CHARACTERISING THE ROLE OF SUBSTANCE P FOLLOWING TRAUMATIC SPINAL CORD INJURY

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DECLARATION

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PUBLICATIONS AND PRESENTATIONS

The following articles have been published or accepted for publication or presentation during the period of my PhD candidature. Sections thereof have been included in the present thesis with the permission of the copyright owner.

Publications

Leonard AV, Blumbergs P, Vink R (2012). The effect of an NK1 receptor antagonist on blood spinal cord barrier permeability following a balloon compression model of spinal cord injury. acta neurochir (suppl). In press 2012.

Leonard A, Corrigan F, Ghabriel M, Van den Heuvel C, Vink R (2012). A Substance P antagonist improves outcome in female Sprague Dawley rats following diffuse traumatic brain injury. CNS Neuroscience and Therapeutics, Jun;18(6):513-5.

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Thornton E, Ziebell JM, **Leonard AV** and Vink R. (2010). Kinin receptor antagonists as potential neuroprotective agents in central nervous system injury. Molecules, 15 p6598-6618.

Abstracts

Leonard AV, Vink R, (2012), Characterizing the role of substance p as a mediator of neurogenic inflammation in a balloon compression model of spinal cord injury. Society for Neuroscience. New Orleans, Louisiana.

Leonard AV, Blumbergs PC, Vink R (2012), The effect of an NK1 Receptor following traumatic spuibnal cord injury. Proc. Aust. Neurosci. Soc, 32nd.

Leonard AV, Newcombe REA, Blumbergs PC, Vink R (2011), Alterations in substance P and neurokinin-1 receptor expression in response to traumatic spinal cord injury: human and animal studies Proc. Aust. Neurosci. Soc, 31st

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ABBREVIATIONS

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
ANOVA	analysis of variance
APP	amyloid precursor protein
AQP	aquaporin
ASIA	American spinal cord injury association
ATP	adenosine triphosphate
BBB	blood brain barrier
BBB	Basso Beattie Bresnahan motor score
BF	burst fracture
BSCB	blood spinal cord barrier
C	cervical
Ca^{2+}	calcium
CBF	cerebral blood flow
CGRP	calcitonin gene-related peptide
Cl^-	chloride
CNS	central nervous system
COX	cyclooxygenase
CPP	cerebral perfusion pressure
CSF	cerebrospinal fluid
CT	X-ray computed tomography
DAB	diaminobenzidene
DNA	deoxyribose nucleic acid
EB	Evan's blue
F	female
FD	fracture dislocation
GFAP	glial fibrillary associated protein

H&E	hematoxylin and eosin
H_2O_2	hydrogen peroxide
HRP	horseradish peroxidase
HV	high velocity
ICP	intracranial pressure
IMVS	institute of medical and veterinary sciences
ITP	intrathecal pressure
i.v.	intravenous
K^+	potassium
L	lumbar
M	male
MABP	mean arterial blood pressure
MP	methylprednisolone
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MVA	motor vehicle accident
Na^+	sodium
NaCl	sodium chloride
NaOH	sodium hydroxide
NASCIS	National Acute Spinal Cord Injury Studies
NAT	N-acetyl L-tryptophan
NeuN	neuronal nuclei
NHS	normal horse serum
NKA	neurokinin A
NKB	neurokinin B
NMDA	N-methyl-D-aspartate
NO	nitric oxide

NOS	nitric oxide synthase
O_2	oxygen
PBS	phosphate buffered solution
PNS	peripheral nervous system
PPTA	preprotachykinin A
PPTB	preprotachykinin B
ROS	reactive nitrogen species
S	sacral
SA	South Australian
SCBF	spinal cord blood flow
SCI	spinal cord injury
SCPP	spinal cord perfusion pressure
SEM	standard error of the mean
SP	substance P
SPC	streptavidin peroxidase conjugate
T	thoracic
TAA	thoracoabdominal aortic aneurysm
TBI	traumatic brain injury
TNF-α	tumor necrosis factor-α
VO	vehicle occupant

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ABSTRACT

Spinal cord injury (SCI) is a devastating injury that commonly results in permanent physical disability. The highest incidence of SCI occurs in younger populations, causing an enormous financial burden to both individuals and society amounting to almost $1 billion annually within Australia spent on hospitalisation, treatment and rehabilitation of spinal cord injured individuals. To date, an effective clinical treatment for SCI remains elusive, highlighting the need for research aimed at developing therapeutic interventions that improve functional outcome. Spinal cord edema is recognised as a common complication of SCI which continues to develop, spreading in a rostrocaudal direction days after injury, resulting in greater tissue damage and functional deficits. Reducing edema following SCI is therefore of utmost importance and represents an attractive target for therapeutic intervention.

Substance P (SP) is a neuropeptide known to facilitate the process of neurogenic inflammation, which has previously been shown to result in blood brain barrier (BBB) disruption and subsequent edema development following both traumatic brain injury (TBI) and stroke. Furthermore, inhibition of the high-affinity SP receptor, the tachykinin NK1 receptor, resulted in reduced BBB permeability, edema and improved functional outcome in both of these conditions. Accordingly, the current thesis sought to determine whether SP played a similar role as a mediator of neurogenic inflammation following traumatic SCI.

Immunohistochemical assessment of human SCI demonstrated a loss of SP from the dorsal horn region, suggesting that SP release increased following injury. NK1 receptor immunoreactivity was also initially increased post-injury before declining, indicating that receptor activation and subsequent internalisation occurred. Assessment of various open experimental injury models, including the weight drop, hemisection and clip compression models, demonstrated similar SP immunoreactivity as that observed in human tissue, although NK1 receptor immunoreactivity varied in localisation and response to injury. These results highlighted the need for experimental models to accurately replicate the primary injury mechanisms observed clinically, especially the closed environment rather than the open nature of most experimental models. The balloon compression model was subsequently employed for the remainder of the study, given its closed nature and its ability to mimic primary injury mechanisms such as an initial impact followed by persisting compression. This model also proved to replicate many other facets of human injury such as severe hemorrhage, axonal injury, neuronal loss, microglial activation, as well as increased BSCB disruption, edema, intrathecal pressure (ITP) and reduced functional outcome.

Balloon compression induced SCI was also associated with reduced SP immunoreactivity, suggesting increased SP release, and increased NK1 receptor immunoreactivity. Such observations implicate a potential role for SP in mediating neurogenic inflammation following SCI. However, administration of an NK1 receptor antagonist, n-acetyl tryptophan (NAT), resulted in no improvement in any assessed outcomes, suggesting that SP mediated neurogenic inflammation might not play a major role in the development of BSCB disruption following SCI. Indeed, the physiological effects of SP on the barrier may be outweighed by the substantial mechanical disruption observed. Interestingly, changes in the immunoreactivity of the water channel protein, aquaporin 4 (AQP4), were observed following both human SCI and the balloon compression model. These alterations implicate the involvement of AQP4 in facilitating the removal of excess water from the spinal cord. As such, modulation of AQP4 may represent a novel therapeutic intervention following SCI.

We conclude that SP mediated neurogenic inflammation is a minor player in the injury cascade after SCI. At times, NAT administration resulted in worsened outcomes and as such raises the question as to whether SP might be beneficial following SCI rather than detrimental. Further investigation of the role of SP following SCI, especially a later time points, is required to better elucidate its potential role. Nonetheless, substantial edema development remained a serious consequence following SCI and given the observed changes in AQP4 immunoreactivity, investigation of AQP4 modulation following SCI is warranted.