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

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Declaration

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Kate Lewis

Date:
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.


Submitted manuscripts:

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>µL</td>
<td>Micro Litres</td>
</tr>
<tr>
<td>µm</td>
<td>Micro Metres</td>
</tr>
<tr>
<td>AQP-4</td>
<td>Aquaporin-4</td>
</tr>
<tr>
<td>AQP-1</td>
<td>Aquaporin-1</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-Brain Barrier</td>
</tr>
<tr>
<td>CCA</td>
<td>Common Carotid Artery</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral Perfusion Pressure</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>d</td>
<td>Days</td>
</tr>
<tr>
<td>DAB</td>
<td>3,3'-diaminobenzidine</td>
</tr>
<tr>
<td>Dex</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>ECA</td>
<td>External Carotid Artery</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial Fibrillary Acidic Protein</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>IBA1</td>
<td>Ionized Calcium Binding Adaptor Molecule 1</td>
</tr>
<tr>
<td>ICA</td>
<td>Internal Carotid Artery</td>
</tr>
<tr>
<td>ICP</td>
<td>Intra-cranial Pressure</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon Gamma</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IL-11</td>
<td>Interleukin-11</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible Nitric Oxide Synthase</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
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<tr>
<td>mg</td>
<td>Milligrams</td>
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<tr>
<td>mL</td>
<td>Millilitres</td>
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<tr>
<td>mm</td>
<td>Millimetres</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>n</td>
<td>Number</td>
</tr>
<tr>
<td>NAT</td>
<td>n-acetyl L-tryptophan</td>
</tr>
<tr>
<td>NHS</td>
<td>Normal Horse Serum</td>
</tr>
<tr>
<td>OA</td>
<td>Ophthalmic Artery</td>
</tr>
<tr>
<td>PPT-A</td>
<td>Pre Protachykinin-A</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per Minute</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SP</td>
<td>Substance P</td>
</tr>
<tr>
<td>STA</td>
<td>Superior Thyroid Artery</td>
</tr>
<tr>
<td>TJ</td>
<td>Tight Junction</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor Alpha</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>--------</td>
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<tr>
<td>wk</td>
<td>Weeks</td>
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</table>
# Table of Contents

DECLARATION ........................................................................................................ II  
PUBLICATIONS ....................................................................................................... III  
ACKNOWLEDGEMENTS ........................................................................................... V  
ABBREVIATIONS .................................................................................................... VI  
TABLE OF CONTENTS .......................................................................................... IX  
LIST OF TABLES AND FIGURES: .................................................................... XIII  
ABSTRACT .............................................................................................................. XV  

## 1 INTRODUCTION ........................................................................................................ 1  

1.1 EPIDEMIOLOGY OF BRAIN METASTASES .............................................................. 1  
1.1.1 Incidence ................................................................................................... 1  
1.1.2 Organ of origin ........................................................................................... 2  
1.2 LOCATION OF METASTATIC TUMOUR ................................................................. 3  
1.3 CLINICAL RELEVANCE ................................................................................... 3  
1.4 TUMOUR ASSOCIATED MORBIDITY ...................................................................... 4  
1.4.1 Seizures ...................................................................................................... 4  
1.5 CURRENT TREATMENTS FOR METASTATIC BRAIN TUMOURS .................. 5  
1.5.1 Chemotherapy ........................................................................................... 5  
1.5.2 Radiation therapy ...................................................................................... 7  
1.5.3 Dexamethasone .......................................................................................... 7  
1.6 BLOOD BRAIN BARRIER ................................................................................... 10  
1.6.1 Cerebral capillary endothelial cells ........................................................ 10  
1.6.2 Tight junctions ........................................................................................... 10  
1.6.3 Basement membrane ................................................................................ 11  
1.6.4 Astrocytes ................................................................................................ 11  
1.6.5 Pericytes .................................................................................................. 11  
1.7 FORMATION OF BRAIN METASTASES ............................................................. 12  
1.7.1 Immune system interactions ..................................................................... 14  
1.8 EXPERIMENTAL MODELS OF BRAIN METASTASES ....................... 16  
1.8.1 Tumour injection into the internal carotid artery .................................. 16  
1.8.2 Syngeneic model ...................................................................................... 17  
1.8.3 Walker 256 breast carcinoma ................................................................. 17  
1.9 FEATURES OF METASTATIC BRAIN TUMOURS ...................................... 19  
1.9.1 Tumour morphology ................................................................................. 19  
1.9.2 Tumour border ........................................................................................... 19  
1.9.3 Blood-tumour barrier .............................................................................. 19  
1.9.4 Angiogenesis ............................................................................................ 21  
1.9.5 Peri-tumoral environment ....................................................................... 22  
1.10 PERITUMORAL OEDEMA ................................................................................. 22  
1.10.1 Tumour size ............................................................................................. 23  
1.10.2 Complications of cerebral oedema ........................................................ 23  
1.10.3 Clearance of oedema .............................................................................. 24  
1.11 SUBSTANCE P ................................................................................................. 24  
1.11.1 Immunoreactivity in the brain ................................................................. 25  
1.11.2 Substance P effects on the blood-brain barrier .................................... 26
1.11.3 Substance P and oedema formation .................................................... 26
1.11.4 Substance P and NK1 expression in cancer cells ................................. 28
1.11.5 Role of substance P and NK1 receptors on cancer growth .................. 33
1.11.6 Substance P effects on angiogenesis ................................................... 40
1.11.7 Substance P interactions with radiotherapy of cancer ........................ 40
1.11.8 Potential effects of Substance P on tumour cell extravasation into the brain 41

