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The Effect of Selective Hypothermia on Stroke Volume

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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(Thesis format: Integrated Article)

by

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Abstract

Total body hypothermia is an established neuroprotectant in global ischemia. Applying hypothermia selectively could provide more rapid cooling and eliminate systemic side effects. We studied the effect of selective hypothermia in a novel stroke model in adult domestic swine.

Via craniotomy under general anesthesia, one middle cerebral artery branch was occluded for 3 hours followed by 3 hours of reperfusion. In half the animals, hypothermia was induced during reperfusion via a dual-lumen balloon occlusion catheter placed in the carotid ipsilateral to the ischemic region. Following reperfusion, the animals were sacrificed. Brain MRI and histology were evaluated blinded to the intervention.

In this series of 28 animals, the mean temperature achieved was 26.5C. Mean time from start of perfusion to attainment of moderate hypothermia (< 30 C) was 25.4 minutes. Mean histologic stroke volume was reduced by 38.4-44.2% (p=0.292). Percentage stroke seen on MRI showed a significant reduction(p=0.042)

Selective moderate hypothermia was rapidly induced using endovascular technology. A promising reduction in stroke volumes is seen. Further study is warranted.

Keywords

Selective hypothermia, Focal cerebral ischemia, Stroke, Endovascular, Porcine

Co-Authorship Statement

Conception and Design: Lownie, Lee, Pelz

Acquisition of data: Mattingly, Das, Lopez-Ojeda, Denning, Siroen, Lehrbass, Leiber, Solar, Lownie, Lee, Ang,

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Table of Contents

List of Tables

List of Figures

List of Plates (where applicable)

List of Appendices (where applicable)

Chapter 1

1 Background

1.1 Introduction.

As a form of neuroprotection, hypothermia is the only one that has stood the test of time. Numerous animal studies and in recent years even some human ones point to its protective effect. In the application of hypothermia during surgical procedures, early investigators recognized both its potential power but also the systemic risks associated with its use.

A surge of interest has recently arisen in the application of hypothermia to ischemic stroke. This stems from developments in endovascular catheter technology, which can provide hypothermia more rapidly than traditional surface cooling techniques (DeGeorgia, Froehler), and also provide it to more selective regions of the body including the brain. Our purpose was to evaluate catheter technology as a means of selectively cooling an ischemic region of brain. Focal cooling potentially avoids the systemic complications of hypothermia, while providing moderate hypothermia rapidly and efficiently.

1.2 The history of total-body and selective hypothermia

Interest in hypothermia as a protection against hypoxic-ischemic injury during surgery dates back to the 1950s (Bigelow, Botterell, Lougheed). At that time, efforts to improve surgery on both the heart and the brain led to investigations as to how to improve tolerance to circulatory arrest and resultant hypoxia. For cardiac surgeons the issue was with direct surgery on the heart, which would require cardiac standstill, and which without some form of protection would result in cerebral ischemia (Bigelow). For neurosurgeons the tantalizing possibility lay in being able to operate in a bloodless field in the depths of the cranium to precisely reconstruct diseased blood vessels. This led to

interest in methods to arrest cerebral blood flow while improving the brain's tolerance to ischemia (Botterell, Lougheed).

These lines of investigation were focused on two different forms of cerebral ischemia. The cardiac surgeons were interested in the prevention of global ischemia due to cardiac standstill. For the neurosurgeons the interest was in preventing focal ischemia due to temporary occlusion of a single brain artery (Lougheed, Drake, Lawton). Temporary occlusion provides the advantage of softening of the brain aneurysm sac, allowing manipulation and obliteration with a reduced risk of aneurysm rupture. But temporary occlusion comes at the cost of potential stroke in the territory supplied by the artery. Providing hypothermia could decrease the metabolic demand of neural tissue and decrease the risk of stroke.

The metabolic effect of hypothermia was established in canine experiments the 1950s by Lougheed, who found a 50% reduction in cerebral metabolic rate at 28°C, and a 75% reduction at 25°C (Lougheed). Given the degree to which cerebral metabolism was reduced by moderate hypothermia, it seemed logical that it would be protective during induced ischemia. Initially cooling was performed by whole body ice water bath, but cardiac dysrrhythmias limited the degree of cooling to 30°C (Botterell). Later studies utilized full cardiac bypass with profound hypothermia(10-15°C), but untoward bleeding was a significant problem (Drake, Lawton).

The recognition of these systemic side-effects led to interest in focal or selective hypothermia applied to the area at risk for ischemia. Lougheed et al first proposed local or selective hypothermia in 1955 as a way of circumventing the cardiac arrhythmia problem (Lougheed). Using a bypass pump attached to a carotid artery in dogs, he was able to reduce brain temperature to 20°C in 20 minutes, while the systemic body temperature only dropped to 35°C. The approach was abandoned when all the dogs developed fatal arrhythmias. However, additional canine experiments by Verdura were able to overcome this issue with impressive clinical results (Verdura). Of 16 animals treated with femoral-carotid bypass hypothermia and concurrent ischemia, all survived

and only 2 had permanent deficits, whereas none of the 5 normothermic controls survived.

Subsequently, a human trial of selective catheter-based brain cooling during surgery for aneurysms and tumours was undertaken in the 1960s (Willams). Although 10 patients achieved deep hypothermia of less than 20°C, bleeding complications led to a 50 per cent mortality rate. It was concluded that selective brain cooling was inferior to open chest cardiopulmonary bypass techniques. However the excessive mortality may have been due in part to the technique. The surgical procedure involved the isolation of all 4 arteries to the brain in order to perfuse the brain through one of them, the other three being clamped.Supra-normal infusion pressures were then used to achieve total brain cooling in Williams' study.

Despite these early challenges, the scientific allure of hypothermia for brain ischemia continues, due to repeated demonstrations of reduced stroke volume in animal studies. Animal studies have included both global and focal ischemic models, in both small and large animals, and using different timing of induction of hypothermia in relation to the onset of ischemia. In one study, a global ischemic model in swine with a full 20 minute period of cardiac arrest, femoral–to–carotid bypass using cooled blood for 12 hours led to brain temperatures of 32-34°C with drops in body temperature to only 35°C (Mori, Mori 2). Histological grading in the CA1 region of the hippocampus, an area known for ischemia-sensitive neurons, demonstrated significantly better scores in animals that received hypothermia. In a canine model of global ischemia, clamping off 3 vessels and perfusing the brain solely through the right vertebral artery with cold Ringer's lactate led to marked drops in brain temperature with no evidence of cerebral ischemic damage either clinically or histologically (Ohta).

The above were studies of global ischemia during cardiac arrest. However with neuroscientists' interest in focal ischemia, many studies have used focal ischemic models instead. One common model utilizes rodents in which one middle cerebral artery and the ipsilateral or both carotids are occluded. In many such studies, infarct volumes have been found to be smaller compared with normothermic controls (Huang, Kozlowski, Xue,

Onesti, Goto). These studies differ in the degree of hypothermia and in the timing of hypothermia in relation to the ischemic insult. Hypothermia was generally induced by whole body cooling, and the method, although considered focal, involves a more significant loss of blood flow to the whole brain than a focal model would in a larger animal. Also, rats probably tolerate a greater degree of hypothermia without cardiac arrhythmias than higher order mammals such as humans. In one study, whole body cooling to less than 24°C was tolerated (Onesti).

The timing of the induction of hypothermia relative to the ischemic insult is an interesting area of study (Onesti). While ischemic insults applied during hypothermia have shown promise, the reverse also appears to be true (Goto, Xue, Huang). In other words, hypothermia can be induced hours after the ischemic insult or even during reperfusion, and stroke volumes will still be reduced compared with normothermic controls. Proposed mechanisms for this benefit include the protection of "penumbra" neurons at the peripheral zone of ischemia, suppression of excitatory neurotransmitters, and suppression of leukotrienes (Onesti). During reperfusion with hypothermia there are measurable reductions in ischemic metabolites such as lactate and high energy phosphates (Koslowski). Hypothermia during reperfusion also leads to reduction in one form of blood-brain-barrier disruption (Huang). Thus based on rodent studies it would appear that hyperacute stroke may benefit from hypothermia applied during reperfusion.

Although rodents are easily handled, tolerate moderate to deep hypothermia with whole body cooling, and have provided insight into the mechanisms behind hypothermia's benefit, the transferability of these findings to higher order mammals is not clear. Humans do not tolerate temperatures much below 32°C without significant cardiac effect (Botterell). Furthermore, whole body cooling can take hours to induce, and thus its applicability to acute stroke is limited, where even an hour's delay can be critical (Froehler).

In swine, focal ischemic models have become more established in recent years. Sakoh and colleagues used full size pigs to compare cerebral metabolic rate of oxygen ($CMRO_2$) using positron emission (PET) scanning with activated diffusion coefficient (ADC)

changes using magnetic resonance (MR) imaging. Their model was proximal occlusion of the middle cerebral artery (MCA) (Sakoh). Smaller models using either infant or miniature piglets have also been described, which allow study of permanent or temporary MCA occlusion (Imai, Kuluz, Cooper). Unlike rodents, no additional carotid clamping is required, making swine models much more like the human acute stroke situation.

