

# NK1 receptor antagonists and dexamethasone as anticancer agents *in vitro* and in a model of brain tumours secondary to breast cancer

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Emend, an NK1 antagonist, and dexamethasone are used to treat complications associated with metastatic brain tumours and their treatment. It has been suggested that these agents exert anticancer effects apart from their current use. The effects of the NK1 antagonists, Emend and *N*-acetyl-L-tryptophan, and dexamethasone on tumour growth were investigated *in vitro* and *in vivo* at clinically relevant doses. For animal experiments, a stereotaxic injection model of Walker 256 rat breast carcinoma cells into the striatum of Wistar rats was used. Emend treatment led to a decrease in tumour cell viability *in vitro*, although this effect was not replicated by *N*-acetyl-L-tryptophan. Dexamethasone did not decrease tumour cell viability *in vitro* but decreased tumour volume *in vivo*, likely to be through a reduction in tumour oedema, as indicated by the increase in tumour cell density. None of the agents investigated altered tumour cell replication or apoptosis *in vivo*. Inoculated animals showed increased glial fibrillary acidic protein and ionized calcium-binding adapter molecule 1 immunoreactivity indicative of astrocytes and

microglia in the peritumoral area, whereas treatment with Emend and dexamethasone reduced the labelling for both glial cells. These results do not support the hypothesis that NK1 antagonists or dexamethasone exert a cytotoxic action on tumour cells, although these conclusions may be specific to this model and cell line. *Anti-Cancer Drugs* 24:344–354 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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## Introduction

Malignancies of the central nervous system have shown an increased incidence in recent years, although mortality rates have plateaued [1]. Breast cancer is the most prevalent cancer type in women and the second most common cancer type to cause metastatic brain tumours after lung cancer, accounting for ~20% of all secondary brain tumours [2–4] and causing a significant patient morbidity and mortality, with survival time commonly in the order of months [5].

Although curative treatments for metastatic brain tumours remain elusive, anti-inflammatory agents are commonly prescribed to patients with secondary brain tumours to control the symptoms associated with tumour complications and to treat side effects. Dexamethasone is a synthetic glucocorticoid administered to patients with brain metastases to reduce neurological symptoms related to the mass effect of peritumoral oedema. Since it was first used in 1962, it has contributed towards a significant reduction in mortality in patients with brain tumours [6]. However, the beneficial effects of dexamethasone treatment are limited by its associated side effects, including suppression of the immune system [7], hyperglycaemia [8], psychosis [9] and avascular necrosis [10,11].

Aprepitant, also known as L-754 030, and its intravenous prodrug fosaprepitant dihydrochloride, also termed L-758 298 (Emend), is an NK1 receptor antagonist used as an antiemetic to control chemotherapy-induced nausea in many cancer patients; it is the only NK1 antagonist that has been approved for use in humans [12–15]. The mechanism of chemotherapy-induced nausea is believed to be through neurotransmitter release in the gastrointestinal tract and in the central nervous system, with the vomiting centre and chemoreceptor trigger zone in the medulla oblongata being particularly affected [16]. It is believed that NK1 antagonism exerts inhibitory activity on this process both centrally and peripherally [17]. Emend is coadministered commonly with dexamethasone, and the maximum benefit is observed when combined with the 5-HT<sub>3</sub> receptor antagonist [18]. Despite the common use of dexamethasone and Emend in cancer patients, their effect on tumour growth remains controversial.

It has been suggested that dexamethasone may also act to control cancer growth [19,20]. Dexamethasone has been shown to reduce brain tumour volume *in vivo* in murine models of brain tumours [19–23], although it remains unclear whether this results from decreased oedematous

fluid or decreased tumour cell viability and proliferation [20–23]. Indeed, the effect of dexamethasone on tumour cell proliferation and apoptosis *in vivo* has not been investigated. Several *in-vitro* studies have found that dexamethasone exerts an antiproliferative, proapoptotic effect on cancer cells [24–26], whereas in other apparently conflicting *in-vitro* studies, dexamethasone was not always able to inhibit tumour cell growth [20,21]. Despite the consistent use of dexamethasone as the standard treatment for peritumoral brain oedema for many years, its exact mechanism of action remains unclear. Furthermore, dexamethasone treatment is often investigated in conjunction with other chemotherapy treatments, meaning that its mechanism of action alone remains unclear [19,27–29].

