± 29.4
$PaCO_2$, mmHg	24.4 ± 12.8	-	37.4 ± 14.6

table 2 Measured vital parameters and blood gas analysis of both experimentation groups for the 24-H group.

	t = 0	t = 4 h	t = 24 h
heart rate,/min	373 ± 35.6	355 ± 32	397.6 ± 40.7
respiratory rate,/min	104 ± 8.3	99.1 ± 7.4	103.7 ± 8.4
temperature, ° C	36.9 ± 0.9	37.9 ± 0.3	38 ± 0.3
$PbrO_2$, mm Hg	36.6 ± 15.6	34.4 ± 17.2	-

figure 2 Example of the oxygen readings taken within the first 1 h after insertion. The oxygen measurements fall from the ambient room oxygen value of 154 mmHg to the actual brain parenchyma values. Initial low values, later stabilizing to slightly higher values were observed in six out of 19 experiments. Five of these catheters stabilized within 1 h, the sixth after 2 h.

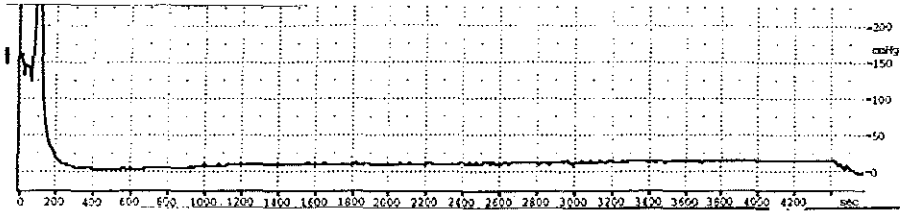
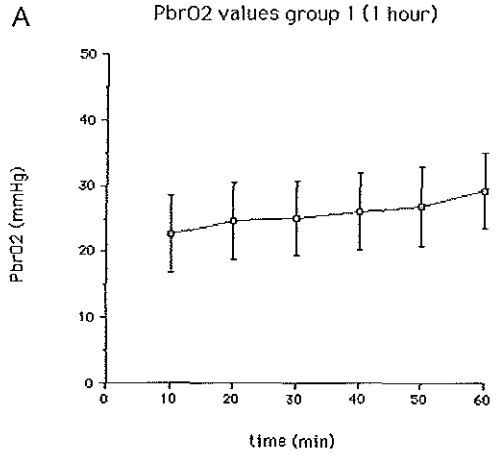
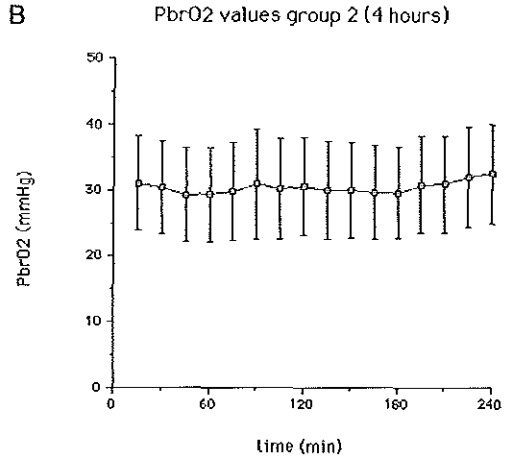


figure 3 Oxygen measurements with SEs of all experiments. The slight mean increase during the first hour (A), is absent in the 4 h / 24-h group (B).





One-Hour Experiments

Histologic analysis: In a number of sections the probe tract was not visible, as the probe had entered the ventricular system. In adjacent slices a tract both on the left and right side could always be evaluated. Around the hole created by the catheter itself, a zone of edema was seen. This zone was characterized by a bleaching of the pink stain and a spongy appearance of the neuropil. Neurons in this area were smaller showing hyperchromatic nuclei, usually regarded as a sign of hypoxia. Figure 4 shows a typical example of such a probe tract with surrounding edema. Precisely delineating the edematous tissue from normal brain was difficult, but using the mentioned digital image measurement technique sufficient standardization was obtained in this process. The measured areas are listed in table 3. Some extravasation of erythrocytes was usually found. In four rats a substantially larger area ($> 100 * 10^3$ µm^2) of blood near the catheter was observed [figure 4]. In eight rats, blood was also found in the ventricular system. The amount of blood present seemed to be inversely related to the average measured $PbrO_2$ [table 4].

figure 4 Example of the probe tract with a surrounding zone of edema. Pycnotic cells, indicative of local ischemia, are present in the edematoous layer of 120 µm. Bar = 100 µm.

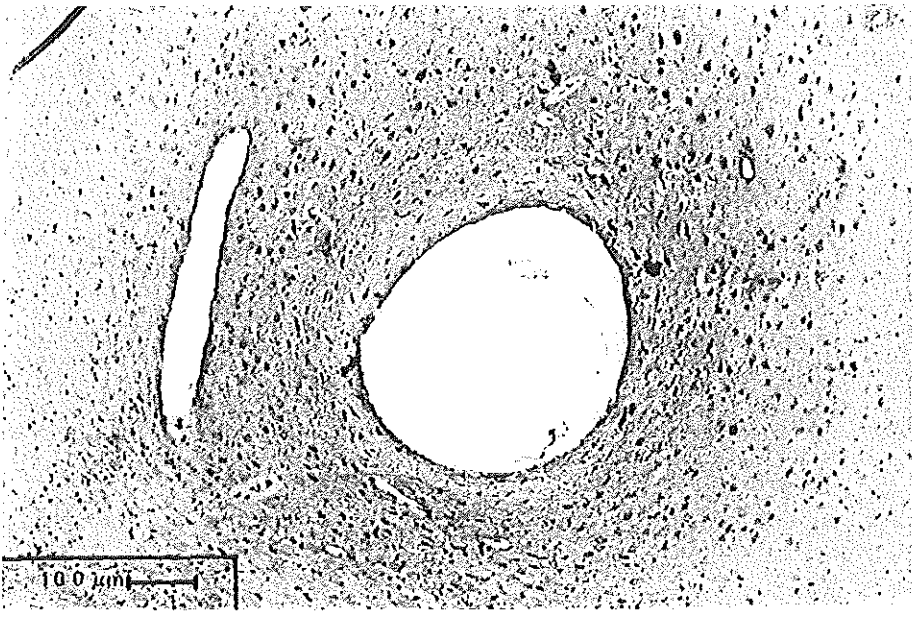


table 3 Inter individual variability in $PbrO_2$. The 1-h averaged oxygen tension of individual rat brains. The inter individual variation with this method is clear. A relationship of $PbrO_2$ with PaO_2 or $PaCO_2$ is not observed.

experiment no.	$PbrO_2$	PaO_2	$PaCO_2$
1	20	149	23
2	25	115	27
3	24	115	22
4	33	-	-
5	13	100	37
6	12	113	33
7	35	117	33
8	12	90	48
9	13	85	55
10	18	137	21
11	68	141	17
12	63	120	16

table 4 Digital image analysis provides a surface area for each histological slice. Areas were recalculated as diameters for the probe tract and the edema. Except for the larger probe tract diameter after 24 h, no significant difference among groups was found.

	1-H group		24-H group	
	measured	sham	measured	sham
Radius probe tract in μm ± sd	162 ± 40	151 ± 32	188 ± 30	173 ± 70
Shell of edema in μm ± sd	117 ± 38	137 ± 31	124 ± 47	130 ± 41
Area of blood in 10^3 μm^2 ± sd	74 ± 124	13 ± 8	61 ± 109	111 ± 144

24-Hour Experiment

All animals remained appropriately sedated at the end of the 24 hours period, with only three rats showing minor hindpaw withdrawal on firm manual squeezing. During this period two unexpected deaths after several hours were encountered, probably due to drug overdose. Histologic analysis: The histologic aspect of brain surrounding the tissue probe showed the same abnormalities as described in the one hour group. The probe caused a slightly larger tract (radius mean ± sd 188 ± 30 μm) compared to the one hour group (162 ± 40 μm). The difference is statistically significant. However no difference in zone of edema or area of blood surrounding the tissue probe compared to the one hour group was present. In four rats there was an area of blood larger than 100. 10^3 μm^2, and two rats had a small amount of blood in the ventricular system.

Discussion

The animals measured for a one hour period had an average $PbrO_2$ of 27.8 mmHg (range 12 to 68), in rats studied for 24 hours the average $PbrO_2$ was 32.8 mmHg. This $PbrO_2$ difference is not significant and can possibly be explained by a higher arterial PO_2 consistent with the higher respiratory rate observed in this group of animals. The measured $PbrO_2$ values correspond well with data from other studies [table 5]. The observed variability between animals however is larger than in other investigations. In this experimental protocol the main focus was on the histological analysis. Ventilation and oxygenation of the rat was not standardized by mechanical ventilation or changes in FiO_2. The variability in $PbrO_2$ can not be explained completely by the differences in arterial PO_2 or PCO_2 however. A plausible explanation for the variability is the relatively large length of the $PbrO_2$ probe inserted into the small rat brain, carrying the risk of penetration of the ventricular system. PO_2 values of the CSF are higher than brain tissue $PbrO_2$[10, 11]. If part of the probe is in contact with CSF this will lead to higher values of measured $PbrO_2$. The larger the length of the measurement probe residing in the ventricular system, the larger the influence of the CSF on the measurement. The fact that we could demonstrate the presence of blood in CSF of more than half of the animals studied supports this hypothesis. Following discussion of these observations with the manufacturer, a specially designed rat probe with a measurement length of 1 mm has been developed.

table 5 Effect of the presence of blood on the $PbrO_2$ measurement in the 1-H group. A considerable amount of blood was defined as an area of blood > $100 * 10^3$ μm^2 or a ventricular system filled with blood. In these cases, the $PbrO_2$ readings were significantly lower than in the absence of blood. Fischer exact test: p = 0.01.

	Average $PbrO_2$ < 30 mm Hg	Average $PbrO_2$ > 30 mm Hg
Blood		
Considerable	7	0
Small amount	1	4

Although for the reasons mentioned this 5 mm probe is less suitable for evaluation of physical or pharmacological interactions on brain tissue PO_2 in small animals, for the clinical situation in patient monitoring the probe has deserved its merit in relation to the catheter stability and the extremely low complication rate[8, 33]. Lübbers and Cruickshank employed a micro-electrode needle tip histogram measurement[4, 16]. This type of measurement combines a high spatial resolution with little histologic damage at the time of measurement, resulting in absolute PO_2 values[11]. However, the technique only yields momentary values and does not permit continuous monitoring. In our experiments $PbrO_2$ remained constant over the measuring period, even over a four hour time window, in contrast to the findings of Tomida et al[29]. This group reported that insertion of an electrode in the rodent cortex can cause a decrease in

cerebral blood flow in the punctured hemisphere for a period of six to 24 hours. This would be expected to have some effect on $PbrO_2$, being low initially and subsequently rising slowly in response to increasing blood flow, as was shown in six of our experiments. In the clinical situation in head injured patients initial low values, increasing towards a plateau, is often observed[31-33]. This study indicates that in the uninjured brain, with minimal traumatic introduction, the time after which the catheter is stabilized is less than one hour in by far the most cases. The possibility of an increase in $PbrO_2$ occurring after the four hour period, although unlikely, can however not be completely excluded.

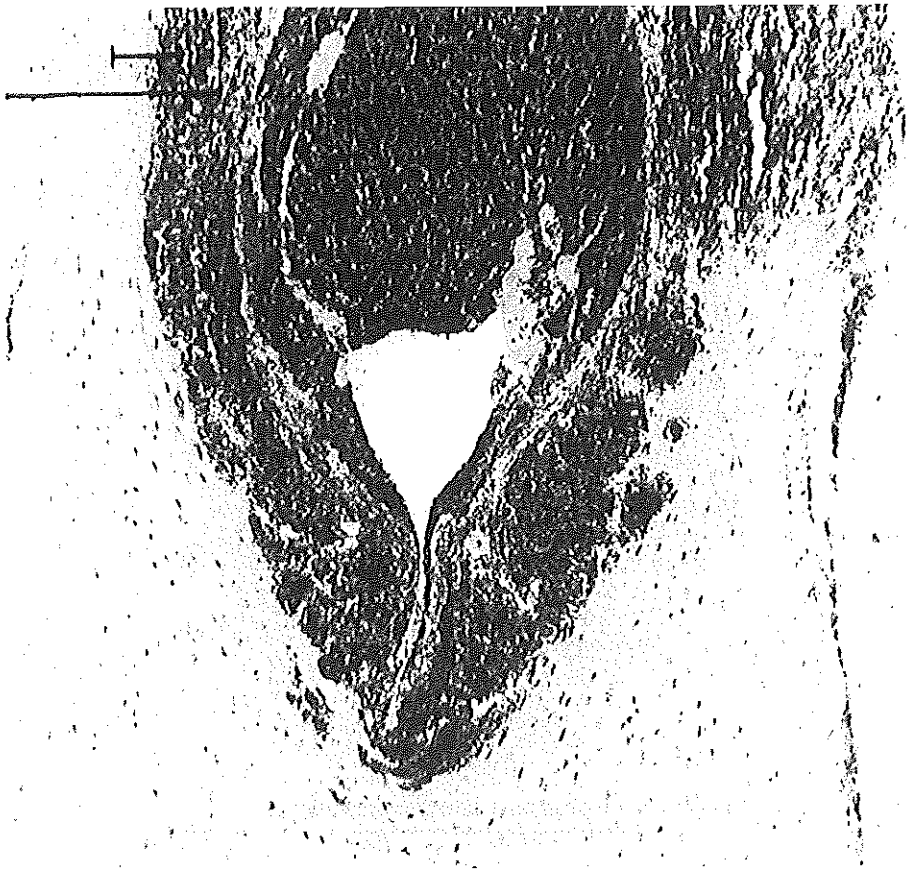
The presence of blood in the vicinity of the probe or in the ventricular system seems to influence measured $PbrO_2$. We dichotomized the results in animals having a $PbrO_2$ -value above or below 30 mmHg. Lower $PbrO_2$ -values were indeed significantly associated with the presence of a considerable amount of blood ($p < 0.05$). A possible reason for this phenomenon could be the fact that blood around the probe slows down the transport of oxygen. In contrast to circulating blood, clotted blood has a higher diffusion coefficient (W. Erdmann, personal communication, 1996). The large stationary probe is very sensitive to the diffusion coefficient of the tissue around the measuring surface. Another possible reason for low $PbrO_2$ readings in the presence of blood could be vasoconstriction due to oxy-hemoglobin in the subarachnoid space, as observed in subarachnoid hemorrhage. This effect, however, usually occurs after several days, and would not seem applicable to our acute experiments. Although the areas of blood identified in some cases in this study had a significant effect on measured $PbrO_2$ it is highly unlikely that such small amounts of blood can be detected in the clinical situation on either CT or MRI scans. Inference can be made that if a low $PbrO_2$ is measured in an individual patient, it can not be excluded that this value is caused by the local presence of blood. Care should be taken in interpreting such low values as indicative of local, or even global, cerebral hypoxia. It would appear that in the clinical situation the trend over time is more valuable in interpreting the condition of brain oxygenation and reactions to therapeutic interventions.

In this study the amount of observed histologic damage caused by the $PbrO_2$ catheter was very limited; probe insertion resulted in a tract with an average radius of 151 to 188 µm. The difference with the 250 µm radius of the probe is caused by the fixation process, shrinking the tissue. Surrounding the probe a zone of 120 µm edema was noted. This edema could be identified by less intense pink staining and some spongy appearance of the neuropil. In contrast to results reported in liver tissue[1], where the sturdy intrinsic capsule and architecture cause 'organ stiffness' no distortion of capillaries was seen in our experiments. Although this would suggest a normal local oxygen delivery, pycnotic cells were seen in and closely around edematous zone. The irreversibility of neuronal damage can not be assessed from these sections, but the zone is so small that it is not considered clinically relevant. The zone of edema (120 µm) is larger than the 70 µm zone of cellular damage, observed in

muscle and liver tissue[1, 24]. In these experiments however a needle probe was used, which was advanced through the tissue with the use of a micro-manipulator, and the duration the probe remaining in the tissue was shorter. Whether the larger zone of edema observed, can be explained by different tissue characteristics, or is due to the method of introduction or duration of the measurements, remains to be determined. Zauner et al,[31] utilized a probe, simultaneously measuring PO_2, PCO_2, pH and temperature in the cat. The probe diameter was similar to the Clark-type probe used in our study. After six to eight hours of measurement in the feline brain they found no histological damage beyond 40 µm from the probe tract. Our method of establishing edema by means of a digital image analysis system, resulting in 100 µm edematous zone, might be more sensitive than the methods used by these authors. The minimal local damage is however confirmed. No probe tract bleeding, nor ventricular penetration, was reported in these experiments however. The discrepancy between the sensors diameter and the rodent or feline brain sizes may explain the observed differences. With respect to tissue reaction no difference was observed between the one hour and 24 hour groups, suggesting that neither time, nor microvoltage, current, or oxygen reduction influences the observed damage.

$PbrO_2$ is measured at the surface of the $PbrO_2$ electrode. To accurately measure tissue PO_2, oxygen needs to diffuse from normal brain tissue through the edematous zone to the $PbrO_2$ electrode. Assuming diffusion constants to be similar in normal and edematous brain tissue the transit time for diffusion of oxygen through a 120 µm radius of edema can be calculated to be approximately three seconds [figure 6]. This is considerably shorter than the reaction time of the $PbrO_2$ electrode (T90 = 20 seconds). Keeping in mind that the brain parenchymal oxygen tension measurement is a local measurement, as opposed to the jugular oximetry, approximation of the measured sample surface of the virtual plane surrounding the oxygen sensitive tip of the catheter would be worthwhile. This virtual plane within the tissue surrounding the catheter is however not constant. It depends on the blood flow, carrying oxygen molecules, it depends on local cellular metabolism consuming the oxygen and it depends on the movement of the interstitial fluids[25]. If the catheter would be placed for instance in a certain volume of tissue in which the cells will not consume oxygen, the catheter will measure oxygen that diffuses from distant sites through this volume of non-consuming cells. In this situation the so-called 'sample area' is larger than it would be if the catheter would be placed in an area in which cells have a normal metabolism, but the cerebral bloodflow does not meet the demand. In this situation the measured value will be lower and the 'measured sample volume' will be smaller. Although difficult to measure reliably a sample areas of 17 mm^2 has been reported[13]. Recalculating this surface value of 17 mm^2 with a probe length of 5 mm and a probe diameter of 0.5 mm, the 'capture distance' would be 440 µm from the surface of the probe. This is considerably larger than the observed zone of edema surrounding the probe tract.

figure 5 Example of probe tract partially filled with and surrounded by a considerable amount of blood. Such an amount of blood is too small however to be detected by modern patient imaging systems. Bar = 100 μm.



Consequently, other than the presence of local blood around the catheter tip, no histologic changes were noted at the probe tissue interface, which could significantly influence the results of local tissue measurements of $PbrO_2$ in brain tissue. The method can therefore be considered clinically applicable without increased risk to the patient.

figure 6 Oxygen transit time through tissue. This graph shows the average time it takes for oxygen to travel through a layer of brain tissue. Mean transit time (T_D) in brain tissue [5]. $T_D = X^2/_3$. Dox = diffusion coefficient for oxygen: $1.8 * 10^{-5}$ cm^2/s.