While small porcine models are considered advantageous for ease of handling and for craniotomy (Imai), their vasculature is too small to evaluate the use of endovascular tools designed for humans. Larger pigs have been used for endovascular equipment evaluation and training for close to three decades (Massoud, Guglielmi, Lee). Thus a stroke model in domestic swine would be ideal to study endovascular approaches to selective brain cooling.

1.3 The Argument for Selective Hypothermia

The failure of selective brain cooling observed in humans during the 1960s has been noted above. However based on animal studies and on the avoidance of cardiac and bleeding complications, further investigations into this promising technique continue. In one study in baboons, a femoral-to-carotid circuit was used to cool the ipsilateral cerebral hemisphere to under 25°C via one carotid artery. This was rapidly performed within a mean of 12 minutes, and maintained for 3h with only minimal systemic cooling to 34°C (Schwartz). No hemodynamic complications were noted, although 2 of 12 animals died due to other complications. A second study involved swine, first as a feasibility study, in which brain temperatures were reduced to 30°C in 30 min without reducing core temperature below 34°C (Mori). Subsequently, the same group induced a global ischemic insult in the form of controlled cardiac arrest for 20 minutes, then randomly provided selective cooling for 12 hours. There was a statistically significant improvement in histological scores in the cooled group compared with controls (Mori 2). Brain

temperatures in this study were more in the moderate range (32-34°C) while core temperatures dropped to 35°C. Most recently, a small study in baboons demonstrated significant reductions in stroke volume by MRI in selective hypothermia maintained over 12hours (Schwartz 2).

Recently, ice-cold saline has been proposed as a method for selective cooling. A theoretical study predicted that brain temp less than 35°C would be achieved in 10 minutes at infusion rates of 20ml/min (Konstas). A clinical pilot study in humans demonstrated internal carotid artery infusions of ice-cold saline at 33 ml/min for 10 minutes led to a statistically significant drop in jugular venous bulb temperatures compared with core temperatures (Choi). The magnitude of the drop was small (0.84°C versus 0.15°C). However, the authors suggested that this form of selective brain cooling might be useful in acute stroke as a bridge to recanalization and to prevent reperfusion complications.

Finally, Lownie et al utilized a femoral-carotid bypass to induce selective hypothermia during the evacuation and clipping of a giant middle cerebral artery aneurysm (Lownie). Both safety and utility were demonstrated in this case report, with temporary occlusion times of approximately 20 minutes, and a brain temperature of 22°C, without evidence of stroke, hemodynamic or cardiac changes, or coagulopathy.

1.4 Potential Utility of Selective Hypothermia in Acute Ischemic Stroke

Given the demonstrable effect of hypothermia in reducing stroke volumes in both small and large animal models, as well as significant human data suggesting benefit of hypothermia in preventing ischemic injury, the utility of hypothermia for treatment after a focal ischemic insult is an area of active research. In a rodent model, delaying hypothermia until 1.5 hours after the ischemic insult still showed decreased infarct volumes, although not as dramatically as with concurrent hypothermia (Xue). In clinical trials, hypothermia is beneficial after cardiac arrest (Holzer). There is some disagreement

about the length of time required for benefit but depth of hypothermia may not be as important (Yenari). In acute ischemic stroke, multiple authors suggest that hypothermia may be useful as a bridge to recanalization (Choi, Yenari, Schwartz, Froehler).

One barrier to clinical utility is the rapidity of hypothermia induction. This is not an issue for rodent studies, but whole body cooling in humans takes a significant amount of time. The rate of cooling may be an important determinant for clinical utility in stroke (Froehler). The COOLAID study demonstrated that endovascular cooling combined with a drug cocktail (to prevent shivering) achieved target temperature in just over an hour, compared with surface cooling technology which can take over 4 hours (De Georgia). However, stroke volume was not reduced.

A subsequent human clinical trial called ICTuS-L evaluated the combination of FDA-approved intravenous tPA and endovascular hypothermia for the treatment of acute ischemic stroke (Hemmen). This was the first trial to evaluate a demonstrated stroke therapy with hypothermia, but unfortunately could not enroll enough patients to evaluate whether hypothermia might extend the treatment window for intravenous therapy. Like COOLAID, there were no significant differences in clinical outcome or mortality, although pneumonia was more commonly seen in the hypothermia treatment arm.

Despite the negative results in COOLAID and ICTUS-L, endovascular methods would seem to be ideal for the application of hypothermia to acute ischemic stroke. Endovascular cooling is significantly more rapid than whole body or surface cooling methods. By being selective, endovascular cooling should reduce or avoid the systemic side effects of cardiac dysfunction and coagulopathy, while providing a greater depth of hypothermia. Finally, endovascular techniques are already in use as recanalization strategies for stroke, so adding catheter-delivered hypothermia to the mix makes sense as a “neuroprotective bridge” to recanalization (Choi, Yenari, Schwartz, Froehler).

A new catheter technology to administer selective cooling has become available. This is the “Duo-Flo” dual lumen catheter (Thermopeutix Inc., San Diego, CA). The catheter consists of a 9.5 French balloon catheter coaxially introduced through a 14 French outer catheter. The outer catheter is positioned in the aorta, and is used to withdraw blood to

an extracorporeal perfusion unit, wherein the blood is cooled and then reinfused via the balloon catheter into the common carotid artery ipsilateral to the ischemic zone. The Duo-Flo has received 510k approval for human use in 2010 from the U.S. Food and Drug Administration (personal communication, Thermopeutix Inc.).

The ideal animal model provides both a reproducible ischemic stroke and a relative size similar to humans for endovascular catheter work. Endovascular training models in pigs weighing 30-45kg are well established (Guglielmi, Massoud, Lee). The senior author has extensive experience with such models for endovascular device evaluation. Also, stroke models in pigs are well established (Sakoh, Imai, Cooper). Pigs have a relatively thick skull that has led investigators to use either transorbital approaches (Sakoh), or smaller specimens (Imai, Cooper). However, miniature or immature pigs would not allow the use of human-size endovascular instruments.

A large animal model of acute focal ischemia with which to test theory and application of selective hypothermia is therefore required.

There remains a significant mismatch between the potential promise of hypothermia for acute stroke and actual human data. Despite decades of successful animal experimentation using hypothermia in both global and focal ischemia models, and recent clinical trials showing benefit in both adults and neonatal global ischemia, there is no evidence that supports the use of hypothermia for stroke in humans (Heleen, Yenari, Froehler). This could be due to the prolonged time to induce whole body hypothermia, uncertainties regarding the depth and duration of hypothermia required for clinical benefit, and limitations to whole body hypothermia imposed by systemic complications. While some of these factors could be addressed using selective hypothermia, the benefit of hypothermia in human stroke remains hypothetical. A large animal model could address these issues in a transferable way prior to further human trials.

Chapter 2

2 Integrated Manuscript

2.1 Introduction

The extensive history of hypothermia, including successes in both animal series and human clinical trials, suggests that it may be effective in acute ischemic stroke. The hurdles include the speed of application, as well as uncertainties regarding the depth and duration of hypothermia (Froehler). The COOLAID and ICTUS-L trials, as well as animal selective hypothermia series, suggest that combining selective hypothermia with an endovascular approach could allow rapid induction of therapeutic hypothermia within a time frame meaningful in acute stroke (DeGeorgia, Ohta, Mori, Schwartz)

We wished to test two hypotheses. First, that moderate hypothermia can be induced rapidly in a swine model of focal ischemia through percutaneous catheter technology. Second, that ischemic stroke volumes in the swine model can be reduced through catheter-delivered hypothermia. We utilized an adult porcine model of temporary occlusion of the middle cerebral artery, followed by the percutaneous introduction of an endovascular dual-lumen cooling catheter (Duo-Flo, Thermopeutix Inc. San Diego, CA) to provide an aorta-to-carotid bypass circuit for selective hypothermia ipsilateral to the ischemic territory. We evaluated both the utility and efficacy of this method in a series of animals, alternating normothermic controls with others undergoing intervention with hypothermia during the reperfusion phase.

2.2 Methods

2.2.1 Set up and craniotomy

Animals were used under an approved protocol of the Schulich School of Medicine and Dentistry AUP 2009-079 at Western University. 50 kg adult swine were anesthetized using 1-2 mL subcutaneous Telazol (Fort Dodge, IA), then intubated and maintained with a nitrous oxide-isofluorane mixture. Arterial and central venous monitoring is obtained via bilateral transfemoral 6F sheath placement. A frontal-orbital craniotomy is performed

(Fig 1). This requires a perforator and Hudson brace or other hand drill for 2 burr holes, and Leksell rongeurs to thin the intervening cranium. Kerrison punches are used to connect the burr holes and widen the craniotomy. Once the frontal and temporal dura is exposed, the microscope is used for orbital roof resection.