Several studies have shown an increase in NK1 receptor expression in human surgical specimens of astrocytoma and brain metastases from breast carcinomas and melanoma [30–35]. Substance P (SP) is a proinflammatory tachykinin that acts preferentially on NK1 receptors. Recent studies have implicated SP in the proliferation and progression of many cancer types [36]. In cell culture studies, SP has consistently been shown to induce tumour cell mitogenesis, with NK1 antagonists causing apoptosis [31,37–44] and decreased mitogenesis, particularly on NK1 receptor-expressing human cancer cell lines, whereas non-neoplastic cell lines did not show these effects [33]. Thus, it has been suggested that the NK1 receptor antagonist Emend may not only be useful in the treatment of chemotherapy-induced emesis, but may also inhibit SP-induced tumour cell proliferation. Therefore, Emend may aid in the treatment of cancer itself, as well as the side effects of chemotherapeutic agents [45–48]. However, the literature shows conflicting results. Although some studies have shown that NK1 antagonists or inhibition of SP decrease tumour growth [49–51], almost an equal number of studies have shown that SP inhibits tumour growth through its stimulatory effects on the immune system [52–55].

The current study was carried out to determine the effects of NK1 antagonism and dexamethasone on breast carcinoma cell growth *in vitro* and *in vivo* at clinically relevant doses.

## Materials and methods

### Cell viability assay

Walker 256 rat breast carcinoma cells were obtained from the Cell Resource Centre for Medical Research at Tohoku University. Culture was performed in complete culture medium consisting of Sigma RPMI-1640 containing 10% sterile fetal bovine serum and 1% penicillin and streptomycin (Sigma 10 000 U penicillin and 10 mg of streptomycin/ml; Sigma, Sydney, Australia). Fosaprepitant dimeglumine (Emend<sup>®</sup> Merck & Co. Whitehouse Station, New Jersey, USA), *N*-acetyl-L-tryptophan (NAT)

and DBL dexamethasone sodium phosphate were used in this assay. To assess the response of Walker 256 tumour cells to differing doses of NK1 antagonists (Emend and NAT) and dexamethasone, a trypan blue cell viability assay was used. A sample of  $10^5$  cells were seeded into each well of a 12-well tissue culture plate with 2 ml of complete culture medium. Cells were allowed to grow for 24 h, after which the drugs of interest or saline as a vehicle control were added for a further 24 h. Each treatment was applied at three different concentrations, and each concentration was applied to three wells on four different occasions ( $n = 12$ ). The three concentrations used were 10, 100 and 1000  $\mu\text{g/ml}$  for each of the three agents (equivalent to 9.95, 99.52 and 995.22  $\mu\text{mol/l}$  for Emend; 40.61, 406.07 and 4060.7  $\mu\text{mol/l}$  for NAT; 19.36, 193.65 and 1936.5  $\mu\text{mol/l}$  for dexamethasone). Cells were detached using 0.02% EDTA and transferred into tubes that were subsequently centrifuged for 5 min at 1500 rpm. The cells were then resuspended in 1 ml of fresh medium with 1 ml of 0.4% trypan blue. Cells capable of excluding the trypan blue dye were counted as viable cells. The percentage of viable Walker 256 cells was calculated in relation to the total cell count using a haemocytometer.

### Cell culture for inoculation

Cells growing in 150  $\text{cm}^2$  culture flasks were passaged when more than 90% confluence was reached using 3.5 ml of 0.02% EDTA. The cells were spun down in a centrifuge (5 min at 1500 rpm) and resuspended in serum-free media. The cells were counted using a haemocytometer and then diluted so that  $\sim 10^6$  cells/8  $\mu\text{l}$  were used for direct injection into the brain.

### Animals

Animal procedures were performed in accordance with the National Health and Medical Research Council (NHMRC) guidelines and were approved by the animal ethics committees of the University of Adelaide and the Institute of Medical and Veterinary Science, Adelaide. Animals were group housed and allowed free access to food and water. Six male albino Wistar rats between 250 and 350 g were randomly selected for each treatment group. Male animals were used to eliminate the possible confounding effects of oestrogen, a known neuroprotective agent [56].

### Tumour inoculation

The direct inoculation model of secondary brain tumour induction was used in this study, as it produces tumour growth of consistent size and location. This was appropriate to the current study, as tumour growth was of particular interest rather than the invasion of tumour cells through the blood–brain barrier (BBB) and into the brain. Animals were stereotaxically inoculated with  $10^6$  Walker 256 breast carcinoma cells in 8  $\mu\text{l}$  of sterile culture medium or culture medium alone as a control.