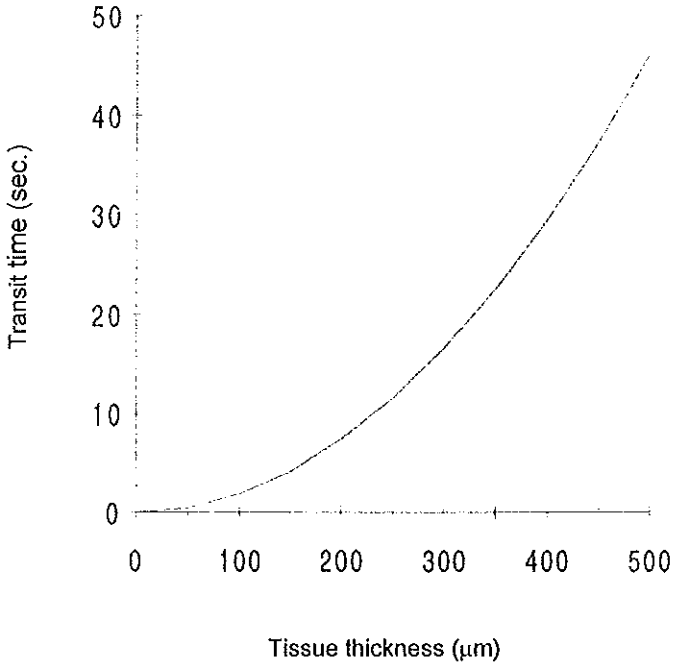


table 6 studies without compromised cerebral circulation

	subject	$PbrO_2$	PaO_2	Type of probe
This study	Rat	27.8 ± 19.6	116.7 ± 27.1	Clark electrode 500 µm
Fennema et al., 1989	Rabbit	-	9	Electrode 10 µm
Sick et al., 1982	Rat	12.9 ± 0.9	> 110	Electrode 10 µm
Liu et al., 1995	Rat	24 ± 3	-	EPR spectroscopy
Lübbers et al., 1994	Guinea pig		23.3	Electrode 1 µm
Crockard et al., 1975	Baboon	23.8 ± 12	400	Electrode 100-150 µm
Maas et al., 1993	Dog	28.2 ± 7.5	112.2 ± 6.3	Clark electrode 500 µm
v/d Brink , 1996 unpub.	Monkey	28.8 ± 8.9	110.8 ± 16.6	Clark electrode 500 µm
Seyde et al., 1986	Rat	29 ± 5	128 ± 5	Electrode 1-2 µm
Nair et al., 1987	Gerbil	35.4 ± 1.7		Electrode <2 µm
Hoffman et al., 1996	Human	37 ±12	169 ± 49	Clark electrode 500 µm
Meixensberger, 1993	Human	47.9 ± 13.1	170.3 ± 28.1	Surface electrode

Conclusions

Introduction of a $PbrO_2$ probe into brain tissue causes a small zone of surrounding edema. The size of this zone is too small to influence response time and reliability of measurements. Introduction of the probe can produce small microhemorrhages around the catheter tip, beyond the detection limits of modern patient imaging systems. Lower values of $PbrO_2$ were seen in animals with more extensive local hemorrhage. Therefore, low $PbrO_2$ pressures may indicate the presence of small hemorrhages, instead of an actual low partial pressure of oxygen in the brain tissue. A trend over time however provides the clinician valuable information on the cerebral oxygenation and treatment response of patients monitored.

References

Paper:
van den Brink WA, Haitsma IK, Avezaat CJJ, et al: The brain parenchyma-PO_2 catheter interface, a histopathological study in the rat. **J Neurotrauma 15**:813-824, 1998

1. Bossen W: Morphologische veranderungen bei messungen mit einstichsonden aus der gewebe - pO2 - **histographie in der rattenleber.** Lübeck, 1995

2. Clark LC: Monitor and control of blood and tissue oxygen tensions. **Trans Am Soc Artif Int Org** 2: 41-45, 1956

3. Crockard HA, Symon L, Branston NM, et al: Changes in regional cortical tissue oxygen tension and cerebral blood flow during temporary middle cerebral artery occlusion in baboons. **J Neurol Sci** 27: 29-44, 1976

4. Cruickshank GS, Rampling R: Peri-tumoural hypoxia in human brain: Peroperative measurement of the tissue oxygen tension around malignant brain tumors. **Acta Neurochir Suppl** 60: 375-377, 1994

5. Cserr HF: Physiology of the choroid plexus. **Phys Rev** 51: 273-311, 1971

6. Davies PW, Brink F: Microelectrodes for measuring local oxygen tensions in animal tissues. **Rev Sci Instrum** 13: 524-533, 1942

7. Dearden NM, Midgley S: Technical considerations in continuous jugular venous oxygen saturation measurements. **Acta Neurochir Suppl** 59: 91-97, 1993

8. Dings J, Meixensberger J, Roosen K: Brain tissue pO2-monitoring: Catheter stability and complications. **Neurol Res** 19: 241-245, 1997

9. Fennema M, Wessel JN, Faithfull NS, et al: Tissue oxygen tension in the cerebral cortex of the rabbit, in Rakusan K, Biro GP, Goldstick TK, et al (eds): **Oxygen transport to tissue XI.** New York, London, Plenum press: 451-460, 1989

10. Fleckenstein W, Maas AIR, Nollert G, et al: Oxygen pressure in cerebrospinal fluid. Part 2: Effects of hypocapnia and hypercapnia, in Ehrly AM (ed): **Clinical Oxygen Pressure Measurement II.** Berlin, Blackwell Ueberreuter Wissenschaft: 386-395, 1990

11. Fleckenstein W, Maas AIR, Nollert G, et al: Oxygen pressure in cerebrospinal fluid. Part1: Dynamics of oxygen transfer at normal, increased and decreased oxygen offer, in Ehrly AM (ed): **Clinical Oxygen Pressure Measurement II.** Berlin, Blackwell Ueberreuter Wissenschaft: 368-385, 1990

12. Hoffman WE, Charbel FT, Edelman G: Brain tissuye oxygen, carbon dioxide, and pH in neurosurgical patients at risk for ischemia. **Anesth Analg** 82: 582-586, 1996

13. Kiening KL, Unterberg AW, Bardt TF, et al: Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO_2 versus jugular vein oxygen saturation. **J Neurosurg** 85: 751-757, 1996

14. Kunze K: Die lokale, kontinuierliche sauerstoffdruckmessung in der menslichen muskulatur. **Pflugers Arch** 292: 151-160, 1966

15. Leniger-Follert E, Lubbers DW, Wrabetz W: Regulation of local tissue pO_2 of the brain cortex at different arterial O_2 pressures. **Pflugers Arch** 359: 81-95, 1975

16. Lubbers DW, Baumgartl H, Zimelka W: Heterogenity and stability of local pO_2 distribution within the brain tissue. **Adv Exp Med Biol** 345: 567-574, 1994

17. Maas AIR, Fleckenstein W, de Jong DA, et al: Monitoring cerebral oxygenation: Experimental studies and preliminary clinical results of continuous monitoring of cerebrospinal fluid and brain tissue oxygen tesion. **Acta Neurochir Suppl 59**: 50-57, 1993

18. Meixensberger J, Baunach S, Amschler J, et al: Influence of body position on tissue-pO_2, cerebral perfusion pressure and intracranial pressure in patients with acute brain injury. **Neurol Res 19**: 249-253, 1997

19. Meixensberger J, Dings J, Kuhnigk H, et al: Studies of tissue PO_2 in normal and pathological brain cortex. **Acta Neurochir Suppl 59**: 58-63, 1993

20. Nair PK, Buerk DG, Halsey JH: Comparison of oxygen metabolism and tissue pO_2 in cortex and hippocampus of the gerbil brain. **Stroke 18**: 616-622, 1987

21. Robertson CS, Contant CF, Narayan RK, et al: Cerebral blood flow, $AVDO_2$, and neurological outcome in head injured patients. **J Neurotrauma 9**: S349-S358, 1992

22. Robertson CS, Narayan RK, Gokaslan ZL, et al: Cerebral arteriovenous oxygen difference as an estimate of cerebral blood flow in comatose patients. **J Neurosurg 70**: 222-230, 1989

23. Russ JC: **The image processing handbook**. Boca Raton, FL., C. R. C. Press: 1995

24. Schramm U, Fleckenstein W, Weber C: Morphological assessment of skeletal muscular injury caused by pO_2 measurements with hypodermic needel probes, in Ehrly AM (ed): **Clinical Oxygen Pressure Measurement II**. Berlin, Blackwell Ueberreuter Wissenschaft: 38-50, 1990

25. Schäffler A, Weiss Ch, Fleckenstein W: On the differences between muscle pO_2 measurements obtained with hypodermic meedle probes and with multiwire surface probes. **Functions analyse biologische systeme 19**: 169-177, 1989

26. Seyde WC, Longnecker DE: Cerebral oxygen tension in rats during deliberate hypotension with sodium nitroprusside, 2-chloroadenosine, or deep isoflurane anesthesia. **Anesthesiology 64**: 480-485, 1986

27. Sick TJ, Lutz PL, lamanna JC, et al: Comparative brain oxygenation and mitochondrial redox activity in turtles and rats. **J Appl Physiol 53(6)**: 1354-1359, 1982

28. Stocchetti N, Paparella A, Bridelli F, et al: Cerebral venous oxygen saturation studied with bilateral samples in the internal jugular veins. **Neurosurgery 34**: 38-44, 1994

29. Tomida S, Wagner HG, Klatzo I, et al: Effect of acute electrode placement on regional CBF in the gerbil: A comparison of blood flow measured by Hydrogen clearance, [3H] Nicotine, and [14c] Iodoantipyrine techniques. **J Cereb Blood Flow Metabol 9**: 79-86, 1989

30. Unterberg AW, Kiening KL, Bardt TF, et al: Multimodal monitoring in patients with head injury: evaluation of the effects of treatment on cerebral oxygenation. **J Trauma 42**: s32-s37, 1997

31. van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Monitoring brain oxygen tension in severe head injury: The Rotterdam experience, in Marmarou A, Bullock R (eds): **Acta Neurochirurgica Supplementum**. Springer-Verlag: 1998

32. van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Monitoring brain oxygen tension in severe head injury, early cerebral hypoxia is related with an unfavorable outcome. **Neurosurgery 46**: 868-878, 2000

33. van Santbrink H, Maas AIR, Avezaat CJJ: Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. **Neurosurgery 38**: 21-31, 1996

34. Zauner A, Bullock R, Di X, et al: Brain oxygen, CO_2, pH, and temperature monitoring: evaluation in the feline brain. **Neurosurgery 37**: 1168-1177, 1995

Chapter 10

Experimental Closed Head Injury marginally impairs brain oxygenation, systemic parameters are of influence on brain oxygen values

By hyperventilation the average $PaCO_2$ of the cats was reduced within minutes; CPP, MAP, and ICP were reduced and only slightly reduced blood flow. Although the PaO_2 was elevated, the PO_2 of the Cerebro Spinal Fluid was significantly decreased. ***Wolfgang Fleckenstein, Clinical Oxygen Pressure Measurement II, 1990***

Abstract

Object: In patients with severe head injury, decreased brain oxygen tensions ($PbrO_2$), as well as increased reactivity of brain oxygenation to increases in FiO_2, are related with poor outcome. We investigated whether experimental closed head injury (CHI), under controlled conditions, causes similar impairment of brain oxygenation and oxygen reactivity, to elucidate the underlying mechanisms. A standardized model of diffuse CHI, with and without subsequent systemic hypoxia (FiO_2: 14% O_2, 30 min.), was utilized to induce traumatic brain injury. Intraparenchymal micro catheters were used to study brain tissue oxygenation.

Methods: Rats were submitted to CHI using a standardized impact acceleration weight drop model. Either CHI alone (severe: n=8, very severe: n=28), or a combination of severe CHI and hypoxia (FiO_2 = 14% during thirty minutes, n=19) was induced. Two sham groups, without hypoxia (n=5), or with 30 minutes hypoxia (n=5) were used as controls. Rats submitted to hypoxia were artificially ventilated and paralyzed. $PbrO_2$ was monitored for 4, 5 hours after CHI using an Intraparenchymal Clark type oxygen sensor. At regular intervals after CHI, FiO_2 was increased to 66%, and $PbrO_2$-reactivity indices were calculated.

Conclusions: Severe CHI causes statistically significant lower $PbrO_2$ values, related to the severity of trauma. The $PbrO_2$ decrease might be caused by systemic parameters such as lower arterial blood pressure, $PaCO_2$, and PaO_2. During the thirty minutes systemic hypoxia deep cerebral hypoxia was observed, and augmented in combination with CHI. The depth of cerebral hypoxia in this episode was related to post traumatic survival rate. Levels generally considered hypoxic (<10 mmHg) were only observed in rats just before brain death. Differences in brain oxygen reactivity indices were not observed during the 4, 5 hour experiment. This study confirms the importance of adequate oxygenation and blood pressure management in head injured patients.

Introduction

Intracranial pressure (ICP) and cerebral perfusion pressure (CPP) therapy are currently the main therapeutic pillars in the treatment of severe head injury[18,22]. The aim is to ensure adequate cerebral blood flow (CBF) and tissue oxygenation. Our group has previously reported on continuous monitoring of partial pressure of brain tissue oxygen ($PbrO_2$) in severely head injured patients[29-31]. In these reports the presence of low $PbrO_2$ values at tissue level, was demonstrated in the early phase after head injury. Early cerebral hypoxia was significantly associated with unfavorable outcome and death. Furthermore reactivity tests to hyperoxygenation were performed. High oxygen reactivity indices, in the early phase after head injury, were related to unfavorable outcome [31]. Low $PbrO_2$ levels are in accordance with reports of decreased CBF[1], and ischemia[8,9] after severe head injury. The underlying mechanism of increased O_2 reactivity in patients with an unfavorable outcome, however, is as yet unexplained. We questioned whether a decrease in $PbrO_2$ would be reproducible in experimental head injury, which $PbrO_2$ patterns are related to outcome, and if the increased reactivity to temporary increases if the fraction of inspired oxygen (FiO_2) would be related to head injury or to secondary insults. To this end the weight drop model of diffuse Closed Head Injury (CHI) in the rat[16] was used with and without secondary hypoxia in a 4, 5 hour experiment in which $PbrO_2$ was monitored utilising an intraparenchymal microcatheter[28].

Materials and methods

Experimental protocol

preparation and monitoring

Studies were performed in male Wagrij rats weighing 256 ± 29 grams (means ± sd). Anesthesia was induced with ether using the chamber technique and maintained with isoflurane through an endotracheal tube (1 to 2 - 2.5% isoflurane, in an oxygen: nitrogen mixture of 33%: 66%). Arterial pressure was monitored with a femoral artery catheter connected to a strain gauge pressure transducer (Baxter, Uniflow, Utrecht, The Netherlands). Heart rate and respiration were monitored by means of subcutaneous needle electrodes. At regular intervals arterial blood samples were drawn and analyzed (ABL 500, Radiometer Copenhagen, Denmark). Core body temperature, was monitored by a rectal probe and maintained at 37° C. Vital signs were monitored for 4.5 hours after trauma using an ICU monitor (Hewlett Packard, Model 56S, Boeblingen, Germany). Animals not submitted to secondary injury breathed spontaneously. While inducing secondary injury by means of hypoxia ('secondary hypoxia': 14% FiO_2 for 30 min.), however, reflex hyperventilation was abolished by pharmacological paralysis (pavulon 0.1 mg IM), and these animals were artificially ventilated. The experiments were terminated by Euthasate (sodiumpentobarbital 300 mg IA) injection. Brains were removed

after death and inspected on the presence of blood and the position of the catheter tracts. The protocols were approved by the Institutional Animal Care and Use Committee, Erasmus University Rotterdam.

Trauma Model

The mechanics and pathophysiology of the CHI model employed, have been reported in detail elsewhere[16]. Briefly; traumatic brain injury is induced by weight drop on the intact skull. A stainless steel disk is cemented onto the vertex to prevent skull fractures and evenly distribute the impact forces of the falling weight. During impact the head is partially restrained on a foam head support, allowing for standardized skull acceleration. To minimize and standardize anesthesia effects, isoflurane was withdrawn before inducing CHI, the weight was not released until a hindpaw withdrawal on squeezing was observed. After weight drop, rebounding is prevented by quickly removing the foam bed with the rat sideways after the first contact, and isoflurane anesthesia was maintained. 'Severe' injury, causing a mortality rate of 50% was induced with an impactor mass/height combination of 450 gram/2 m. Using a higher impactor mass of 550 gram resulted in much higher mortality rate of 89%.

$PbrO_2$ Monitoring

$PbrO_2$ was monitored using a Clark type microcatheter adapted for use in small volumes (C1r, oxygen sensitive tip: 1 mm) and a Licox PO_2 measuring computer (Licox, GMS, Kiel-Mielkendorf, Germany). Before and after each experiment the catheters were tested for zero drift (= zero display error), using an absolute zero solution ($Na_2S_2O_4$) and sensitivity drift at room air level (= PO_2 display error). Actual room air PO_2 was calculated from local barometric pressure and humidity (ambient PO_2 = 20.9% of dry barometric pressure). The $PbrO_2$ and temperature probes were placed through frontal burr holes using a stereotactic needle holder. The temperature probe was used for automatic correction of the temperature sensitive oxygen catheter. Care was taken not to penetrate the ventricles. All data monitored were acquired with a custom developed software package (CDAI, Dijkzigt University Hospital Rotterdam, The Netherlands), and stored onto a hard disk. Files were scrutinized for artifacts due to animal handling, or blood withdrawal.

Reactivity tests

At 30 minutes, and at hourly intervals after CHI the reactivity of the cerebral oxygenation to increases of FiO_2 from 33% to 66% oxygen was tested. Just before and at the end of each test, which lasted 15 minutes to allow for complete stabilization of $PbrO_2$ values, bloodgas analysis and vital parameters were recorded.

The reactivity index was calculated as proposed by van Santbrink [31]:

$$\frac{PbrO_2\,(66\%) - PbrO_2\,(33\%)}{PbrO_2\,(33\%)} * \frac{1}{PaO_2\,(66\%) - PaO_2\,(33\%)} * 100\,\% = \text{react. index}$$

experimental groups

Sixty-five experiments were performed in five separate groups, in which the effects of trauma, hypoxia, and a combination of the two were investigated. Thirty-six rats were submitted to CHI

(8 severe injury: 450 gram/2 m. = group Ia, and 28 very severe injury with the 550 gram impactor mass = group Ib). Five rats served as sham-controls, (group II) in which all procedures except for traumatization were performed. Nineteen animals were traumatized and submitted to 30 minutes hypoxia by decreasing FiO_2 to 14 % (group III), and five rats served as hypoxia-controls (group IV). Experimental group specifications are presented in table 1.

table 1 Protocol groups, in which the effects of trauma, severity thereof, and secondary hypoxia on cerebral oxygenation were studied.

	number (survivors)	trauma (level)	hypoxia & ventilation
group I-a	8 (4)	CHI (severe)	no
group I-b	28 (3)	CHI (very severe)	no
group II	5 (5)	sham	no
group III	19 (5)	CHI (severe)	yes
group IV	5 (5)	sham	yes

Statistics

Mean brain oxygenation and reactivity indices after CHI were compared with data from sham animals, presented over time in representative graphs, and tested for statistical significance using the F-test for unpaired means with Datadesk software (version 4.2, Data Description Inc., Ithaca, NY, USA). P-values less than 0.05 were considered significant.

Results

Mortality and skull fractures

Mortality after trauma without secondary hypoxia was 50% using the 450 gram/ 2 m. impactor parameters. This weight/height combination caused no skull fractures. Higher impactor weight/height combinations resulted in 4 skull fractures, and a mortality rate of 89% in animals without skull fracture. Death occurred within ten minutes in all but two animals. Hypoxic and ventilated

animals traumatized with the 450 gram/2 m. weight/height combination had a considerably higher mortality rate of 74%. Seven of the 14 non-surviving animals died within ten minutes, the remaining seven survived for 3-4 hours after injury, after which brain oxygen values decreased to zero-levels. The animals subsequently became hypotensive; indicating impending brain death. In five of these animals an intracranial pressure (ICP) transducer was inserted just prior to death. ICP in these rats was severely increased (> 30 mmHg) resulting in impaired perfusion pressures (< 10 mmHg).