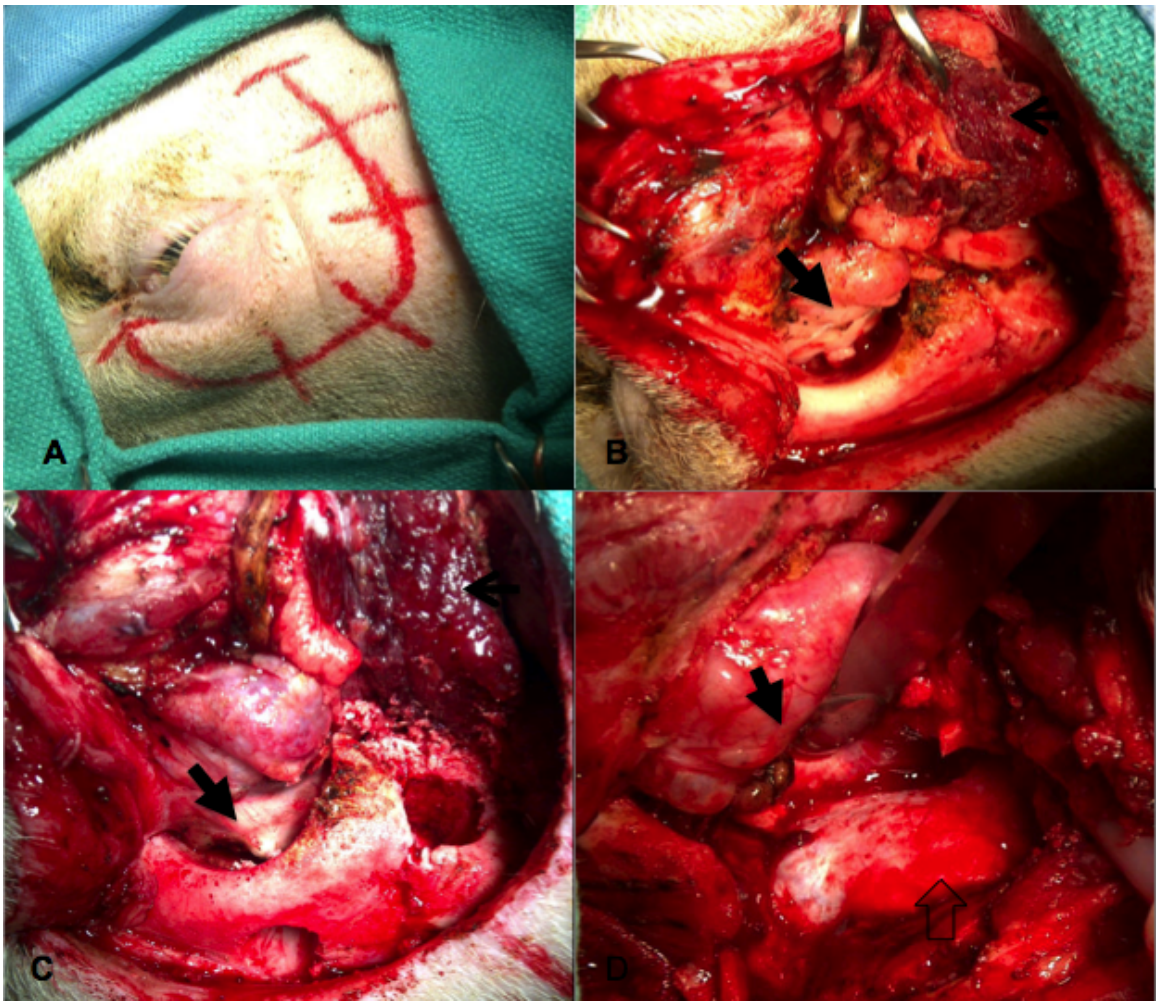

Figure 1Craniotomy. A. Right skin incision extending from the medial orbit superiorly then posteriorly over the calvarium, coursing inferiorly to the zygoma, anterior to the ear. B. Superficial exposure, with temporalis muscle reflected inferiorly, and the periorbita depressed. C. Two burr holes in the skull, with periorbita depressed. An emissary vein is typically found here. D. Full exposure of frontal and temporal dura, with retractor depressing the completely unroofed periorbita. Arrowheads=temporalis muscle, Black arrow = periorbita, Open arrow=dura

Once the orbital roof has been removed, the dura is opened in a cruciate fashion (Fig 2). The arachnoid is opened to release CSF, and the head of the bed elevated to allow maximal brain relaxation without the use of mannitol. Under high magnification, the middle cerebral arteries (MCA) were located along the posterior frontal lobe. Unlike humans, there are 2 or 3 MCA branches arising from the internal carotid artery as it continues anteriorly to become the anterior cerebral artery (Imai). A temporary mini-clip is applied to a single branch. This occlusion is continued for 3 hours. At the conclusion of 3 hours of temporary clipping, the microscope is brought back into the field, and the temporary clip is removed from the MCA branch. The surgical field is then irrigated, and any remaining cottonoids are removed, but Gelfoam is allowed to remain. The skin incision is closed with interrupted nylon suture.

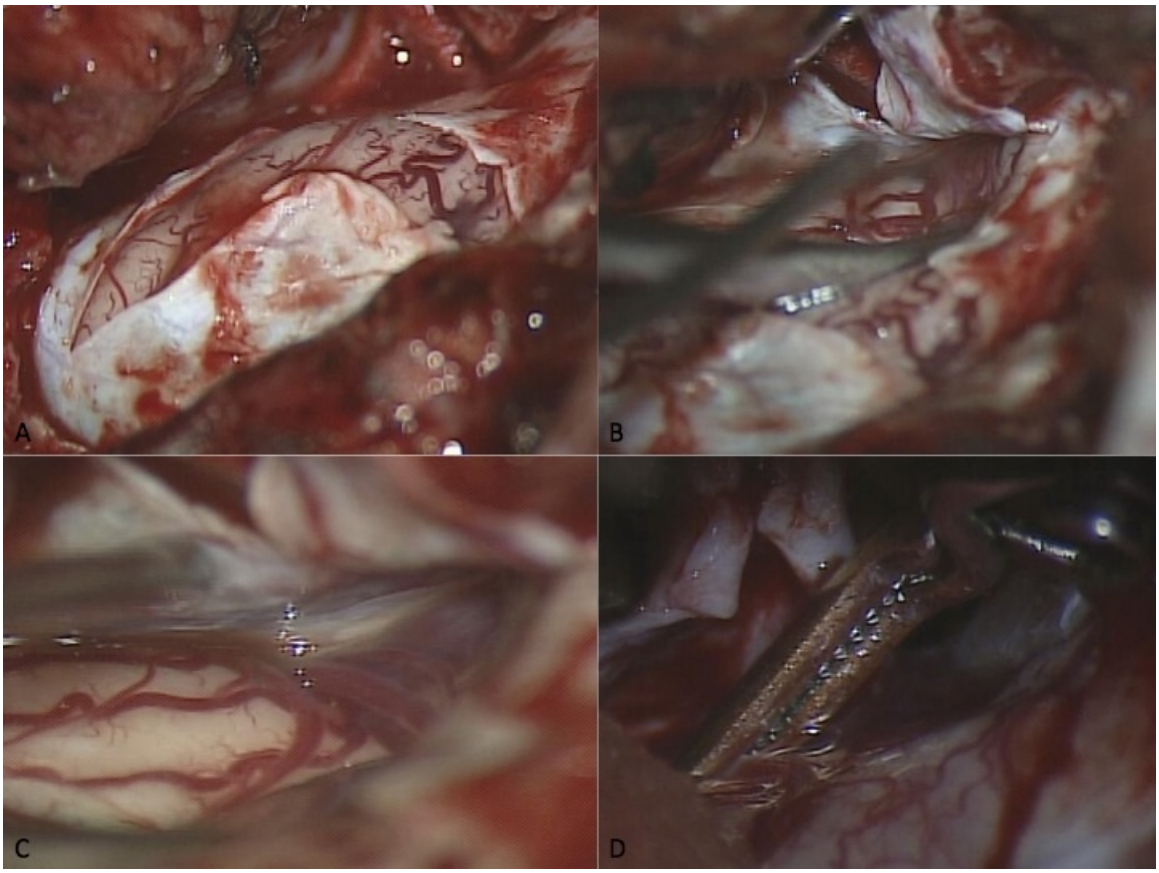

Figure 2 Intradural Exposure A. Microscope view, cruciate dural incision. B and C. Exposure of the MCAs (usually 2-3 are seen). D. Final temporary clip placement across a single MCA.

At this point, there is a 3-hour period of reperfusion. If the animal is a normothermic control, it remains in the lateral decubitus until the last 30 minutes of a 3-hour reperfusion period. If the animal is a hypothermic intervention, it is turned supine immediately on skin closure.

2.2.2 Selective cooling technology

Thermopeutix Inc. (San Diego, CA) provided the catheters used in the study. Prior to the conclusion of temporary clipping, the Thermopeutix catheter is prepared on the backtable. This catheter consists of a 14F Outer Flow Lumen (OFL) which is placed in

the descending aorta, and a 9.5F Inner Flow Lumen (IFL) balloon catheter placed in the ipsilateral common carotid artery (Fig 3). There are two ports on the OFL and a check valve (Fig 4). One port is attached to the outflow portion of an extracorporeal circuit, while the other is for continuous heparinized drip and flushing. The IFL has 3 ports, flushed as usual, and a rotating hemostatic valve which is attached to a heparinized drip. One port is attached to the extracorporeal circuit for infusion of cooled blood. The second port is the balloon inflation lumen, which is prepped with 50:50 contrast and saline. The third port is a pressure transducer distal to the balloon for infusion pressure measurement. All ports are attached to 3 way stopcocks.

