

# **A Surgical Model of Middle Cerebral Artery Occlusive Stroke in the Sheep**

Adam James Wells

Faculty of Health Sciences, Division of Anatomy and Pathology  
The University of Adelaide

Submitted as part requirement for the degree of Doctor of Philosophy, October 2013

*Dedicated to the memory of my father, Christopher John Wells.  
Gone but never forgotten, your legacy of hard work and discipline will live on  
forever*

*And for my incredible wife Vanessa Rose, and my two beautiful daughters,  
Hannah Rose and Charlotte May*

# **TABLE OF CONTENTS**

<b>ABSTRACT</b>	<b>IV</b>
<b>AUTHOR CONTRIBUTIONS</b>	<b>VII</b>
<b>ACKNOWLEDGEMENTS</b>	<b>IX</b>
<b>ABBREVIATIONS</b>	<b>XI</b>
<b>STYLE CONVENTIONS</b>	<b>XIV</b>
<b>FINANCIAL SUPPORT</b>	<b>XIV</b>
<b>LIST OF TABLES</b>	<b>XV</b>
<b>LIST OF FIGURES</b>	<b>XV</b>
<b>1. INTRODUCTION</b>	<b>1</b>
1.1 STROKE	1
1.1.1 HISTORY	1
1.1.2 CLINICAL MANIFESTATIONS AND BASIC NEUROVASCULAR ANATOMY	2
1.1.3 EPIDEMIOLOGY AND RISK FACTORS	7
1.1.4 ISCHAEMIC STROKE	9
1.1.5 PATHOPHYSIOLOGY OF CEREBRAL ISCHAEMIA	9
1.1.6 NORMAL BRAIN METABOLISM AND SUBSTRATE TRANSPORT	10
1.1.7 MEASURING CEREBRAL BLOOD FLOW	12
1.1.8 CEREBRAL HYPOXIA AND PbtO <sub>2</sub>	15
1.1.9 THE PENUMBRA CONCEPT	18
1.1.10 CELLULAR RESPONSE TO INTERRUPTED ARTERIAL BLOOD FLOW	20
1.1.11 THE BIG PICTURE: NEUROIMAGING OF ACUTE ISCHAEMIA	21
1.1.12 BRAIN TOLERANCES TO ISCHAEMIA	23
1.1.13 TEMPORARY CLIP OCCLUSION IN NEUROVASCULAR SURGERY	24
1.1.14 STROKE TREATMENT CONCEPTS	27
1.1.15 THE BLOOD-BRAIN BARRIER, CEREBRAL OEDEMA, THE MONRO-KELLIE DOCTRINE AND RAISED INTRACRANIAL PRESSURE	30
1.1.16 MALIGNANT MCA STROKE	36
1.1.17 REPERFUSION INJURY AND HAEMORRHAGIC TRANSFORMATION	42
1.1.18 CURRENT THERAPEUTIC GUIDELINES	43
1.1.18.1 STANDARD MEDICAL THERAPY	44
1.1.18.2 REPERFUSION THERAPIES	45
1.1.18.3 NEUROPROTECTION THERAPIES	46
1.1.18.4 SURGERY AND MANAGEMENT OF ISCHAEMIC CEREBRAL OEDEMA	46
1.1.19 SUMMARY	47
1.2 STROKE RESEARCH	47
1.2.1 HISTORY OF STROKE RESEARCH	48
1.2.2 ANIMAL MODELS OF CEREBRAL ISCHAEMIA	50
1.2.3 OTHER MODELS	56
1.2.4 FAILINGS AND RESEARCH PRIORITIES	57
1.2.5 POTENTIAL TARGETS FOR FUTURE THERAPIES	61
1.2.6 SUMMARY	62
<b>2. AIMS AND HYPOTHESES</b>	<b>63</b>
2.1 AIMS	63
2.2 HYPOTHESES	64
<b>3. PRELIMINARY EXPERIMENTAL DESIGN</b>	<b>65</b>
<b>4. EXPERIMENTS: MANUSCRIPTS PUBLISHED OR BEING CONSIDERED FOR PUBLICATION</b>	<b>70</b>
<b>4.1 A SURGICAL MODEL OF PERMANENT AND TRANSIENT MIDDLE CEREBRAL ARTERY STROKE IN THE SHEEP</b>	<b>70</b>