$PbrO_2$ measurement technique

All calibrations performed after the experiment showed a minimal zero drift of less than 0,3 mmHg. On macroscopically post mortal evaluation, five rats had a considerable amount of blood in the catheter tract. Data from these experiments were not used for analysis. A considerable amount of blood in the catheter tract could be predicted by very low readings and unresponsiveness of $PbrO_2$ to increases in FiO_2. Small traces of blood in the tract did not influence $PbrO_2$ readings.

$PbrO_2$ values

An example of the $PbrO_2$ tracing during the entire 4.5 hour experiment, of a rat submitted to both CHI and hypoxia, is shown in figure 1. After introduction of the $PbrO_2$ microprobe in sham animals, values were increasing from 20 ± 2 mmHg after 30 minutes to 34 ± 3 mmHg after two hours. This increase could not be explained by an increase in PaO_2 during this period. Thereafter a steady plateau was reached. This run in time was similar among all experimental groups. Mean $PbrO_2$ values are presented in table 2, traumatized animals had a significantly lower overall $PbrO_2$ than sham operated animals. These $PbrO_2$ values remain lower during the entire 4,5 hour experiment, and were related to the severity of trauma [figure 2 a and b]. Thirty minutes of systemic hypoxia resulted in severely hypoxic brain oxygen values during this episode, with rapid recovery after normalization of FiO_2. Significantly lower values (3 ± 1 mmHg) were observed after the combination of CHI and hypoxia (group III), than in control group IV (6 ± 2 mmHg). Furthermore the depth of hypoxia in traumatized animals was significantly lower in animals that did not survive the four hours of experimentation [figure 3].

Figure 1 Example of time course of $PbrO_2$ after severe CHI and hypoxia. The introduction artifact, rapid decrease during hypoxia, and minimal overshoot after reinstitution of normal FiO_2 are clearly visible. During the experiment there is a stable baseline $PbrO_2$ after two hours with reproducible increases during the reactivity tests.

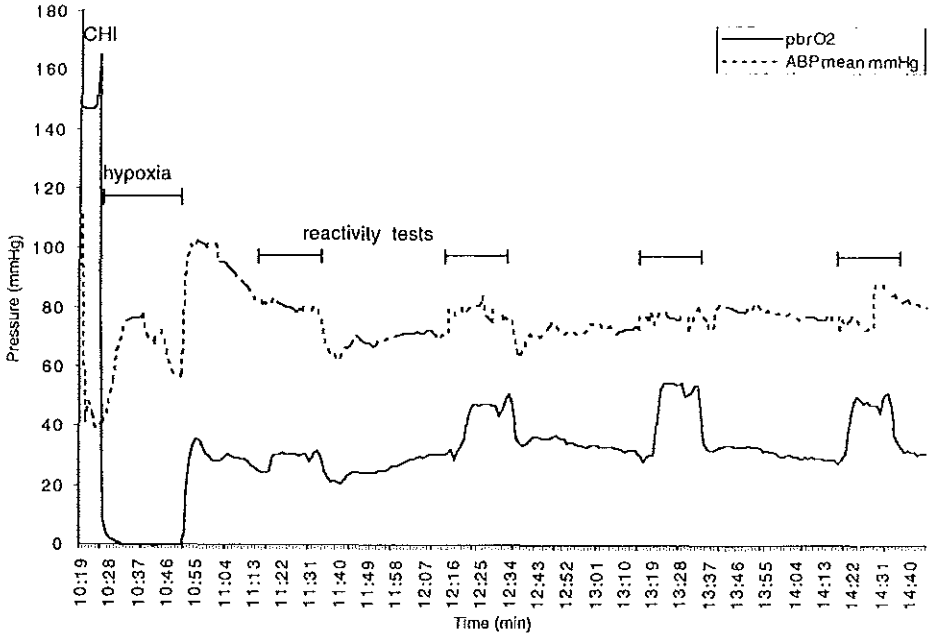
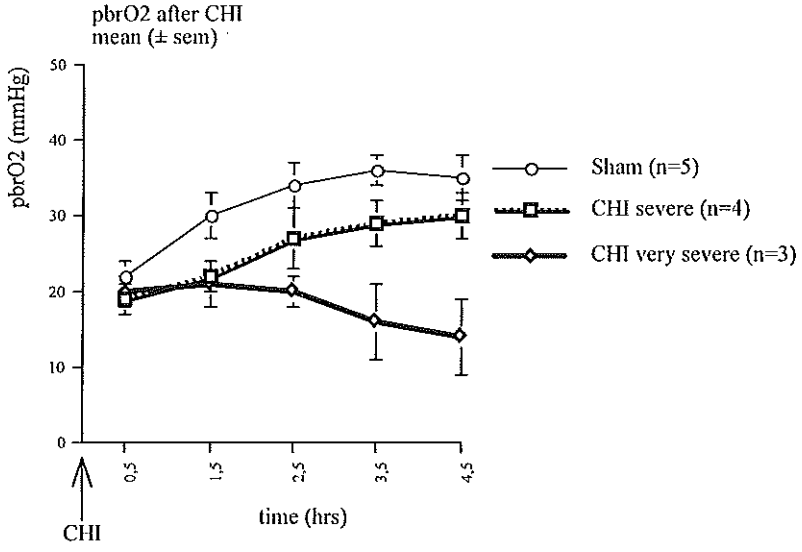


table 2 Factors of potential influence on $PbrO_2$ values. After recovery from both CHI and the hypoxic episode, overall $PbrO_2$ values are significantly lower in traumatized animals as compared to sham controls. MAP was lower in rats that were traumatized. $PaCO_2$ is slightly decreased after CHI, indicating spontaneous hyperventilation. Moreover PaO_2 is higher in ventilated animals, as PaO_2/FiO_2 ratio is higher in these animals. These higher PaO_2 values are reflected in higher $PbrO_2$ values in ventilated animals.

	MAP	(mmHg)	PCO_2	(mmHg)	PaO_2	(mmHg)	$PbrO_2$	(mmHg)
	mean	std	mean	std	mean	std	mean	std
Trauma (severe)	86	11	54	5	103	8	25, 2	4, 3
Trauma (very severe)	74	15	43	8	98	13	17, 9	3, 3
Sham	97	8	56	8	107	17	31, 1	4, 5
Trauma & hypoxia	84	13	49	8	143	25	28, 1	6, 2
Sham & hypoxia	86	14	49	10	150	18	34, 2	8, 4

Figure 2 Time course of $PbrO_2$ in rats not submitted to hypoxia (figure 2-a) and those submitted to secondary hypoxia with subsequent artificial ventilation (figure 2-b). Traumatized rats showed lower $PbrO_2$ values during the entire experiment, which was even more outspoken in the very severely injured animals (figure 2-a). (asterix displays statistical significant differences $p < 0.05$)

A



B

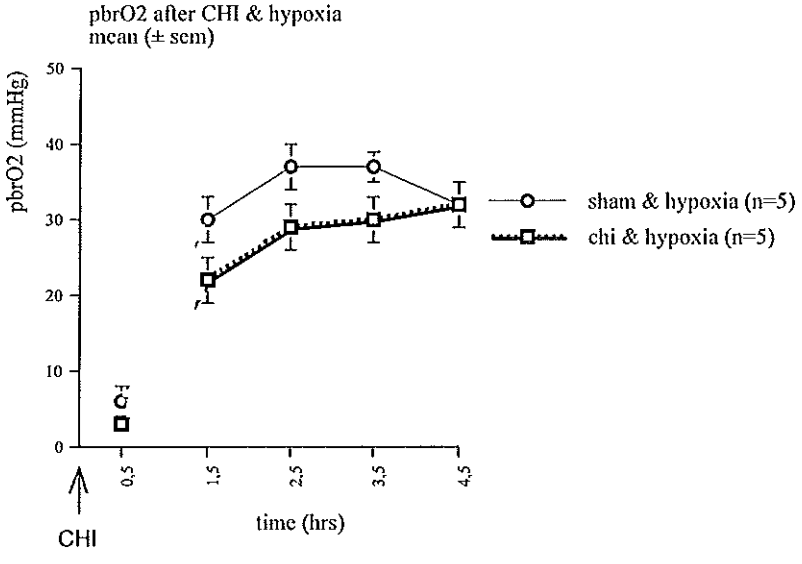
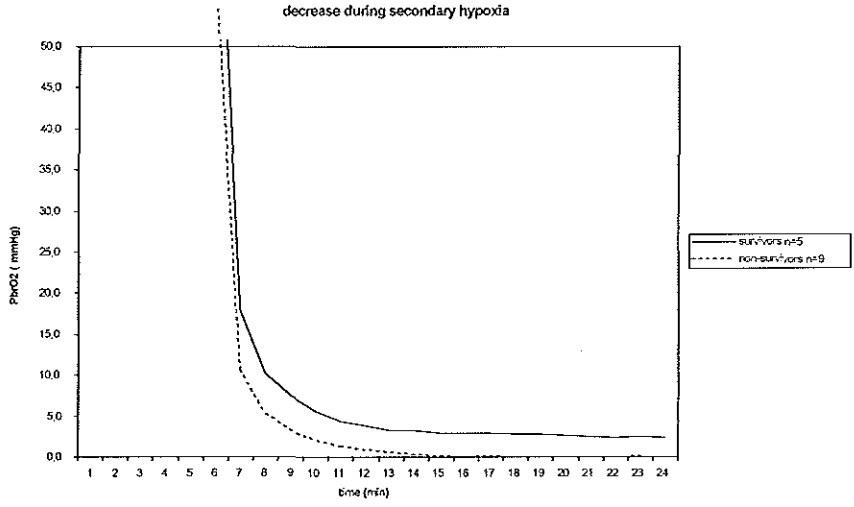


Figure 3 Mean values of the initial $PbrO_2$ time course as measured in injured rats submitted to secondary hypoxia. Rats that did not survive showed significantly lower $PbrO_2$ values during this episode of induced systemic hypoxia. The slope of the descending curve was similar in both survivors and non survivors.



Physiological parameters of influence on tissue oxygenation

Tissue perfusion, arterial oxygen pressure PaO_2, hemoglobin concentration are the main parameters influencing (brain) tissue oxygenation. Brain tissue perfusion, is dependent on arterial blood pressure, Cerebral Perfusion Pressure (CPP) and vascular resistance, which in its turn is related $PaCO_2$. The most important effect on blood pressure was seen early after CHI and during hypoxia. After CHI mean arterial pressure (MAP) decreased and recovered to values slightly lower than baseline. During the thirty minute episode of secondary hypoxia, which resulted in a PaO_2 level of 43 ± 8 mmHg, MAP decreased in both sham (11 mmHg decrease to 84 ± 3 mmHg) and traumatized animals (17 mmHg decrease to 72 ± 5 mmHg) with rapid recovery after FiO_2 normalization. After recovery from both CHI and secondary hypoxia, systemic parameters continued to be different to a certain extent, as shown in table 2. Some spontaneous hyperventilation was seen in traumatized rats that were not ventilated. $PaCO_2$ was thus lower in these rats as compared to their sham controls. As ventilation had to be artificial in animals submitted to 30 minutes of hypoxia, $PaCO_2$ could be kept in a range of 50 ± 2 mmHg for traumatized as well as sham control animals. Arterial oxygenation, however, was higher in artificially ventilated rats, despite of the fact that FiO_2 was kept constant at 33%. The PaO_2/FiO_2 ratio was higher in these animals. Therefore a direct comparison of $PbrO_2$ values between spontaneously breathing and artificially ventilated rats is not possible. Rats submitted to CHI did show significantly lower $PbrO_2$ values, though, in survivors, measured values in this posttraumatic episode were not lower than 10 mmHg.

$PbrO_2$ reactivity on FiO_2 increases

Increase of FiO_2 from 33% to 66% during 15 minutes resulted in a PaO_2 increases from 103 ± 4 to 210 ± 7 mmHg in spontaneously breathing animals. Ventilated animals had higher baseline PaO_2 values, in these rats PaO_2 rose from 146 ± 5 mmHg to 305 ± 5 mmHg. Increases of PaO_2 were the same in both trauma and sham-control groups. $PbrO_2$ increased immediately after FiO_2 increase and reached a plateau within seven minutes. During the four hours experimentation no time related differences in these responses were observed. As the $PbrO_2$ values just prior to the test were variable, this variability was adjusted for by expressing the values as a percentage of the baseline values. Figures 4 a and b display the averages of these reactivity tests for both spontaneous breathing and artificially ventilated rats. CHI did not cause a different response, and reactivity indices were equal in either of the two groups [table 3]. Larger increases in PaO_2, in artificially ventilated rats, caused by the higher PaO_2/FiO_2 ratio, resulted in lower reactivity indices than in spontaneously breathing animals for both CHI and sham groups.

Figure 4 Mean values of all reactivity tests during the experiment for rats not submitted to hypoxia (figure 4-a) and those submitted to secondary hypoxia with subsequent ventilation (figure 4-b). Values are expressed as percentage of baseline.

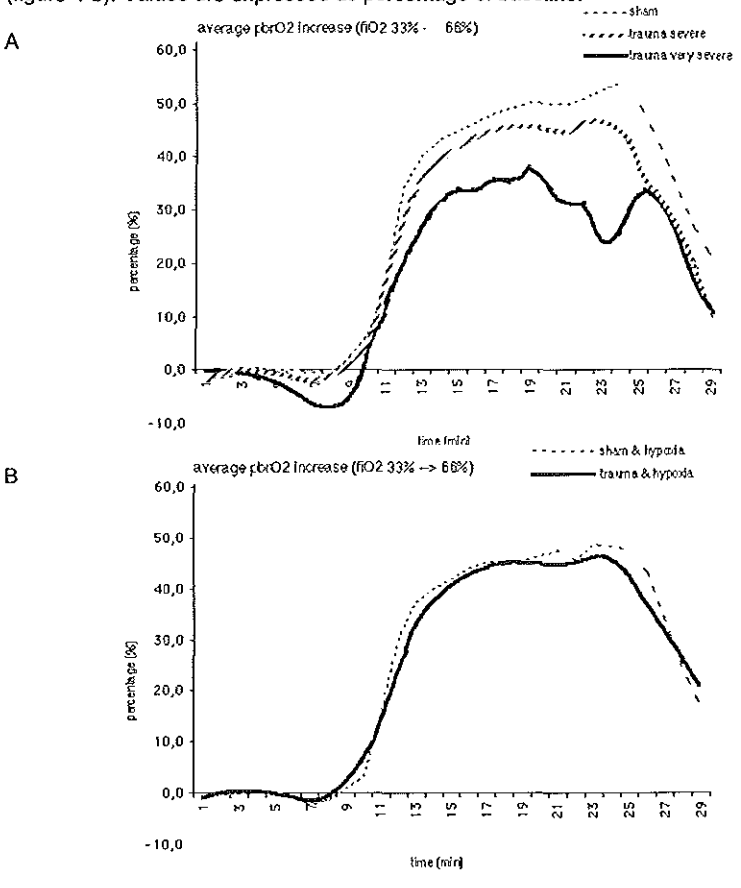


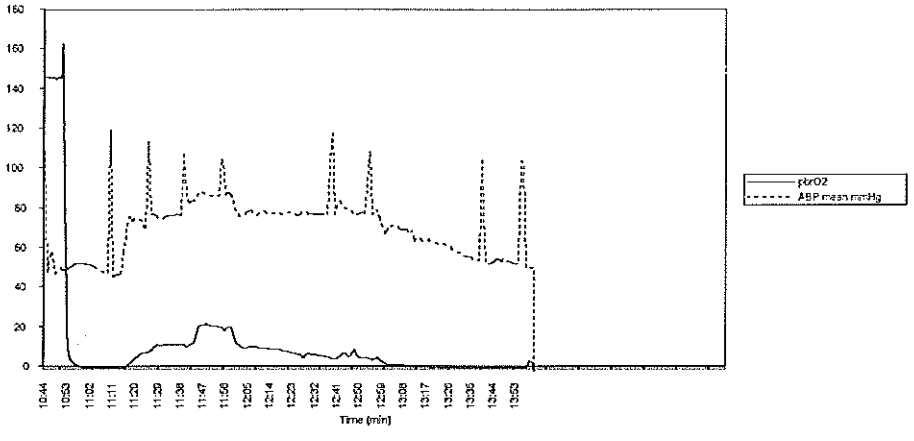
table 3 Reactivity indices (means of all tests during the experiment) do not differ among traumatized or sham control animals. A time related trend was not observed. The difference observed between spontaneous breathing, and artificial ventilated rats is caused by larger PaO_2 increments in the latter group.

reactivity index	mean	sem
Trauma severe	0, 47	0, 03
Trauma very severe	0, 50	0, 07
Sham	0, 50	0, 02
Trauma & hypoxia	0, 31	0, 02
Sham & hypoxia	0, 32	0, 02

Discussion

After experimental CHI significantly lower values of $PbrO_2$ are observed during the 4, 5 hour experiments. Values lower than 10 mmHg, which are considered hypoxic and related to poor outcome in clinical studies [11, 30, 31], were only observed during induced systemic hypoxia, and just prior to brain death [figure 5]. In the latter situation very high levels of ICP were recorded at termination of the experiment. Thirty minutes of secondary systemic hypoxia did not alter the oxygen reactivity during the hyperoxia tests in the course of the experiments. CHI did increase the depth of cerebral hypoxia during systemic hypoxia, furthermore the depth of cerebral hypoxia in this period was related to mortality. Systemic factors, known to influence brain oxygenation, were slightly impaired in traumatized animals. The additional effects of these basic physiological parameters can to a certain extent be held responsible for the observed lower brain oxygenation values.

Figure 5 Example of a MAP and $PbrO_2$ tracing of an animal that was submitted to severe CHI and secondary hypoxia, but did not survive the experiment despite of initial recovery. Brain death occurred 2, 5 hours after CHI. ICP at that moment was severely increased.



$PbrO_2$ measurement technique

The polarographic intraparenchymal technique measures local partial pressure of oxygen, reflecting the balance of oxygen offer and demand. Although the issue of local heterogeneity remains a matter of concern[14], in the absence of local complicating factors, recorded values can be considered indicative of global cerebral oxygenation. $PbrO_2$-values measured are dependent on the relation between the oxygen probe to the capillary mesh, and the diameter of microvascular vessels. The extremely low values of $PbrO_2$, measured in brains with a hematoma in the catheter tract, confirms the caveat of a hemorrhage around the oxygen probe[28]. The C1r probe used in this study was more sensitive to the disturbing effects of local hemorrhages than the previously used C1 probe. Although we cannot quantify this observation it is clear that the smaller the oxygen sensitive tip, the more susceptible the measurements will be to local tissue disturbances. During the 'run in time' after introduction of the C1r probe, the increase of measured values levelled off after approximately 2 hours, slight increase occasionally continued thereafter. Thus $PbrO_2$ values measured shortly after introduction of the catheter are lower than the real local tissue oxygenation due to equilibration of the probe tissue interface, and should be regarded with caution. After this stabilization period reliable values are obtained and drift of this Clark type catheter was negligable. Using this type of indwelling oxygen probes, this equilibration process takes more time than is the case using needle probes[7, 14, 24].

