

**The Cerebrovascular Response to Metastatic
Melanoma and *Clostridium perfringens* Type D
Epsilon Toxin**

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Dedication

For Peter John & Patricia May Mander

Declaration

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Kimberley Anne Mander

Date

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Publications and Presentations

Publications

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Corrigan, F, **Mander, KA**, Leonard, AV, Vink, R (2016) Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. *Journal of Neuroinflammation*.

Mander, KA, Williams, R., Finnie, JW (2016) Clostridium perfringens type D epsilon toxin produces a rapid and dose-dependent cytotoxic effect on cerebral microvascular endothelial cells *in vitro*. *Anaerobe (in review)*

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Abstracts/Presentations

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Abbreviations

ABC	ATP-Binding Cassette
AMT	Absorptive-Mediated Transport
AQP4	Aquaporin-4
BBB	Blood-Brain Barrier
CAV-1	Caveolin-1
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
Da	Dalton
DAB	3,3'-diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
EBA	Endothelial Barrier Antigen
EC	Endothelial Cell
ECM	Extracellular Matrix
EEL	External Elastic Lamina
EGFR	Epithelial Growth Factor Receptor
EGM	EndoGRO-MV Complete Media
ELISA	Enzyme-Linked Immunosorbent Assay
ETX	Clostridium perfringens type D
GBM	Glioblastoma Multiforme
GLUT1	Glucose Transporter 1
hCMEC/D3	Human Cerebral Microvascular Endothelial Cells
HER2	Human Epidermal Growth Factor Receptor 2
HIF	Hypoxia Inducible Factor
ICAM-1	Intercellular Adhesion Molecule-1
ICP	Intracranial Pressure
IEL	Internal Elastic Lamina
IL-1	Interleukin-1
kDa	Kilodalton
MTT	3-(-4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
MRI	Magnetic Resonance Imaging
NBF	Neutral Buffered Formalin
NHS	Normal Horse Serum
NK-1R	Tachykinin Receptor 1

NSCLC	Non-Small Cell Lung Carcinoma
PAS	Periodic Acid-Schiff
PBS	Phosphate Buffer Solution
PECAM-1	Platelet Endothelial Cell Adhesion Molecule-1
PET	Positron Emission Tomography
Pgp	P-Glycoprotein
rh	Recombinant Human
RMT	Receptor-Mediated Transport
SP	Substance P
TBS	Tris-Buffered Saline
TEER	Transendothelial Electrical Resistance
TEM	Transmission Electron Microscope
TJ	Tight Junction
VAM-1	Vascular Cell Adhesion Molecule-1
VEGF	Vascular Endothelial Growth Factor
ZO-1	Zona Occludens

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Thesis Format

The format of my thesis includes two main themes and is arranged as follows: a general introduction, five research chapters, a second background chapter, three research chapters, a general discussion and references.

Broadly, my thesis is focused on the, fundamental and important role of the cerebral vasculature in two disease processes, namely cerebral metastatic melanoma and *Clostridium perfringens* type D epsilon neurotoxicity. The first theme aims to characterise the extent of vascular alteration following metastatic progression in human tissue, giving rise to the first two research chapters (chapter 3 and 4). Together, these chapters form the scope of the remaining research chapters pertaining to the development of reliable *in vitro* and *in vivo* models for the study of malignant transmigration of the blood-brain barrier and targeted therapeutic approaches. This is reported in chapters 5, 6 & 7). The second theme relates to the investigation of several vascular features of the neurological disorder produced by *Clostridium perfringens* type D epsilon toxin (ETX), giving rise to an additional three primary research chapters (chapters 8, 9 and 10).

Abstract

The principal focus of this thesis is the cerebral vasculature and, more specifically, its fundamental and important role in two disease processes, namely cerebral metastatic melanoma and *Clostridium perfringens* type D epsilon neurotoxicity. Firstly, blood vessels are critical for both impeding and facilitating the penetration, colonisation, and spread of metastatic tumours such as melanomas in the brain and, secondly, the microvasculature is the major target of the potent bacterial neurotoxin, *Clostridium perfringens* type D epsilon toxin, which causes a severe, and frequently fatal, naturally-occurring, neurological disorder in domestic livestock and is a potential bioterrorism agent for human populations.

