CHARACTERISING THE ROLE OF SUBSTANCE P IN ACUTE ISCHAEMIC STROKE

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DECLARATION

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 and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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Renée Turner

Date:

DEDICATION

This thesis is dedicated in memory of Jack Byrne.

PUBLICATIONS AND PRESENTATIONS

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

Publications

Turner RJ, Blumbergs, PC, Sims, NR., Helps, SC, Rodgers, KM, Vink, R. (2006) Increased substance P immunoreactivity and edema formation following reversible ischemic stroke. *Acta Neurochir (Suppl)*, 96:263-266.

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Turner RJ. (2007) New hope for stroke. *Australasian Science*. 28(1):38-40.

Abstracts

Turner, RJ., Blumbergs, PC., Vink, R. (2005) A substance P antagonist improves outcome following reversible middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab*, 25(Suppl):32.

Turner, RJ., Blumbergs, PC., Sims, NR., Helps, SC., Rodgers, KM., Vink, R. (2005) Increased substance P immunoreactivity and edema formation following g reversible ischaemic stroke. Abstracts of the *XIIIth International Symposium on Brain Edema and Tissue Injury*. Ann Arbor, MI, USA, May 31-June 3, p70.

Turner, RJ., and Vink, R. (2006) Novel approaches to the management of oedema following stroke. *Stroke Society of Australasia – Annual Scientific Meeting.* Adelaide, Australia, October 11-13, p27.

Turner, RJ., Vink, R. (2007) Capsaicin pre-treatment in cerebral ischaemia. *XXIIrd International Symposium on Cerebral Blood Flow, Metabolism and Function.* Osaka, Japan, May 20-25, In press.

Patents:

Vink R, Nimmo A, Whitfield K, **Turner RJ**. (2007) *Methods and composition for reducing reperfusion injury*. Australian Provisional Patent Number 2006906859.

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ABBREVIATIONS

Ab	antibody
ACE	angiotensin converting enzyme
AF	atrial fibrillation
ANOVA	analysis of variance
APP	amyloid precursor protein
APP-α	amyloid precursor protein α
ATP	adenosine triphosphate
BBB	blood brain barrier
BGL	blood glucose levels
bFGF	basic fibroblast growth factor
BI	barthel index
BP	blood pressure
CGRP	calcitonin gene-related peptide
cAMP	cyclic adenosine monophosphate
CA neurons	cornu ammonis neurons
CAST	Chinese Acute Stroke Trial collaborative group
CBF	cerebral blood flow
CCA	common carotid artery
CSF	cerebrospinal fluid
cm	centimetres
CNS	central nervous system
COX	cyclooxygenase

CPP	cerebral perfusion pressure
d	day
DAB	diaminobenzidene
DCC	dark cell change
DISC	death inducing signalling complex
DPX	mounting medium
EB	Evan's Blue
ECA	external carotid artery
ECASS	European Cooperative Acute Stroke Study
ECM	extracellular matrix
ED-1	anti- CD-68 antibody
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
FJC	Fluoro Jade C
FR	free radicals
G	gauge
GOS	Glasgow outcome scale
GFAP	glial fibrillary associated protein
h	hour
HDL	high-density lipoproteins
H&E	haemoatoxylin and eosin
HRP	horseradish peroxidase
i.c.v.	intracerebroventricular
ICA	internal carotid artery

ICH	intracerebral haemorrhage
ICP	intracranial pressure
IFN-γ	interferon-γ
iNOS	inducible nitric oxide synthase
1L-1β	interleukin-1β
Il-6	interleukin-6
IMVS	Institute of Medical and Veterinary Science
IP_3	inositol 1,4,5-triphosphate
IST	international stroke trial collaborative group
I.U/ml	international units per millilitre
kDA	kilodaltons
L 760 735	NK_1 receptor antagonist
LDL	low-density lipoprotein
L/min	litres per minute
LRP	low-density lipoprotein-related receptor protein
M	molar
m	metre
MCA	middle cerebral artery
MCAO	middle cerebral artery occlusion
mins	minutes
mg/ml	milligrams per millilitre
MK-801	NMDA receptor antagonist
ml	millilitre
mm	millimetre
mmHg	millimetres of mercury