$PbrO_2$ after severe CHI and secondary hypoxia

Following severe experimental CHI, lower brain oxygenation is observed as compared to sham control animals, which remains for the entire duration of the experiment. These statistically significant lower values were not in the ischemic range. Values below 10 mmHg were only observed in brains that showed a local haematoma, during induced systemic hypoxia, or just before brain death. The influence of $PaCO_2$, CPP, and mainly arterial oxygenation on CSF-oxygenation was shown by Maas et al[15]. After traumatic brain injury, potentially disturbing auto regulatory mechanisms, such systemic parameters might be even more important in ensuring adequate brain tissue oxygenation. These physiologic parameters were evaluated, and did show a difference in the traumatized animals, though not always significant. One might argue however that both the lower MAP and lower $PaCO_2$ influence cerebral oxygenation in a negative way. This observation stresses the prime importance of stable systemic parameters in the management of head injured patients. In artificially ventilated animals arterial oxygenation was higher during the post-hypoxic period. As a result of higher PaO_2, $PbrO_2$ was also significantly higher. A conclusion as to the potential benefit of increasing arterial oxygen content after head injury can not be drawn from these experiments, since only animals with secondary hypoxia were artificially ventilated. During induced systemic hypoxia $PbrO_2$ was as low as 7 mmHg in non traumatized animals, and even

lower in animals that were traumatized just prior to hypoxia. A decrease in CBF[20] or derangement of autoregulation[19], as observed in this model, could account for this added deleterious effect. After resetting FiO_2 to 33%, after 30 minutes of hypoxia, the new, higher, plateau was reached within a few minutes. The depth of brain tissue hypoxia, during the episode of systemic hypoxia, was related to late mortality. Although $PbrO_2$ values were normalized during the 4,5 hour experiment, our behavioral studies, which will be reported elsewhere, indicate that the added secondary hypoxia results in significant impairment of motor and memory function. Early systemic hypoxia after CHI results in irreversible functional deficits, even when $PbrO_2$ values in the episode after resuscitation are in the normal range.

Oxygen reactivity after severe CHI and secondary hypoxia

When systemic oxygenation is increased, even from normal to supernormal levels, brain tissue oxygen will also increase. Thus the measured value of $PbrO_2$ is directly related to PaO_2. Van Santbrink coined the term 'oxygen reactivity index' to describe the height of increase related to baseline $PbrO_2$ value and PaO_2 increment[31]. He found a relationship between the reactivity index and patient outcome, which was attributed to an impairment of the intrinsic oxygen regulatory mechanism. Leniger-Follert et al[13] reported an overshoot phenomenon of $PbrO_2$ values when returning FiO_2 from hypoxia to room air ventilation, caused by an increase in cerebral blood flow (CBF). This overshoot CBF was attributed to a temporary decrease in vascular resistance. In this CHI experiment we intermittently increased FiO_2 during the 4, 5 hours after trauma. An overshoot oxygen reactivity, related to temporary hyperemia, could not be observed in rats submitted to either CHI, hypoxia, or the combination of the two insults. In the pilot phase of the experiment two rats were accidentally hypoventilated due to problems with the ventilator. This resulted in $PaCO_2$ values over 70 mmHg. In these rats much higher $PbrO_2$ values, and oxygen reactivities were observed. This observation, discarded from statistical analysis in this report, indicate the importance of $PaCO_2$ with respect to the interpretation of both absolute $PbrO_2$, and oxygen reactivity indices. The cause of high $PbrO_2$ during hypoventilation could be both direct vasodilation or a shift of the oxygen dissociation curve due to increased $PaCO_2$. Several explanations for the lack of correspondence between the clinical observation and the absence of any such difference in this experiment can be given. At first our experiments were performed for a 4.5 hour duration, while the average time of introduction of the $PbrO_2$ catheter in head injured patients was 7 hours after injury. Secondly all patients were hyperventilated, while normocarbia was aimed at in these experiments. Furthermore the effect of isoflurane on both regional CBF and blood brain barrier function[5, 12], might mask any trauma induced impairment of the more subtle phenomenon of oxygen mediated cerebral vasoactivity[3, 7].

Relation with clinical $PbrO_2$ monitoring

Cerebral ischemia is common after severe head injury. In patients dying from head injury, ischemic changes have been demonstrated in over 90% of patients[9]. Bouma et al[2] have demonstrated the occurrence of low cerebral blood flow (CBF) in the ultra early posttraumatic period. Secondary systemic insults, i. e. hypotension and hypoxia are frequently observed in the early period after injury[10]. The adverse influence of systemic insults on outcome has been well documented[4, 6, 21]. In order to monitor cerebral homeostasis, particularly when sedation impairs the accuracy of clinical assessment, several techniques have been developed. Continuous monitoring of brain oxygenation is a means of accurately investigating the balance of oxygen supply and demand of the brain. If inserted in relatively undamaged tissue, the obtained values can be regarded as indicative of global oxygenation. Recent neurosurgical literature has shown the practical and additive value of this intraparenchymal monitoring technique in the evaluation and NICU monitoring of head injury patients[11, 26, 29-31, 33]. It has been shown that severe head injury can be accompanied by cerebral ischemia[2,9] and hypoxia[11, 17, 27, 30]. In a clinical study covering 101 patients we have demonstrated the relationship of both depth and duration of cerebral hypoxia with outcome [30]. The determinators of cerebral hypoxia and the treshold values of cerebral hypoxia, as monitored by intraparenchymal catheters, remain to be determined. Similar to the observations in these clinical studies with head injured patients, impaired brain tissue oxygenation after experimental closed head injury was demonstrated in this study. Only minor deterioration of systemic parameters such as blood pressure and $PaCO_2$ can, to some extent, be held responsible for this impairment, an observation that emphasizes the importance of accurate management of these parameters in head injured patients. Although an increased oxygen reactivity was observed in patients with a poor outcome, an explanation for this observation was not provided by van Santbrink et al [31]. As we anticipated a relationship with secondary insults to the brain, we first chose to implement systemic hypoxia, since this resulted in an overshoot phenomenon to rebound normoxia in other experimental studies [13]. During the course of this experiment, no effect of the added systemic hypoxia could be found however. Further exploration of other added systemic insults, such as hypotension or hypoventilation, needs to be performed to elucidate the underlying mechanism of overshoot oxygen reactivity. Cerebral vasodilation, as sometimes occurring in severe head injury due to decreased vascular tone and decreased CO_2 reactivity[23, 25, 32] might be an explanation for the findings of van Santbrink et al [31]. From a therapeutic point of view, one could argue that artificially increasing $PbrO_2$ by instituting supernormal PaO_2 values can benefit patient outcome, since increments of PaO_2 linearly increase $PbrO_2$. The Richmond group did indeed observe improvement of substrates obtained by cerebral microdialysis in hyperoxic patients, indicating potential benefit from this therapy[17]. The therapeutic value of brain oxygen targeted treatment remains to be proven, and requires a controlled study with patient outcome as main outcome parameter.

Conclusion

This experiment shows lower $PbrO_2$ values after severe traumatic brain injury, and confirms results of clinical observational studies. Systemic parameters can be held at least partially responsible for this observation and suggesting the increased susceptibility of injured brain for low CPP, $PaCO_2$, and PaO_2. This report stresses the importance of meticulous management of these systemic parameters in head injured patients. Furthermore, active management of $PbrO_2$ by artificially increasing FiO_2, or MAP, might be a promising therapy which requires further investigations.

References

Paper:
van den Brink WA, ter Horst M, van den Heuvel A, et al: **Brain oxygenation is marginally impaired in experimental closed head injury.** (submitted)

1. Bouma GJ, Muizelaar JP, Choi SC, et al: Cerebral circulation and metabolism after severe traumatic brain injury: The elusive role of ischemia. **J Neurosurg 75**: 685-693, 1991

2. Bouma GJ, Muizelaar JP, Stringer WA, et al: Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. **J Neurosurg 77**: 360-368, 1992

3. Brown MM, Wade JPH, Marshall J: Fundamental importance of arterial oxygen content in the regulation of cerebral blood flow in man. **Brain 108**: 81-93, 1985

4. Chesnut RM, Marshall LF, Klauber MR, et al: The role of secondary brain damage in determining outcome from severe head injury. **J Trauma 34**: 216-222, 1993

5. Chi OZ, Anwar M, Sinha AK, et al: Effects of isoflurane on transport across the blood-brain barrier. **Anesthesiology 76**: 426-431, 1992

6. Fearnside MR, Cook RJ, McDougall P, et al: The Westmead head injury project outcome in severe head injury. A comparative analysis of prehospital, clinical, and CT variables. **Br J Neurosurg 7**: 267-279, 1993

7. Fennema M, Wessel JN, Faithfull NS, et al: Tissue oxygen tension in the cerebral cortex of the rabbit, in Rakusan K, Biro GP, Goldstick TK, et al (eds): **Oxygen transport to tissue XI**. New York, London, Plenum press: 451-460, 1989

8. Graham DI, Adams JH, Doyle D: Ischaemic brain damage in fatal non missile head injuries. **J Neurosci 39**: 213-234, 1987

9. Graham DI, Ford I, Adams JH, et al: Ischaemic brain damage is still common in fatal non missile head injury. **J Neurol Neurosurg Psychiatry 52**: 346-350, 1989

10. Jones PA, Andrews PJ, Midgley S, et al: Measuring the burden of secondary insults in head injured patients during intensive care. **J Neurosurg Anest 6**: 4-14, 1994

11. Kiening KL, Unterberg AW, Bardt TF, et al: Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO_2 versus jugular vein oxygen saturation. **J Neurosurg 85**: 751-757, 1996

12. Lee JG, Hudetz AG, Smith JJ, et al: The effects of halothane and isoflurane on cerebrocortical microcirculation and autoregulation as assessed by Laser-Doppler flowmetry. **Anest Analg 79**: 58-65, 1994

13. Leniger-Follert E, Lubbers DW, Wrabetz W: Regulation of local tissue pO_2 of the brain cortex at different arterial O_2 pressures. **Pflugers Arch 359**: 81-95, 1975

14. Lubbers DW, Baumgartl H, Zimelka W: Heterogenity and stability of local pO_2 distribution within the brain tissue. **Adv Exp Med Biol 345**: 567-574, 1994

15. Maas AIR, Fleckenstein W, de Jong DA, et al: Monitoring cerebral oxygenation: experimental studies and preliminary clinical results of continuous monitoring of cerebrospinal fluid and brain tissue oxygen tension. **Acta Neurochir Suppl 59**: 50-57, 1993

16. Marmarou A, Abd-Elfattah Foda MA, van den Brink WA, et al: A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. **J Neurosurg 80**: 291-300, 1994

17. Menzel M, Doppenberg EM, Zauner A, et al: Increased inspired oxygen concentration as a factor in improved brain tissue oxygenation and tissue lactate levels after severe human head injury. J **Neurosurg 91**: 1-10, 1999

18. Miller JD: Intracranial pressure. **Curr Opin Neurol Neurosurg 1**: 8-13, 1988

19. Nawashiro H, Shima K, Chigasaki H: Immediate cerebrovascular responses to closed head injury in the rat. J **Neurotrauma 12(2)**: 189-197, 1995

20. Prat R, Markiv V, Dujovny M, et al: Failure of cerebral autoregulation in an experimental diffuse brain injury model. **Acta Neurochir Suppl 71**: 123-126, 1998

21. Robertson CS, Contant CF, Narayan RK, et al: Cerebral blood flow, $AVDO_2$, and neurological outcome in head injured patients. J **Neurotrauma 9**: S349-S358, 1992

22. Rosner MJ, Rosner SD, Johnson AH: Cerebral perfusion pressure: management protocol and clinical results. J **Neurosurg 83**: 949-962, 1995

23. Saunders ML, Miller JD, Stablein D, et al: The effects of graded experimental trauma on cerebral blood flow and responsiveness to CO_2. J **Neurosurg 51**: 18-26, 1979

24. Seyde WC, Longnecker DE: Cerebral oxygen tension in rats during deliberate hypotension with sodium nitroprusside, 2-chloroadenosine, or deep isoflurane anesthesia. **Anesthesiology 64**: 480-485, 1986

25. Tenjin H, Yamaki T, Nakagawa Y, et al: Impairment of CO_2 reactivity in severe head injury patients: An investigation using thermal diffusion method. **Acta Neurochir 104**: 121-125, 1990

26. Unterberg AW, Kiening KL, Bardt TF, et al: Multimodal monitoring in patients with head injury: evaluation of the effects of treatment on cerebral oxygenation. J **Trauma 42**: s32-s37, 1997

27. Valadka AB, Gopinath SP, Contant CF, et al: Relationship of brain tissue PO_2 to outcome after severe head injury. **Crit Care Med 26(9)**: 1576-1581, 1998

28. van den Brink WA, Haitsma IK, Avezaat CJJ, et al: The brain parenchyma-PO_2 catheter interface, a histopathological study in the rat. J **Neurotrauma 15**: 813-824, 1998

29. van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Monitoring brain oxygen tension in severe head injury: The Rotterdam experience, in Marmarou A, Bullock R (eds): **Acta Neurochirurgica Supplementum**. Springer-Verlag: 1998

30. van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Brain oxygen tension in severe head injury, early cerebral hypoxia is related with an unfavorable outcome. **Neurosurgery 46**: 868-878, 2000

31. van Santbrink H, Maas AIR, Avezaat CJJ: Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. **Neurosurgery 38**: 21-31, 1996

32. Yoshida H, Bandoh K, Marmarou A: Cerebrovascular carbon dioxide reacivity assessed by intracranial pressure dynamics in severe head injured patients. J **Neurosurg 82**: 386-393, 1995

33. Zauner A, Doppenberg EMR, Woodward JJ, et al: Continuous monitoring of cerebral substrate delivery and clearance: Initial experience in 24 patients with severe acute brain injuries. **Neurosurgery 41**: 1082-1093, 1997

Chapter 11

Brain oxygen tension in severe human head injury

A crucial issue is the relationship between intracranial hypertension and brain damage. Does brain damage cause high ICP, or does high ICP cause brain damage? Somewhat unsatisfactorily, the answer to both of these questions is affirmative. ***J. Douglas Miller, Journal of Neurosurgery, 1977***

Abstract

Objectives: Ensuring adequate cerebral oxygenation and perfusion is of fundamental importance in the treatment of patients with acute cerebral disorders. Online continuous monitoring of brain oxygenation is possible with a parenchymal micro-electrode, measuring local brain oxygen tension ($PbrO_2$). The ultimate question is whether therapeutic approaches can be targeted based on such monitoring. Before this question can be addressed, the technique requires validation in the clinical setting. The occurrence of low values, and the relation to outcome need to be established.

Methods: 101 comatose head-injured patients (GCS $\leq$ 8) were studied. $PbrO_2$ probes were inserted in an undamaged part of the frontal region. Patients were treated conforming to the European Brain Injury Consortium guidelines. Six months outcome was determined according to the Glasgow Outcome Scale.

Results: Early brain tissue hypoxia was frequently observed, despite aggressive ICP and CPP treatment. Values lower than 15 mmHg, with a duration longer than 30 minutes, were observed in 57 cases. Values lower than 10 mmHg in 42, and lower than 5 mmHg in 22 cases, during the first 24 hours. Depth and duration of tissue hypoxia were related to outcome and were proven to be an independent predictor of unfavorable outcome and death.

Conclusion: Local cerebral tissue $PbrO_2$ monitoring is a safe and reliable method to monitor cerebral oxygenation. Because brain tissue hypoxia occurs frequently, and is significantly related to poor outcome, future efforts should be aimed at the treatment of brain tissue hypoxia. The effects of such 'brain hypoxia targeted treatment' needs to be established in a multicenter study.

Introduction

Cerebral ischemia is common after severe head injury. In patients dying from head injury, ischemic changes have been demonstrated in over 90% of patients[5, 21]. Bouma et al have demonstrated the occurrence of low cerebral blood flow (CBF) in the ultra early posttraumatic period[2]. Cerebral ischemia results from intrinsic pathophysiologic pathways and from systemic insults, i.e. hypotension and hypoxia. Such systemic insults are frequent in the early posttraumatic period[27] as well as in the intensive care setting[9]. The adverse

influence of systemic insults on outcome has been well documented[3, 4, 21]. Intracranial pressure (ICP) and cerebral perfusion pressure (CPP) therapy are currently the main therapeutic pillars in the treatment of severe head injury[19, 23]. The aim is to ensure adequate cerebral blood flow (CBF) and oxygenation, meeting metabolic demands. Multimodality monitoring and preliminary results of microdialysis in severe head injury have increased our understanding of metabolism and oxygenation. The focus of multimodality monitoring in head injury is primarily on CBF and oxygenation. Our group has previously reported on the feasibility, safety, and initial experience of continuous monitoring of $PbrO_2$[31]. In this report on 22 patients with severe head injury we demonstrated the presence of early ischemia at the tissue level. Questions however were raised whether the observed initial low $PbrO_2$-values actually reflected oxygen pressure at the tissue level or were possibly caused by a technical catheter related phenomenon. We have extended our series to 101 patients, with GOS evaluation after six months. This report focusses on the occurrence, depth, and duration of initial low values, and its relationship with clinical and CT-scan characteristics and patient outcome.

Materials and methods

Patients and treatment protocols

Data from multimodality monitoring, including brain tissue oxygen pressure were collected prospectively in a series of 101 patients with severe non penetrating head injury (GCS < 8) admitted to the neurosurgical intensive care unit of the Academic Hospital Rotterdam from September 1992 until November 1997. In all patients an early CT-scan was obtained at admission or from the referring hospital. Routinely a second CT-scan was acquired within 24 hours, or earlier if indicated. The initial CT-scans were coded according to the TCDB-protocol[17]. The ventricles were scored as asymmetrical or symmetrical, the status of the cisterns were scored in three grades: normal, compressed, absent. The presence of intraventricular blood was noted. The extent of midline shift was evaluated in three categories: 0-5 mm, 5-10 mm and more than 10 mm. The presence, localization and extent of subarachnoid blood was scored and the presence of intracranial lesions according to their space occupying effect and their nature. Finally the intracranial diagnosis was scored according to the 6 point-scale of Marshall[17]. All patients received standard intensive care treatment and monitoring, conforming to the principles of recently published EBIC-guidelines[11]. Outcome was evaluated at three and six months after trauma, according to the GOS[8]. For purposes of analysis the GOS was dichotomized into unfavorable outcome (D, VS, SD) and favorable outcome (MD, GR).

Monitoring, data acquisition and analysis

Heart rate, respiratory rate, mean arterial blood pressure (MABP), peripheral oxygen saturation, ICP and CPP as well as $PbrO_2$ were monitored in all patients.

MABP was monitored with a pressure transducer calibrated at the level of the heart. Intracranial transducers were inserted into the brain parenchyma through a modified three channel intracranial bolt with an outer diameter of 6 mm. Unless a skull fracture, hemorrhage, or contusion was present the bolt was inserted at the right frontal side. ICP was monitored using the Camino® fiberoptic device (Camino, San Diego, CA, USA). $PbrO_2$ was monitored using a Clark type microcatheter and a Licox $PbrO_2$ measuring computer (Licox, GMS, Kiel Mielkendorf, Germany). In six patients a second $PbrO_2$-catheter was introduced through the third channel in the bolt. All physiologic monitored data were acquired with a custom developed software package (CDAI, University Hospital Rotterdam, The Netherlands), stored using a Novell network onto a 3 GB hard disk. All files were scrutinized for contamination and artifacts due to nursing care, or patient transport by one experienced research nurse (W. J.). At 24 hour intervals the reactivity of the cerebral oxygenation to 100% oxygen and hyperventilation was tested. These episodes were cleared from the data reported here, and will be reported elsewhere. After ending the measurements the catheters were tested for zero drift (= zero display error), using an absolute zero solution ($Na_2S_2O_4$) and sensitivity drift at room air (= PO_2 display error). Actual room air PO_2 was calculated from local barometric pressures, obtained from the meteorological institute and measured humidity (ambient $PO_2 = 20.9\%$ of dry barometric pressure).

Statistical analysis

Data are presented as means plus or minus standard deviation. Statistical analysis was performed by means of the chi-square statistic when testing for associations of nominal data. The relation between an unfavorable outcome and death and initial low $PbrO_2$-values was analyzed with a restricted cubic spline function[6], as implemented in S-plus software (Data-analysis Products, Division of Mass Soft, Inc., Seattle, USA). For this analysis time periods with $PbrO_2$ lower than respectively 5, 10 and 15 mmHg were labeled. If the cumulative period was longer than 30 minutes, these patients were considered to have has low initial $PbrO_2$-values. Correlations of clinical and CT-scan parameters with $PbrO_2$-values were expressed as Spearman Rank correlation coefficients. The prognostic value of $PbrO_2$-measurements was determined by correlating outcome with the main clinical and CT-scan variables using a multivariable logistic regression analysis (SPSS Sofware, SPSS Inc., Chicago, II, USA). A p-value lower than 0.05 was considered statistically significant.