There are important structural and functional differences between blood vessels in the brain and other tissues and the regional distribution is inhomogeneous. These features also have consequences for patterns of disease expression, for example lodgement of tumour emboli. Moreover, the dynamic microvascular interface between blood and brain parenchyma, termed the blood-brain barrier (BBB), differs in important structural detail from capillaries elsewhere and is critical in maintaining homeostasis in the central nervous system.

In Part A of this thesis, the different patterns of neovascularisation in archival, human melanomas metastatic to the brain were characterised, given that

acquisition of a new vascular supply is essential for these neoplasms to survive, proliferate, and disseminate. These new blood vessels are frequently structurally and functionally aberrant and those examined in the metastatic melanoma cohort herein were classified using histological and immunohistochemical techniques. It was also determined whether there was any correlation between vascular subtype and histological category of melanoma, mitotic index, extent of tumour necrosis, and intratumoural haemorrhage.

Since the substance P (SP)/NK-1 receptor (NK-1R) system plays an important role in tumour survival, proliferation, and progression, its distribution was examined immunohistochemically in these metastatic melanomas, both in tumour-associated blood vessels and melanocytes. The NK-1 receptor was expressed by most melanocytes and endothelium in a small subset of tumour blood vessels, but there was no detectable immunoreactivity of the tachykinin peptide, SP, in tumour cells or blood vessels. The distribution of caveolin-1, the main structural component of caveolae, was also examined in these melanomas. Its immunoexpression was reduced in tumour-associated blood vessels, concordant with increased neoangiogenesis, and CAV-1 was commonly expressed in melanocytes, particularly in cell membranes, reflecting its important role in both tumour progression and suppression.

Since melanomas generally metastasise via the haematogenous route and finally encounter the BBB when they reach the brain, it was decided to

examine the transendothelial migration of melanocytes using *in vitro* and *in vivo* models. In a culture system, the migration of melanocytes from a melanoma cell line across a membrane representing a “blood-brain barrier” was quantified and the manner of their passage across this endothelial barrier examined by light and electron microscopy, the ultrastructural assessment being one of the very few studies of this type conducted to date. In order to examine how melanocytes in the systemic circulation enter the brain, a melanoma cell line was injected into rat carotid arteries and the distribution of melanocytes in the brain assessed at different time intervals post-injection. Unfortunately, very few tumour cells penetrated into the brain parenchyma and this technique proved to be unsatisfactory for examining transendothelial migration of metastatic melanocytes and evaluation of drugs that might impede this process.

In Part B of this thesis, several vascular features of the neurological disorder produced by *Clostridium perfringens* type D epsilon toxin (ETX) were studied. In the principal, and novel, study, the aim was to determine whether ETX produced a direct and damaging effect on cerebral microvascular endothelial cells *in vitro*. While previous histological and ultrastructural studies suggested that the fundamental lesion in this neurotoxicity was ETX-induced microvascular injury, with subsequent BBB breakdown, increased vascular permeability and severe, generalised cerebral vasogenic oedema, the effect of ETX on brain-derived endothelial cells in culture had not been examined. The present study found, for the first time, that EXT produces a dose-

dependent cytopathic effect on cultured human brain microvascular endothelial cells, confirming the importance of microvascular endothelial damage in the pathogenesis of this neurological disorder.

In an animal model of ETX neurotoxicity using Sprague-Dawley rats, extravasation of endogenous albumin was used as a surrogate immunohistochemical marker of increased vascular permeability; loss of endothelial barrier antigen was evaluated after exposure to ETX as it is a marker of an intact BBB in this species; and the role of the major water channel protein in the brain, aquaporin-4, in the development/resolution of ETX-induced cerebral oedema was studied. Since the BBB is a prime target for ETX-induced brain damage and the blood-retinal barrier (BRB) resembles the BBB in many respects, the action of ETX on the BRB was also examined in rats using albumin immunohistochemistry to assess enhanced vascular permeability and electron microscopy to study retinal blood vessels. Retinal microvascular endothelial damage resembled that found in ETX-disrupted BBB and there was widespread retinal oedema as indicated by diffuse albumin extravasation.

Studies carried out in this thesis aimed to better characterise the cerebral microvasculature alterations and the associated mechanisms, in response to two distinct insults; metastatic melanoma, and ETX. A range of investigative modalities facilitated the detailed exploration of vascular reactions in these 2

neuropathological states and findings from this thesis will direct further research in the field of cerebrovascular pathology.