The Potential for Substance P Antagonists as Anti-Cancer Agents in Brain Tumours

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The Potential for Substance P Antagonists as Anti-Cancer Agents in Brain Tumours

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Abstract: Despite recent advances in cancer treatment and diagnosis, the prognosis for patients with CNS tumours remains extremely poor. This is, in part, due to the difficulty in completely removing tumours surgically, and also because of the presence of the blood brain barrier, which can prevent the entry of chemotherapeutic agents typically used in cancer treatment. Despite the presence of the blood brain barrier, tumour cells are capable of entering and colonising the brain to form secondary brain tumours. Additionally, tumour related disruption of the blood brain barrier is associated with the clinical presentation of many patients, with accompanying increases in intracranial pressure due, in part, to the development of vasogenic oedema. Vasogenic oedema results because the newly formed angiogenic vessels within brain tumours do not retain the highly selective properties of the blood brain barrier, and thus allow for the extravasation of plasma proteins and water into the brain parenchyma. Tachykinins, and in particular substance P, have been implicated in blood brain barrier disruption and the genesis of cerebral oedema in other CNS insults via a process known as neurogenic inflammation. Recent evidence suggests that substance P may play a similar role in CNS tumours. It has been well established that an upregulation of substance P and its receptors occurs in a number of different cancer types, including CNS neoplasms. In addition to disrupting blood brain barrier permeability, substance P and the NK1 receptors facilitate promotion of tumour growth and the development of cerebral oedema. Accordingly, recent patents describe the potential of NK1 receptor antagonists as anti-cancer agents suggesting that substance P may provide a novel cancer treatment target. This review will examine the role of substance P in the development of CNS tumours.

Keywords: Blood-brain barrier, cerebral oedema, CNS tumours, glioma, substance P, tachykinins.

INTRODUCTION

Cancer causes significant death and disability worldwide, with 10 million new cases and more than 6 million deaths occurring as a result of cancer growth and complications each year [1]. Tumours of the central nervous system (CNS) are particularly devastating, given that the unique nature of the brain can complicate their diagnosis and treatment. The presence of the blood brain barrier (BBB) makes the use of chemotherapeutic agents difficult, because they are unable to cross an intact BBB. Furthermore, complete surgical resection of infiltrative brain tumours is extremely challenging without compromising neurological function.

Primary brain tumours arise from cells native to the brain, with glial tumours accounting for the majority of adult CNS cancers; these cells retain their ability to proliferate throughout life and accordingly have more opportunities for neoplastic transformation [2]. Brain metastases are up to 10 times more common than primary tumours of the CNS [3] and are malignancies that invade the brain from tumours originating outside the CNS. Typically, primary brain neoplasms are more infiltrative in nature, whilst metastatic brain tumours compress the surrounding brain tissue, rather than invade it. However, both primary and secondary brain tumours exhibit similar symptoms, with the tumour being confined to the skull and increased pressure developing within this closed system. Symptoms associated with elevated intracranial pressure (ICP) are some of the most common first signs of brain tumours and include headache, nausea and vomiting, and visual disturbances [4]. Another non-specific symptom is seizures, which generally occur in association with long standing compression and irritation of the adjacent brain. Consequently, seizures are typically a symptom of relatively slow growing benign tumours. Other symptoms depend on the specific location of the tumour within the brain. Weakness or hemiparesis may indicate compression of the motor cortex, loss of sensory modalities the result of a tumour of the sensory cortex while behavioural changes are often associated with frontal lobe tumours [5-7].

Despite advances in the diagnosis and treatment of CNS cancers, both primary and secondary brain tumours tend to have a poor prognosis; the majority of cases cannot be cured. At present, the standard treatment for brain tumours is surgical resection followed by radiation therapy. Surgery has long been advocated for the treatment of single brain tumours or in metastases patients with controlled systemic disease [8]. It is typically considered in any patient with a single brain malignancy in an accessible location, particularly when it is large and the mass effect of the lesion is significant. Complete surgical resection of a single tumour allows immediate
relief of neurological symptoms such as intracranial hypertension, seizures and a reduction in focal neurological deficits. However, unless the tumour is extremely well encapsulated, it is difficult to remove entirely. Often microscopic foci of tumour cells not visible on MRI are left behind. Thus, surgery tends to be followed by a course of postoperative radiotherapy.