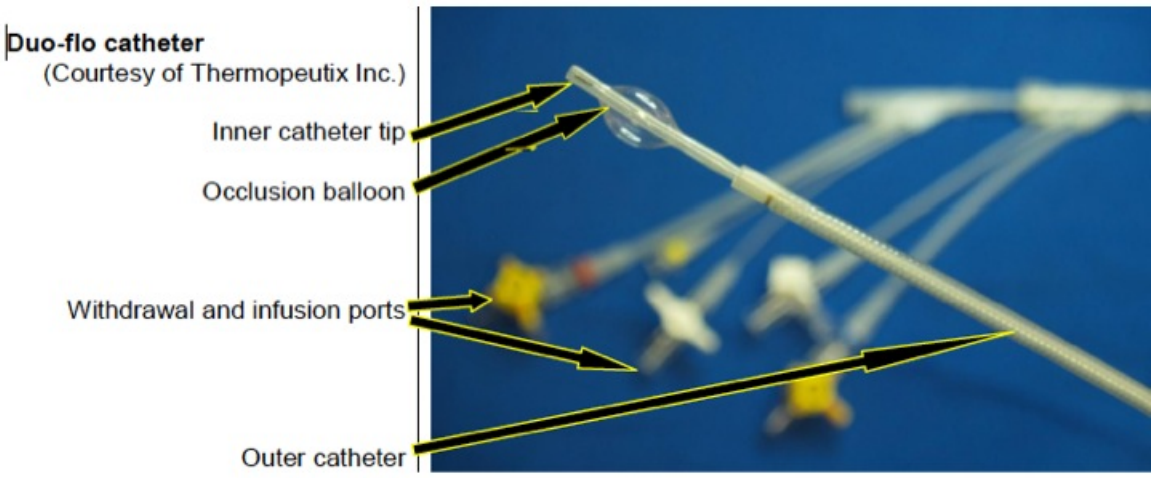

Figure 3 Thermopeutix catheter. Distal end of the inner and outer Duo-Flo catheters showing the aortic outflow catheter with the carotid inflow balloon occlusion catheter

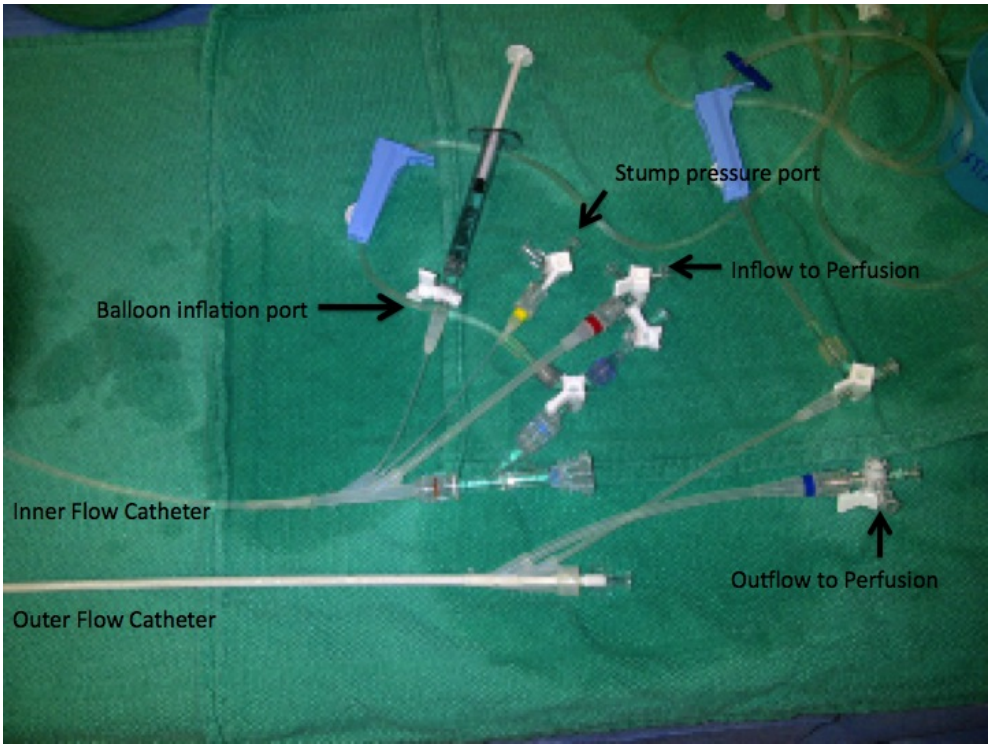

Figure 4 A. Layout of the access ports for the Outflow and Inflow components of the Duo-Flo catheter

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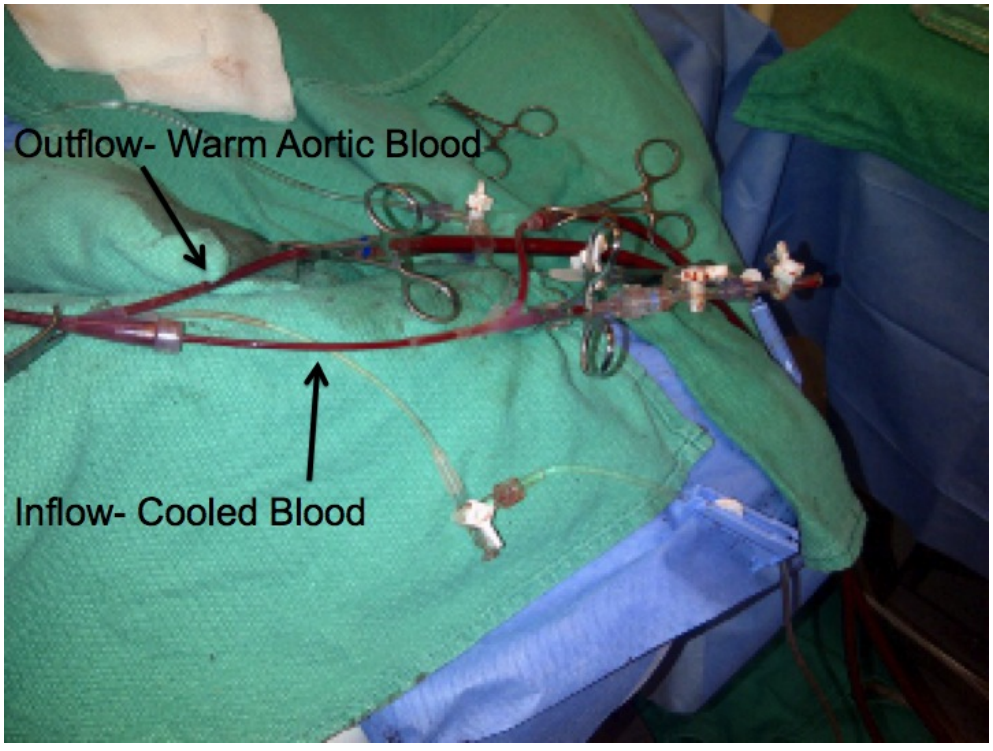

Figure 5 Final setup

2.2.3 Selective Cooling

With the skin incision closed, the pig is then turned supine for the duration of the case. Under continuous fluoroscopy, one of the 6F sheaths is exchanged over a J wire for a 12F Coons dilator, followed by the 14F OFL catheter with its dilator. The OFL is positioned in the thoracic portion of the descending aorta, the dilator and J-wire are removed, and double flushing performed. The Activated Clotting Time (ACT) is doubled by intravenous heparin bolus and maintained at least two times normal for the remainder of the experiment. A 5F diagnostic H1H catheter is introduced over an exchange-length 0.038 Terumo Glidewire through the OFL and navigated into the right common carotid artery. The H1H diagnostic catheter is exchanged for the IFL. Once positioning is satisfactory, the OFL outflow port and IFL inflow port are attached to the extracorporeal circuit. Extracorporeal circulation is established with a starting temperature of 25°C (Fig 5). The balloon is then inflated under fluoroscopy until occlusion (Fig 6). Pressure monitoring from the distal end of the IFL and inflow rates at the extracorporeal circuit are checked to ensure that excessive pressures or flow rates are avoided, and that hemodynamic parameters are stable. Inflow temperature is decreased in increments of

5°C to rapidly achieve moderate hypothermia while maintaining hemodynamic stability. The perfusion is terminated once 3 hours of reperfusion has occurred.

