Characterising the Role of Substance P in Human and Experimental Brain Tumours

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Dedication

For Andrew Weldon and Jennifer Harford

Declaration

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Publications and Presentations

Publications

Lewis, KM, **Harford-Wright E**, Vink R, Ghabriel MN (2012) Targeting classical but not neurogenic inflammation reduces peritumoral oedema in secondary brain tumours. *J Neuroimmunology*, 250(1-2) 59-65.

Lewis, KM, **Harford-Wright E**, Vink R, Ghabriel MN (2012) Walker 256 tumour cells increase substance P immunoreactivity locally and modify the properties of the blood-brain barrier during extravasation and brain invasion. *Clinical Experimental Metastasis,* [Epub ahead of print].

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Harford-Wright E, Thornton E and Vink R. (2010) Angiotensin-converting enzyme (ACE) inhibitors exacerbate histological damage and motor deficits after experimental traumatic brain injury. *Neuroscience Letters, 481 p26-29*

Abstracts/Presentations

Harford-Wright, E, Lewis KM, VInk R, Ghabriel M. (2012) Substance P as a novel therapeutic target in the treatment of peritumoural oedema. *Proc. Aust Soc for Medical Research.*

Lewis KM, Harford-Wright E, Vink R, Ghabriel M. (2012) Effect of NK1 Receptor Antagonist Treatment on Tumor Growth In Vitro and in a Rat Model of Brain Metastases. *Proc. Aust Soc for Medical Research.*

Harford-Wright E, Lewis KM, Vink R, Ghabriel M. (2012) Characterising the role of substance P in the genesis of peritumuoral oedema. *Proc. Aust Neurosci Soc,* 32nd annual meeting

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Harford-Wright, E, Lewis KM, Vink R, Ghabriel M. (2011) Characterising the expression of Substance P in human and experimental brain tumours. *Society for Neuroscience*

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Abbreviations

5'HT	Serotonin
ACE	Angiotensin Converting Enzyme
AQPs	Aquaporins
AQP-1	Aquaporin-1
AQP-4	Aquaporin-4
BBB	Blood-Brain Barrier
BSA	Blood Serum Albumin
Ca^{2+}	Calcium
CCM	Complete Culture Medium
CGRP	Calcitonin Gene Related Peptide
Cl^-	Chlorine
CNS	Central Nervous System
CO_2	Carbon dioxide
CPP	Cerebral Perfusion Pressure
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DAB	3'3'-diaminobenzidine
DMEM	Dulbeccos Modified Eagles Medium
DWI	Diffusion Weighted Imaging
EB	Evans Blue
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
GBM	Glioblastoma Multiforme
GFAP	Glial Fibrillary Acidic Protein
H_2O	Water
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulfuric Acid
H&E	Haematoxylin and Eosin
HRP	Horseradish Peroxidase
ICP	Intracranial Pressure
IL	Interleukin
K^+	Potassium

LDH	Lactate Dehydrogenase
MRI	Magnetic Resonance Imaging
Na^+	Sodium
NAT	N-acety-L-tryptophan
NBF	Neutral Buffered Formalin
NEP	Neutral Endopeptidase
NHS	Normal Horse Serum
O_2	Oxygen
PBS	Phosphate Buffered Saline
PNS	Peripheral Nervous System
PPTA	Preprotachykinin A
PPTB	Preprotachykinin B
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SD	Standard Deviation
SEM	Standard Error of Mean
SP	Substance P
SPC	Streptavidin Peroxidase Conjugate
SRS	Stereotactic Radiosurgery
TAM	Tumour Associated Macrophages
TADC	Tumour Associated Dendritic Cells
TBI	Traumatic Brain Injury
TBS	Tris-Buffered Saline
TCA	Trichloroacetic Acid Solution
TNF	Tumour Necrosis Factor
WBRT	Whole Brain Radiotherapy

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Abstract

Currently within Australia, brain tumours account for one death every eight hours. They are inherently difficult to treat, and even with the best current treatments, prognosis for these patients remains extremely poor. At present, treatment options for patients with either primary or secondary brain tumours are limited. Indeed most brain metastasis patients have a short survival time, despite the fact that initial treatment is often effective in reducing neurological deficits and tumour size.

One of the most serious complications of brain tumours is cerebral oedema, which is typically vasogenic in nature due to disruption of normal blood brain barrier function. Cerebral oedema is associated with many life-threatening complications such as raised intracranial pressure, reduction in cerebral perfusion pressure and in severe cases can result in brain herniation and death. Although treatments for cerebral oedema currently exist, they are associated with many deleterious side effects, prompting the search for alternative treatments.

The neuropeptide substance P and its NK1 receptor are reported to be upregulated in a number of different cancer types. This increase is thought to correspond with SP mediated increases in cellular proliferation, impairment of apoptosis and increased angiogenesis. Furthermore, SP has recently been implicated in the development of neurogenic inflammation in the brain, where it is associated with vasodilation, plasma extravasation and genesis of oedema. Accordingly, the current thesis sought to determine whether SP may provide a novel treatment target in both tumour associated oedema and tumour growth.

Immunohistochemical assessment of human primary and secondary brain tumour tissue demonstrated a marked increase in SP and the NK1 receptor in all tumour types investigated, confirming what has been previously observed in the literature. Of the mestastatic tumours, melanoma demonstrated particularly elevated levels of SP and its receptor. Correspondingly, a human melanoma cell line, A-375, was examined *in vitro* and found to express both SP and the NK1 receptor. Furthermore, treatment with NK1 antagonists resulted in decreased cell viability of A-375 melanoma cells and an increase in cell death in this cell line *in vitro*, implicating SP in the facilitation of tumour growth.

An *in vivo* model of brain tumours using the same cell line, demonstrated increased SP and the NK1 receptor not only within the tumour, but also in peritumoural vessels. This increase corresponded

with a significant increase in brain water content and BBB permeability, implicating SP in the genesis of tumour associated oedema. Subsequent administration of the NK1 antagonist, Emend decreased BBB dysfunction and oedema formation and was more efficacious than the current clinical treatment choice, dexamethasone. Furthermore, treatment with Emend resulted in a decrease in tumour volume and in the number of proliferating cells, as well as an increase in apoptotic cells, an effect that was not seen with dexamethasone treatment. This indicates that SP may play multiple roles in tumour pathogenesis within the brain.

In conclusion, the present studies have identified a role for SP in tumour-associated oedema as well as in tumour growth and progression. Moreover, treatment with the NK1 antagonist Emend was more effective than the currently available clinical treatment, dexamethasone. Treatment with an NK1 antagonist may thus provide a novel therapeutic option, able to treat these two very different aspects of brain tumour pathogenesis.