Briefly, tumour cells were implanted into the striatum at coordinates 0.5 mm anterior and 3 mm lateral to the bregma over the right hemisphere, under isoflurane inhalation general anaesthesia at 3%, as was described previously [57].

### Treatment

All drugs used were dissolved in a 0.9% sodium chloride solution, and this was also used as a vehicle control. Unless otherwise stated, each animal group was treated with an intraperitoneal injection of either the NK1 antagonist Emend<sup>®</sup> (3 mg/kg/day), the NK1 antagonist NAT (7.5 mg/kg/day), DBL dexamethasone sodium phosphate (8 mg/kg/day) or equal volume of saline on days 4–6 following tumour inoculation. The dose used for dexamethasone treatment was determined by that reported previously in the literature for use on murine models of brain tumours [58,59]. The Emend dose was based on that recommended for intravenous injections clinically and previously shown to have a central effect in animal models [60], whereas the NAT dose was determined based on that previously used to alter the permeability of the BBB [61]. In addition, a dose response was performed for Emend to determine whether 3, 30 or 300 mg/kg/day on days 4–6 following tumour inoculation altered tumour volume. All animals were killed on day 7 following tumour inoculations.

### Tumour volume

To determine tumour volume, animals ( $n = 6$ /group) were transcardially perfused with 10% formalin under terminal anaesthesia induced by an intraperitoneal injection of pentobarbitone sodium (60 mg/kg). Brains were embedded in paraffin wax and 5  $\mu$ m serial coronal sections were cut every 400  $\mu$ m to be used for immunostaining and haematoxylin and eosin staining. The haematoxylin and eosin-stained sections were scanned using a Hamamatsu Nanozoomer (Hamamatsu, Hamamatsu City, Japan) and the images were used to calculate the tumour volume by determining the area of tumour in each section using the NDP viewer program and multiplying the area by the distances between sections. The same technique was used to determine the volume of haemorrhage and necrosis within the tumour mass.

### Immunostaining

Slides from two levels from each brain in each treatment group ( $n = 6$ /group) were stained for NK1 receptors (1:8000; Biocore, Sydney, Australia), Ki67 (1:2000; Abcam, Cambridge, Massachusetts, USA), caspase 3 (1:400; Bio Vision, Milpitas, California, USA), glial fibrillary acidic protein (GFAP) (1:40000; Dako, Campbellfield, Australia) and ionized calcium-binding adapter molecule 1 (IBA1) (1:50000; Dako). Immunohistochemistry was performed using the standard streptavidin procedure used regularly in our laboratory with 3,3'-diaminobenzidine (DAB) for visualization and

haematoxylin counterstaining. Slides were scanned using the Nanozoomer.

### Analysis of NK1 receptor, GFAP and IBA1 immunostained sections

Images were exported from the Nanozoomer files for all immunostained sections. From each slide, four images were taken from each of the following areas: the tumour, the peritumoral area and the striatum. Nonsubjective estimation of the immunocytochemical staining was carried out using colour deconvolution techniques to reveal the %DAB in the scanned slides as described previously [62,63]. The %DAB from the four fields of view were averaged to determine the mean immunoreactivity in each area for each stain used. For GFAP and IBA1 immunostained slides, in addition to colour deconvolution, labelled cells in the images were counted to determine the effect of treatment on the number of GFAP-labelled and IBA1-labelled cells.

### Tumour cell replication, density and apoptosis

Ki67-labelled cells were counted in four fields of view, each equalling 0.0678 mm<sup>2</sup> as representative for the tumour, and the percentage of labelled cells in relation to the total number of tumour cells in the tumour mass was calculated. The same method was used to determine the percentage of caspase 3-labelled cells indicative of apoptotic tumour cells. Similarly, the density of tumour cells was determined by counting tumour cells within six fields of view 0.0678 mm<sup>2</sup> each within the tumour from haematoxylin and eosin-stained slides for each brain ( $n = 6$ ).

### Statistical analysis

Data were expressed as mean  $\pm$  SEM. To determine statistical significance, either an unpaired *t*-test (two groups) or a one-way analysis of variance (more than two groups), followed by Bonferroni's post-hoc tests was carried out as appropriate. A value of *P* less than 0.05 was considered significant.