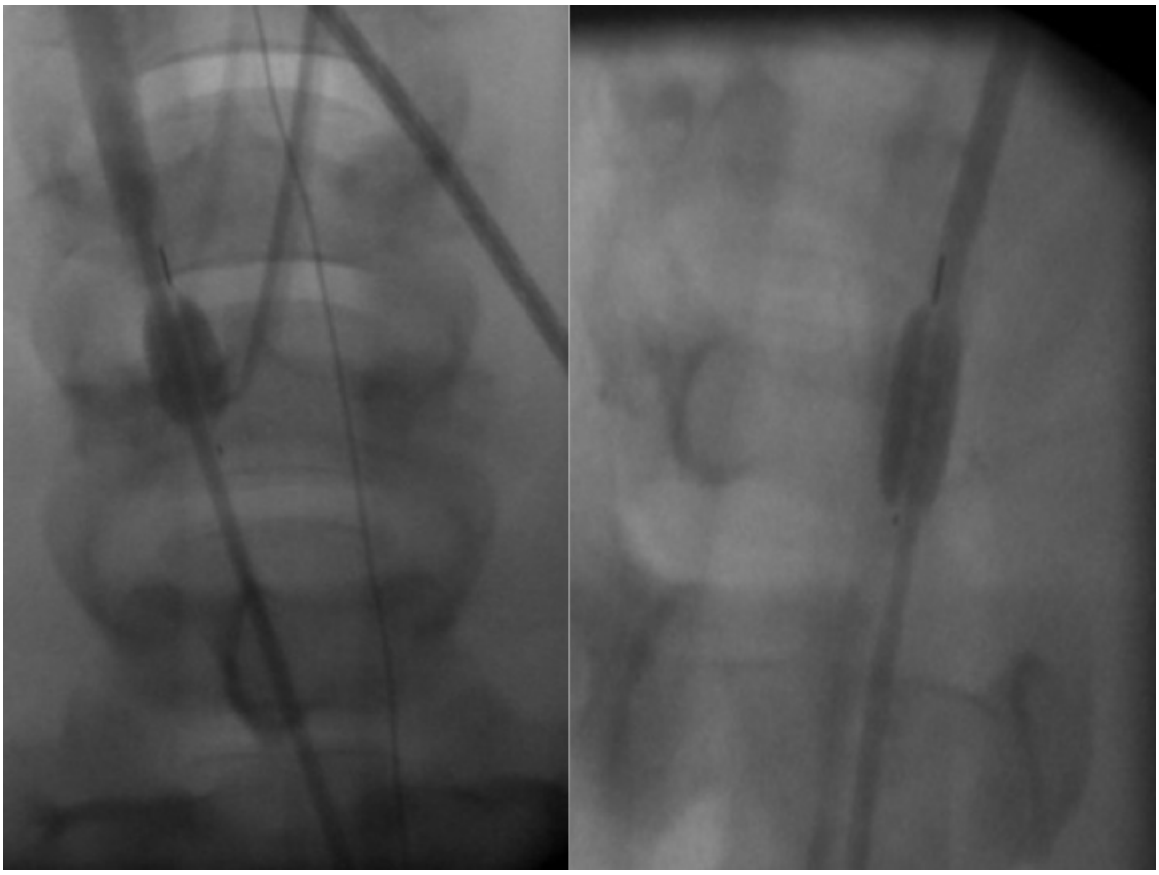

Figure 6 Angiographic view of right and left balloon occlusion catheter with distal contrast stasis during inflation.

2.2.3.1 Temperature Measurements

The core temperature is measured by both rectal and esophageal thermometers. For the most part, rectal temperatures were felt to represent the most accurate core temperature, but in some cases (e.g. Pig #0008) only esophageal temperatures were available. For brain and head temperatures, in an initial case brain and nasal temperatures followed the same trend and were within 2 degrees of the other (Pig #0003, unusable due to cerebral contusion). Thereafter, only nasal temperatures were used because of concern of additional brain trauma and/or bleeding from brain probe insertion during the full anticoagulation required for the endovascular procedure. This is consistent with at least one other selective hypothermia study in the literature (Mori). A cutoff temperature of

30C was chosen. This is significantly below what can be routinely tolerated by humans during whole body cooling (usually 32-34C) prior to cardiac arrhythmia onset.

2.2.3.2 Euthanasia and brain fixation

At the conclusion of the experiment, *in situ* fixation of the brain is achieved using the IFL balloon catheter. The animal is euthanized with 54 mg/kg Euthanyl Forte (Bimeda-MTC, Cambridge, ON). The balloon is inflated, occlusion is confirmed by contrast stasis, and then 500 mL saline, followed by 500 mL 10% neutral buffered formalin (EMD, Baltimore, MD) is infused through the distal port. The catheter is then navigated into the contralateral common carotid artery where the procedure is repeated.

2.2.4 Analysis

The brain is then removed for imaging and histology. The postmortem animal is turned prone and an H-type incision is made in the scalp, centered on the midline. A craniectomy is performed by connecting multiple burr holes with Leksell and Kerrison rongeurs, extending to the vertical walls of the cranial vault. The dura is opened on both sides of the sagittal sinus and reflected back, with the sagittal sinus and falx being divided and reflected. The brain is elevated anteriorly and attachments are divided, working posteriorly. These include the olfactory nerve, the optic and oculomotor nerve, the pituitary stalk, and the brainstem. The brain is lifted out of the cranial vault and placed in a plastic sling with slits in a plastic container filled with 4% Formalin.

2.2.4.1 Neuroradiology

MRI is performed postmortem, and therefore DWI sequences are not possible. T2 volumetric sequences and T1 axial slices are examined by a neuroradiologist blinded to the intervention (Fig. 7). The area of T2 signal abnormality on each slice is calculated using Osirix™, and then all contiguous slices with T2 signal abnormality are summated to give a volume. An identical method of calculating hemispheric volume, excluding the midbrain, is performed, and the ratio of T2 signal abnormality to hemisphere volume is calculated to give a percentage of stroke in the hemisphere.

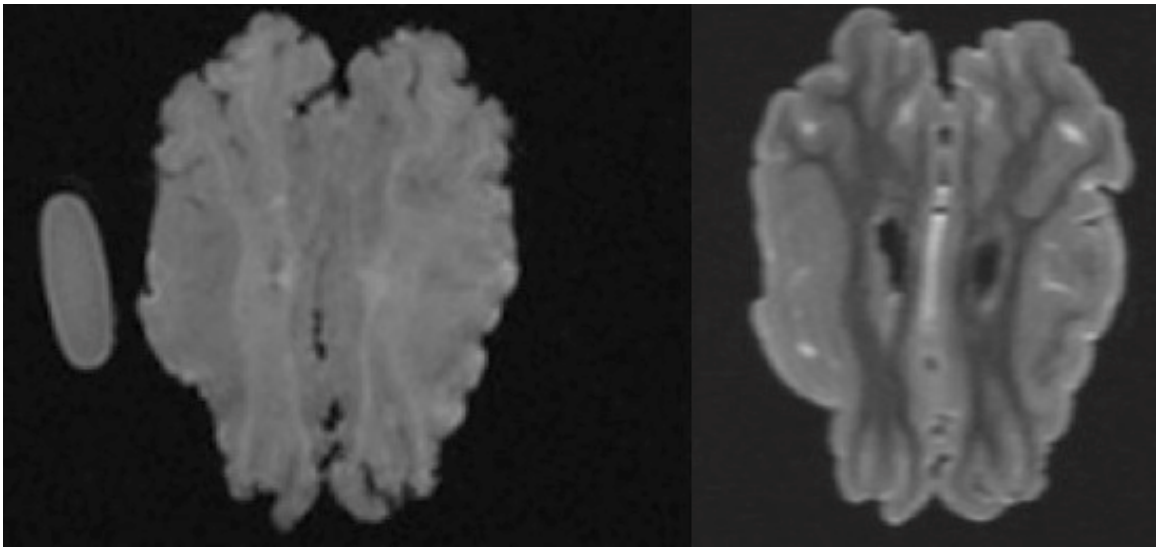

Figure 7.T1 (Left) and T2 (Right) axial MRI images showing ischemic change on the side with the Vitamin E pellet.

2.2.4.2 Neuropathology

The fixed cerebrum is sliced into 1cm thick slices, placed in cassettes, and paraffin wax is applied. Pathological examination is conducted by a blinded neuropathologist. Blocks of coronal slices of the cerebrum were stained with hematoxylin and eosin. This stain was chosen for availability and consistency with histologic studies done in selective hypothermia animal models (Mori). The slices were scanned under light microscopy for evidence of ischemic injury. A definite change was defined as the presence of both dark neuronal change and perineuronal vacuolation. In order to maintain consistency, if only one of the aforementioned changes were present without the other, then this was not regarded as definite ischemic change. Two-dimensional measurements of the affected regions were taken manually and the areas were calculated from those measurements. Volumes of affected region were calculated using the area multiplied by the thickness of the block that showed the defined ischemic change (each block was 1.0 cm thick).