4.1.1 ABSTRACT	72
4.1.2 INTRODUCTION	74
4.1.3 MATERIALS AND METHODS	75
4.1.3.1 EXPERIMENTAL PROCEDURE	75
4.1.3.2 ANIMALS AND EXPERIMENTAL DESIGN	75
4.1.3.3 SURGICAL APPROACH TO THE MCA	76
4.1.3.3.1 NEUROVASCULAR ANATOMY	77
4.1.3.3.2 MCA OCCLUSION	78
4.1.3.4 HISTOLOGICAL EXAMINATION	79
4.1.3.5 MAGNETIC RESONANCE IMAGING	79
4.1.3.3.6 STATISTICAL ANALYSIS	80
4.1.4 RESULTS	80
4.1.4.1 SURGERY	80
4.1.4.2 PHYSIOLOGICAL PARAMETERS	80
4.1.4.3 POST MORTEM AND GROSS PATHOLOGICAL CHANGES	81
4.1.4.4 HISTOPATHOLOGY AND INFARCT AREA	82
4.1.4.5 MAGNETIC RESONANCE IMAGING	82
4.1.5 DISCUSSION	83
4.1.6 CONCLUSIONS	91
4.1.7 ACKNOWLEDGEMENTS	91
4.1.8 FIGURES	92
4.1.9 SUPPORTING INFORMATION	100
<b>4.2 REGIONAL BRAIN TISSUE OXYGEN RESPONSE TO TEMPORARY ANEURYSM CLIP OCCLUSION OF THE PROXIMAL MIDDLE CEREBRAL ARTERY</b>	<b>101</b>
4.2.1 ABSTRACT	104
4.2.2 INTRODUCTION	105
4.2.3 METHODS	107
4.2.3.1 EXPERIMENTAL PROCEDURE	107
4.2.3.2 ANIMALS AND EXPERIMENTAL DESIGN	107
4.2.3.3 STATISTICAL ANALYSIS	108
4.2.4 RESULTS	109
4.2.4.1 SURGERY AND BASIC PHYSIOLOGICAL PARAMETERS	109
4.2.4.2 PBT <sub>2</sub>	109
4.2.3.3 HISTOPATHOLOGY	110
4.2.4 DISCUSSION	111
4.2.5 CONCLUSIONS	116
4.2.6 ACKNOWLEDGEMENTS	117
4.2.7 DISCLOSURE/CONFLICT OF INTEREST	117
4.2.8 TITLES AND LEGENDS TO FIGURES	118
<b>4.3 MALIGNANT MIDDLE CEREBRAL ARTERY STROKE IN AN OVINE MODEL: RAISED INTRACRANIAL PRESSURE FOLLOWING PERMANENT BUT NOT TRANSIENT PROXIMAL MIDDLE CEREBRAL ARTERY OCCLUSION</b>	<b>123</b>
4.3.1 ABSTRACT	125
4.3.2 INTRODUCTION	126
4.3.3 MATERIALS AND METHODS	128
4.3.3.1 EXPERIMENTAL PROCEDURE	128
4.3.3.2 ANIMALS AND EXPERIMENTAL DESIGN	128
4.3.3.3 MAGNETIC RESONANCE IMAGING	129
4.3.3.4 HISTOLOGICAL EXAMINATION	129
4.3.3.5 STATISTICAL ANALYSIS	130
4.3.4 RESULTS	131
4.3.4.1 SURGERY AND BASIC PHYSIOLOGY	131
4.3.4.2 INTRACRANIAL PRESSURE	131
4.3.4.3 BRAIN TISSUE OXYGEN	132
4.3.4.4 MRI	132
4.3.4.5 TTC AND HISTOLOGY	133