Whole brain radiation therapy (WBRT) is the standard treatment for most patients with brain malignancies, as it remains the best alternative for patients with single metastases that are not surgically or radiosurgically resectable [9]. The majority of patients treated with WBRT have multiple intracranial tumours at the time of diagnosis making surgical or focal treatments ineffective [10]. The median survival of patients with brain metastases treated with WBRT is generally 3 to 6 months, with only 10% to 15% surviving beyond a year [9]. However, most patients treated with WBRT die of a progressive systemic disease and not as a direct result of secondary brain tumours [11]. Complications of WBRT include temporary loss of hair and short term transient worsening of neurological symptoms. Unfortunately, due to the short survival time, long-term effects remain unknown.

Stereotactic radiosurgery (SRS) is increasingly being used in the treatment of brain tumours. SRS delivers a single, relatively high dose of radiation to a tumour with great accuracy. It aims to maximize the dose to the tumour whilst minimizing the effect on surrounding tissue [9]. Typically, SRS is reserved for patients with between one and three cerebral tumours that are less than 3 cm in diameter, highly radioresistant and are unable to be surgically resected [12]. Large tumours or tumours with extensive oedema have been found to be difficult to control with SRS because of a high risk of radiation-associated necrosis and/or neurological deterioration at biologically effective doses [12]. Thus, more than one course of SRS is rarely administered to the same site because of the increased risk of radiation necrosis.

Both primary and metastatic brain tumours are inherently difficult to treat by pharmacological means because the BBB prevents most chemotherapeutic agents from reaching the cancerous tissue. In particular, brain metastases tend to respond poorly to chemotherapeutic agents, which may in part be due to the differences in chemosensitivity between primary and metastatic tumours. In metastases of previously treated tumours, clonal selection of chemoresistant cells may have already occurred [13]. In small cell lung carcinoma, the response rate of cerebral metastases to chemotherapeutic agents without radiotherapy is only 53% when compared to a 79% response rate in the primary tumour [14]. Many methods of drug delivery that bypass the BBB and blood-tumour barrier have been investigated. Intraventricular administration of drugs is traumatic and further complicated by the blood cerebrospinal fluid barrier. More recently the biodegradable 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) polymer wafers (Gliadel) have been developed for the use in malignant high-grade gliomas. These wafers allow the delivery of high doses of chemotherapeutic agents directly to the tumour tissue whilst bypassing the BBB. Following surgical resection of the glioma, up to 8 Gliadel wafers are placed directly on the resection cavity and release chemotherapeutic agents over the following 2-3 weeks [15]. Clinical trials have demonstrated a statistically significant improvement in survival following treatment with Gliadel wafers post surgery, however their use has been limited by the high rate of complications [16, 17].

Increasingly, research has been directed towards finding specific mediators that can be targeted in the treatment of cancer. Of particular interest are tachykinins and their receptors, which have been implicated in many aspects of cancer growth and progression, as well as in the development of cerebral oedema. The extensive research on the role of SP in cancer growth and the potential anti-tumour action of NK1 antagonists in various systemic cancer types has led to the filing of patents [18]. Additionally, the revelation that tachykinin receptors, and in particular substance P (SP) receptors, are upregulated on brain tumour cells has stimulated research into the potential of SP-mediated interventions in the treatment of brain malignancies [19-21]. As such the aim of the current paper is to build on our previous review and give a broad outline of some of the different potential roles of SP in brain tumours that are currently under investigation [22]. In particular, this review will focus on the potential applications of therapies directed at modulating the inflammatory effects of SP at the BBB and the associated complications in the setting of brain tumours.

THE TACHYKININ FAMILY

Tachykinins are a group of small structurally related peptides characterized by the specific C-terminal sequence, Phe-X-Gly-Lue-Met-NH₂, and include SP, neurokinin A (NKA) and neurokinin B (NKB), as well as neuropeptide K (NPK) and neuropeptide γ (NPγ) [23]. The mammalian tachykinins are encoded for by two distinct genes, preprotachykinin A (PPTA) and preprotachykinin B (PPTB). The PPTB gene gives rise to NKB, whilst alternate splicing of the PPTA gene results in four different forms of mRNA: α-PPT, β-PPT, γ-PPT and δ-PPT [23]. The α-PPT and δ-PPT forms encode for SP alone, whilst β-PPT and γ-PPT encode SP, NKA, NPK and NPγ [23, 24]. Tachykinins are produced in both neuronal and glial cell bodies, and are widely distributed throughout the CNS and PNS [25]. Specifically, within the PNS, SP is found in areas of immunologic importance such as the skin, gastrointestinal and respiratory tracts [26, 27]. In the CNS levels of SP are higher in the grey rather than white matter [28] with the highest concentrations of SP occurring in the basal ganglia, hypothalamus, amygdala, locus coeruleus and dorsal root ganglia of the spinal cord [29].