Results

I. Demographics and CT-scan characteristics

In total 101 patients were enrolled in the study. Eighty-three patients were male; mean age was 34 years (range 11-82). Seventy-two patients were victim of a road traffic accident. This distribution is typical to a population of severe head injury. Detailed demographic and clinical data of the patients are displayed in table 1. CT characteristics are shown in table 2. The severity of injuries in the population studied is illustrated by the occurrence of pupillary abnormalities in 50%, partial or complete obliteration of basal cisterns in 61% of patients and by an overall mortality of 39%.

II. Duration and reliability of $PbrO_2$ monitoring

$PbrO_2$ monitoring was started as soon as possible after injury (mean 7.0 ± 3.5 hours). The duration of $PbrO_2$ monitoring was intended to last five days; monitoring was terminated earlier because of early death, or if ICP monitoring was no longer considered indicated. Eighty-three of the patients were monitored more than 24 hours. Three patients were monitored, but post hoc analysis was not possible due to computer related errors. The average duration of monitoring was 86 hours (range 4-180 hours).

Adverse events and complications related to catheter introduction and monitoring were not seen. Postmeasurement calibration resulted in an average zero display error of 0.42 ± 0.85 mmHg. PO_2 display error (sensitivity drift) calibrated at a mean room air PO_2 of 157.6 ± 1.5 mmHg was 0 ± 6%.

III. Time course and pattern of $PbrO_2$

Mean $PbrO_2$ time course with standard deviations averaged over all patients is shown in figure 1. Low initial values were common in the first 12 to 24 hours after injury, occurring in over 50% of patients. A detailed analysis of the frequency of occurrence of such low values during the first 24 hours after injury was performed. Values lower than 15 mmHg were observed in 57 cases, values lower than 10 mmHg in 42 cases and values lower than 5 mmHg in 22 cases. Patients with documented hypoxia on admission did not necessarily show lower values during the measurement period. In 30 of the patients with initial low values an 'overshoot phenomenon' was observed with a mean high value of $PbrO_2$ of 46 mmHg in the time period 36 to 48 hours after injury. No relation between presence or absence of an overshoot phenomenon and documented hypoxia or hypotension at admission could be demonstrated. Neither was the occurrence of overshoot related to outcome.

table 1 Demographic data of the 101 patients enrolled in this study.

Sex	
Male	83
Female	18
Type of injury	
Road Traffic Accident	
- motor vehicle occupant	32
- other	40
Fall	21
Other	8
Mean age	34 ± 16 (11-82 years)
Glasgow Coma Score	
GCS 3-5	42
GCS 6-8	59
Pupils	
both reactive	51
one non reactive	30
both non reactive	20
Outcome 6 months	
unfavorable	54
dead	39
PVS	1
severely disabled	14
favorable	47
moderately disabled	17
good	30

IV. Evaluation of 'run in' time

In six patients comparative measurements of $PbrO_2$ were performed with two catheters introduced through the three-way bolt. The second catheter was introduced after a variable period of 4 to 24 hours. Absolute values of $PbrO_2$ between the two catheters differed considerably [table 3]. After introduction of the second catheter initially lower values were seen, despite a stable $PbrO_2$ recording obtained from the first catheter. These initial low values quickly increased to stable values. The duration of this stabilization was variable, but in all cases within a two hour run-in period [figure 2]. When both catheters displayed stable $PbrO_2$ values the absolute values were not necessarily equal, but fluctuations of both catheters were simultaneous and in the same direction.

Table 2 CT-scan characteristics as scored according to the TCDB protocol, Intracranial diagnosis (ICD) as the Marshall classification17 (n = 101)

ventricular asymmetry	40
basal cisterns	
present	40
compressed	35
absent	26
intraventricular blood present	23
midline shift	
0-5 mm	75
5-10 mm	14
≥ 10 mm	12
subarachnoid blood	
none	52
cisterns	7
convexity	28
both cisterns and convexity	14
intracranial diagnosis	
1. diffuse injury, no CT-pathology	10
2. diffuse injury, normal cisterns	24
3. diffuse injury with swelling	27
4. diffuse injury with shift	1
5. mass lesion surgically evacuated	24
epidural hematoma	9
acute subdural hematoma	15
6. mass lesion not surgically evacuated	15
acute subdural hematoma	1
intracerebral hematoma	3
contusion	11

Table 3 Mean values of $PbrO_2$ of catheter 1 and catheter 2 during the entire measurement on the second day after injury. The second catheter is not necessarily measuring lower values than the first catheter.

	Catheter 1	Catheter 2
Pat. 1	27	32
Pat. 2	40	29
Pat. 3	38	21
Pat. 4	24	58
Pat. 5	16	20
Pat. 6	41	20

Figure 1 Average $PbrO_2$ measurements of 100 patients during the first 120 hours. Mean and standard deviation lines of values measured in hours after injury are displayed. Note the initial low values with a rapid increase during the first hours and a slower increase over the first day. Stabilization occurs at 25 to 30 mmHg.

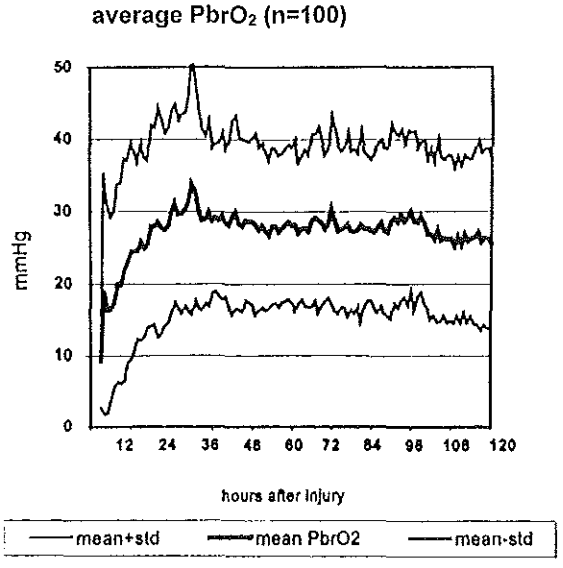
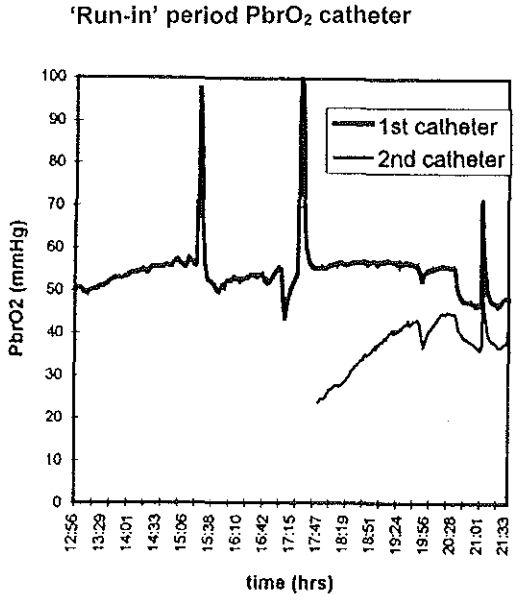


Figure 2 Example of comparative measurements of two catheters. During a stable period of the first catheter, a run in rime of two hours of the second catheter can be observed. Hereafter this second catheter is also stable, and the $PbrO_2$-fluctuations are similar in both time and relative height.



V. Relation to $PbrO_2$-values to clinical and CT scan characteristics

The association between the occurrence of low values in the first 24 hours after trauma and clinical variables, such as age, GCS, pupils, ICP and presence of multiple injuries, as well as CT characteristics, were investigated with the Spearman Rank coefficient. No significant correlations were found for clinical variables; of the CT characteristics the only parameter significantly correlating with initial low $PbrO_2$ values was compression of the cisterns. For analysis of the relation between $PbrO_2$ and ICP, patients were dichotomized into two groups, according to ICP levels measured within the first 24 hour period: in the first group ICP was consistently below 25 mmHg (n = 46); the second group consisted of patients in whom ICP was raised above 25 mmHg for a period of 60 minutes or longer (n = 54). No correlation was found between patients with and without raised ICP and presence or absence of low $PbrO_2$ values. Results of the analysis are summarized in table 4.

Table 4 Correlation $PbrO_2$< 10 mmHg > 30 minutes with patient characteristics (r)

Clinical variables	r	p-value
Age	.04	.69
GCS	-.08	.55
Pupil	.08	.46
multi trauma	-.06	.54
ICP	.09	.38
CT variables		
shift	.13	.21
cisterns	.34	.001
TSAH	.05	.64
CT classification	.13	.19

VI. Predictive value of $PbrO_2$. Prognostic value of $PbrO_2$ in relation to known prognostic variables.

Higher $PbrO_2$ values were found during the entire measurement period in survivors [figure 3]. The association between initial low values ($PbrO_2 \leq 10$ mmHg) with mortality or unfavorable outcome is shown in table 5. 24 of 43 patients with low initial values died, in contrast to 14 out of 66 patients without these low values. If the patient outcome was dichotomized into unfavorable versus favorable a similar significant association was found. The odds ratio for death was 3.8 (p = 0.002) and the odds ratio for an unfavorable outcome was 2.8 (p = 0.015).

Figure 3 Mean $PbrO_2$ values in patients that were dead (thin line) or alive (fat line) after six months. The patients that died had a lower $PbrO_2$ during the entire monitoring period, most pronounced during the first 36 hours.

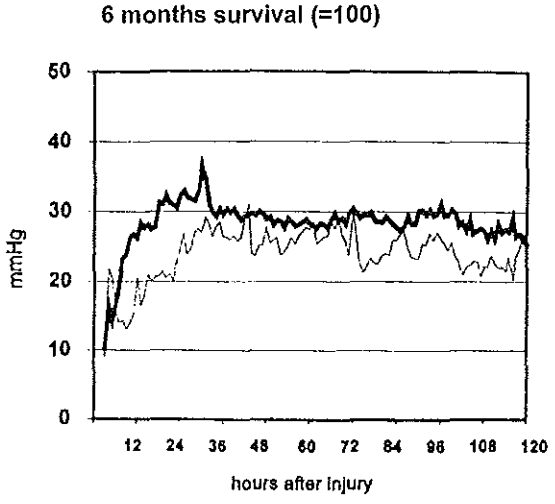


Figure 4 Restricted cubic spline functions of the relative risk of death, related to initial low values categorized into < 5 mmHg (solid line), < 10 mmHg (dotted line) and < 15 mmHg (stripe-dot line). The ordinal characterization follows from the layering of the curves, < 5 mmHg being worse than < 10 mmHg, being worse than < 15 mmHg. Note that the curves stabilize at long durations of hypoxia.

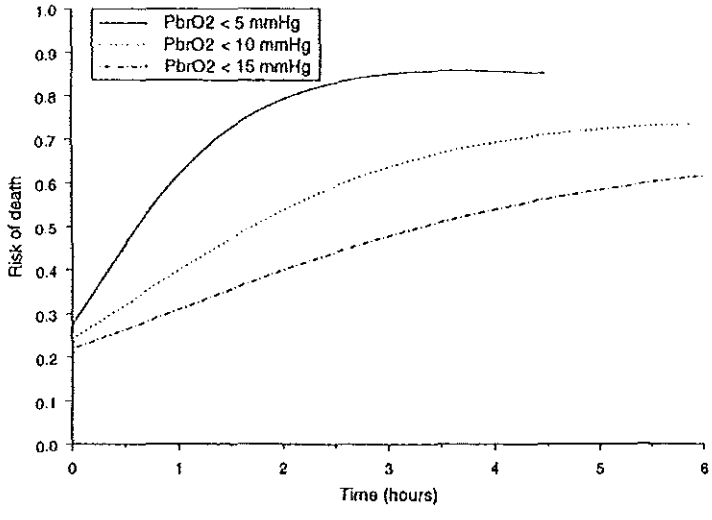


Table 5 The multiple logistic regression analysis shows that the low initial $PbrO_2$-values have an independent prognostic value concerning death or unfavorable outcome. When clinical variables, CT scan variables, or combinations were taken into account the odds ratios of the $PbrO_2$ values [lower/upper 95% confidence interval] remained significant. Only the compression of the basal cisterns adjusted the predictive value significantly.

Relation of duration of $PbrO_2$ < 10 mmHg with outcome at 6 months.

	alive	dead	favorable	unfavorable
< 30 min	42	14	32	24
≥ 30 min	19	24	14	29

Table 6 Initial values < 10 mmHg for longer than 30 minutes are significantly associated with mortality and unfavorable outcome. A similar significant association with unfavorable outcome is found. The odds ratio for an unfavorable outcome is 2.8 (p = 0.015).

Multiple logistic regression analysis. Independent contribution of low $PbrO_2$ ($PbrO_2$ < 10 mmHg > 30 minutes) taking relevant parameters into account:

	odds ratio for death [95% CI]	odds ratio for unfavorable outcome [95% CI]
low $PbrO_2$	**3.8 [1.6-8.4]**	**2.8 [1.2-6.3]**
adjusted for clinical variables:		
Age	3.7 [1.6-8.9]	2.7 [1.2-6.3]
GCS	3.8 [1.6-9.0]	2.8 [1.2-6.6]
Pupil	4.0 [1.6-10]	3.0 [1.2-7.5]
multi trauma	3.7 [1.6-8.8]	2.9 [1.2-6.7]
ICP	4.1 [1.7-10]	3.0 [1.2-7.5]
adjusted for CT scan variables:		
CT classification	3.9 [1.6-9.7]	2.7 [1.1-6.4]
cisterns	3.6 [1.3-9.4]	1.8 [0.7-4.5]
shift	3.7 [1.5-9.0]	2.6 [1.1-6.1]
TSAH	4.3 [1.7-11]	3.0 [1.2-7.5]
adjusted for combinations:		
age + GCS + pupil	3.8 [1.4-9.9]	2.8 [1.0-7.4]
clinical + ICP+CT var.	6.0 [1.8-20]	5.3 [1.4-20]

For further analysis of the predictive significance of low $PbrO_2$ values, including adjustment for other predictive variables, the presence of low values within the first 24 hours were categorized into < 5, < 10, and < 15 mmHg. Time periods during which low values were measured were summed. In figure 4 we observe that lower $PbrO_2$ values are related to higher risks of death. There is no exact threshold point in time after which the risk of death increases steeply. After several hours there is no increase of the risk of death indicating that the duration of low values shorter than this period is indicative of

impending death. During the first 1.5 hours several cut-off points of time were evaluated. The odds ratio for death was 3.8 (95% confidence intervals at 1.6 and 8.9) at 30 minutes duration. The odds ratios increased and were even more significant at 45 minutes and 60 minutes duration. Even a period of larger than 10 minutes of a local $PbrO_2$-value of less than 10 mmHg showed a statistically significant risk of death. We chose the cut-off point at a duration of 30 minutes or more for further analysis. In logistic regression models the low initial $PbrO_2$ remained an independent prognostic factor as shown in table 6. Only the status of the perimesencephalic cisterns had a clear relation with $PbrO_2$ and reduced the prognostic value. We note that the large odds ratio for $PbrO_2$ with adjustment for a combination of clinical and CT-scan variables in table 6 may reflect overfitting, as a relatively large number of variables is used, in comparison to the number of patients dying of severe head injury.

Discussion

Reliability of $PbrO_2$ measurements

Intraparenchymal monitoring of partial oxygen pressure of brain tissue (local $PbrO_2$) provides the clinician with additional information on the local oxygen status of the injured brain. As no complications such as hemorrhage or infection, were observed, the method is considered safe. The catheters show a negligible zero drift and low sensitivity drift, confirming the technical reliability of the method in the clinical setting. The differences in absolute values of $PbrO_2$ measured in patients with two inserted probes reflect the heterogeniety of oxygenation at the tissue level[13]. These observations, although in a small study sample, emphasize the limitations of focussing too much emphasis on absolute values of $PbrO_2$, and attempts to identify a critical threshold value of $PbrO_2$ should be viewed with caution. Local factors, such as the presence of micro hemorrhages around the oxygen probe may influence values measured[29]. That this may also occur in the clinical setting was observed in one of our patients in whom the oxygen probe was inadvertently placed in a small hemorrhagic contusion: despite low $PbrO_2$ recordings the patient made an excellent recovery. This observation illustrates the potential limitations of a local monitoring technique. We therefore recommend routine CT evaluation of the position of the oxygen sensor. If the investigator chooses to insert the oxygen probe in an undamaged area of the brain and local complicating factors are absent the recorded values can be considered representative for the other undamaged areas of brain tissue, taking the known heterogeneity of brain oxygenation into account. A local measure of oxygenation indicates changes in global oxygenation, as a global ischemic insult will reduce $PbrO_2$ in all areas of the brain. Values measured are dependent on the relation of the catheter to the capillary mesh, being influenced by both the diameter of microvascular vessels and the diffusion distance between the capillary mesh and the oxygen probe.

Causes of tissue hypoxia, implications for monitoring techniques

Cerebral ischemia is generally considered to result from insufficient oxygen supply in relation to demand. With this concept in mind, monitoring of jugular venous saturation is considered appropriate for detecting global cerebral ischemia. Low jugular saturation values indicate a higher extraction of oxygen and thus are indicative of ischemia. However, the causes of tissue hypoxia are not easily explained. In an overview on the oxygen status of the blood, Siggaard-Andersen et al[26] identified potential causes of tissue hypoxia. The main categories, their causes and expected change in arteriovenous oxygen extraction are summarized in table 7. From this table it becomes evident that monitoring of venous oxygen saturation will not detect all causes of tissue hypoxia. Moreover, it demonstrates that tissue hypoxia may be present despite normal or increased venous saturation values. We have previously reported that low $PbrO_2$ values were observed in the presence of normal or even high oxygen saturation values in the cerebral venous blood[31]. Local $PbrO_2$ can provide additive information of brain oxygenation, also when jugular bulb oximetry is already available. The results of the study by Kiening et al[12] support this opinion.

Table 7 Causes of tissue hypoxia

Type of hypoxia	Cause	Expected change in arteriovenous oxygen extraction ($AVDO_2$)
Ischemic hypoxia	Decrease in flow	Increase
Low extractivity hypoxia	Low arterial pO_2 (hypoxemic hypoxia)	No change
	Low Hb concentration (anemic hypoxia)	
	Low half saturation tension P50	
	(high affinity hypoxia)	
Shunt hypoxia	Arteriovenous shunting	Decrease
Dysperfusion hypoxia	Increased mean diffusion length from erytrocytes to mitochondria, caused by intracellular or interstitial edema	Decrease
Histotoxic hypoxia	Toxic agents	Decrease
Uncoupling hypoxia	Agents interfering with synthesis of ATP	No change
Hypermetabolic hypoxia	Mitochondrial dysfunction	Increase
	Increased demand	

Low initial $PbrO_2$

Real or artifact?

Clinical studies have shown critically reduced cerebral blood flow following head injury[1, 2, 7, 16, 21, 25]. Microdialysis studies have shown the presence of lactacidosis indicating ischemia at the tissue level[20]. In studies utilizing transcranial doppler in patients with head injury we have observed low flow velocities in the early phase after trauma[30]. The observation of low brain tissue $PbrO_2$ in the first 24 hours after injury is consistent with these reports. However, comparative measurements indicate an artifactual low value during the first two hours of recordings. Some equilibration time before attaining steady state measurements of the probe tissue interface is required; this time will probably be dependent on the type of catheter and the introducing method used. The catheters used in our studies have a small diameter (0.5 mm) and are very flexible. For insertion a stiff introducer (outer diameter 1.1 mm) is used, through which the catheter is passed into the brain tissue. The use of this introducer causes a larger degree of local tissue damage than would occur when simply passing the catheter itself into the brain tissue and it is conceivable that microvascular flow surrounding the tip of the catheter is disturbed due to this method. This hypothesis would explain the longer run in time, observed in the clinical setting, compared to our experience in the experimental situations, where the microcatheters are introduced directly into the brain without help of an introducer. In the laboratory settings a maximum equilibration time of one hour was found in the animal experiments [29].