4.3.5 DISCUSSION	135
4.3.6 CONCLUSIONS	140
4.3.7 DISCLOSURE/CONFLICT OF INTEREST	140
4.3.8 FIGURES AND LEGENDS	141
<b>4.4 INVASIVE ARTERIAL AND INTRACRANIAL PRESSURE MONITORING USING A MILLAR TYPE STRAIN GAUGE TIP PRESSURE TRANSDUCER</b>	<b>151</b>
4.4.1 ABSTRACT	152
4.4.2 INTRODUCTION	152
4.4.3 METHOD	154
4.4.4 FIGURES	158
<b>5. GENERAL DISCUSSION</b>	<b>160</b>
5.1 THE SHEEP AS A MODEL OF MCAO STROKE	160
5.2 COMPARISONS WITH EXISTING MODELS	164
5.3 LIMITATIONS OF THE CURRENT MODEL	167
5.4 METHODS AND DURATION OF ARTERIAL OCCLUSION	169
5.5 DEVELOPING A 24-HOUR MODEL OF MCAO	172
5.6 ANAESTHESIA AND NEUROPROTECTION	174
5.7 MALIGNANT MCA STROKE	176
5.8 REGIONAL TISSUE OXYGENATION IN MCAO	178
5.9 PROPOSED USES FOR THE CURRENT MODEL	181
5.10 FUTURE DIRECTIONS	183
<b>6. CONCLUSIONS</b>	<b>186</b>
<b>7. APPENDICES</b>	<b>187</b>
7.1 PUBLICATIONS	187
7.2 PAPERS PREPARED AND/OR SUBMITTED FOR PUBLICATION	187
7.3 PRESENTATIONS AT SCIENTIFIC MEETINGS	188
7.4 PRIZES	189
7.5 TECHNIQUES	190
7.5.1 TTC PREPARATION AND USE	190
7.5.2 MODIFIED SWANSON CALCULATION FOR CALCULATING STROKE VOLUME	190
<b>8. REFERENCES</b>	<b>192</b>

## **ABSTRACT**

Background: Stroke is an acute neurological injury secondary to vascular pathology, and is the second biggest killer of Australians and the leading cause of adult disability. The rationale of current therapy for occlusive stroke is rapid reperfusion of the ischaemic brain to limit the size of the injury. However, there are no standard neuroprotective therapies that have proven to be beneficial in clinical stroke, despite in excess of 1000 novel drugs showing promise in preclinical rodent studies. The consistent failure of clinical translation in rodent models suggests that they are perhaps not the best choice to simulate the intracranial pathophysiological changes that occur following human cerebral ischaemia, and that a better representative animal model with similar neuroanatomical features is required. Small ruminants such as the sheep have proven to be valuable in traumatic brain injury models, and a surgical model of permanent middle cerebral artery occlusion (MCAO) has recently been developed in the sheep. However, the existing model has a number of shortcomings and is in need of further characterisation before its widespread use in preclinical testing. The aim of this study was therefore to characterise the pathophysiological and radiological response to both temporary and permanent MCAO using a sheep model.

Methods: Several different studies were performed. In the first to determine the feasibility of the project, 18 adult male and female Merino sheep were randomised to sham surgery (n=6), permanent MCAO (n=6) or 2 h temporary MCAO (n=6), and animals had intracranial pressure (ICP) and regional brain tissue oxygen (PbtO<sub>2</sub>) monitored for 4 h. 6 further animals had magnetic resonance imaging (MRI) after permanent (n=3) or temporary (n=3) MCAO. In the second study, 10 adult Merino sheep were randomised to sham surgery (n=5) or temporary MCAO (n=5), with continuous monitoring of PbtO<sub>2</sub> to determine the relationship between duration of temporary MCAO and the development of regional hypoxia. In the third study, 28 adult female Merino sheep were randomised to sham surgery (n=6), permanent

MCAO (n=10) or temporary MCAO (n=12), and monitored for 24 h under light general anaesthesia. MRI was performed in 12 animals (permanent MCAO n=6, temporary MCAO n=6). Stroke volume was calculated after staining fresh brains with 2,3,5-triphenyltetrazolium chloride (TTC).

