The role of substance P in the progression and complications of secondary brain tumours

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Declaration

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Publications

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

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

Lewis KM, Harford-Wright E, Vink R, Nimmo AJ, 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 and Experimental Metastases.* Paper accepted on 8th May 2012, DOI: 10.1007/s10585-012-9487-z

Harford-Wright E, Lewis KM, Vink R. (2011) Towards drug discovery for brain tumours: interaction of kinins and tumours at the blood brain barrier interface. *Recent Patents in CNS Drug Discovery*; 6: 31-40.

Submitted manuscripts:

Lewis KM, Harford-Wright E, Vink R, Ghabriel MN. NK1 receptor antagonists and dexamethasone as anticancer agents in vitro and in a model of brain tumours secondary to breast cancer. *Anti-Cancer Drugs.*

Lewis KM, Harford-Wright E, Vink R, Ghabriel MN. Tumorigenicity of Walker 256 breast carcinoma cells from two tumour cell banks as assessed using two models of secondary brain tumours. *Cancer Cell International.*

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Abbreviations

ºC	Degrees Celsius
µL	Micro Litres
µm	Micro Metres
AQP-4	Aquaporin-4
AQP-1	Aquaporin-1
BBB	Blood-Brain Barrier
CCA	Common Carotid Artery
CNS	Central Nervous System
CPP	Cerebral Perfusion Pressure
CSF	Cerebrospinal Fluid
d	Days
DAB	3,3′-diaminobenzidine
Dex	Dexamethasone
ECA	External Carotid Artery
GFAP	Glial Fibrillary Acidic Protein
H & E	Haematoxylin and Eosin
h	Hours
IBA1	Ionized Calcium Binding Adaptor Molecule 1
ICA	Internal Carotid Artery
ICP	Intra-cranial Pressure

IFN-γ	Interferon Gamma
IL-6	Interleukin-6
IL-11	Interleukin-11
iNOS	Inducible Nitric Oxide Synthase
IU	International Units
MAP	Mean Arterial Pressure
mg	Milligrams
mL	Millilitres
mm	Millimetres
MRI	Magnetic Resonance Imaging
n	Number
NAT	n-acetyl $_{L}$-tryptophan
NHS	Normal Horse Serum
OA	Ophthalmic Artery
PPT-A	Pre Protachykinin-A
RPM	Revolutions per Minute
SEM	Standard Error of the Mean
SP	Substance P
STA	Superior Thyroid Artery
TJ	Tight Junction
TNF-α	Tumour Necrosis Factor Alpha

VEGF	Vascular Endothelial Growth Factor
wk	Weeks

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Abstract

Secondary brain tumours occur when cancer cells enter the circulation from their primary site and colonise the brain, previously shown to occur across the blood-brain barrier (BBB). Substance P (SP), a neurogenic inflammatory mediator, acting predominantly through NK1 receptors plays a role in opening the BBB and in the formation of oedema following stroke and brain trauma. It is hypothesised that SP may also promote the extravasation of tumour cells through the BBB, formation of peritumoral oedema and progression of secondary brain tumours.

Walker 256 rat breast carcinoma cells obtained from the Centre for Medical Research, Tohoku University had superior tumorigenic properties compared to cells from the American Type Culture Collection, and were therefore subsequently used in two albino Wistar rat models of tumorigenesis.

Firstly, internal carotid artery tumour cell injection was used to establish the effect of tumour cell extravasation across the BBB on brain albumin, endothelial barrier antigen (EBA) and SP immunoreactivity. I then determined if NK1 receptor antagonists could prevent tumour cell extravasation, by evaluating tumour incidence and volume.

Secondly, a stereotaxic direct inoculation model was used to investigate the effect of NK1 receptor antagonists on brain tumour growth and peritumoral oedema, compared with dexamethasone treatment. Evan's blue extravasation and albumin immunoreactivity were used to assess BBB permeability, and brain water content to evaluate cerebral oedema. Tumour volume, Ki67 immunoreactivity, caspase-3 immunoreactivity and tumour cell density were used as measures of tumour growth. Furthermore, cell viability and cell death assays determined if NK1 antagonists or dexamethasone treatment cause alterations in tumour cell growth in vitro.

In the carotid model, SP and albumin immunoreactivity increased in the brain during the extravasation of tumour cells, and in the peritumoral area of established tumours. The invaded blood vessels lacked EBA immunoreactivity, indicating loss of BBB properties. However, NK1 antagonists administered in the first three days following tumour cell injection failed to reduce tumour incidence or volume, suggesting that

extravasation may be a multifactorial process, and that NK1 receptor antagonism alone is not sufficient to prevent tumour extravasation and growth.

In the direct inoculation model, NK1 receptor antagonists did not reduce peritumoral oedema or decrease tumour growth when used to treat established brain metastases. In contrast, dexamethasone, the standard treatment for peritumoral oedema, caused a reduction in brain water content and decreased tumour volume, but not tumour growth. The decrease in tumour volume with dexamethasone reflects reduced fluid content, as there was increased tumour cell density with no change in immunoreactivity to Ki67 (marker for proliferation) or caspase-3 (marker for apoptosis). Furthermore, in vitro studies showed no effect for dexamethasone on tumour cell viability. These results suggest that peritumoral oedema is driven by classical inflammation rather than neurogenic inflammation in the direct inoculation model.

In conclusion, in these models of secondary brain tumours, SP does not appear to play a role sufficient to promote NK1 receptor antagonism as an appropriate preventative treatment for brain metastasis, as an anticancer agent, or as an alternative to dexamethasone for the management of peritumoral oedema.