## Results

### Cell viability assay

Treatment with NK1 antagonists *in vitro* showed varying results on Walker 256 cell viability. Although Emend treatment at both 100 and 1000  $\mu$ g/ml caused a significant reduction in viable cells that excluded trypan blue dye ( $P < 0.001$ ; Fig. 1), NAT treatment exerted no effect on the percentage of viable tumour cells at any concentration (Fig. 1). Similarly, dexamethasone did not alter the percentage of viable tumour cells after 24 h of treatment at any of the concentrations used (Fig. 1).

### NK1 receptor expression *in vivo*

The induced Walker 256 tumours expressed NK1 receptors *in vivo*, as evidenced by the significant increase in NK1 receptor immunoreactivity within the tumour

mass when compared with the peritumoral area of tumour inoculated brains 7 days following surgery ( $P < 0.05$ ; Fig. 2a and b). The immunostaining was localized within the tumour cell cytoplasm (Fig. 2b).

### Tumour growth

Following Walker 256 tumour cell inoculation, treatment with the NK1 receptor antagonist Emend or NAT did not cause a significant difference in tumour volume when compared with vehicle-treated animals (Fig. 3a, c–e). Similarly, at higher doses of 30 and 300 mg/kg/day on days 4–6 following tumour inoculation, there was still no significant difference in tumour volume from the vehicle-treated group (Fig. 3g). Conversely, dexamethasone treatment resulted in a considerable decrease in tumour volume when compared with the vehicle-treated and other treated groups (Fig. 3a, c–f). Furthermore, dexamethasone treatment also caused a reduction in necrosis within the tumour mass when compared with the vehicle-treated group (Fig. 3b). NK1 antagonists did not exert any effect on the percentage of necrosis or haemorrhage

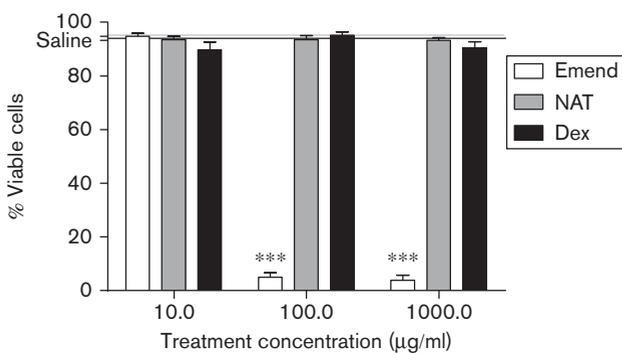
within the tumours compared with the vehicle control (Fig. 3b).

Neither of the NK1 receptor antagonists, Emend or NAT, exerted any effect on tumour density or Ki67 immunoreactivity, which indicates replicating cells (Fig. 4a and b). Similarly, neither Emend nor NAT altered the percentage of caspase 3-positive cells, which indicates apoptotic tumour cells (Fig. 4c). Dexamethasone treatment caused a significant increase in the density of tumour cells within the tumour mass when compared with the vehicle-treated group ( $P < 0.01$ ; Fig. 4a). However, dexamethasone treatment did not alter the percentage of Ki67-positive tumour cells or caspase 3-positive cells (Fig. 4b and c). Therefore, the dexamethasone-treated group showed tumour masses with cancer cells more tightly packed together, but with no change in replication or apoptosis.

### Brain microenvironment

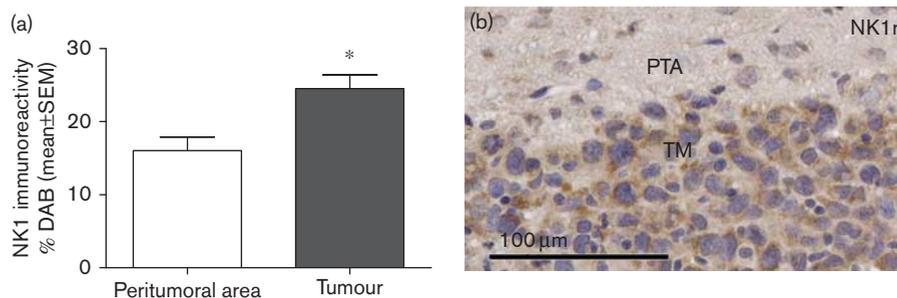
GFAP and IBA1 immunoreactivity were used as indicators of astrocytic and microglial response, respectively, and thus represented the interaction of the tumour cells and treatment agents with these components of the brain microenvironment. Inoculation with Walker 256 breast carcinoma cells and subsequent tumour growth caused a significant increase in GFAP and IBA1 immunoreactivity in the peritumoral area when compared with the same location in culture medium control animals ( $P < 0.001$  and  $P < 0.05$ , respectively; Fig. 5a–e). GFAP immunoreactivity was not present within the tumour, and thus showed significantly reduced %DAB when compared with the peritumoral area ( $P < 0.001$ ; Fig. 5a and b). Furthermore, the tumour mass also showed less GFAP immunoreactivity than the same location in the brains of control animals, injected with culture medium without Walker 256 tumour cells (Fig. 5a). However, IBA1 immunoreactivity was significantly elevated within the tumour mass when compared with that evident in the striatum