1.12 CONCLUSION ................................................................................................ 41

2 MATERIALS AND METHODS ........................................................................ 42

2.1 CELL CULTURE ............................................................................................. 42
2.1.1 Walker 256 cells from American Type Culture Collection ..................... 42
2.1.2 Walker 256 cells from Cell Resource Centre at Tohoku University ......... 42
2.1.3 Cell viability assay .................................................................................. 43

2.2 ANIMALS ...................................................................................................... 43

2.3 INTERNAL CAROTID ARTERY INJECTION ................................................ 44

2.4 DIRECT INTRACEREBRAL INOCULATION ............................................. 47

2.5 DRUG TREATMENTS .................................................................................. 47
2.5.1 Emend ...................................................................................................... 47
2.5.2 NAT .......................................................................................................... 48
2.5.3 Dexamethasone ....................................................................................... 48

2.6 ASSESSMENT OF BRAIN HISTOLOGY ...................................................... 48

2.7 TUMOUR VOLUME ...................................................................................... 49

2.8 IMMUNOHISTOCHEMISTRY ................................................................... 49

2.9 BRAIN WATER CONTENT ......................................................................... 50

2.10 EVANS BLUE extravasation .................................................................. 50

2.11 STATISTICAL ANALYSIS ......................................................................... 51

3 TUMORIGENICITY OF WALKER 256 BREAST CARCINOMA CELLS FROM TWO DIFFERENT TUMOUR CELL BANKS AS ASSESSED USING TWO MODELS OF BRAIN METASTASES .......................................................... 52

3.1 ABSTRACT .................................................................................................... 52

3.2 INTRODUCTION ........................................................................................ 53

3.3 METHOD ...................................................................................................... 54
3.3.1 Cell Culture ............................................................................................. 54
3.3.2 Animals .................................................................................................... 54
3.3.3 Internal Carotid Artery Injection ............................................................ 54
3.3.4 Direct Inoculation ................................................................................... 55
3.3.5 Tumour Volume ....................................................................................... 55
3.3.6 Immunohistochemistry ......................................................................... 55
3.3.7 Statistical Analysis ................................................................................ 56

3.4 RESULTS ...................................................................................................... 57
3.4.1 Cell Morphology ...................................................................................... 57
3.4.2 Tumorigenicity ........................................................................................ 58
3.4.3 Tumour Interactions with the BBB ........................................................ 63
3.4.4 Brain Microenvironment ....................................................................... 65

3.5 DISCUSSION ............................................................................................... 69

4 WALKER 256 TUMOUR CELLS INCREASE SUBSTANCE P IMMUNOREACTIVITY LOCALLY AND MODIFY THE PROPERTIES OF
7 NK1 RECEPTOR ANTAGONISTS AND DEXAMETHASONE AS ANTICANCER AGENTS IN VITRO AND IN A MODEL OF BRAIN TUMOURS SECONDARY TO BREAST CANCER............................................. 124

7.1 ABSTRACT .................................................................................................. 124
7.2 INTRODUCTION ........................................................................................... 125
7.3 MATERIALS AND METHODS ........................................................................ 128
  7.3.1 Cell Viability Assay ............................................................................... 128
  7.3.2 Cell Culture for Inoculation .................................................................. 128
  7.3.3 Animals .................................................................................................. 128
  7.3.4 Tumour Inoculation ............................................................................... 128
  7.3.5 Treatment ............................................................................................... 128
  7.3.6 Tumour Volume ..................................................................................... 129
  7.3.7 Immunostaining ..................................................................................... 129
  7.3.8 Analysis of NK1 receptor, GFAP and IBA1 immunostained sections... 129
  7.3.9 Tumour cell replication, density and apoptosis .................................... 130
  7.3.10 Statistical Analysis ............................................................................ 130

7.4 RESULTS ..................................................................................................... 131
  7.4.1 Cell Viability Assay ............................................................................... 131
  7.4.2 NK1 receptor expression ....................................................................... 133
  7.4.3 Tumour Growth ..................................................................................... 134
  7.4.4 Brain microenvironment ........................................................................ 138

7.5 DISCUSSION .............................................................................................. 143

8 GENERAL DISCUSSION .................................................................................. 149
  8.1 PURPOSE ..................................................................................................... 149
  8.2 MODELS USED ............................................................................................ 150
  8.3 PRINCIPAL FINDINGS ................................................................................... 151
  8.4 FURTHER RESEARCH ................................................................................... 159
  8.5 CONCLUSION .............................................................................................. 161