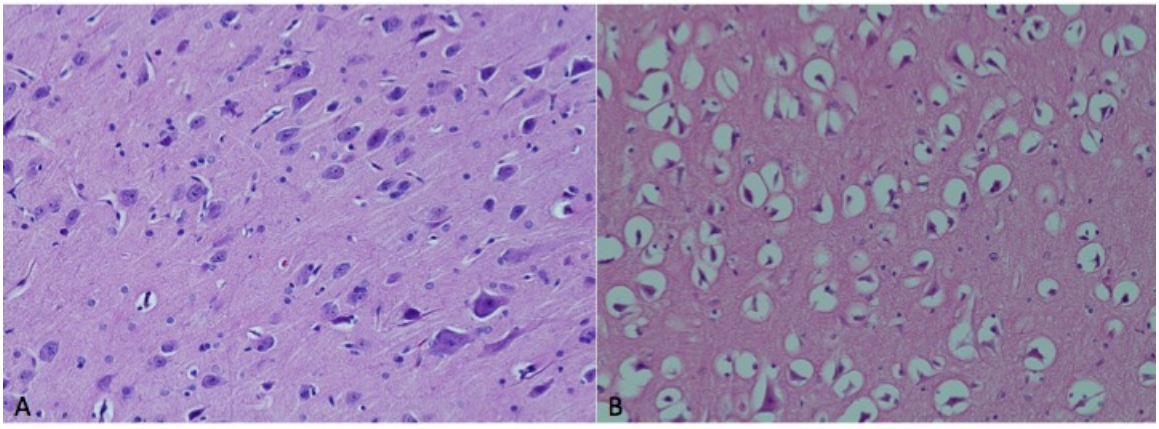

Figure 8 Histopathology A. Normal Swine Neocortex B. Ischemic Swine Neocortex

2.2.4.3 Statistics

Data were analyzed by an independent biostatistician (Mr L. Stitt, MSc, Statistical Services; lwstitt.com). Calculations included means, ranges, standard deviations, and standard error of the mean. MRI results were analyzed and compared between 13 controls and 12 selective hypothermia cases. Pathological results were compared between 11 controls and 10 hypothermia cases. Student's t-test was used for comparison of means. Variances were not equal; therefore a square root transformation was used to improve equality of variances. Non-parametric Wilcoxon 2-sample test was also performed.

2.3 Results

2.3.1 Animals available for analysis

Twenty-eight pigs were utilized. One death (#0025) occurred due to arrhythmia approximately 1 hour into clip occlusion time. Autopsy demonstrated a preexisting pulmonic stenosis and hypertrophic cardiomyopathy. Two pigs (#0003 and #0011) were deemed unusable because of brain contusions and subsequent edema during exposure. Femoral cutdown was required in 1 case (#0021). Advancement of the 14F OFL into the very small femoral artery resulted in aortic dissection. Ultimately, the true lumen was entered and cooling successfully applied cooling for a limited time of 36 minutes. The neuroradiology data is completely analyzed at this time. Histopathology data with statistics is presented for 11 animals in the normothermic group (control), and 10 animals in the hypothermic (neuroprotection) group.

2.3.2 Selective cooling in this model

Temperature trends during selective hypothermia are demonstrated in Fig 8 and 9, and in Table 1. Figure 8 is a typical set of temperature curves generated during selective hypothermia. Core temperature is rectal (or esophageal temperature in #0008). The right and left temperatures are nasal temperatures. Nasal temperatures follow the brain temperature closely, as also observed by others (Mori 2). During hypothermia, the mean ipsilateral (right) temperature dropped from 38°C to 26°C, with contralateral (left) temperature dropping to 31.6°C. Core temperature did drop during the experiment, but never below mild hypothermia levels (32-34°C).

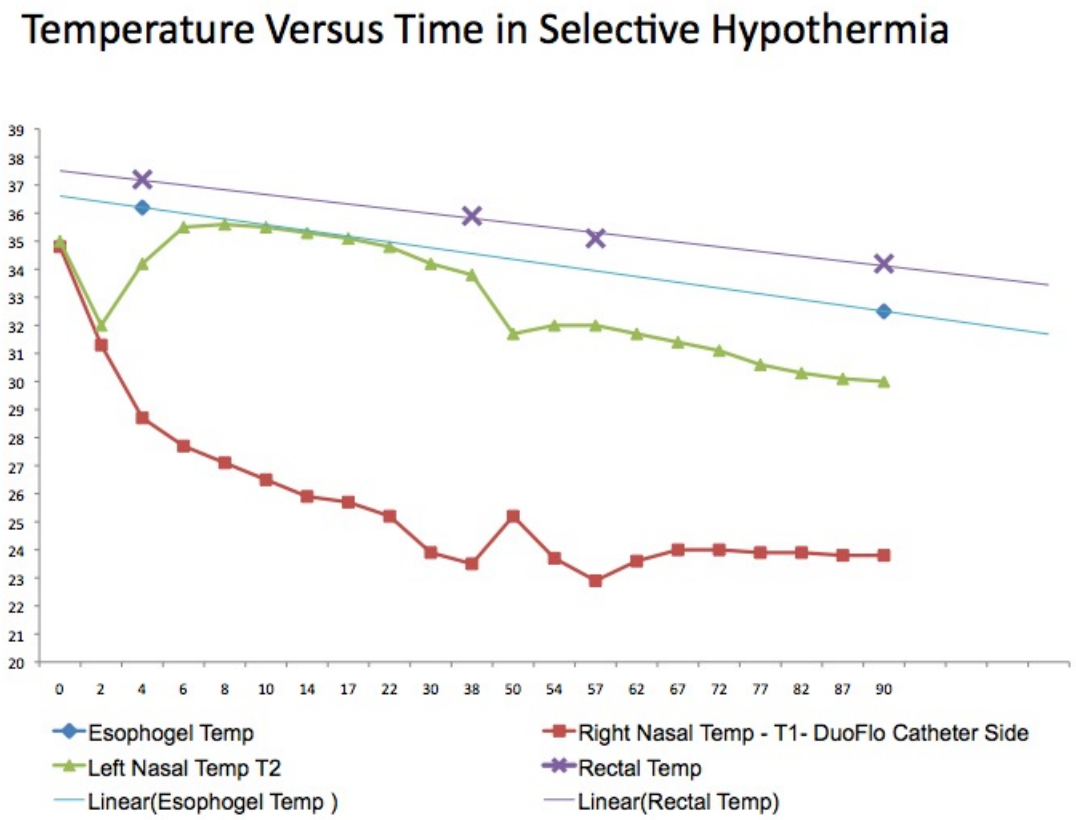

Figure 9. A typical temperature profile during hypothermia

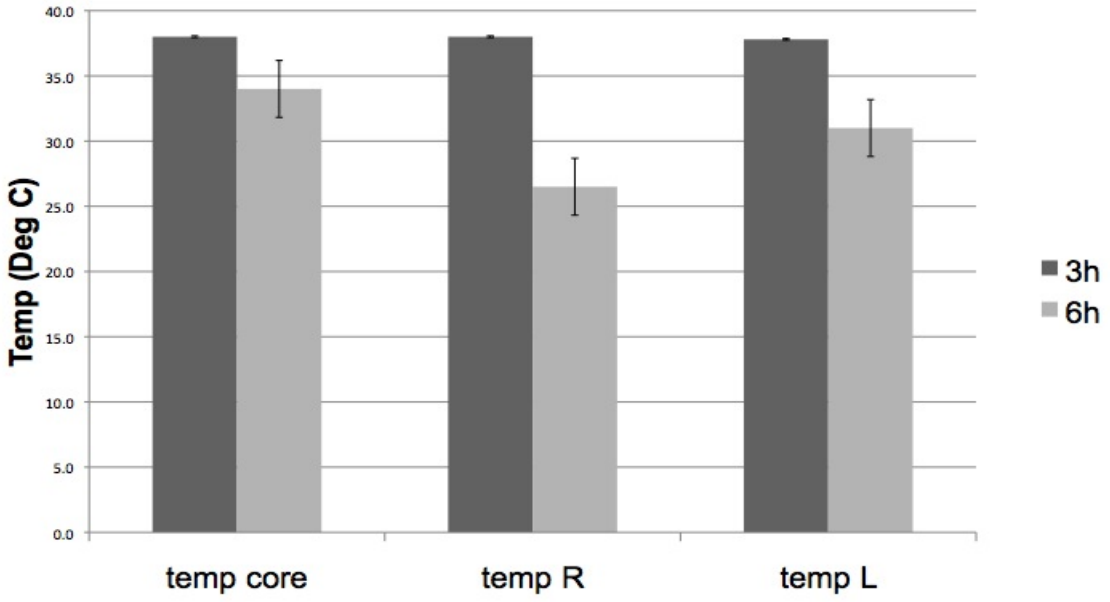

Figure 10.Changes in rectal temperature (temp core), right nasal (temp R) and left nasal (temp L) with hypothermia

	3 hours	6 hours	
Temperature	Mean +/- SEM	Mean +/- SEM	Mean Change
Core	38.0+/-0.2	34.0+/-0.4	4.0
Right	38.0+/-0.2	26.5+/-0.8	11.5

Left	37.8+/-0.3	31.6+/-1.0	6.1

Table 1 Changes in mean temperature in the hypothermia cohort

2.3.3 Metrics related to the model

The design of the experiment attempted to replicate the realities in establishing vascular access and extracorporeal circulation once three hours of ischemia had already taken place. The time needed to set up the system and begin cooling, the overall length of cooling time achieved during the second 3 hour window, and the time to achieve moderate hypothermia are shown in Table 2. We also looked at the lowest temperature recorded ipsilaterally (the temperature nadir). The decision as to the depth of hypothermia was made on clinical grounds, based on the animal's tolerance during the experiment. The average length of time to establish the extracorporeal circulation or bypass was over 1 hour but highly variable. Once extracorporeal circulation was established, moderate hypothermia (less than 30C) could be achieved in less than 30 minutes. Thus the total time from reperfusion to moderate hypothermia was just below an hour and a half, so falling within the 3-4.5 hour window following ischemic insult.

The lowest temperature achieved was often quite lower than what was sustained for the duration of cooling.