ml/100g/min	millilitres per 100g of tissue per minute
mmol/L	millimoles per litre
MMP	matrixmetalloproteinase
mNSS	modified neuroseverity score
mRNA	messenger riboneucleic acid
MRS	modified rankin scale
mw	molecular weight
n	number
Na^+/K^+ATPase	sodium/potassium ATP-dependent ion pump
NAT	n-acetyl-tryptophan
NEP	neutral endopeptidase
NF-κB	nuclear factor κB
NGF	nerve growth factor
NH_2	amino terminus
NH&MRC	National Health and Medical Research Council
NIHSS	National Institute of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NK_1	neurokinin 1
NK_2	neurokinin 2
NK_3	neurokinin 3
NKA	neurokinin A
NKB	neurokinin B
NKγ	neurokinin γ
NMDA	N-methyl-D-aspartate
Nm	nanometres

nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
O_2	oxygen
PAI-1	plasminogen activator inhibitor 1
PBS	phosphate buffered saline
PET	Positron Emission Tomography
PKC	protein kinase C
PLA_2	phospholipase A2
PLC	protein lipase C
pMCAO	permanent middle cerebral artery occlusion
PNS	peripheral nervous system
PPT	preprotackykinin
RCC	red cell change
RP 67580	NK_1 receptor antagonist
rpm	revolutions per minute
s	seconds
SAH	subarachnoid haemorrhage
SEM	standard error of the mean
s/min	strokes per minute
SP	substance P
SPC	streptavidin peroxidase conjugate
SR 140333	NK_1 receptor antagonist
TBI	traumatic brain injury
TGF-β	transforming growth factor-β

TIA	transient ischaemic attack
tMCAO	transient middle cerebral artery occlusion
TMB	3,3'-5,5'-tetramethylbenzidine
TNF-α	tumour necrosis factor-α
tPA	tissue plasminogen activator
TRPV1	transient receptor potential V1
TTC	2,3,5-triphenyltetrazolium chloride
UV	ultraviolet
VEGF	vascular endothelial growth factor
WHO	World Health Organization
WIN 51 708	NK_1 receptor antagonist
ww dw	wet weight dry weight
μl	microlitres
μmol/kg	micromoles per kilogram
ºC	degrees celcius

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ABSTRACT

More than 15 million people worldwide will suffer a stroke each year two thirds will die or be left permanently disabled. Accordingly, stroke represents an enormous financial burden on the community, due to the cost of hospitalisation, treatment and rehabilitation of stroke patients. Despite the significance of this public health problem, a safe and widely applicable stroke therapeutic remains elusive. Cerebral oedema is widely recognised as a common and often fatal complication of stroke that is associated with worsened outcome. However, the exact mechanisms of oedema formation remain unclear, with current therapies largely ineffective in addressing the mechanisms of cerebral swelling, and also being associated with their own negative side-effect profile.

This thesis characterises the role of neurogenic inflammation and the neuropeptide, substance P (SP), in mediating the development of blood brain barrier breakdown, cerebral oedema and resultant functional deficits following stroke, using a rodent model of reversible cerebral ischaemia. The findings of this thesis demonstrate that increased SP immunoreactivity, particularly of the penumbral tissue vasculature, is a feature of tissue perfusion following stroke, but not in non-reperfused infarcts. The central role for SP in the breakdown of the BBB following stroke and the associated deleterious effects of such breakdown was confirmed by studies using an NK_1 receptor antagonist. These antagonists conferred a profound attenuation of BBB breakdown, cerebral oedema formation, neuronal death and injury, and the associated development of functional deficits following reversible stroke. Similarly, depletion of all neuropeptides by capsaicin pre-treatment also reduced

both histological abnormalities and functional deficits following stroke, confirming the central role of neuropeptides in the secondary injury process after stroke.

The NK_1 receptor antagonist was able to be safely combined with the currently approved treatment for stroke, tPA, producing a synergistic effect of greater protection from the ischaemic insult. In particular, histological and functional outcome were markedly improved, as well as a reduction in the risk of intracerebral haemorrhage and death. Furthermore, the NK_1 receptor antagonist was effective even when administered up to 8 h following the onset of ischaemia, and in a variety of stroke severities.

We conclude that SP plays a central role in the secondary injury that occurs following stroke, in particular, the genesis of BBB breakdown and cerebral oedema. Accordingly, combination therapy of tPA and an NK_1 receptor antagonist may offer a novel therapeutic strategy for the clinical management of ischaemic stroke of varying severity.