Results: The first study demonstrated the feasibility of performing surgical MCAO, with significantly larger ischaemic lesion areas on histology and MRI following permanent versus temporary occlusion. The second study demonstrated that  $P_{btO_2}$  fell from a mean baseline of  $45.0 \pm 14.1$  mmHg to a predefined hypoxic threshold of 15 mmHg after  $42.4 \pm 11.2$  minutes of temporary MCAO, at a rate of 1.3 mmHg/min. The third study showed a significantly elevated ICP, infarct volumes of  $27.4 \pm 6.4\%$ , evidence of space occupying cerebral oedema on MRI and a 30% mortality rate following permanent MCAO monitored for 24 h.

Conclusions: A surgical model of temporary and permanent proximal MCAO stroke has been developed in the sheep. The response of the sheep brain to cerebral ischaemia shares many features with the human brain, particularly following permanent proximal occlusion and the development of space occupying cerebral oedema. The sheep as a representative model of human occlusive stroke appears highly promising for use in preclinical testing, for drugs that demonstrate efficacy in the sheep model may be more likely to successfully translate to clinical stroke.

## DECLARATION

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Adam James Wells and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent for this copy of my thesis when deposited in the University Library, being available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

1. **Wells A**, Vink R, Blumbergs P, Brophy B, Helps S, Knox S, Turner R. A Surgical Model of Permanent and Transient Middle Cerebral Artery Stroke in the Sheep. *PLoS One*, 2012; 7(7):e42157. doi: 10.1371/journal.pone.0042157. Epub 2012 Jul 27

Adam James Wells

October, 2013



## **AUTHOR CONTRIBUTIONS**

The following people have contributed to authorship of the manuscripts enclosed in this thesis (in alphabetical order): Peter C Blumbergs, Brian P Brophy, Stephen C Helps, Stephen J Knox, Anna V Leonard, Renée J Turner, Robert Vink.

The individual contributions of each author can be summarised as:

Conceptualisation of the work: AJW, RJT, RV, SCH.

Realisation of the work: AJW, RJT, SCH, RV, AVL.

Documentation of the work: AJW, RJT, RV, SCH, BPB, PCB, SJK.

I give my consent for any manuscript(s) in which I am a co-author to be included in this thesis:

Peter C Blumbergs

Brian P Brophy

Stephen C Helps

Stephen J Knox

Anna V Leonard

Renée J Turner

Robert Vink

## **ACKNOWLEDGEMENTS**

There are many people who have contributed significantly to this study over the past three years. First I need to thank my three supervisors, Robert Vink, Renée Turner and Stephen Helps, as well as all of the staff and students of the Adelaide Centre for Neuroscience Research “Team Neuro”. It was Professor Vink’s principle supervision and direction, Dr Turner’s vision, hard work and dedication, and Dr Helps’ considerable experience in animal experimentation and data analysis, that got this project off the ground.

The staff at LARIF at Gilles Plains, headed by Tim Kuchel and Loren Matthews, need high praise in helping to establish the sheep stroke surgery, particularly the overnight experiments and radiology. Diana Pilkington from the Royal Adelaide Hospital MRI department must take credit for establishing the imaging protocol and for performing the studies, and radiologist Steve Knox was instrumental in their interpretation. Establishing an overnight protocol for maintaining a large animal under general anaesthesia was akin to running an intensive care unit and was not an easy task, however was made a lot easier with the help of intensivist Matt Maiden who was concurrently undertaking his own PhD also in a sheep model.