Unlike classical neurotransmitters, tachykinins are produced in the cell soma rather than in the nerve endings, and are released following calcium dependent mechanisms in response to stimuli [26]. Following release, tachykinins act on the transmembrane G-protein coupled receptors NK1, NK2 and NK3 to exert a myriad of effects, with each tachykinin demonstrating some degree of affinity for each tachykinin receptor due to the common COOH-terminal sequence [30]. However, SP preferentially binds the NK1 receptor, NKA the NK2 receptor and NKB the NK3 receptor.
Although the NK receptors are structurally closely related, they are pharmacologically distinct, which has allowed detailed investigation of the structure and function of the NK1 receptor. Binding to the NK1 receptor occurs at the second and third membrane domains and results in rapid endocytosis and internalisation of the receptor [31]. Within the CNS, NK1 receptors are found on neurons and glia [32], with expression of NK1 receptors highest in the caudate putamen and superior colliculus [29]. The widespread expression of SP and the NK1 receptor throughout the CNS has resulted in it being implicated in a number of neurological diseases, including CNS tumours. Accordingly, recent research has been focused on the potential of this particular tachykinin as a treatment target in numerous facets of cancer development.

**SP IN TUMOUR GROWTH**

The last decade has seen a substantial rise in research into the expression and secretion of peptides by tumours [33], and in particular the tachykinins. Tachykinins and their receptors are thought to be heavily involved in the development and progression of many neoplasms, given that they are able to facilitate cell growth via induction of DNA synthesis and cellular proliferation. In addition, tachykinins have a stimulating or potentiating effect on lymphocyte proliferation and differentiation, cytokine secretion, and immunoglobulin production [34]. More specifically, SP and NK1 receptors have been implicated in numerous facets of cancer initiation and progression, with recent research implicating the PPT-1 gene and NK receptors in breast cancer development [35]. Furthermore, NK1 receptors have also been found in intratumoural and peri-tumoural blood vessels, suggesting a potential role for SP in tumour angiogenesis [36].

An increase in SP and the NK1 receptor expression has now been reported in a number of tumour types (Table 1) including glioma, retinoblastoma, colon, metastatic melanoma, in situ melanoma, keratocystic odontogenic tumours and squamous cell and larynx carcinoma [37-45]. In a number of glial tumour cell lines, the presence of NK1 receptors correlated with a SP and/or NK-A mediated increase in DNA synthesis and cellular proliferation [46, 47]. This increase in SP receptors *in vivo* is therefore thought to correspond with increases in tumour growth.

Oncogenes are genes that assist in the transformation of normal cells into cancerous ones, if mutated or expressed at high levels. Under normal physiological conditions, any cellular genetic damage activates one or more of the programmed cell death pathways. In order to avoid this, tumour cells must set up ways to neutralise the multiple pathways leading to cell death. It has been proposed that the increase in the expression of NK1 receptors may play a role in malignant cells evasion of apoptosis [33, 34]. Such increased expression makes tumour