Therefore the slow gradual increase of $PbrO_2$ over periods up to 12 to 24 hours, can not be explained by the short run in time of two hours. We firmly believe that the observed low values of $PbrO_2$ occurring after the run in time of two hours reflect the presence of local tissue hypoxia. This opinion is strengthened by the clear correlation between the presence of low values in the first 24 hours and poorer outcome. Whether the low $PbrO_2$ value early after injury reflects a change of cerebral blood flow, or an increase in utilization, resulting in a decrease of O_2 concentration, can only be investigated by concurrent measurements of CBF and metabolism and this issue warrants further research.

Low initial $PbrO_2$

Relation with clinical parameters, CT-scan parameters and outcome

Clinical variables known to be associated with the outcome of head injured patients, such as age, Glasgow Coma Scale score on admission, pupillary reactivity, ICP and the presence of significant systemic trauma, are not significantly correlated with the initial low values. Of CT-scan variables, known to have a prognostic value, only the status of the basal cisterns correlates with early tissue hypoxia. Since there is no straight forward correlation between clinical or CT-scan variables and the new monitoring technique, this technique might well give an additional value as is demonstrated with the multiple logistic

regression model. After a period of 10 minutes measuring $PbrO_2$ values < 10 mmHg, a significant correlation with death or unfavorable outcome was observed. Highest significance was reached after 60 minutes, but after 30 minutes the odds ratio for death was already 3.8. Addition of the separate or combined clinical and CT-scan variables to the logistic regression model did not cause an important adjustment of predictive value of low $PbrO_2$. Thus low initial $PbrO_2$-values are an independent predictor of death or unfavorable outcome.

Statistical analysis using cubic spline have the advantage of being very flexible, while the continuous character of a variable is preserved, resulting in smooth functional relationships. The relative risk of death for the three threshold values is graded [figure 4]. In cases of deep local tissue hypoxia ($PbrO_2$ < 5 mmHg) the risk of death of 50% occurs at about 30 minutes. For moderate hypoxia (<10 mmHg) the same risk of death of 50% occurs at one hour and 45 minutes, while for mild tissue hypoxia (< 15 mmHg) this point is reached at less than four hours. Jones et al [10] demonstrated in the experimental situation the well-known effect of depth and duration of tissue hypoxia on neurological outcome. Our present study, utilizing this novel monitoring technique, confirms such a relationship in the traumatized human brain. Furthermore, this significant correlation of low $PbrO_2$ to poorer outcome supports our earlier conclusions that initial low values are not due to an artifact, but are evidence of a patho-physiological phenomenon frequently observed after head injury. Our data confirm our earlier report on the relationship of low $PbrO_2$ and outcome[31]. Valadka et al[28] evaluated 43 head-injured patients in whom different cut-off points were used in search of a clinical treshold. Using a Tobit regression analysis he concluded that there is a relationship of both depth and duration of cerebral hypoxia with mortality. The results of our analysis are in agreement with his findings and the restricted cubic spline analysis visualizes these important relationships even more.

Low initial $PbrO_2$

Implications for therapy

We conclude that tissue oxygenation in severe head injury can be reliably monitored on a continuous basis with the described technique utilizing an intraparenchymally introduced Clark type PO_2 sensor. We have further shown that low values frequently occur in the first 24 hours after injury, and that depth and duration of this low values are related to unfavorable outcome and death. Evidence therefore supports the concept of targetting therapy towards improvement of cerebral oxygenation. Such improvement can be obtained either by increasing the oxygen content of the arterial blood, or by improving delivery through increase of flow. The latter concept is the basis for cerebral perfusion pressure therapy[23]. In a recent study by Robertson et al[22] however no overall benefit on outcome was seen in patients treated with a CBF targeted regime, aiming at a mean arterial pressure ≥ 90 mmHg when compared to patients treated according to an ICP targeted regime. In this study however all

patients were treated according to one of the specified regimes, and CBF management was not specifically targeted to patients at risk for low CBF or cerebral hypoxia. Brain oxygen tension monitoring may aid in determining in which patients CBF targeted therapy might be appropriate and what level of critical perfusion pressure is required in individual patients.

Correcting anemia and ensuring optimal saturation of the arterial blood is the most important method for improving oxygen content of the arterial blood. Increasing FiO_2 in patients already adequately oxygenated to supranormal values will only increase the physiologically resolved O_2 in plasma, which represents only 2-3% of overall oxygen content. Yet PaO_2 is the driving force of oxygen flux into the tissue. Significant increases of brain tissue $PbrO_2$ after increases of arterial PaO_2 from normal to supranormal levels have frequently been observed. The PaO_2 level was linearly related to the $PbrO_2$ level in both animal and patient studies [15, 31]. The Richmond group has shown that such an increase of brain tissue $PbrO_2$ after simply increasing arterial PaO_2 results in a concurrent decrease of tissue lactate[18, 32]. These authors suggest a shift from anaerobic to aerobic metabolism in these injured brains due to the influence of supranormal levels of PaO_2. A potential disadvantage of increasing arterial PaO_2 to very high levels could be a reduction in CBF due to flow regulatory mechanisms[11], but it seems unlikely that this will be of clinical concern as tissue oxygenation is improved. The potentially harmful effects of FiO_2 levels higher than 60%, used for longer than 24 hours prohibits long term use of this treatment.

In our opinion sufficient pathophysiologic evidence currently exists to warrant targetting therapy in patients with severe head injury towards improvement of cerebral oxygenation, guided by continuous monitoring of cerebral $PbrO_2$. We do not wish to debate extensively the relative value of brain oxygen tension measurements and jugular oximetry, but feel that both techniques yield complementary information and should preferably be used in conjunction to each other.

Conclusions

Continuous measurement of cerebral oxygenation, by means of an intraparenchymally placed Clark type catheter, is a technically reliable methodology, clinically applicable, and in our experience safe.

Stabilization of the probe tissue interface with the methodology used, is obtained within a maximum of two hours after placement of the catheter.

If inserted in undamaged brain tissue local $PbrO_2$ values can be considered representative for larger areas of undamaged tissue, as opposed to insertion of the probe in the so-called 'penumbra zone', in which it can be considered indicative of oxygenation of tissue at risk.

Hypoxia of relatively undamaged brain tissue, as evidenced by low $PbrO_2$ recordings, is frequent in the first 24 hours after head injury.

Depth and duration of local tissue hypoxia is independently related to outcome at six months.

It may be appropriate to focus intensive care management in severe head injury not only on increasing cerebral perfusion pressure, but more specifically on attempts to increase brain tissue oxygen levels.

Intensive care research in severe head injury should focus on a potential clinical benefit of brain tissue oxygen management.

References

Paper:
van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Monitoring brain oxygen tension in severe head injury, early cerebral hypoxia is related with an unfavorable outcome. **Neurosurgery 46**: 868-878, 2000

Presentation, abstract:
van den Brink WA, van Santbrink H, Avezaat CJJ, et al: Monitoring brain oxygen tension in severe head injury: The Rotterdam experience, in Marmarou A, Bullock R (eds): **Acta Neurochirurgica Supplementum.** Springer-Verlag:1998 (Abstract)

1. Bouma GJ, Muizelaar JP, Choi SC, et al: Cerebral circulation and metabolism after severe traumatic brain injury: The elusive role of ischemia. **J Neurosurg 75**: 685-693, 1991

2. Bouma GJ, Muizelaar JP, Stringer WA, et al: Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. **J Neurosurg 77**: 360-368, 1992

3. Chesnut RM, Marshall LF, Klauber MR, et al: The role of secondary brain damage in determining outcome from severe head injury. **J Trauma 34**: 216-222, 1993

4. Fearnside MR, Cook RJ, McDougall P, et al: The Westmead head injury project outcome in severe head injury. A comparative analysis of prehospital, clinical, and CT variables. **Br J Neurosurg 7**: 267-279, 1993

5. Graham DI, Ford I, Adams JH, et al: Ischaemic brain damage is still common in fatal non missile head injury. **J Neurol Neurosurg Psychiatry 52**: 346-350, 1989

6. Harrell FE: Design: SS-plus functions for biostatistical/epidemiological modelling, testing, estimation, validation, graphics, prediction, and typesetting by storing enhanced model design attributes in the fit, in **Programs available at Internet: http: //www. lib. stat. cmu. edu/**. 1997

7. Jaggi JL, Obrist WD, Gennarelli TA, et al: Relationship of early cerebral blood flow and metabolism to outcome in acute head injury. **J Neurosurg 72**: 176-182, 1990

8. Jennett B, Bond M: Assessment of outcome after severe brain damage. A practical scale. **Lancet 1**: 480-484, 1975

9. Jones PA, Andrews PJ, Midgley S, et al: Measuring the burden of secondary insults in head injured patients during intensive care. **J Neurosurg Anest 6**: 4-14, 1994

10. Jones TH, Morawetz RB, Crowell RM, et al: Tresholds of focal cerebral ischaemia in awake monkeys. **J Neurosurg 54**: 773-782, 1981

11. Kety SS, Schmidt CF: The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. **J Clin Invest 27**: 484-492, 1948

12. Kiening KL, Unterberg AW, Bardt TF, et al: Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO_2 versus jugular vein oxygen saturation. **J Neurosurg 85**: 751-757, 1996

13. Lubbers DW, Baumgartl H, Zimelka W: Heterogenity and stability of local pO_2 distribution within the brain tissue. **Adv Exp Med Biol 345**: 567-574, 1994

14. Maas AIR, Dearden NM, Teasdale E, et al: EBIC-Guidelines for management of severe head injury. **Acta Neurochir 139**: 286-294, 1997

15. Maas AIR, Fleckenstein W, de Jong DA, et al: Effect of increased ICP and decreased cerebral perfusion pressure on brain tissue and cerebrospinal fluid oxygen tension, in Avezaat CJJ, van Eijndhoven JHM, Maas AIR (eds): **Intracranial pressure VIII**. Berlin, Springer-Verlag: 233-237, 1993

16. Marion DW, Darby J, Yonas H: Acute regional cerebral blood flow changes caused by severe head injuries. **J Neurosurg 74**: 407-414, 1991

17. Marshall LF, Bowers Marshall S, Klauber MR, et al: A new classification of head injury based on computerized tomography. **J Neurosurg 75 (suppl)**: S14-S20, 1991

18. Menzel M, Doppenberg EM, Zauner A, et al: Increased inspired oxygen concentration as a factor in improved brain tissue oxygenation and tissue lactate levels after severe human• head injury. **J Neurosurg 91**: 1-10, 1999

19. Miller JD: ICP monitoring - Current status and future directions. **Acta Neurochir 85**: 80-86, 1987

20. Persson L, Hillered L: Chemical monitoring of neurosurgical intensive care patients using intracerebral microdialysis. **J Neurosurg 76**: 72-80, 1992

21. Robertson CS, Contant CF, Narayan RK, et al: Cerebral blood flow, $AVDO_2$, and neurological outcome in head injured patients. **J Neurotrauma 9**: S349-S358, 1992

22. Robertson CS, Valadka AB, Hannay HJ, et al: Prevention of secondary ischemic insults after severe head injury. **Crit Care Med 27**: 2086-2095, 1999

23. Rosner MJ, Rosner SD, Johnson AH: Cerebral perfusion pressure: management protocol and clinical results. **J Neurosurg 83**: 949-962, 1995

24. Ross DT, Graham DI, Adams JH: Selective loss of neurons from the thalamic reticular nucleus following severe human head injury. **J Neurotrauma 10**: 151-165, 1993

25. Salvant JB, Muizelaar JP: Changes in cerebral blood flow and metabolism related to the presence of subdural hematoma. **Neurosurgery 33**: 387-393, 1993

26. Siggaard-Andersen O, Fogh-Andersen N, Gothgen IH, et al: Oxygen status of arterial and mixed venous blood. **Crit Care Med 23**: 1284-1293, 1995

27. Stocchetti N, Furlan A, Volta F: Hypoxemia and arterial hypotension at the accident scene in head injury. **J Trauma 40**: 764-767, 1996

28. Valadka AB, Gopinath SP, Contant CF, et al: Relationship of brain tissue PO_2 to outcome after severe head injury. **Crit Care Med 26(9)**: 1576-1581, 1998

29. van den Brink WA, Haitsma IK, Avezaat CJJ, et al: The brain parenchyma-PO_2 catheter interface, a histopathological study in the rat. **J Neurotrauma 15**: 813-824, 1998

30. van Santbrink H, Maas AIR, Avezaat CJJ: Serial transcranial doppler measurements in patients with severe head injury; The early posttraumatic period, in Nagai H, Kamiya K, Ishii S (eds): **Intracranial Pressure IX**. Tokyo, Spinger -Verlag: 585-586, 1994

31. van Santbrink H, Maas AIR, Avezaat CJJ: Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. **Neurosurgery 38**: 21-31, 1996

Zauner A, Doppenberg EMR, Soukop J, et al: Extended neuromonitoring: New therapeutic opportunities? **Neurol Res 20**: s85-s90, 1998

Summary

Judge: "I have read your case Mr. Smith,
and I am no wiser now than I was when I started."
F.E.S.: "Possibly not, My Lord, but far better informed."
From: The life of F.E. Smith, the first Earl of Birkenhead.
Written by his son, the second Earl of Birkenhead.

This thesis deals with head injury. To put the effort of the experimental work in perspective to the magnitude of the problem, three introductory chapters precede the experimental studies. The development of a new head injury model is described. The model of Closed Head Injury in the rat has been characterised physically, and physiologically. Furthermore the effects of secondary systemic hypoxia on cerebral hypoxia, and neurological outcome in the experimental model was investigated. The last neuro-intensive care study, showing the importance of a novel monitoring technique in a large group of head injured patients, brings us back to the final aim of these basic studies: improvement of patient care.

Introductory chapters, clinical relevance

Chapter 1 General introduction

This chapter provides epidemiological data of accidental injuries in general and head injury in particular, as provided by official institutions in the USA (CDC), in the Netherlands (CBS). In well developed countries, injury is the leading cause of death and disability among young adults. Over the years a gradual decline in the total number of deaths can be observed. This is mainly caused by a decrease in lethal road traffic accidents. Each year 48.800 head injured patients are assessed at a hospital emergency room in the Netherlands. 12.300 of them will be admitted with an average admission time of 7.3 days. In the Rotterdam University Hospital an annual average of 251 head injured patients were admitted during the last five years. 52% of these patients were severely injured enough to be admitted to the Intensive Care Unit. Overall mortality rate was 15%.

About 50% of severely head injured patients has an unfavorable outcome. The most important predictors of outcome are: age, depth of coma, GCS motor score, pupillary reactivity, and CT scan characteristics.

After impact to the head, damage to certain areas of the brain are permanent and beyond cure ('primary injury'). Other, less damaged areas, which are potentially salvageable, are suspect to damage by 'secondary injuries'. Adverse systemic or intracranial events, require early recognition and treatment. Basic ABC treatment, important for any traumatized patient, is even more valuable for the head injured patients. Unequivocally national and regional agreements on referral policy should ensure timely referral to a centre with neurosurgical expertise and capacity for emergency surgery.

After traumatic brain injury, several cascades of secondary injuries, active at a sub-cellular basis, have been identified. Theoretically these pathways should be amenable to pharmacological intervention, ameliorating its effects, and improving patient outcome. However clinical trials have not succeeded in proving beneficial effects of promising pharmaceutical agents in the overall population of patients with severe head injury.

Chapter 2 Pathophysiology of traumatic brain injury

In the second chapter the most important pathophysiological sequelae of traumatic brain injury are summarized. The knowledge gathered the last years, which has survived the 'experimental' stages, and which has found its place in current consensus, or which remain under serious debate, is discussed. The causes for severe head injury are various , each mechanism inflicting the head can cause several types of primary injury. These are classically divided in focal brain injury, and diffuse brain injury. Often a admixture of focal and diffuse pathology is present in one patient.

In brain contusions, disruption of the blood brain barrier, results in vasogenic brain edema and areas of perivascular hemorrhage, seen as mixed density lesions on the CT scan. Diffuse brain injury is hallmarked by axonal damage. Concomitant vascular injuries result in punctate hemorrhages only visualized by high resolution CT scanners and MR Imaging.

An increase in one of the three main constituents of the skull, brain tissue, blood or cerebrospinal fluid must be balanced by an equal loss of volume of the others, or ICP will increase. Although increased ICP is related to poor patient outcome, it remains unknown whether or not high ICP is the cause of, rather than the result from, irreversible brain damage. Obviously in some patients the first, in others the latter mechanism will predominate.

Because an energy storage in the brain does not exist, maintenance of cerebral blood flow (CBF) to meet metabolic demand is pivotal. The driving force of CBF is cerebral perfusion pressure (CPP). Both a low arterial pressure and a high ICP compromise CPP after head injury. The intrinsic mechanism of autoregulation assures the brain of adequate CBF at variable levels of cerebral perfusion pressure. After head injury, this mechanism is often impaired, rendering the brain vulnerable to fluctuations in arterial blood pressure.

Several putative mechanisms of cellular damage have been postulated, and shown to be active in the secondary phase after head injury. Which intervention of these mechanisms is useful in the active treatment of head injury remains to be proven.

Chapter 3 Animal models of traumatic brain injury

This last introducing chapter provides a historical perspective of the diversity of head injury models. As head injury is diverse by nature, a variety of head injury models will remain required to unravel parts of the patho-physiological events occurring after traumatic brain injury. The development of one head

injury model covering all pathophysiological entities encountered in the clinical situation is impossible, and not desirable. For example studies of penetrating head injury require different models than that of diffuse axonal injury. The objective of the investigator, and his scientific questions, will shape the development of the model used.

The following hypothesis is implicit to brain injury modelling: *"Part of the spectrum of human traumatic brain injury can be duplicated in non-humans"*. This hypothesis allows for the assumption that inferences from one specific animal model can be made to the human condition. These inferences have their limitations caused by difference in neuraxic structure, and the inability to test complex behavioral deficits. Another important prerequisite for experimental modelling remains: *"The induced response to trauma must be reproducible, and quantifiable covering a clinically relevant continuum"*. Of pivotal importance is the strive for minimal variability caused by animal characteristics, physiological parameters, anesthesia, and loading forces.

Traumatic brain injury can be induced by whole head loading or localized brain loading. Both can result in diffuse injury, brain-stem injury, or localized contusions. Acceleration of the brain, occurring after whole head loading, results in tissue strains. Magnitude and location of these strains are dependent on direction of applied forces and dimensions of the skull.

Utilizing non human primates, Denny-Brown and Russell pioneered experimental injury by means of impact to the unrestrained head. They emphasized the importance of acceleration of the brain as cause of cerebral concussion. The so called 'Penn injury devices' induce highly controlled rotational acceleration. It was shown that widespread diffuse axonal damage results mainly of rotational acceleration in the coronal plane. Concussive states were attributed to widespread axonal damage.

Using small animals in impact-acceleration experiments several limitations were observed. The most important one being the fracture rate when inducing accelerations severe enough to cause concussive brain injury. To overcome this problem Marmarou developed a model in which the forces directed to the skull are evenly distributed over a larger surface by means of a cemented metal disc. Placing the rodent head on a foam support, provided acceleration and partial constraint. Chapters four through ten characterize this model of CHI in the rat.