9 REFERENCE LIST ........................................................................................ 162
LIST OF TABLES AND FIGURES:

TABLE 1.1  EXPRESSION OF SUBSTANCE P (SP) AND NK1 RECEPTORS IN HUMAN CANCER SPECIMENS .................. 29
TABLE 1.2  EXPRESSION OF SUBSTANCE P (SP) AND NK1 RECEPTORS IN CANCER CELL LINES .......................... 31
TABLE 1.3  EFFECT OF EXOGENOUS SUBSTANCE P (SP) APPLICATION AND NK1 ANTAGONIST TREATMENT ON CANCER CELLS IN VITRO ......................................................... 36
TABLE 1.4  SUBSTANCE P (SP) AND NK1 ANTAGONIST EFFECTS ON CANCER IN VIVO ........................................................ 39
FIGURE 2.1  INTERNAL CAROTID ARTERY INJECTION ......................... 46
FIGURE 3.1  TUMOUR CELL MORPHOLOGY IN VITRO AND IN VIVO 58
TABLE 3.1  TUMOUR INCIDENCE IN ANIMALS INJECTED VIA THE INTERNAL CAROTID ARTERY OR DIRECTLY INOCULATED INTO THE BRAIN WITH WALKER 256 BREAST TUMOUR CELLS OBTAINED FROM THE CELL RESOURCE CENTRE FOR MEDICAL RESEARCH AT TOHOKU UNIVERSITY (CRCTU) OR THE AMERICAN TYPE CULTURE COLLECTION (ATCC) ........................................ 60
FIGURE 3.2  TUMOUR VOLUME IN MODELS OF BRAIN METASTASES ........................................................................................................... 61
FIGURE 3.3  EXTRACRANIAL TUMOUR GROWTH FOLLOWING INTERNAL CAROTID ARTERY INJECTION OF CRCTU WALKER 256 CELLS ................................................................. 62
FIGURE 3.4  ALBUMIN IMMUNOREACTIVITY IN METASTATIC BRAIN TUMOUR MODELS ................................................................. 64
FIGURE 3.5  GFAP IMMUNOREACTIVITY IN METASTATIC BRAIN TUMOUR MODELS ................................................................. 66
FIGURE 3.6  IBA1 IMMUNOREACTIVITY IN METASTATIC BRAIN TUMOUR MODELS ................................................................. 68
TABLE 4.1  TUMOUR INCIDENCE OVER TIME ......................................... 80
FIGURE 4.1  TUMOUR GROWTH OVER TIME ............................................. 81
FIGURE 4.2  ALBUMIN IMMUNOREACTIVITY OVER TIME ................... 83
FIGURE 4.3  ENDOTHELIAL BARRIER ANTIGEN (EBA) IMMUNOREACTIVITY ....................................................................................... 85
FIGURE 4.4  SUBSTANCE P (SP) IMMUNOREACTIVITY WITH TUMOUR INVASION ......................................................................................... 87
FIGURE 4.5  SUBSTANCE P (SP) IMMUNOREACTIVITY SURROUNDING ESTABLISHED BRAIN METASTASES ..................................... 88
TABLE 5.1  EFFECT OF TREATMENT ON INCIDENCE OF METASTATIC BRAIN TUMOURS ................................................................. 99
FIGURE 5.1  EFFECT OF TREATMENT ON TUMOUR VOLUME IN MM3 SHOWING NO SIGNIFICANT DIFFERENCE IN TUMOUR VOLUME AMONG THE GROUPS ................................................................. 100

FIGURE 5.2  ALBUMIN AND SUBSTANCE P (SP) IMMUNOREACTIVITY .............................................................................................................................. 101

FIGURE 5.3  ANIMAL WEIGHT CHANGE FOLLOWING TUMOUR CELL INOCULATION AND TREATMENT *P<0.05................................. 102

FIGURE 6.1  SUBSTANCE P (SP) IMMUNOREACTIVITY WITH METASTATIC BRAIN TUMOUR GROWTH ...................................................... 114

FIGURE 6.2  BRAIN WATER CONTENT ........................................................................... 115

FIGURE 6.3  EVANS BLUE EXTRAVASATION .................................................................. 117

FIGURE 6.4  ALBUMIN IMMUNOREACTIVITY WITH TUMOUR INOCULATION AND TREATMENT ................................................................. 118

FIGURE 7.1  CELL VIABILITY ASSAY ........................................................................... 132

FIGURE 7.2  NK1 RECEPTOR IMMUNOREACTIVITY .................................................. 134

FIGURE 7.3  TUMOUR GROWTH .................................................................................. 136

FIGURE 7.4  TUMOUR GROWTH CHARACTERISTICS .................................................. 138

FIGURE 7.5  TUMOUR AND PERITUMORAL GLIAL REACTION .................. 140

FIGURE 7.6  BRAIN MICROENVIRONMENT REACTION TO TUMOUR GROWTH ...................................................................................... 142
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.