<table>
<thead>
<tr>
<th>Table 1. SP and NK1 Receptor Expression in Tumours</th>
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<tbody>
<tr>
<td><strong>Human Specimens</strong></td>
</tr>
<tr>
<td>SP Expression</td>
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<tr>
<td>Present in:</td>
</tr>
<tr>
<td>Astrocytoma [65],</td>
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<tr>
<td>Meningioma [65]</td>
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<td>Breast carcinoma [66],</td>
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<td>Melanoma [37]</td>
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<tr>
<td>NK1 Receptor Expression</td>
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<tr>
<td>Present in:</td>
</tr>
<tr>
<td>Astrocytoma [36, 67]</td>
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<tr>
<td>Breast carcinoma [36, 63, 66, 67]</td>
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<tr>
<td>NK1 Antagonist Treatment</td>
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<td>N/A</td>
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<tr>
<td><strong>Tumour cell lines</strong></td>
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<tr>
<td>SP Expression</td>
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<td>Evident in:</td>
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<tr>
<td>Breast carcinoma (ZR-75–30, BT-474, T-47D,</td>
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<tr>
<td>MDA-MB-330, 184B5, CP-96 345-1, DU4475, BT</td>
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<tr>
<td>483, MDA-MB-231, MDA-MB-231 Dox, MCF7,</td>
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<tr>
<td>LCC1, LCC2, SK-BR-3) [66, 68-70]</td>
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<tr>
<td>NK1 Receptor Expression</td>
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<tr>
<td>Evident in:</td>
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<tr>
<td>Astrocytoma (UC11MG, U373MG, SNB-19, DBTRG-05</td>
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<td>MG, GAMG) [20, 38, 71-74]</td>
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<tr>
<td>Breast carcinoma (ZR-75–30, BT-474, T-47D,</td>
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<tr>
<td>MDA-MB-330, MDA-MB-231, 184B5, CP-96 345-1,</td>
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<td>DU4475, BT 483, MCF7, LCC1, LCC2, SK-BR-3)</td>
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<td>[48, 63, 66, 69, 70]</td>
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<tr>
<td>Melanoma(COLO 858, MEL HO, COLO 679) [75]</td>
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<td>NK1 Antagonist Treatment</td>
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<td>Induced apoptosis in: Astrocytoma (GAMG, SNB-19,</td>
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<td>DBTRG-05 MG, U373 MG) [20, 38, 74, 76],</td>
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<tr>
<td>Breast carcinoma (MDA-MB-231, T47D, MDA-MB-468,</td>
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<tr>
<td>MDA-MB-453, SKBR3 MCF7, BT474) [48, 63, 77]</td>
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<tr>
<td>Melanoma (COLO 858, MEL H0, COLO 679) [75, 76]</td>
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<tr>
<td><strong>Animal Models</strong></td>
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<tr>
<td>SP Expression</td>
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<tr>
<td>Present in breast carcinoma [78]</td>
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<td>NK1 Antagonist Treatment</td>
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<tr>
<td>Causes differential tumour response.</td>
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<tr>
<td>Caused decreased tumour growth in astrocytoma</td>
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<td>(U373 MG) in the flank of female nude mice</td>
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<td>[38], but did not affect tumour growth in</td>
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<tr>
<td>breast carcinoma (MDA-MB-231) in the flank of</td>
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<td>female nude mice [48].</td>
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cells highly dependent on SP stimulus, a known potent mitotic signal that counteracts the different death signal pathways [34]. Indeed, administration of an NK1 antagonist for 2 weeks in a nude mouse model of breast cancer was found to effectively inhibit tumour growth [48], whilst administration of the SP receptor antagonist, L-733, 060, was found to inhibit the metastatic progression of NK1 expressing human glioma cancer cells [49]. Thus, antagonism of the NK1 receptor may allow cells to become more receptive to apoptotic death signals, leading to the death of mutated cells.

As well as exhibiting mitogenic and anti-apoptotic activity, SP is known to stimulate the release of cytokines. The role of cytokines in cellular communication, coordination of cell growth and maturation, wound healing, the immune responses and neoangiogenesis have been well documented [50]. Many of these processes can be utilised by tumour cells to facilitate their growth, thus supporting their own growth and facilitating their metastatic spread [50-52]. Furthermore, such cytokine release from malignant cells may also induce normal cells to synthesise additional cytokines perpetuating tumour progression [50]. Specifically, granulocyte-macrophage colony stimulating factor (GM-CSF) is released following SP mediated protein kinase C (PKC) activation and is responsible for the macrophage aggregation observed in malignant gliomas and the regulation of cellular growth and migration [21, 35]. Cytokines also feedback onto the release of SP, with leukemia-inhibiting factor (LIF), a neuropoietin cytokine responsible for neurogenesis and neuronal differentiation, able to stimulate glioma proliferation by induction of SP and NK1 receptor expression [21]. LIF is produced and released by neuronal cells following exposure to proinflammatory cytokines leading to increased SP production and NK1 expression [53].

Cytokines and other mediators are also responsible for the process of tumour angiogenesis, thereby enhancing tumour growth that is dependent upon a corresponding increase in vascularization [54]. Given the presence of its NK1 receptor in tumour vessels, it is hypothesised that SP may also facilitate tumour growth by mediating angiogenesis. Indeed, an increase in SP NK1 receptors has been demonstrated in tumoural and peri-tumoural vessels in glioblastomas, which is thought to be significant due to the known vasodilative and angiogenic effects of SP [36]. It has been reported that SP enhances capillary growth in an in vivo rabbit cornea assay, an effect that was abolished following administration of a SP receptor antagonist [55]. Furthermore, SP stimulates migration and proliferation of some types of endothelial cells, thus enhancing the angiogenic process in human tumours [54]. Thus, SP may play a major role in development of tumour stroma and facilitate tumoral blood supply.