Staff from the Royal Adelaide Hospital, The Memorial Hospital and the Wakefield Hospital were extremely helpful in aiding the acquisition of consumables and instruments vital to the success of the experiments. Neurosurgeon Brian Brophy assisted in establishing the surgical approach and with the interpretation of brain tissue oxygen data, and anaesthetist Tony Barnard helped greatly in establishing an inhalational anaesthetic protocol to avoid the neuroprotective qualities of isoflurane.

Jim Manavis’ staff and laboratory in SA Pathology were excellent in tissue preparation, cutting and staining. Peter Blumbergs’ interpretation of the microscopy was insightful, as were his contributions to the stroke model manuscripts.

The amazing artwork from the first stroke model and the arterial blood pressure methodology manuscripts were produced with flair and aplomb by Joshua Burton. Further artwork and figure preparation were contributed to by Tavik Morgenstern. Chris Leigh was incredibly helpful in establishing a protocol for creating a vascular cast of the sheep cerebral arteries; although this never made it into the final thesis, it proved to be a powerful method that would benefit from further exploration.

Neurosurgeon and President of the Neurosurgical Research Foundation, Brian North, provided the support for me to convert my candidature to a PhD. The generosity of my scholarship sources, the NRF, the Neurosurgical Society of Australasia and the National Health and Medical Research Council, will not be forgotten. Further encouragement from neurosurgeon Peter Reilly helped both stimulate my work and inspire an academic surgical career.

In addition, there are surely people who have helped the success of this project who I haven't named above, and to all those people I say thank you.

Finally I must thank my family for their unconditional love and support, and especially for putting up with my long absences from home whilst performing the overnight sheep experiments.

## **ABBREVIATIONS**

ACA – Anterior cerebral artery

AComA – Anterior communicating artery

ADP – Adenosine diphosphate

AMP – Adenosine monophosphate

ATP – Adenosine triphosphate

ATPases – ATP hydrolases

BBB – Blood-brain barrier

Ca<sup>2+</sup> – Calcium

CBF – Cerebral blood flow

CCT – Central conduction time

CMR – Cerebral metabolic rate

CMRGlc – Cerebral metabolic rate of glucose

CMRO<sub>2</sub> – Cerebral metabolic rate of oxygen

COW – Circle of Willis

CPP – Cerebral perfusion pressure

CSF – Cerebrospinal fluid

CVA – Cerebrovascular accident

CVD – Cerebrovascular disease

CT – Computerised tomography

CTP – Computerised tomography perfusion

DVT – Deep venous thrombosis

DWI – Diffusion weighted imaging

EEG – Electroencephalogram

GLUT – Glucose transporter protein

ICA – Internal carotid artery

ICP – Intracranial pressure

LMWH – Low molecular weight heparin

MAC – Minimum alveolar concentration

MAP – Mean arterial pressure

MCA – Middle cerebral artery

MCAO – Middle cerebral artery occlusion

MRI – Magnetic resonance imaging

Na<sup>+</sup> – Sodium

NIHSS – National Institutes of Health Stroke Scale

NMDA – *N*-methyl-d-aspartate

NO – Nitric oxide

NOS – Nitric oxide synthase

PbtO<sub>2</sub> – Partial pressure of brain tissue oxygen

PbtCO<sub>2</sub> – Partial pressure of brain tissue carbon dioxide

PCA – Posterior cerebral artery

PComA – Posterior communicating artery

PDM – Perfusion diffusion mismatch

PE – Pulmonary embolism

PET – Positron emission tomography

PWI – Perfusion weighted imaging

rt-PA – Recombinant tissue plasminogen activator

SPECT – Single photon emission computerised tomography

SSEP – Somatosensory evoked potentials

T1WI – T1-weighted imaging

T2WI – T2-weighted imaging

TBI – Traumatic brain injury

TCD – Transcranial Doppler

THAM – tris-hydroxy-methyl-aminomethane

t-PA – Tissue plasminogen activator

TTC – 2,3,5-triphenyltetrazolium chloride

## **STYLE CONVENTIONS**

The abbreviations, punctuations and reference style used in this thesis conform with the guidelines of the *AMA Manual of Style* and the *Style Manual*. The spelling is Australian English and conforms with *The Australian Concise Oxford Dictionary*, except for manuscripts submitted to scientific journals in which spelling is American English.