Fig. 1



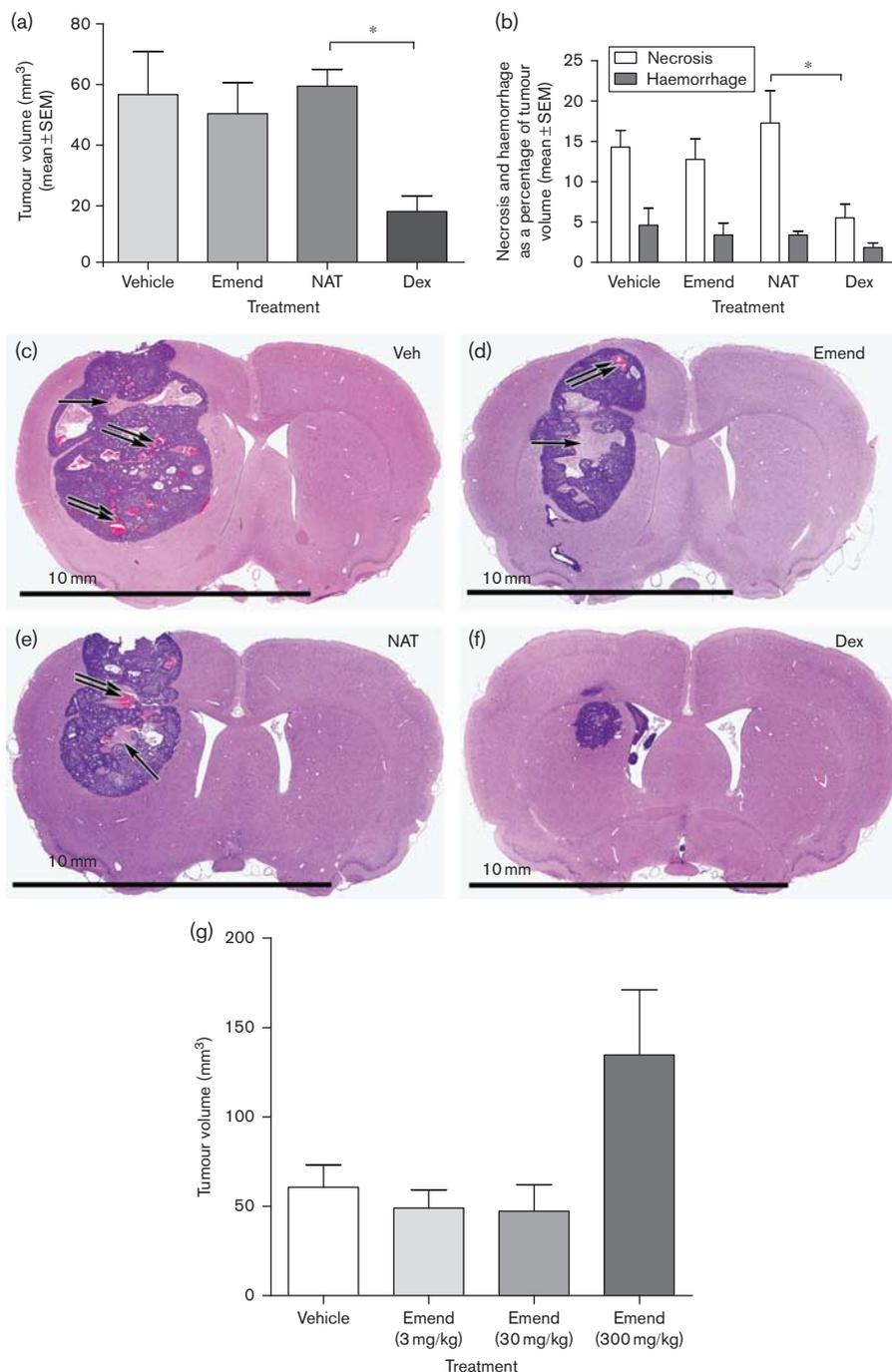
Walker 256 cell viability as indicated by trypan blue exclusion assay. NAT, *N*-acetyl-L-tryptophan; Dex, dexamethasone. \*\*\* $P < 0.001$ .