Experimentally, a number of NK1 antagonists have been identified as anti-cancer agents (Table 2). However, there is only one NK1 antagonist that is currently approved for human use [56-58], and specifically approved for use in cancer patients. Fosaprepitant diglutemide (L-758,298) is the intravenous prodrug of aprepitant (L-754,030), and is commonly known as Emend. The application of SP in vivo is known to induce emesis, confirming that SP is involved in the emetic reflex pathway [59]. Emend is accordingly used as an antiemetic to ameliorate the nausea and vomiting frequently associated with the use of many chemotherapeutic agents [60-62]. Of interest, however, is that the anti-emetic effects of NK1 receptor antagonists are centrally mediated [63]. Furthermore, Emend is barrier permeable and has the added benefit of being able to cross the BBB [64]. Thus, Emend

<table>
<thead>
<tr>
<th>NK1 antagonists</th>
<th>Evidence as anticancer agents in vitro</th>
<th>Molecular Formula</th>
<th>Chemical name</th>
<th>Indication</th>
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<tr>
<td>L-733,060</td>
<td>glioma [20, 74, 76], neuroblastoma [20, 76], melanoma [41], gastrointestinal tract cancer [40], acute lymphoblastic leukemia [33], retinoblastoma [20, 79], laryngeal cancer [45]</td>
<td>C$_2$H$_5$F$_3$NO</td>
<td>3-((3,5-Bis(trifluoromethyl)phenyl)methyloxy)-2-phenylpiperidine</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Aprepitant</td>
<td>glioma [80], neuroblastoma [80], melanoma [80], breast carcinoma [81], gastrointestinal tract carcinoma [80], pancreatic carcinoma [80], retinoblastoma [80], larynx carcinoma [80]</td>
<td>C$_2$H$_6$F$_3$N$_2$O$_3$</td>
<td>5-[[2R,3S]-2-((R)-1-[[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)morpholino)methyl]-1H-1,2,4-triazol-3(2H)-one</td>
<td>Preclinical</td>
</tr>
<tr>
<td>L-732,138</td>
<td>retinoblastoma [79], acute lymphoblastic leukemia [33], laryngeal cancer [44]</td>
<td>C$_2$H$_6$F$_3$N$_2$O$_3$</td>
<td>N-Acetyl-L-tryptophan 3,5-bis(trifluoromethyl)benzyl ester</td>
<td>Preclinical</td>
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<tr>
<td>MEN 11467</td>
<td>glioma [82], breast carcinoma [48]</td>
<td>C$_9$H$_8$N$_2$O$_4$</td>
<td>N-[2-[(4-Methylphenyl)acetyl]-N-methyl-(3-naphthyl)-L-alanine N-[2-(1H-indol-3-yl)carboxamido]cyclohexyl]amide</td>
<td>Preclinical</td>
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may potentially be able to exert other effects on CNS tumours, given that SP plays a role in tumour growth as well as in the development of peri-tumoural oedema.

**SP IN BRAIN TUMOURS**

It has been suggested that substance P and the NK receptors are particularly significant in the development and progression of malignant brain tumours [35]. Increased expression of NK1 receptors compared to the normal surrounding tissue has been well documented in human gliomas and malignant brain tumours [19, 20, 35, 83]. Indeed, in human gliomas there is a correlation between increased density of NK receptors and the degree of tumour malignancy. A study by Hennig et al. [36] reported the presence of NK1 receptors on 100% of glioblastoma biopsy specimens and 75% of astrocytomas using autoradiography. Furthermore, SP receptor expression was observed in blood vessels regardless of tumour grade or type. The increased expression of SP in astrocytic tumours is consistent with the widespread distribution of SP and its precursors throughout the CNS. Moreover, the high levels of NK1 receptor within primary astrocytic tumours can be attributed to the fact that astrocytes harbour functional SP receptors and retain this with malignancy [38]. SP is known to mediate tumour growth in a number of glial tumour cell lines; the presence of tachykinin NK receptors correlated with a SP and/or an NK-A mediated increase in DNA synthesis and cellular proliferation [46]. Following activation of the NK1 receptor, a number of biochemical reactions take place that are associated with cancer progression. Binding to the NK1 receptor leads to activation of protein kinase C (PKC). PKC then phosphorylates a number of proteins that stimulate the induction of DNA synthesis and cell proliferation [84]. Indeed, growth regulation via PKC stimulation has been noted on the human glioma line, U373 MG [85]. Apart from its potential role in the promotion of tumour growth, SP may also be integral in the development of peri-tumoural oedema. SP has been implicated in the disruption of the BBB and subsequent development of oedema in both traumatic brain injury (TBI) and stroke [86, 87]. Given the well-documented disruption to the BBB associated with brain tumours, SP may provide a novel therapeutic target in the treatment of tumour-associated oedema.

