Development of Novel Pharmacological Treatments for Intracranial Pressure Using Appropriate Experimental Models of Traumatic Brain Injury

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Doctor of Philosophy

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution in my name 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Dedication

To those who give love and support to children and put the right seeds into their souls to grow.

Publications and Presentations

The following articles have been published or in preparation for publication or presentation during the period of my PhD candidature and sections of these articles have been included in the present thesis.

Journal papers:

Gabrielian, L., Willshire, L. W., Helps, S. C., van den Heuvel, C., Mathias, J. and Vink, R. (2011). Intracranial pressure changes following traumatic brain injury in rats: lack of significant change in the absence of mass lesions or hypoxia. Journal of neurotrauma, 28, 2103-2111.

Byard, R. W., **Gabrielian, L.,** Helps, S. C., Thornton, E. and Vink, R. (2012). Further investigations into the speed of cerebral swelling following blunt cranial trauma. Journal of forensic sciences, 57, 973-975.

Gabrielian, L., Helps, S. C., Thornton, E., Turner, R. J., Leonard, A. V. and Vink, R. (2013. Substance P antagonists as a novel intervention for brain edema and raised intracranial pressure. Acta neurochirurgica. Supplement, 118, 201-204.

Abstracts and Posters:

S. C. Helps, **L. Gabrielian,** L. W. Willshire and R. Vink (2009). Poster for ANS – 2009. Effects of hypoxia on ICP and PbtO2 following experimental traumatic brain injury in rats.

L. Gabrielian, S. C. Helps, J. Mathias, C. Van Den Heuvel, and R. Vink (2009). Only mass lesions increase intracranial pressure in rats after lateral fluid percussion brain injury and secondary hypoxia. INTS-2009 International conference, CA, USA.

S. C. Helps, **L. Gabrielian**, R. J. Turner, D. P. Amato, L. W. Willshire, P. L. Reilly, and R. Vink (2009). Choice of Species for Therapeutic Treatment of Intracranial Hypertension. Abstract for 2009 ANS Annual Scientific Meeting, 52-53.

L. Gabrielian, S. Helps, C. Van Den Heuvel, and R. Vink (2010. Poster for ANS – 2010. Only mass lesions increase intracranial pressure after lateral fluid percussion brain injury and secondary hypoxia in rats.

Manuscripts in Preparation:

L. Gabrielian, A. Melkumyan, N. Melkoumian, and R. Vink (2013). The Dynamics between Intracranial Pressure, Mean Arterial Blood Pressure, Cerebral Perfusion Pressure, and Cerebral Oxygenation after Traumatic Brain Injury in Sheep: Reconsidering Critical Thresholds of ICP and the CPP Formula.

L. Gabrielian and R. Vink (2013). Effects of NK1 Antagonist-NAT, versus Mannitol, on Intracranial Pressure, Mean Arterial Pressure, Cerebral Perfusion Pressure, and Brain Oxygenation Changes after Traumatic Brain Injury in Sheep.

L. Gabrielian and R. Vink (2013). Effects of NK1 Antagonist-EU-C-001, versus Hypertonic Saline, on Intracranial Pressure, Mean Arterial Pressure, Cerebral Perfusion Pressure, and Brain Oxygenation Changes after Traumatic Brain Injury in Sheep.

L. Gabrielian and R. Vink (2013). Effects of Magnesium and Progesterone on Intracranial Pressure, Mean Arterial Pressure, Cerebral Perfusion Pressure, and Brain Oxygenation Changes after Traumatic Brain Injury in Sheep.

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Abbreviations

ACE	Angiotensin-Converting Enzyme
AI	Axonal Injury
AMPA	Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
ANOVA	Analysis of Variance
APP	Amyloid Precursor protein
ATP	Adenosine Triphosphate
ATPase	Adenosine Triphosphate-Hydrolases
BBB	Blood-Brain Barrier
Ca^{2+}	Calcium
CBF	Cerebral Blood Flow
CCI	Controlled Cortical Injury
CGRP	Calcitonin Gene Related Peptide
Cl^-	Chloride
CNS	Central Nervous System
CPP	Cerebral Perfusion Pressure
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DAB	Diaminobenzidine Tetrahydrochloride
DAI	Diffuse Axonal Injury
DC	Decompressive Craniectomy
DNA	Deoxyribonucleic Acid
DVA	Diffuse Vascular Injury

EDH	Extradural Haemorrhage
ETCO2	End Tidal Carbon Dioxide
FPI	Fluid Percussion Injury
GCS	Glasgow Coma Scale
GPCR	G Protein-Coupled Receptor
GPs	Gaussian Processes
H+L	Heavy and Light
HTS	Hypertonic Saline
ICD	International Classification of Diseases
ICH	Intracerebral Haemorrhage
ICP	Intracranial pressure
IgG	Immunoglobulin G
IL	Interleukin
K^+	Potassium
LFP	Lateral Fluid Percussion
LFPI	Lateral Fluid Percussion Injury
MABP	Mean Arterial Blood Pressure
Mg^{2+}	Magnesium
mRNA	Messenger Ribonucleic Acid
Na^+	Sodium
NAT	N-Acetyl L-Tryptophan
NEP	Neutral Endopeptidase
NHS	Normal Horse Serum