Characterization of an experimental Closed Head Injury model

Chapter 4 A new model of diffuse brain injury in rats, Pathophysiology and biomechanics

This chapter describes the development of the CHI model, producing diffuse brain injury in the rodent. Adult rats were injured utilizing the weight drop device consisting of a segmented brass weight free-falling through a plexiglass guide tube. Skull fracture was prevented by cementing a small stainless-steel

disc on the calvaria. Fracture threshold and the primary cause of death at severe injury levels were determined. A 450-gm weight falling from a 2-m height (0.9 kg-m) resulted in a mortality rate of 44% with low incidence (12.5%) of skull fracture. Impact was followed by apnea, convulsions, and moderate hypertension. The surviving rats developed decortication flexion deformity of the forelimbs, with behavioral depression and loss of muscle tone. The cause of death was due to central respiratory depression and the mortality rate decreased markedly in animals mechanically ventilated during the impact. Analysis of mathematical models showed that this mass-height combination resulted in a brain acceleration of 900 G and a brain compression gradient of 0.28 mm. It is concluded that this simple model is capable of producing a graded brain injury in the rodent without a massive hypertensive surge or excessive brain-stem damage.

Chapter 5 The Closed Head Injury model, standardization of acceleration mechanisms

As standardization is especially difficult in acceleration models in small animals, the CHI model was approached in a physical way. The contribution of mechanisms acting during the process of acceleration of the brain, were sequentially approached. In the Closed Head Injury model, acceleration of the brain is caused by impact of a falling weight onto the rodent skull. Movement of the skull is provided by means of a foam head support. Changes of foam properties could play a role in the variability of acceleration. Acceleration in this model is a fast dynamic process. An ISO normation based dynamic foam tester, to be used as a calibration procedure to standardize foam properties, was developed. Three foams with different firmness were tested. The 'dynamic compressibility', covering the visco-elastic properties of the foam, did not correlate with static compression tests. In animal experiments an influence of properties of these three foams on mortality, brain edema, blood glucose, -lactate and -gases could not be demonstrated. From this study the conclusion can be drawn that if one considers meticulous standardization necessary, the visco-elastic properties of the foam should be tested in a dynamic procedure. Pragmatically however, foam characteristics are an over-criticized issue, without direct pathophysiological consequences.

Chapter 6 Cortical dysfunction, preservation of brain-stem function

Because models of Fluid Percussion Injury (FPI) are hampered by predominant brain-stem damage, even at moderate levels, the extent of brain-stem and cortical dysfunction associated with this model of CHI were examined. Rats were submitted to severe or moderate CHI. Brain-stem Auditory Evoked Potentials and Somato Sensory Evoked Potentials, measuring brain-stem and cortical function, were acquired at regular intervals up to 24 hours after trauma. Sham groups, undergoing all manipulations except for the actual injury, served as controls. Results obtained in the CHI model were compared to results obtained in severe FPI. In contrast to traumatic brain injury induced by FPI,

primary brain-stem dysfunction was not observed in survivors of CHI. By 24 hours, there were indications for mild brain-stem impairment, probably due to secondary mechanisms such as brain-stem compression or ischaemia. At moderate levels of CHI, cortical function showed mild initial impairment with rapid recovery to baseline. At high level CHI, cortical function was severely impaired and did not completely recover within 4 hours.

These evoked potential studies revealed that Brain-stem Potentials remained unaltered immediately after CHI. Somato Sensory Evoked Potential, evaluating cortical function, deteriorated after CHI. This impairment was more outspoken, and longer lasting in severely injured animals.

Chapter 7 Blood brain barrier dysfunction and edema formation

The integrity of the Blood Brain Barrier and temporal course of edema formation were studied in the CHI model. In contrast to FPI the CHI model does not produce a post-traumatic hypertensive surge. Using an albumin-bound radioactive tracer it was shown that blood brain barrier disruption is of short duration and occurs early after head injury. A subsequent rise in arterial pressure further increases the magnitude of this dysfunction. Microgravimetrical techniques, used to study brain water content, showed that brain edema gradually increased during the first 24 hours after injury.

These studies indicate that vascular damage might play a role in the initiation of cerebral edema. Subsequent cellular edema, must be held responsible for the continuation of edema formation, being an important contributor to post-traumatic brain swelling in diffuse traumatic brain injury.

Experimental head injury and oxygen

Chapter 8 Hypoxia augments behavioral deficits in CHI

Traumatic brain injury is often followed by secondary insults, which are deleterious with respect to patient outcome. Hypoxia is one of the most important secondary insults in head injury. In animals models of traumatic brain injury, the observation of behavioral outcome parameters could be facilitated by the addition of secondary insults. To this end effects of secondary hypoxia on spontaneous behavior, motor, and cognitive function were investigated in the CHI model. Rats were submitted to either severe CHI alone, or a combination of severe CHI and hypoxia ($FiO_2 = 14\%$ during thirty minutes). Two sham groups, one without hypoxia, and one with 30 minutes hypoxia were used as controls. In surviving animals, the time of recurrence of normal reflexes, which were abolished by trauma, was recorded. The week after CHI, tests of spontaneous behavior, and motor function were performed daily. During the second week after CHI, visuo-spatial memory and learning capability was evaluated using the Morris Water Maze.

Severe CHI alone causes minimally impaired motor and cognitive function. Secondary hypoxia, administered after CHI, results in significantly augmented impairment of both motor function and learning capabilities. These larger

deficits can be employed in the evaluation of new treatment regimens. Such augmentation of behavioral deficits with a 'double insult' paradigm provides the neuroscientist with better measurable outcome parameters to evaluate new treatment strategies. Extrapolated to the clinical situation, this implicates the importance of early detection, and aggressive treatment of secondary insults.

Chapter 9 The brain parenchyma-$PbrO_2$ catheter interface

Monitoring brain tissue oxygen is a promising means for evaluating the course of cerebral oxygenation after head injury. Local cerebral oxygenation can be monitored continuously using an intraparenchymal Clark-type PO_2 sensitive catheter. Measured values of brain tissue PO_2 ($PbrO_2$) not only depend on the clinically interesting balance between oxygen offer and demand, but also on catheter properties and characteristics of the probe tissue interface. Microdamage surrounding PO_2 sensitive needles, inserted into various tissues, has been reported; we evaluated histological changes at the probe tissue interface after insertion of PO_2 probes, suitable for clinical use, in the rat brain. The effect of insertion of the probe itself (mechanical damage), the application of micropotential during the measurements and the effect of time was evaluated using digital image analysis of Haematoxiline-Eosine stained histological slices. Surrounding the probe tract a zone of edema with an average radius of 126.8 µm was seen; microhemorrhages with an average surface area of 56.2 x 103 μm^2 were observed in nearly all cases. The area of edema and the presence of microhemorrhages were not influenced by performed measurements or by time. Intraventricular blood was observed in 10 of 19 rats studied. The presence of a microhemorrhage in either probe tract or ventricles was related to low $PbrO_2$ values measured.

Tissue damage and the amount of edema surrounding the probe does not influence the accuracy or response time of the PO_2 probe. Low $PbrO_2$ readings, however, could be caused by local micro-hemorrhages, undetectable on CT or MRI. This observation has implications for the clinician, who should be aware of this pitfall of artificial low measurements.

Chapter 10 Brain oxygenation marginally impaired in CHI

In patients with severe head injury, decreased brain oxygen tensions ($PbrO_2$), as well as increased reactivity of brain oxygenation to increases in FiO_2, are related with poor outcome. We investigated whether experimental closed head injury causes similar impairment of brain oxygenation and oxygen reactivity. CHI, with and without subsequent systemic hypoxia, was utilized to induce traumatic brain injury. Either CHI alone, or a combination of CHI and hypoxia (FiO_2 = 14% during thirty minutes), was induced. Two sham groups, without hypoxia, or with 30 minutes hypoxia were used as controls. Rats submitted to hypoxia were artificially ventilated and paralyzed. $PbrO_2$ was monitored for 4,5 hours after CHI using an Intraparenchymal Clark type oxygen sensor. At regular intervals after CHI, FiO_2 was increased to 66%, and $PbrO_2$-reactivity indices were calculated.

Severe CHI causes statistically significant lower $PbrO_2$ values, related to the severity of trauma. Clinically hypoxic values however were not observed. The $PbrO_2$ decrease might be caused by systemic parameters such as lower arterial blood pressure, $PaCO_2$, and PaO_2. During the thirty minutes systemic hypoxia deep cerebral hypoxia was observed, and augmented in combination with CHI. The depth of cerebral hypoxia in this episode was related to post traumatic mortality, implicating the occurrence of an irreversible event. Levels generally considered hypoxic (<10 mmHg) were only observed just before brain death. Differences in brain oxygen reactivity were not observed.

Back from model to man: human head injury and oxygen

Chapter 11 Brain oxygen tension in severe human head injury

Ensuring adequate cerebral oxygenation and perfusion is of fundamental importance in the treatment of head injury. On-line continuous monitoring of brain oxygenation is possible with a parenchymal micro-electrode, measuring local brain oxygen tension ($PbrO_2$). The ultimate question is whether therapeutic approaches can be targeted based on such monitoring. Before this question can be addressed, the technique requires validation in the clinical setting. The occurrence of low values, and the relation to outcome was established in 101 comatose head-injured patients (GCS $\leq$ 8). $PbrO_2$ probes were inserted in an undamaged part of the frontal region. Patients were treated conforming to the European Brain Injury Consortium guidelines. Six months outcome was determined according to the Glasgow Outcome Scale. Early brain tissue hypoxia was frequently observed, despite aggressive ICP and CPP treatment. Depth and duration of tissue hypoxia were related to outcome and were proven to be an independent predictor of unfavorable outcome and death.

Local $PbrO_2$ monitoring is a safe and reliable method to monitor cerebral oxygenation. Because brain tissue hypoxia occurs frequently, and is significantly related to poor outcome, future efforts should be aimed at the treatment of brain tissue hypoxia. The effects of such 'brain hypoxia targeted treatment' needs to be established in a multicenter study.

Samenvatting

Dit proefschrift beschrijft een aantal studies op het gebied van traumatisch hersenletsel. Teneinde het experimentele werk in perspectief te plaatsen, zijn er drie introductie hoofdstukken opgenomen die de omvang en de aard van het probleem beschrijven. De clinicus wordt vaak geconfronteerd met vragen die slechts te beantwoorden zijn met behulp van een gestandardiseerd proefdier model. De ontwikkeling van zo'n nieuw traumatisch hersenletsel model wordt beschreven. Dit model wordt gekarakteriseerd met betrekking tot de belangrijkste fysische en fysiologische parameters. In de hierop volgende sectie worden de effecten van gegeneraliseerde hypoxie op cerebrale oxygenatie en neurologische uitkomst beschreven. De relatie tussen cerebrale hypoxie en uiteindelijke uitkomst, welke wordt beschreven in een klinische studie, brengt ons terug bij het eigenlijke doel van het experimentele werk: verbetering van mogelijkheden voor optimale behandeling van de patiënt met traumatisch hersenletsel.

Hoofdstukken ter introductie, klinische relevantie

Hoofdstuk 1 Algemene introductie, epidemiologie

Dit hoofdstuk geeft een overzicht van de epidemiologische gegevens met betrekking tot ongevallen, met name traumatisch hersenletsel. De gegevens betreffende de Verenigde Staten zijn afkomstig van het 'Centre of Disease Control'; de Nederlandse gegevens zijn afkomstig van de doodsoorzaken database van het Centraal Bureau voor de Statistiek, en van de Landelijke Medische Registratie. In ontwikkelde landen zijn ongevallen de belangrijkste doodsoorzaak op de jong volwassen leeftijd. Gedurende de afgelopen jaren is er een gestage vermindering van het totale aantal ongevalslachtoffers waarneembaar, hetgeen geduid zou kunnen worden als succes van preventieve maatregelen. Deze vermindering is met name het gevolg van een vermindering van het aantal dodelijke verkeersongelukken. In Nederland worden ieder jaar 48.800 patiënten met hersenletsel gezien op een afdeling spoedeisende hulp van een ziekenhuis. Van deze groep wordt 12.300 opgenomen met een gemiddelde opname duur van 7.3 dagen. In het Academisch Ziekenhuis Rotterdam, werden gedurende de laatste 5 jaar jaarlijks gemiddeld 251 patiënten met traumatisch hersenletsel opgenomen. 52% van deze patiënten behoefde opname op een Intensive Care, en de mortaliteit bedroeg 15%.

Van de patiënten met ernstig traumatisch hersenletsel heeft 50% een slechte uitkomst, in de zin van overlijden, of ernstige blijvende restverschijnselen. De belangrijkste prognostische variabelen zijn: leeftijd, diepte van coma, GCS motor score, pupil reactiviteit, en CT scan kenmerken.

Nadat het hoofd heeft blootgestaan aan inwerkend geweld raken sommige delen van de hersenen onherstelbaar beschadigd, het zogenaamde 'primaire letsel'. Andere minder beschadigde delen, welke mogelijk kunnen herstellen,

staan bloot aan later optredende factoren die het zogenaamde 'secundaire letsel' kunnen veroorzaken. Bedreigende systemische of intracraniële situaties dienen vroeg te worden herkend en behandeld. Het basale ABC (Airway, Breathing, Circulation), dat belangrijk is voor iedere trauma patiënt, is destemeer belangrijk voor de patiënt met hersenletsel. Landelijke en regionale afspraken dienen verwijzing en opnames op de juiste plaats te waarborgen. Voor patiënten met ernstig hersenletsel is dit een centrum met neurochirurgische expertise en 24 uurs acute operatie capaciteit.

Diverse subcellulaire cascades, die optreden na traumatisch hersenletsel, zijn geïdentificeerd. Theoretisch bieden deze cascades mogelijkheden tot farmacotherapeutische interventie, welke de negatieve aspecten hiervan zouden kunnen verminderen. Tot nu toe echter is het onmogelijk gebleken om in een klinische trial een positief effect van één van de, experimenteel veelbelovende, medicamenten aan te tonen.

Hoofdstuk 2 Pathophysiologie van hersenletsel

In het tweede hoofdstuk worden de belangrijkste pathophysiologische gevolgen van traumatisch hersenletsel samengevat. De belangrijkste onderwerpen waarover de afgelopen jaren consensus is verkregen, alsmede die waarover nog hevig wordt gediscussieerd, worden genoemd. Er zijn vele mechanische oorzaken van hersenletsel. Ieder mechanisme kan verschillende soorten 'primair letsel' veroorzaken. Dit primaire letsel wordt klassiek verdeeld in gelokaliseerd en diffuus letsel. Vaak komen beide soorten tegelijk in één patiënt voor.

In lokale hersenkneuzingen ontstaat een lekkage van de bloed hersen barriËre. Dit heeft een vasogeen hersenoedeem en bloedingen tot gevolg, welke als peper-en-zout afwijkingen op de CT scan zichtbaar zijn. Het diffuse letsel wordt gekenmerkt door wijdverspreid axonaal letsel. De begeleidende puntvormige bloedinkjes zijn alleen met behulp van CT scanners en MRI scanners met hoge resolutie zichtbaar te maken.

Een toename van het volume van bloed, of hersenzwelling, binnen de afgesloten schedel moet worden uitgewisseld tegen een afname van het volume hersenvocht. Hersenvocht kan gemakkelijk uit het intracraniële compartiment weg naar de spinale ruimte en dient zo als volume buffer. Gebeurt dit niet, of raakt de buffercapaciteit uitgeput, dan is een verhoging van de intracraniële druk (ICP) onvermijdelijk. Hoewel deze ICP stijging gerelateerd is aan een slechte uitkomst, is het onzeker of de verhoging van de ICP een oorzaak of gevolg is van onomkeerbaar hersenletsel. Er zijn waarschijnlijk patiënten waarin het eerste, en patiënten waarin het tweede voorop staat.

Aangezien er geen energie voorraad in het hersenweefsel bestaat is de instandhouding van de cerebrale bloeddoorstroming (CBF), teneinde aan de metabole behoefte te voorzien essentieel. De motor achter de bloeddoorstroming is de perfusiedruk (CPP). De hoogte hiervan wordt berekend als het rekenkundig verschil tussen de arteriële bloeddruk en de ICP. Zowel een

lage arteriële bloeddruk, als een verhoogde ICP zijn een bedreiging voor een adequate CPP. De zogenaamde 'autoregulatie' van de hersenen waarborgt onder normale omstandigheden een constante CBF over een bepaalde bandbreedte van CPP. Na hersenletsel is dit mechanisme vaak minder werkzaam, hetgeen het hersenweefsel kwetsbaar maakt voor schommelingen in de arteriële bloeddruk.

Diverse gepostuleerde mechanismen die cellulaire schade veroorzaken zijn actief na traumatisch hersenletsel. Welke interventie van deze mechanismen bruikbaar kan zijn in de actieve behandeling van patiënten met hersenletsel zal nog bewezen moeten worden.

Hoofdstuk 3 Dierexperimentele modellen van hersenletsel

Dit laatste introductie hoofdstuk geeft een historisch overzicht van de diversiteit van hersenletsel modellen. Aangezien hersenletsel van nature zeer heterogeen is zal een aantal modellen benodigd zijn om de diverse mechanismen van hersenletsel na te bootsen, en om onderdelen van de pathophysiologische gevolgen na hersenletsel te ontrafelen. De ontwikkeling van één model, welke alle pathophysiologische entiteiten die men in de kliniek kan tegenkomen beschrijft, is onmogelijk, en niet wenselijk. Bestudering van penetrerend hersenletsel behoeft bijvoorbeeld een heel ander model dan bestudering van de mechanismen van diffuus axonaal letsel. Het doel en de vraagstelling van de onderzoeker zal de ontwikkeling van het model vormgeven.

In de ontwikkeling van een model van traumatisch hersenletsel is aanname van de volgende hypothese een voorwaarde: "*Een deel van het spectrum van het humane traumatische hersenletsel kan worden nagebootst in de gebruikte diersoort*". Deze hypothese waarborgt de mogelijkheid om conclusies van observaties in het diermodel naar de klinische situatie te extrapoleren. Deze aanname wordt beperkt door anatomische verschillen in de neuraxis, en door de moeilijkheid om complexe neurologische uitval te testen. Een tweede belangrijke aanname is de volgende : "*De respons op het geïnduceerde trauma moet reproduceerbaar zijn, en een klinisch belangrijke reikwijdte hebben*". Van essentieel belang blijft het streven naar een zo gering mogelijke variabiliteit, zoals deze veroorzaakt kan worden door de diersoort zelf, fysiologische parameters, anesthesie, en mechanische karakteristieken van het model.

Traumatisch hersenletsel kan worden geïnduceerd door een inwerkende kracht op de intacte schedel, of op een deel van het brein. Beide mechanismen kunnen evenwel diffuus letsel, hersenstam letsel, en gelokaliseerde contusiehaarden tot gevolg hebben. Hoge versnellingen van het hersenweefsel, welke optreden na een inwerkende kracht op de intacte schedel, resulteert in schuif krachten in het hersenweefsel. De locatie van de grootst optredende schuifkrachten zijn afhankelijk van de richting van de krachten van het inwerkend geweld, en van de afmetingen en vorm van de schedel.

Denny-Brown en Russel waren de eersten die systematisch experimenteel hersenletsel induceerden door botsing tegen de intacte schedel. Zij zagen de

versnelling van hersenweefsel als oorzaak van de 'hersenschudding'. Het later ontwikkelde 'Penn-apparaat' induceerde zeer nauwkeurig controleerbare rotatieacceleratie van de intacte schedel. Wijdverbreid diffuus axonaal letsel, verantwoordelijk voor langdurige bewustzijnsstoornissen, bleek met name op te treden bij rotatie in het coronale vlak.