**THE BLOOD BRAIN BARRIER**

CNS tumours are associated with disruption of the BBB in the immediate peri-tumoural regions disrupting normal fluid homeostasis. The BBB is a complex structure consisting of physical, biochemical, enzymatic, pharmacological, electrical and immunological components. It is capable of accelerating the passage of certain substances whilst completely restricting the access of others [4], protecting the brain from fluctuations in plasma composition and from circulating agents capable of disturbing neural function [88]. Structurally, the BBB consists of a continuous endothelium of capillary wall, a thick basal lamina and bulbous astrocytic feet which cling to the outside of the capillaries [89]. Brain capillaries are approximately 50-100 times tighter than peripheral microvessels due to complex tight junctions that restrict the paracellular pathway for diffusion of hydrophilic solutes [88]. These tight junctions have high electrical impedance and low permeability to polar solutes, providing an electric barrier as well as an anatomic one [4]. Therefore, penetration across brain endothelium is essentially confined to transcellular mechanisms. The BBB also has minimal pinocytotic vesicles when compared to the rest of the body and thus provides for the relative exclusion of plasma proteins from the CSF.

Alterations in capillary endothelium are the underlying cause of barrier disruption within tumours, with opening of tight junctions, fenestrations, gap junctions and increases in pinocytotic vesicles all associated with an increase in barrier permeability (see Fig. (1)) [90, 91]. Recent evidence suggests some of the component proteins of tight junctions fail to be expressed or are abnormally regulated in aggressive brain tumours [92]. Indeed, investigation of glioma microvessel endothelium revealed defects in tight junctions, an increase in the number of pinocytotic vesicles and the presence of fenestrations [91], which became more pronounced with increasing malignancy. Alterations to capillary permeability are thought to occur via the secretion of vascular permeability factors from tumour cells and contribute to the opening of the BBB [93].

Metastatic tumours recruit endothelial cells from the surrounding brain tissue to form their blood vessels. Interestingly, the molecular structure of tumour endothelial tight junctions differs from those in a normal brain. As such it may be possible to selectively close or open the blood tumour barrier, without influencing the BBB [92]. In the case of metastatic brain tumours, the new blood vessels within the tumour do not retain the qualities of the BBB and have the characteristics of the tissue of origin, facilitating the development of vasogenic oedema [94]. Furthermore, impairment of the BBB can also be seen in the immediate peri-tumoural area surrounding larger tumours, potentially due to mechanical disruption or due to release of substances, like cytokines and SP, from the tumour [95]. This leads to the development of cerebral oedema, a significant contributor to outcome in patients with brain tumours [93, 96]. However, in smaller brain metastases, the BBB has been found to remain intact, and it has been suggested that the integrity of the barrier around small metastatic lesions may be restored after passage of metastatic cells into the brain parenchyma [97]. Interestingly, SP has been implicated in BBB disruption and development of cerebral oedema in a number of neurological conditions such as TBI and stroke, and thus may also mediate BBB disruption in brain tumours [86, 87].

**PERI-TUMOURAL OEDEMA**

Cerebral oedema, the abnormal accumulation of fluid within the brain parenchyma that produces a volumetric enlargement of brain tissue, frequently occurs around brain tumours and significantly contributes to morbidity and mortality [98]. Tumour-associated oedema is typically vasogenic in nature, resulting from a disrupted BBB that allows the extravasation of water and other plasma constituents into the brain parenchyma. Tumour-associated oedema can also have local effects such as impairment of microcirculation, expansion of the extracellular space, and abnormalities in the fluid microenvironment which all may influence the structure and function of normal brain cells [93]. Oede-
matous fluid accumulates rapidly around aggressive brain tumours and once excess extracellular fluid accumulates, mechanisms must exist to allow absorption, so that the rate of fluid formation and absorption are equal. Oedematous fluid is absorbed by transepidermal flow into the ventricles while proteins are phagocytosed by astrocytes and microglia, resulting in the erosion of the osmotic gradient in the extracellular space and subsequent absorption into microvessels [4, 99].

Corticosteroids have remained the primary treatment approach for tumour-associated oedema for the past 40 years. In a broad sense, they work by reducing the permeability of a compromised BBB, with a number of mechanisms of action having been proposed to account for this action. One proposed mechanism is by inhibition of phospholipase A₂, an enzyme responsible for arachidonic acid release [100]. Arachidonic acid destabilizes membrane lysosomes and has a direct destabilizing effect on cerebral capillaries. Corticosteroids are also known to have the ability to reduce vascular endothelial growth factor (VEGF)-induced BBB permeability, an action reversed by a glucocorticoid receptor antagonist [101]. Therefore, corticosteroids may act to reduce the response of the cerebral capillary endothelial cells (CCEC) to VEGF or reduce the secretion of VEGF by tumour cells [101]. Nonetheless, the exact mechanism of action of corticosteroids is yet to be fully elucidated. Dexamethasone is associated with a large number of potentially serious side effects, the severity of which depends on the dose and duration of steroid treatment [103]. Adverse side effects include immunosuppression, hypertension, fluid retention and mood disturbances. These complications have consequently prompted investigation into alternative treatments for tumour associated brain oedema. Given its role in the development of oedema following other insults to the CNS, the tachykinin SP has become a potential target [104, 105].