## **FINANCIAL SUPPORT**

This work was supported by research scholarships from the Neurosurgical Research Foundation (<http://www.nrf.com.au>), the Neurosurgical Society of Australasia (<http://www.nsa.org.au>), and the National Health and Medical Research Council of Australia (<http://www.nhmrc.gov.au>) Dora Lush Biomedical Postgraduate Fellowship (grant number APP1017721).



## LIST OF TABLES

Table 4.1.1: Physiological data.....	100
Table 4.2.1: Individual PbtO <sub>2</sub> and stroke volume data, MCAO group.....	122
Table 4.3.1: MRI characteristics and TTC stroke volume at 24 hours after MCAO.....	150

## LIST OF FIGURES

Figure 1.1: The circle of Willis.....	3
Figure 1.2: Distribution of the MCA .....	5
Figure 1.3: The Integra™ Licox® system .....	17
Figure 1.4: The ischaemic penumbra.....	19
Figure 1.5: MRI of acute ischaemic stroke.....	23
Figure 1.6: Intracranial pressure waveform .....	33
Figure 1.7: Intracranial pressure/volume curve .....	35
Figure 1.8: Malignant MCA stroke.....	36
Figure 1.9: Outcome after decompressive craniectomy for malignant MCA stroke.....	41
Figure 1.10: Approach to the MCA in and neurovascular anatomy of the sheep.....	55
Figure 1.11: The initial STAIR recommendations .....	58
Figure 4.1.1: Surgical approach to the proximal MCA .....	92
Figure 4.1.2: Neurovascular anatomy of the sheep, anterior circulation, inferolateral view, right side .....	93
Figure 4.1.3: ICP after surgical MCAO, first 4 hours .....	94
Figure 4.1.4: PbtO <sub>2</sub> after surgical MCAO, first 4 hours .....	95
Figure 4.1.5: Histopathology .....	96
Figure 4.1.6: Histopathology .....	97
Figure 4.1.7: Infarct area at 4 hours.....	98
Figure 4.1.8: MRI at 4 hours after MCAO, coronal orientation.....	99
Figure 4.2.1: Mean PbtO <sub>2</sub> , 5-minute intervals, temporary MCAO versus sham .....	118
Figure 4.2.2: PbtO <sub>2</sub> response to temporary occlusion.....	119
Figure 4.2.3: PbtO <sub>2</sub> decline following clip occlusion .....	120
Figure 4.2.4: Histopathology, coronal stack .....	121
Figure 4.3.1: Mean ICP, 24 hours.....	141
Figure 4.3.2: Mean PbtO <sub>2</sub> following MCAO or sham, 24 hours .....	142
Figure 4.3.3: MRI findings at 24 hours, MRA and DWI.....	143
Figure 4.3.4: MRI findings at 24 hours, T1 and T2 weighted imaging .....	144
Figure 4.3.5: TTC at 24 hours, coronal stack .....	145
Figure 4.3.6: Histopathology, coronal section .....	146
Figure 4.3.7: Histopathology, H&E, x200.....	147
Figure 4.3.8: Caspase-3 immunostain for apoptosis, x100.....	148
Figure 4.4.1: Configuration of femoral artery catheter and connectors.....	158
Figure 4.4.2: Waveform measurements .....	159
Figure 5.1: Flow profiles after temporary MCAO.....	171