Bruikbaarheid van kleinere proefdieren werd gehinderd door een aantal factoren. Het frequent optreden van schedel fracturen bij acceleraties die hoog genoeg zijn om hersenletsel te veroorzaken was daar één van. Dit probleem werd opgelost door Marmarou, die een model ontwikkelde waarin de schedel protectie werd gewaarborgd door de fixatie van een roestvast stalen schijf midden over de vertex, zodat de krachten van het vallende gewicht over een groter oppervlak worden verdeeld. De plaatsing van de kop van het proefdier op een bed van schuim zorgde voor de mogelijkheid van acceleratie. De volgende hoofdstukken geven een karakterisering van dit model weer.

Karakterisering van een model voor gesloten schedel hersenletsel

Hoofdstuk 4 Een nieuw model van diffuus hersenletsel in de rat Pathophysiologie en biomechanica

Dit hoofdstuk beschrijft de karakteristieken van een model van gesloten schedel hersenletsel in de rat, waarbij diffuus hersenletsel wordt veroorzaakt. Volwassen ratten werd hersenletsel toegebracht door middel van een vallend gewicht, geleid door een plexiglas buis. Schedelbreuken werden voorkomen door een kleine roestvast stalen schijf op de vertex te bevestigen. Een gewicht van 450 gram, vallend van een hoogte van 2 meter resulteerde in een mortaliteit van 45%, bij een lage fractuur frequentie van 12,5%. Na ernstig trauma traden apneu, convulsies en een lichte stijging van de bloeddruk op. Overlevenden van dit letsel vertoonden pathologische buigreacties van de bovenste extremiteiten, verlies van spiertonus, en gedragsafwijkingen. De oorzaak van overlijden, in dieren die niet overleefden, was centrale ademhalingsdepressie. Ondersteuning van de ademhaling zorgde voor een aanzienlijke verlaging van de mortaliteit. Analyse met behulp van een mathematisch computer model toonde aan dat genoemde combinatie van massa en hoogte zorgde voor een acceleratie van 900G en een hersen compressie gradiënt van 0,28 mm. In conclusie is het met dit relatief eenvoudige model mogelijk om gradeerbaar hersenletsel toe te brengen aan de rat, dit in afwezigheid van schedel fracturen, ernstige hypertensieve crisis, of overweldigend hersenstam letsel.

Hoofdstuk 5 Standaardisatie van acceleratie mechanismen.

De bijdrage van mechanismen gedurende het proces van de acceleratie werden op een natuurkundige wijze geanalyseerd. In dit model werd de acceleratie van het hoofd veroorzaakt door een vallend gewicht op de beschermde schedel. Beweging van de schedel werd mogelijk gemaakt door het hoofd met een schuimrubber blok te ondersteunen. Veranderingen van de eigenschappen van dit schuim onder invloed van tijd en veelvuldig gebruik zouden dit acceleratie

proces kunnen beïnvloeden. Een dynamisch schuim test apparaat, gebaseerd op ISO-normeringen, werd ontwikkeld om als calibratie routine te worden gebruikt. Drie types schuim met verschillende hardheid werden getest. De zogenoemde 'dynamische comprimeerbaarheid', welke de visco-elastische eigenschappen van het schuim beschrijft, correleerde niet met uitkomsten van een statische compressie test. In dier experimenten kon geen invloed van het type schuim op mortaliteit, hersenoedeem, bloed-glucose, -lactaat, of -gassen worden aangetoond. Uit deze studie kan worden geconcludeerd dat, indien meticuleuze standaardisatie noodzakelijk wordt geacht, het schuim op een dynamische wijze moet worden gestandaardiseerd. Voor de pragmatisch ingestelde onderzoeker kan worden gesteld dat de schuim karakteristieken geen belangrijke invloed op pathophysiologische gevolgen hebben.

Hoofdstuk 6 Corticale disfunctie met intacte hersenstam functie

Vloeistof Percussie Letsel modellen (FPI) hebben als nadeel dat ze, zelfs bij matige intensiteit, overweldigende hersenstam schade veroorzaken. De uitbreiding van hersenstam en corticale disfunctie werd onderzocht in ratten blootgesteld aan ernstig en matig hersenletsel geinduceerd met het CHI model. Brain-stem Auditory Evoked Potentials en Somato Sensory Evoked Potentials, welke hersenstam en corticale functie op neurofysiologische wijze in kaart brengen, werden op gezette tijden tot 24 uur na trauma gemeten. De resultaten werden vergeleken met die na FPI. In tegenstelling tot FPI zorgde dit nieuwe CHI model niet voor direct hersenstam letsel. Na 24 uur werden aanwijzingen gevonden voor een verminderde hersenstam functie, waarschijnlijk ten gevolge van secundair optredende mechanismen zoals compressie of ischemie. Matig hersenletsel resulteerde in lichte corticale stoornissen met snel herstel, terwijl na ernstig hersenletsel forse stoornissen werden geobserveerd, zonder volledig herstel binnen 4 uur.

Deze evoked potential studies tonen aan dat hersenstam potentialen na trauma met het CHI model ongewijzigd blijven. De corticale functies verslechterden echter, het meest uitgesproken in ernstig getraumatiseerde proefdieren.

Hoofdstuk 7 Bloed hersen barriëre disfunctie en hersenoedeem

De integriteit van de bloed hersen barriëre en het beloop van oedeem vorming werd bestudeerd na gesloten schedel hersenletsel in het CHI model. In tegenstelling tot FPI treedt er geen hypertensieve crisis op na traumatisering met dit nieuwe model. Met behulp van een radioactieve albumine gebonden tracer werd aangetoond dat de opening van de bloed hersen barriëre van korte duur was. Indien vervolgens een verhoging van de bloeddruk optrad, was er een toename van de disfunctie aantoonbaar. Met behulp van micro-gravimetrische technieken werd aangetoond dat een geleidelijke toename van het hersen water gehalte optreedt gedurende de eerste 24 uur na trauma.

Deze studie toont aan dat vasculaire schade een rol zou kunnen spelen in de vorming van hersen oedeem kort na het letsel. Na deze initiële periode moet cellulair oedeem verantwoordelijk worden gehouden voor de continuering van

de oedeem formatie, welke een belangrijke bijdrage levert aan de post-traumatische hersenzwelling in diffuus hersenletsel.

Experimenteel hersenletsel en zuurstof

Hoofdstuk 8 Hypoxie verergert neurologische uitval na gesloten schedel hersenletsel

Traumatisch hersenletsel wordt vaak gevolgd door secundaire bedreigingen, welke de uitkomst negatief beïnvloeden. Hypoxie is een van de belangrijkste secundaire bedreigingen na hersenletsel. In dierexperimentele modellen zou de observatie van gedragsafwijkingen door toevoeging van secundair letsel vergemakkelijkt kunnen worden. De effecten van secundaire hypoxie op spontaan gedrag, motore functie, en geheugen na hersenletsel toegebracht door middel van het CHI model werden bestudeerd. Ratten werden blootgesteld aan ofwel hersenletsel alleen, ofwel een combinatie van hersenletsel en secundaire hypoxie. Twee controle groepen, één met, en één zonder hypoxie werden voor het overige identiek behandeld. In de overlevenden van het trauma werden de tijden van terugkeer van, door hersenletsel uitgevallen, reflexen genoteerd. Gedurende de volgende twee weken werden het spontane gedrag, de motore functie, en het visuele geheugen getest.

Ernstig hersenletsel alleen veroorzaakte geringe motore en cognitieve uitval. Secundaire hypoxie na trauma verergerde de uitval van motore en geheugen functie aanzienlijk. Deze toename van uitval kan enerzijds door de onderzoeker worden gebruikt in de evaluatie van nieuwe behandelings-mogelijkheden. Anderzijds benadrukt dit onderzoek de ernstige gevolgen van secundaire hypoxie.

Hoofdstuk 9 Het hersen parenchym-$PbrO_2$ katheter raakvlak

De bewaking van de zuurstofspanning van hersenweefsel is een veelbelovende techniek om het verloop van de lokale weefsel oxygenatie na hersenletsel te vervolgen. Lokale zuurstofspanning kan continu bewaakt worden door middel van een intraparenchymateuze PO_2 gevoelige elektrode. De gemeten waardes van brein PO_2 ($PbrO_2$) hangen niet alleen af van de balans tussen zuurstof aanbod en gebruik, maar ook van katheter eigenschappen, en van het raakvlak van katheter en hersenweefsel. In diverse weefsels zijn microbeschadigingen rondom de PO_2 gevoelige naalden beschreven. Wij hebben de histologische veranderingen op het raakvlak van hersenweefsel en een flexibele elektrode in rattenbrein onderzocht. Het effect van de insertie van de katheter (mechanische beschadiging), de toevoeging van de meetpotentiaal, en het effect van tijd werden geëvalueerd. Hiertoe werden de Haematoxiline-Eosine coupes digitaal geanalyseerd. Rondom de katheters was een zone van oedeem met een radius van 126.8 µm zichtbaar. Microbloedingen met een gemiddelde oppervlakte 56.2 x 10^3 μm^2 werden vaak geobserveerd. De grootte van genoemde afwijkingen was niet afhankelijk van tijd, of meting. Intraventriculair bloed werd in 10 van de 19 ratten geobserveerd. De aanwezigheid van een bloed was gerelateerd aan de gemeten $PbrO_2$ waardes.

Weefselschade, of de hoeveelheid oedeem, beïnvloeden de accuratesse of respons tijd van de PO_2 katheter niet. Lage waardes kunnen echter wel veroorzaakt worden door een locale bloeding, waarvan de omvang beneden de CT-detectiegrens ligt. De clinicus die deze techniek gebruikt moet op de hoogte zijn van deze tekortkoming.

Hoofdstuk 10 Experimenteel gesloten schedel hersenletsel vermindert cerebrale oxygenatie marginaal

In patiënten met ernstig hersenletsel zijn lage $PbrO_2$ waarden, en verhoogde reactiviteit van $PbrO_2$ na FiO_2 verhoging, gerelateerd met een slechte uitkomst. Wij onderzochten of experimenteel hersenletsel een soortgelijke vermindering van hersenweefsel oxygenatie en reactiviteit zou laten zien. Hersenletsel met en zonder secundaire hypoxie werd geÔnduceerd met het nieuwe model. Twee controle groepen, één met, en één zonder hypoxie werden voor het overige identiek behandeld. De ratten die secundaire hypoxie ondergingen werden verslapt en beademd. Gedurende 4.5 uur werd de $PbrO_2$ gemeten met een intraparenchymateuze Clark-type elektrode. Op gezette tijden na hersenletsel werd de FiO_2 verhoogd naar 66%, en werd de $PbrO_2$ reactiviteit berekend.

Hersenletsel veroorzaakte geringe doch statistisch significant lagere $PbrO_2$ waardes, welke gerelateerd waren aan de ernst van het letsel. De verlaging van de $PbrO_2$ zou veroorzaakt kunnen zijn door systemische parameters zoals de arteriële bloeddruk, de $PaCO_2$ en de PaO_2. Gedurende de 30 minuten diepe hypoxie werd een diepe cerebrale hypoxie gezien, deze was nog dieper wanneer de hypoxie vooraf gegaan was door hersenletsel. De cerebrale hypoxie in deze periode was gerelateerd aan mortaliteit. Kennelijk treedt er een onomkeerbaar proces op. Cerebrale hypoxie waardes beneden de algemeen geaccepteerde drempel van circa 10 mmHg werden slechts juist voor het intreden van de hersendood geobserveerd. Verschillen in zuurstof reactiviteit werden niet waargenomen.

Terug van experiment tot patient: humaan hersenletsel en zuurstof

Hoofdstuk 11 Bewaking van cerebrale oxygenatie na ernstig traumatisch hersenletsel in de mens

Fundamenteel in de behandeling van patiënten met ernstig hersenletsel is de zorg voor adequate cerebrale oxygenatie en perfusie. Continue bewaking van hersenweefsel oxygenatie ($PbrO_2$) is mogelijk met de recent ontwikkelde intraparenchymateuze micro-elektrode. De uiteindelijke vraag is natuurlijk of de klinische behandeling geleid kan worden met behulp van zulk een bewakingsmodaliteit. Alvorens deze vraag kan worden beantwoord zal de techniek in de kliniek moeten worden geëvalueerd.

Het voorkomen van lage waardes, en de relatie ervan met de uitkomst van de patiënt werd bestudeerd in 101 comateuze patiënten met ernstig hersenletsel. $PbrO_2$ katheters werden ingebracht in een onbeschadigd deel van de frontale kwab. De uitkomst na 6 maanden werd vastgesteld aan de hand van de

Glasgow Outcome Scale. Lage $PbrO_2$ waardes werden, ondanks agressieve ICP en CPP behandeling, in de eerste 24 uur vaak geobserveerd. De diepte en duur van deze hypoxische periodes waren gerelateerd aan de neurologische uitkomst. Door een multipele logistieke regressie analyse werd aangetoond dat lage $PbrO_2$ een onafhankelijke voorspeller van slechte uitkomst en dood is.

Continue meting van de lokale $PbrO_2$ is een veilige en betrouwbare methode om de cerebrale oxygenatie te bewaken. Aangezien cerebrale hypoxie frequent voorkomt, en significant correleert met uitkomst, verdient het de aanbeveling om toekomstige onderzoeken te richten op de behandeling van cerebrale hypoxie. Het effect van zulke 'cerebrale hypoxie gerichte behandelingen' moet in een multicenter studie worden geëvalueerd.

Acknowledgements

To everything there is a season, and time to every purpose under the heaven.... Wherefore I perceive that there is nothing better than that a man should rejoice in his own works; for that is his portion.

Ecclesiastics 3: 1....22

I am greatly indebted to my promotors, collegues and friends, who have given me the opportunity to work and commit myself to science during times in which my attention was also needed on 'the floor'.

Prof. Cees Avezaat, you have given me the opportunity to stay in the United States for quite a period to become engaged in basic research. Furthermore you have provided an excellent training program in the most challenging medical specialty. During training you were always there for education and support. Sometimes short, sometimes long, but always with full attention for the problem.

Prof. Anthony Marmarou, thank you for giving us a very warm welcome when we arrived in Virginia. Despite of the 'neurofog' that could stay with me for days after scientific discussions, there more often than not proved to be a way in the proposed line of thought and future research opportunities. I am especially grateful to you for giving Martha the opportunity to become involved in my work, and part of your office.

Nino Stocchetti has given me a very critical view on life and science, furthermore he has made me addicted to Macintosh Computers very early, this has given me the chance to use computers the way they all work nowadays: intuitive and user friendly. Kazuo Yoshida was the most western Japanese in the Richmond environment these days, I was happy to share two small corners in the B-3 lab with him: one to perform experiments (I even could use his FPI model for some neurophysiological investigations) and one to write our manuscripts (he would even take out his earphones to listen to my remarks). Jana Dunbar was my teacher of rat-science. She has taught me all there is to know about rat brains.

Dr. Andrew Maas, your enormous 'energy-reserve' continuously inspired me to do things beyond my capacity after I returned to Rotterdam. Some people might call it abuse, but then, it did never feel that way to me. Maybe that is why others call it people-management. Dr. Wolfgang Fleckenstein, you have provided our lab with both scientific, organizational, and material input. I feel that neurosurgery in general is indepted to your efforts in the realm of cerebral oxygenation. Dr. Ernst Delwel you were my partner in crime, we made jokes and plans, organized parties and skull base labs. Most of all you have the ability to create an atmosphere so that we could patiently work late on a case without becoming bored, but tired ...yes.

Marie-Lise van Veelen, Henk van Santbrink, and Arnoud Vincent, I feel that we were more than just colleges training to become neurosurgeons. Thank you for giving me the feeling for being at home, when I was at work. We could

mourn and be happy. We could discuss our bad experiences and newly acquired skills freely and without reservedness. I hope we can continue this comradeship.

Dirk de Jong was the continuous sting, posing questions impossible to answer. Probably worthwhile thinking about, although there have been many questions posed during the formation of an answer on the first, making it impossible to answer any. The nature of physicians I presume.

Ed van Beeck has helped me place head injury in perspective on an epidemiological sound basis. Laus Broersen was so kind to show us around in his behavioral laboratories and share his insight in rodent behavior, when the memory studies were started. Ewout Steyerberg took notice first of our problems, and then of our data. I should have met him before I met my stat-software

Bryant Santos, Thomas Fatouros, Leo van der Meer, Saskia Zandee, Marike Zwienenberg, Iain Haitsma, Willem van der Bend, Oscar van Haagen, Maarten ter Horst, Annette van Rooijen, I have had the privilege to teach you, and learn from you, who did outstanding work during your neurosurgical research projects. I am sure each of you will find your way in the medical profession, as you have found your way in my lab.

Angelique van den Heuvel, I have been very fortunate to have had you on the team during the last year of my research, which also was your first year as analyst. Without this year, I could not have finished this work. I am sure that who ever will thrust you with his experiments, will have the best of dedication, and skills.

Wil Kort, and members of the DEC, our plenary discussions on the purpose and need of head injury research were not the easiest, still I want to thank you for your permission to perform the experiments reported in this thesis. Jaap Kasbergen, Ineke Hekking, Henk Dronk, and Rob Meijer, thank you very much for your support in the EDC during times that were not easy for any of you.

Chris Hoogesteeger, Joost Vermeulen, Peter Roodzant, Wilma Jansen, you were the brain oxygen crew in the Neuro ICU, thanks to your commitment to patient care, technical support, and meticulous data gathering, patient related research was possible, and rewarding. Nanda Elink Schuurman organized and edited several manuscripts and abstracts. She was also an often called upon medium between my co-promotor and myself. Not the easiest place to be.

Martha, my wife, followed me to Virginia, and back. Stayed home when I was on call, or worse, working on data. Never complained when she was alone with the kids, always supported me with the goals I've set. I sure hope you will follow me to the North-Eastern part of our beautiful country.

Marie Suzanne, Robbert Jan, and Renee Hélène, I am happy you time and again reminded me that there is more than career. Although often stiff necked, there was always a smile, always a hearty kiss when I came home.

Curriculum Vitae

Wimar van den Brink werd in 1961 geboren in Schiedam - Kethel. Na het behalen van zijn Atheneum diploma werd in 1980 de studie geneeskunde aan de Erasmus Universiteit in Rotterdam aangevangen. Hij werd bevorderd tot arts in 1987. Hierna was hij gedurende twee jaar werkzaam als arts assistent op de afdeling Neurochirurgie van het Academisch Ziekenhuis Rotterdam (hoofd: Prof. dr. C.J.J. Avezaat). Van 1989 tot 1991 was hij gedurende twee jaar verbonden als research fellow aan de Division of Neurosurgery van het Medical College of Virginia, Richmond, VA, USA (hoofd: Prof. H. Young M.D.). Gedurende deze periode is onder leiding van Prof. A. Marmarou Ph.D. een aanvang gemaakt met het experimentele werk dat de basis vormt van dit proefschrift. Na terugkeer in Rotterdam werd de opleiding tot neurochirurg aangevangen. Gedurende deze periode is het onderzoek in het dier-experimenteel laboratorium en de neuro-intensive care voortgezet, hetgeen heeft geleid tot de laatste hoofdstukken van het onderhavige proefschrift. In het jaar 1992 werd de opleidings stage neurologie gevolgd (hoofd: Prof. dr. F.G.A. van der Meché). In 1993 genoot hij het opleidingsjaar algemene heelkunde (opleider: Dr. H.F. Veen). Sinds 1994 is hij lid van de vaste commissie van medewerkers van het Nederlands Tijdschrift voor Intensive Care. Tevens volgde hij de 'Advanced Trauma Life Support' cursus en werd hij in 1996 toegevoegd aan het corps van instructeurs van de stichting ATLS. Na het afronden van zijn opleiding in 1997 is hij twee jaar als junior specialist verbonden geweest aan de afdeling Neurochirurgie van het Academisch Ziekenhuis, en als wetenschappelijk medewerker aan de Erasmus Universiteit te Rotterdam. Hierna vestigde hij zich in het Neurochirurgisch Centrum te Zwolle, alwaar hij zich associeerde met collegae F.C. de Beer, W. Pondaag, en Dr. D.J. Zeilstra.