Tachykinins are known to contribute to cerebral oedema in a number of brain pathologies through a process known as neurogenic inflammation, a neurally elicited inflammatory response characterised by vasodilation, plasma extravasation, mast cell degranulation and tissue swelling [106]. It results from the stimulation of capsaicin sensitive C-fibres causing the release of neuropeptides such as SP and calcitonin gene related peptide (CGRP). Numerous studies have provided evidence for a role for neuropeptides in most immunologic and inflammatory states within the periphery. Activation or damage to neurons can lead to changes in neuropeptide synthesis, which results from the induction of neuropeptide gene expression [24]. Such changes to neuropeptide expression in sensory neurons have been observed in models of acute and chronic inflammation. Furthermore, upregulation of NK1 receptor and PPT mRNA has also been shown in the periphery during noxious stimulation or neurogenic inflammation [25].

SP is thought to be the most potent initiator of neurogenic inflammation because of its association with increased vascular permeability and subsequent plasma protein extravasation [84, 107]. It may also potentiate inflammatory
responses by stimulating the production of inflammatory mediators such as histamine, nitric oxide and kinins, interacting with adhesion molecules and the extracellular matrix causing leukocyte migration [107]. The role of SP in neurogenic inflammation in the periphery has been well documented, particularly in the skin, gastrointestinal and respiratory tracts where administration of SP has been found to induce neurogenic inflammation [108, 109]. This role has been confirmed by findings that NK1 receptor antagonists completely abolished the inflammatory response [110]. In contrast, the concept of neurogenic inflammation in the CNS has remained largely unexplored until relatively recently.

To date, few studies have explored the role of neurogenic inflammation in brain pathologies. As the cerebral arteries have a dense supply of neuropeptide containing sensory neurons, an increase in neuropeptides post CNS damage may be involved in injury pathways [111]. Vink and colleagues (2003) were the first to investigate neurogenic inflammation in the brain following traumatic brain injury (TBI) [111]. They found that depletion of neuropeptides with capsaicin completely attenuated the changes in BBB permeability and subsequent oedema formation usually observed following TBI. Subsequent abolition of neuropeptides also significantly improved functional outcome post trauma. Such findings were rationalised in terms of the high levels of NK receptors found in areas associated with both motor control and memory and learning [112]. Furthermore, successive research has demonstrated a significant upregulation of SP immunoreactivity in perivascular tissue and within the parenchyma occurs by 5 hours post trauma. This increase in SP is associated with significant increases in cerebral oedema and marked functional deficits [86, 113]. Administration of an NK1 antagonist was found to attenuate this oedema formation and improve neurological outcome in both male and female rats [86, 113]. These results are consistent with what is observed in the human condition, with SP immunoreactivity reportedly increased following human TBI [114]. A role for neuropeptides, namely SP, has also been demonstrated in other neurological diseases. Following ischaemic stroke, SP immunoreactivity was increased in the infarcted hemisphere and was associated with profound oedema formation. As was observed in TBI, administration of a SP antagonist resulted in marked improvement in functional outcome following stroke [87]. Several patents have described the use of NK1 antagonists to ameliorate SP induced vasogenic cerebral oedema, particularly following TBI and ischemic reperfusion stroke [115-117]. Furthermore, these efforts to reduce cerebral oedema through NK1 antagonist treatment have led to the filing of patents that also promote this treatment to prevent subsequent increases in ICP [118].

Numerous studies have also demonstrated that SP and NK1 receptor immunoreactivity is increased surrounding brain tumours, particularly within the perivascular region [36, 78]. This localization is suggestive of a role in the formation of tumour-associated oedema. To date, only one study has investigated NK1 antagonist treatment of peritumoural oedema in an animal model of brain metastatic breast cancer. This study proposes that classical inflammation rather than neurogenic inflammation is the predominant mediator of tumour associated oedema, as an NK1 antagonist failed to ameliorate oedema formation, whilst dexamethasone was an effective treatment for peritumoral oedema [78]. However, it should be noted that the outcomes of this study might reflect a varied action of SP depending on tumour type, with further investigation needed on this topic.

