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

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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

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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 ($PbtO_2$) 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 $PbtO_2$ 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 $PbtO_2$ fell from a mean baseline of 45.0 +/- 14.1mmHg to a predefined hypoxic threshold of 15mmHg after 42.4 +/- 11.2 minutes of temporary MCAO, at a rate of 1.3mmHg/min. The third study showed a significantly elevated ICP, infarct volumes of 27.4 +/- 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.

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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

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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

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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.

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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^{2+} – Calcium

CBF – Cerebral blood flow

CCT – Central conduction time

CMR – Cerebral metabolic rate

CMRGlc – Cerebral metabolic rate of glucose

$CMRO_2$ – 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^+ – Sodium

NIHSS – National Institutes of Health Stroke Scale

NMDA – *N*-methyl-d-aspartate

NO – Nitric oxide

NOS – Nitric oxide synthase

$PbtO_2$ – Partial pressure of brain tissue oxygen

$PbtCO_2$ – 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.

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