	Mean	Range
Set up Time (min)	60.3+/-10.3	22-144
Temp Nadir (^{o}C)	24.4+/-0.5	21.8-27.5
Time to <30^{o}C (min)	25.4+/-5.4	6-65
Total Cooling (min)	109.6 +/- 9.8	36-150
Total Time to <30^{o}C (min)	85.7+/-10.5	47-155

Table 2 Hypothermia therapeutic parameters (mean +/- SEM)

2.3.4 Hemodynamic parameters during hypothermia

We evaluated several hemodynamic parameters during the study, with particular attention to possible instability from the hypothermia protocol. Several hemodynamic parameters, (core temperature, heart rate, mean arterial blood pressure) and several arterial blood gas

data points (oxygen, hemoglobin, glucose, and pH) were evaluated for changes in the hypothermia group versus controls over the course of the experiment. Using analysis of covariance, a significant drop was seen only in core temperature ($p<0.001$) and in pH ($p<0.001$) between controls and hypothermia during the last 3 hours of the experiment.

2.3.5 Selective hypothermia and stroke volume

Stroke volumes determined by MRI are presented below. In Table 3 and Figure 10, the data for the means and standard error of the mean for each group (n=13 control, 12 hypothermia) is presented. The hypothermia group mean (stroke as % of hemisphere volume) is smaller than control, with a wide variance. This reached statistical significance using unpaired Student's t test ($p< 0.05$).

	Control	Hypothermia	P Value
Mean+/-SEM	0.050+/-0.016	0.009+/-0.005	0.042
Median	0.050	0.000	

Table 3 MRI-derived stroke % of hemispheric volume

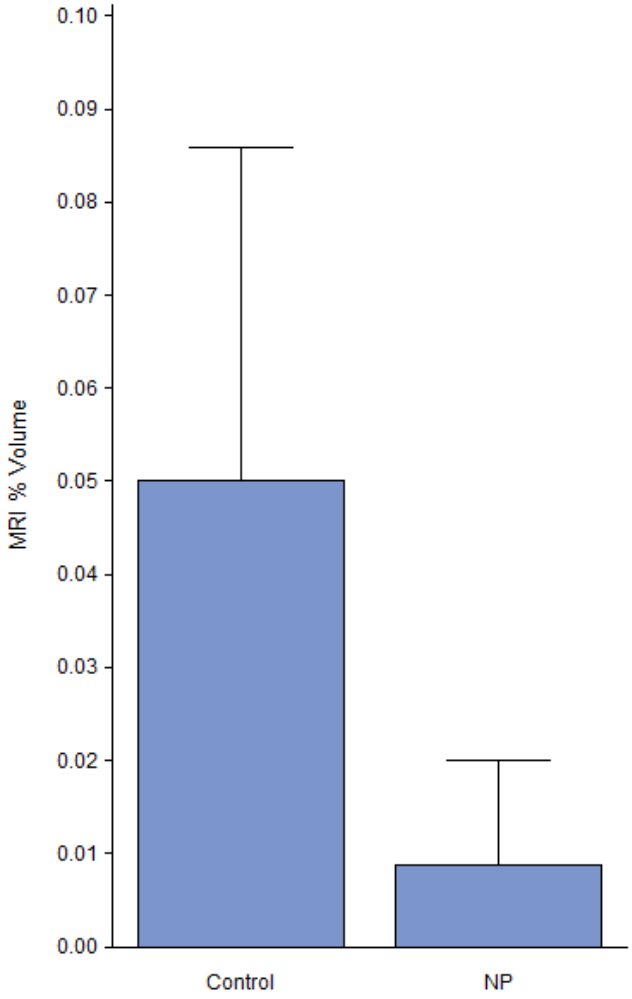

Figure 11 Ratio of stroke volume to hemisphere volume on MRI

Stroke volumes determined by pathology are seen in fig 12 and Table 4. Mean hypothermia total stroke volumes were 61.6% of controls. Mean hypothermia largest stroke volumes were 55.8% of controls. However, in both cases the mean stroke volume difference is not statistically significant, due to the wide variance. We looked for any correlation between duration of cooling and the stroke volume, using two cooling time measures. The first is the total time the perfusion pump was active (actual cooling time). The second is the sum of setup time and time to achieve moderate hypothermia. All r

values were less than 0.25, suggesting no correlation.

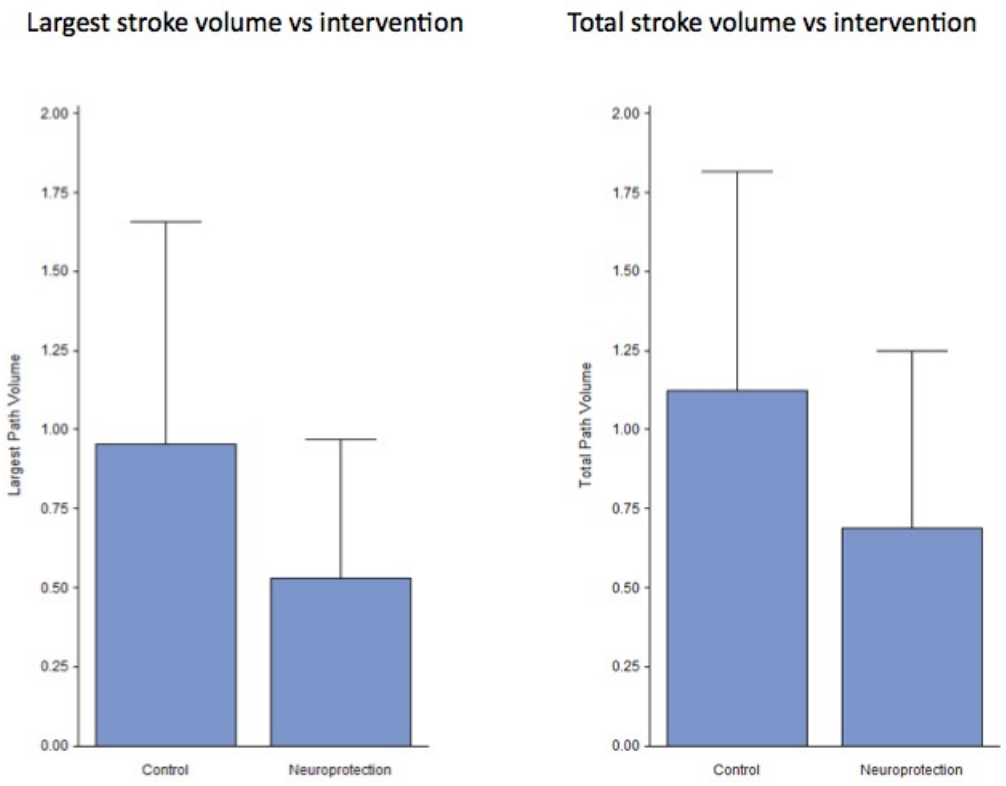

Figure 12. Graphical illustration of mean pathology volumes (expressed as mean +/- standard error)

	Control (n=11)	Hypothermia (n=10)	P Value
Total Path Volume (cm3)	1.12+/-0.31	0.69+/-0.25	0.292
Largest Path Volume (cm3)	0.95+/-0.32	0.53+/-0.19	0.278

Table 4 Pathology-derived stroke volume

2.4 Discussion

2.4.1 Feasibility of rapid-onset moderate hypothermia

This study confirms other work in animals and humans that femoral-to-carotid bypass can rapidly induce hypothermia which is selective to the cranium and particularly to brain tissue. Our mean time to moderate hypothermia (<30C) once the pump was turned on was 25.4 min +/- 5min, and mean final temperature of 26.5°C. Verdura et al were able to cool the brain to 15°C on average by 35 min (dogs), Schwartz achieved 18.5°C in an average of 26 +/- 13 min, and 24.5°C in 12 +/- 6 min (baboons), and Lownie et al were able to drop brain temperature to 26.8°C in 12 minutes (human) (Verdura, Schwartz, Lownie). In the COOLAID trial utilizing endovascular venous cooling, mean time to cooling was 77 +/- 44 minutes to a target of 33°C, which is relatively mild (DeGeorgia). In contrast, whole body surface cooling typically takes hours (deGeorgia, Froehler), and again is limited to 32-34°C typically. In ICTuS-L, median time to target temperature (after catheter placement) was 138.3 +/- 198.9 min

Our experiment is the first to use a purely percutaneous approach to produce selective hypothermia of such a degree. Ice cold saline has been previously reported to reduce temperatures selectively but reductions in a limited safety trial were very modest (Choi). Although cerebral metabolic rate undergoes predictable reduction with hypothermia, it is unknown what degree of hypothermia is necessary to provide meaningful neuroprotection, especially in acute stroke. Some authors suggest that mild hypothermia may be as effective as moderate or deep hypothermia because of the added risk with colder temperatures, but in acute stroke this remains unknown (Yenari). The selective hypothermia method clearly gives reproducible degrees of moderate hypothermia rapidly. Thus, if the degree of hypothermia or the rapidity with which it is introduced are significant variables in determining outcome, then selective hypothermia should produce superior results.