**SUBSTANCE P IN THE EXTRAVASATION PROCESS**

In order to colonise and grow in the brain, cancer cells must first pass through the BBB. Extravasation occurs when cells arrest within the cerebral vasculature and form cytoplasmic protrusions, which interact with and penetrate the BBB allowing tumour cells to enter the brain [119]. However, the precise mechanisms by which tumour cells are able to cross an intact BBB are yet to be fully elucidated. Recent research has been directed towards substances that act directly on the BBB and thus may be possible driving factors for tumour cell extravasation. Identification of such agents may prevent metastatic brain tumour formation; however to date there has been little success. Of these, the VEGF receptor antagonist cediranib AZD2171 was unable to reduce metastatic brain tumour growth following intracardiac left ventricle inoculation of tumour cells [120]. Breast cancer cells transfected with a tissue inhibitor of matrix metalloproteinase 2 reportedly have a propensity for metastases to the brain [121]. Further investigation is required to determine if the manipulation of these substances may be beneficial in preventing human brain metastases.

The well-characterised role of SP in barrier disruption has made tachykinin receptors attractive as potential therapeutic targets to inhibit entry of cancer cells into the brain. Increases in SP and its NK1 receptor have been reported on a variety of different tumour cell lines further supporting this approach. Cerebral blood vessels are particularly well innervated by SP secreting primary sensory nerve fibres. SP has been detected in cerebral capillary endothelial cells and is secreted by these cells in response to treatment with high doses of cytokines such as IL-1β and TNFα [122, 123]. As a result of SP release, the concentration of calcium ions within endothelial cells of the BBB is increased to approximately 10 times above normal, and hence leads to increased permeability of the BBB through endothelial cell contraction [124, 125]. Similarly, using an *in vitro* model of the BBB, treatment with SP decreased tight junction protein concentration, specifically of ZO-1 and claudin-5 [126]. Recently, it has been demonstrated that SP expression is increased locally surrounding tumour-invaded microvessels, in a rodent model of brain metastatic breast cancer [127]. The localised elevation of SP correlated with increased permeability of the BBB and the loss of endothelial barrier antigen staining, indicative of a disrupted BBB [127] (See Fig. (2)). These results suggest tumour cell induced secretion of SP may increase the permeability of the BBB allowing for the movement of tumour cells into the brain, and subsequent development of metastasis. As such, NK1 antagonists are potentially a promising preventative treatment for metastatic brain tumours, particularly in patients who have primary tumours that are known to have a propensity for spread to the brain.

The extensive research on the role of SP in cancer growth and the potential antitumour action of NK1 antagonists in...
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various cancer types has led to the filing of several patents. One patent by Munoz and colleagues [79] described the induction of apoptosis in cancer cells by NK1 antagonists in various types of cancer, but in particular gliomas, melanoma, lung and breast carcinomas. Similarly, the invention of an antibody specific against SP has been suggested for the treatment of cancers expressing NK1 or epidermal growth factor family receptors [18]. In contrast, several patents have also been filed in which SP is proposed as a treatment or preventative strategy for cancer, although these are largely targeting peripheral tissues. Aerosolized SP has been described as a prophylactic treatment for the prevention of cancer in the lung, particularly in response to environmental toxins [128, 129]. Similarly, SP in combination with an immunogenic composition has been proposed for use as a cancer vaccine, acting through tumour antigen manipulation [130]. Clearly, treatment with either an NK1 antagonist or with SP must be applied selectively based on tissue location of the cancer as well as the type of cancer.

CURRENT & FUTURE DEVELOPMENTS

Improved prognosis for patients with CNS tumours will require the development of treatments that target multiple aspects of the disease. Recent research has indicated that SP may play multiple roles in the pathophysiology of CNS tumours, including tumour cell growth, the genesis of peri-tumoural oedema and the extravasation of tumour cells into the brain. Given that NK1 antagonist is already approved for human use, it provides an attractive option for the treatment of this condition. Furthermore, as the drug is routinely used as part of cancer patient management, it would be interesting to investigate whether there are any potential beneficial effects of this treatment on cancer growth and oedema. A retrospective analysis of patient data would be extremely useful to further characterise the potential role of NK1 antagonists in human cancer, and determine whether their efficacy depends on tumour type. To date there has been no such investigation into the effect of the treatment on cancer growth or oedema.

Similarly, further investigations need to be conducted specifically evaluating the effects of NK1 antagonist treatment on BBB dysfunction in the setting of experimental brain tumours. Although the one animal study to date has proved ineffective in reducing peri-tumoural oedema, it is possible that this outcome is related specifically to the tumour cell line used and the concentration of SP within that cell line. Thus, subsequent studies should employ different cell lines to investigate whether this is a universal effect.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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DISCLOSURE

This is an extended and updated version of the authors’ previous manuscript.

REFERENCES

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