NKA	Tachykinin (Neurokinin) A
NKB	Tachykinin (Neurokinin) B
NK1	Tachykinin-1 (Neurokinin) Receptor
NK2	Tachykinin-2 (Neurokinin) Receptor
NK3	Tachykinin-3 (Neurokinin) Receptor
NMDA	N-Methyl-D-Aspartate
NO	Nitric Oxide
NPγ	Neuropeptide γ
NPK	Neuropeptide K
$PaCO_2$	Arterial Blood Carbon Dioxide Tension
PAP	Post-Arteriolar Pressure
PaO2	Arterial Blood Oxygen tension
PBS	Phosphate Buffered Solution
PEEP	Positive Post-Expiratory Pressure
$P_{bt}O_2$	Brain Tissue Oxygenation
PE	Polyethylene
Pg	Progesterone
PGCS	Pediatric Glasgow Coma Scale
PNS	Peripheral Nervous System
PPT-A	Pre-Protachykinin-A
SAH	Subarachnoid Haemorrhage
SD	Standard Deviation
SDH	Subdural Haemorrhage

SEM	Standard Error of Measurement
SP	Substance P
SPC	Streptavidin Peroxidase Conjugate
SP-DE	Substance P Degrading Enzyme
Spm	Stroke per Minute
TAI	Traumatic Axonal Injury
TBI	Traumatic Brain Injury
TGF	Tumor Growth Factor
TNF	Tumor Necrosis Factor
TRPV1	Transient Receptor Potential Vanilloid 1
WDI	Weight Drop Injury
WHO	World Health Organisation

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Abstract

Traumatic brain injury (TBI) is the leading cause of death in the population below 40 years of age. Patients who survive TBI suffer from ongoing physical disabilities as well as mental and emotional deficits that significantly impact their quality of life. While a number of factors have been implicated in the brain injury cascade that is initiated by TBI, increased intracranial pressure (ICP) has been identified as one factor that is strongly associated with outcome. This is largely because increased ICP results in a fall in cerebral perfusion pressure (CPP) and in brain oxygenation ($P_{bt}O_2$), thus starving the brain of essential substrates and oxygen necessary for repair and recovery. Nonetheless, treatments targeting increased ICP are largely ineffective and have not changed for over 40 years. In part, this is because the mechanisms responsible for oedema formation after trauma are unknown and also because existing small animal models of TBI might not duplicate all the pathophysiological features of human TBI. The aim of this thesis was therefore to study changes in ICP and $P_{bt}O_2$ in two different experimental animal models of TBI, both large and small, and subsequently investigate the effects of different pharmacotherapies on these variables following TBI.

The thesis shows that TBI does not consistently produce increases in ICP in rodent models unless a haemorrhagic mass lesion is present. Accordingly, rodents may not be the ideal species for the development of ICP targeted pharmacotherapies. By then studying the effects of TBI on ICP, cerebral perfusion pressure (CPP) and $P_{bt}O_2$ in an ovine, large animal model, we noted that the sheep model of injury produces similar changes in these variables to clinical (human) TBI, and was therefore well suited to the development of ICP targeted pharmacotherapies. The targeted therapy we chose to investigate was the substance P, NK1 antagonists which have been previously shown in our laboratory to reduce blood brain barrier breakdown and oedema

formation following rodent TBI. We characterized the effects of two different NK1 receptor antagonists on ICP, $P_{bt}O_2$ and CPP in an ovine model of TBI at both moderate and severe injury levels, and compared the effects to those to that of the clinically used osmotic agents, mannitol and hypertonic saline. We noted that in contrast to the osmotic agents, the NK1 antagonists consistently reduced ICP and improved $P_{bt}O_2$ irrespective of the severity of injury. As a further comparison, we examined the effects of the putative neuroprotective compounds magnesium and progesterone on ICP and $P_{bt}O_2$ following ovine, moderate TBI, and noted that both agents were ineffective. This finding highlighted the importance of using large animal models of TBI to investigate novel interventional pharmacologies.

Having acquired a considerable amount of physiological data in a large animal model of TBI that largely replicated the temporal changes in ICP and CPP in human TBI, we then applied Gaussian processes for data analyses to investigate the dynamic interrelationship between $P_{bt}O_2$, ICP, and mean arterial blood pressure (MABP) and CPP after trauma. This facilitated the development of a contour plot describing these dynamic interrelationships and enabling the prediction of mean $P_{bt}O_2$ values for any given ICP and MABP, the identification of critical thresholds in ICP, and the physiological basis and refinement of the CPP formula. We noted that $P_{bt}O_2$ had critical thresholds that might be related to the compression of post-capillary venules, capillaries and precapillary met-arterioles, respectively. Real CPP thus depends upon the pressure within the vascular tree, and whether their flow has been restricted by increased ICP.

In conclusion, NK1 antagonists offer a novel intervention for increased ICP and reduced $P_{bt}O_2$ after TBI that is superior to existing, alternative therapies irrespective of injury severity.