What is clear from our data and that of other groups is that endovascular selective hypothermia is very efficient in attaining moderate hypothermia in the brain. Our data also shows that systemic cooling does occur even with selective hypothermia, which is in line with Mori and Schwartz, in which hypothermic perfusion was continued for 12 hours. The degree of mild systemic hypothermia should be tolerable but measures of this such as arrhythmia frequency was not performed. Another potential consequence of systemic hypothermia, coagulopathy, would be difficult to evaluate as the catheterized animals were heparinized during the procedure. The duration and degree of hypothermia required for potential neuroprotection is still unknown.

Statistically significant drops were seen in core temperature (noted above) and in systemic pH. Other parameters were not significantly affected. The drop in systemic pH may be due to lower extremity ischemia from the catheter, although this hypothesis remains to be tested (Thomas Forbes, MD, personal communication). The relative stability of other factors such as mean arterial blood pressure, blood oxygenation, and hemoglobin between normothermic and hypothermic groups suggests that the process of placing the endovascular device and utilizing extracorporeal perfusion-pump to induce selective hypothermia does not destabilize the animal.

2.4.2 Trend to reduction in stroke volumes

Both by MRI and histopathology, the mean stroke volume was decreased by hypothermia. The decrease in mean stroke volume measured by histology was not statistically significant compared with controls but was statistically significant for the MRI measurements. There is a wide range of stroke volumes in both control and hypothermia animals. There are a number of variables that could account for this, but interestingly, it mirrors the experience of the COOLAID study (DeGeorgia). In COOLAID, hypothermia could be rapidly induced in humans, but a statistically significant reduction in stroke volume (measured as % increase from onset to day 3-5) was not seen. Again, their numbers show a difference in mean between controls (108+/-142.4%, n=11)) and well-cooled hypothermia patients (72.9+/-95.2%, n=8) but there was a large variance. However, in the second Schwartz baboon study, a significant reduction in stroke volumes was seen using FLAIR sequence on MRI. The number of animals in each group was very small (4 each) (Schwartz 2). In animal studies, time to hypothermia is controlled, while in the human COOLAID and ICTuS-L trials, up to 8 hours could pass before even mild hypothermia was achieved, which could diminish or even eliminate any clinical benefit.

2.4.3 Limitations of the study

This study has several limitations. First the choice of experimental animal, while helpful from an endovascular experience standpoint, has limitations under anesthesia. The experiment is long (usually about 8h under anesthesia), and pigs can have cardiac issues that develop under anesthesia. By the end of the experiment, the animals are almost universally on medical blood pressure support. This may have contributed to some of the variability in time to cooling, as several experiments required transiently stopping the cooling process during stabilization of the animal. By experimental design, this occurred late in the course, when the animal had already been under anesthesia for many hours.

Second, we chose to selectively clip only one MCA branch. This makes for a smaller and more focal stroke, but also raises the possibility that intracranial collateral flow reduced

the area of significant damage, contributing to the wide variability of stroke volumes. However, this is not unlike the human acute stroke scenario. Furthermore, we did not confirm that removing the clip actually allowed reperfusion of the ischemic territory. We were able to see blanching at the distal to the clip application site, and then return of flow at the occlusion point following removal of the temporary clip, which suggested return of flow in the vessel, but did not elucidate the degree of reperfusion in the affected territory. Confirmation of patent vessels following temporary occlusion by angiography has been reported (Sakoh). NIRS technology has the potential to noninvasively track cerebral blood flow in the hemisphere (Cooper). Another alternative would be indocyanine green fluorescence angiography, which is used clinically to evaluate blood vessel patency, but requires a special filter on the microscope. Another option would be motor evoked potentials (Schwartz).

A third issue relates to our choice of sequences on MRI. Diffusion weighted imaging is the gold standard for determining a volume of stroke, but requires a living creature. Since MRI was obtained postmortem in this study, DWI was not applicable. Thus we used T1 and T2, but DWI is considered more sensitive (Knight). A future experiment might include placing a living animal during or just after cooling to assess the stroke volume.

Finally, since the occlusion ended at 3 hours, reperfusion began at that time. The experimental results may reflect changes in reperfusion-related damage, rather than stroke damage. We measured stroke volumes accurately, but microscopic effects (changes in local chemical substrates) and macroscopic effects (edema or raised intracranial pressure) due to reperfusion were not evaluated. Thus potential beneficial effects of hypothermia on reperfusion injury might have been missed in this model.

2.4.4 Strengths of the study.

There are three major strengths to this study. First, the imaging and pathology review was conducted by qualified personnel who were completely blinded to the intervention.

These specialists are trained to evaluate stroke in humans, but assuming that stroke should follow similar patterns in pigs, they should be able to provide an unbiased evaluation of stroke volumes. We acknowledge the difficulty in transferring pattern recognition from one species to another, and this may reduce the overall sensitivity of analysis. Nevertheless, we feel that the blinded nature of the analysis allows for the highest chance of unbiased assessment of selective hypothermia in acute stroke.

Second, the design of the study evaluated intervention well after the ischemic insult. The three-hour lag time prior to intervention is consistent with typical presentation time frames in human acute ischemic stroke. While it is known that even delayed hypothermia can reduce stroke volumes, the effect is reduced with delay in treatment and animal studies have only examined the period of 1.5 to 2 hours post ischemia. (Xue, Ji). If hypothermia is to act as a bridge to recanalization, it has to be effective in the time frames of recanalization, which for IV tPA are up to 4.5 hours, and for mechanical thrombectomy may be up to 6 hours. Our study suggests that in the 3-6 hour time frame, adding hypothermia to recanalization can reduce stroke volume but the number needed to show significance may be quite large.

Finally, this represents the largest paired study of selective hypothermia in a large animal model. Table 5 shows comparable series comparing normothermia with hypothermia. Our study is unique in its purely endovascular approach to selective cooling, and thus presents the potential advantages of rapid induction as well as selectivity, through the use of novel interventional stroke technology.

Table 5 Comparison Animal Series

Author	Method	Total N	Normothermia	Hypothermia
Ohta	Bypass			12
Imai	None	16	5 MCAO	
Sakoh	None	15	5	

Schwartz	Bypass	12		12
Schwartz	Bypass	8	4	4
Mori	Bypass	12	6	6
Zhang	Epidural	12	4	6
Verdura	Bypass	21	5	16
Mattingly	Bypass	25	13	12

2.4.5 Future directions

This study raises intriguing questions regarding the efficacy of hypothermia in the face of acute stroke. By its design, we have made assumptions regarding both the development of focal cerebral ischemia in this model and its response to hypothermia. We elected to clip only one MCA branch. It would be useful to assess the effect of clipping all MCA branches, in terms of stroke volume and variability. The timing of clip removal, including whether to remove it at all, is another area worthy of exploration. Hypothermia could be effective in reducing stroke volumes even with no recanalization. Furthermore, the hypothermia group was subjected to a significant change in placing the catheter, including repositioning the animal on its back for the exchange of sheath and perfusion. Potentially one could make the two groups more equivalent by performing the same catheter exchange on the controls without perfusion, or perfuse controls with normothermic blood. This would make any observed differences in outcome more directly related to cooling. Finally, it would be interesting to track the effect of hypothermia on stroke volume using real-time MRI. This would require placing the experimental animal in the MRI at least at the end, if not during, cooling. Logistical hurdles to this are substantial, as the entire operation, including life support and catheter would need to be MRI compatible at least at some point, and transferring an animal to

MRI while on inotropic support might destabilize it, causing a premature end to the experiment.

2.5 Conclusion

This experimental protocol demonstrated the feasibility of a focal cerebral ischemia model in 50kg swine via a transcranial approach, without the need for enucleation of the eye. We also developed a unique method of *in situ* perfusion fixation for the brain without the need for thoracotomy. We demonstrated that moderate selective hypothermia is rapidly achievable through a completely percutaneous endovascular approach and our times and temperatures correspond well with the results of other animal studies of selective hypothermia using varying forms of femoral-to-carotid bypass. Using moderate selective hypothermia, there is a trend toward reduced stroke volume on histopathology, and a statistically significant reduction in stroke volume on MRI, within the 3-6 hour postischemic timeframe. These results are promising, and further study is warranted.

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Appendices

Appendix 1 AUP2009-079

Western

Dec. 7, 2009

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2013

Dear Dr. Lownie:

Your Animal Use Protocol form entitled:
Image-Guided Carotid Artery Intervention and Neuroprotection
Funding Agency Heart and Stroke Foundation - Applied for

has been approved by the University Council on Animal Care. This approval is valid from **December 7, 2009 to December 31, 2010.** The protocol number for this project is **#2009-079**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 4 Years

SPECIES & SECT D.5.1 GROUP ID#	STRAIN &/or OTHER SPECIES DETAIL For Rodents, Also Provide Vendor Stock #	AGE or WEIGHT & SEX	4-YEAR TOTAL ANIMAL NUMBER
Pig **ID#** Group A 15 Pigs Hypthermic Group	Domestic Swine	60-70 kg	15
Pig **ID#** Group B 15 Pigs Normothermic Group	Domestic Swine	60-70 kg	15

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

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Mattingly T, Kole MK, Nicolle D, Boulton M, Pelz D, Lownie SP. "Visual Outcomes for Surgical Treatment of Large and Giant Carotid Ophthalmic Segment Aneurysms: A

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