Fig. 2



(a) Graph showing NK1r immunoreactivity in the peritumoral area and within the tumour mass in tumour-inoculated animals 7 days following surgery. (b) NK1r immunostained brain section showing tumour cells labelled brown 7 days following tumour inoculation. DAB, 3,3'-diaminobenzidine; NK1r, NK1 receptor; PTA, peritumoral area; TM, tumour mass. \* $P < 0.05$ .

Fig. 3



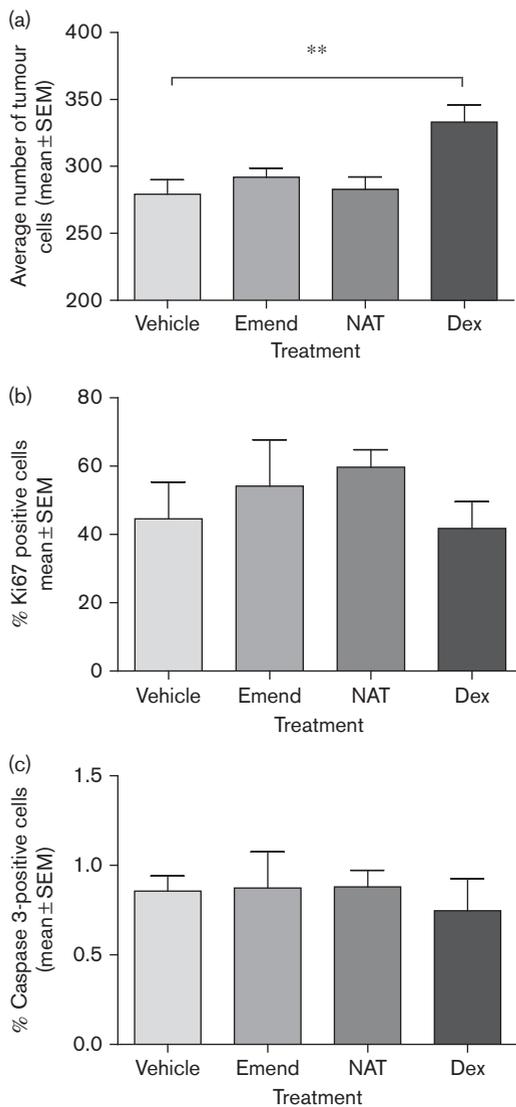
(a) Tumour volume (mm<sup>3</sup>) in Emend, *N*-acetyl-L-tryptophan (NAT), and dexamethasone (Dex)-treated animals compared with vehicle control treatment. (b) The volume of haemorrhage and necrosis within the tumour mass as a percentage of the tumour volume in Emend, NAT and Dex compared with vehicle-treated animals. (c–f) Haematoxylin and eosin-stained coronal section from animals treated with vehicle (Veh) (c), Emend (d), NAT (e) and Dex (f). The sections show large tumour masses in the right hemisphere, with an apparent reduction in size in the Dex-treated animals (f) (necrosis, single arrow; haemorrhage, double arrows). (g) Dose response of tumour volume (mm<sup>3</sup>) to Emend compared with the vehicle control treatment. \**P* < 0.05.

of brains from the culture medium control group (*P* < 0.01; Fig. 5c–e).

In conjunction with the colour deconvolution results, GFAP-immunolabelled and IBA1-immunolabelled cells

were also counted to determine the number of astrocytes and microglia, respectively. The growth of Walker 256 breast carcinoma tumours caused an increase in the number of astrocytes and microglia in the striatum surrounding tumour masses when compared with similar brain locations

Fig. 4



(a) The effect of treatment on tumour cell density. The average number of cells was obtained from four areas within the tumours in animals treated with vehicle, Emend, *N*-acetyl-L-tryptophan (NAT) and dexamethasone (Dex). (b) The percentage of replicating tumour cells as indicated by Ki67 immunoreactivity in animals treated with vehicle, Emend, NAT and Dex. (c) The percentage of caspase 3 immunopositive cells, indicative of apoptosis, in tumour masses from animals treated with vehicle, Emend, NAT and Dex. \*\* $P < 0.01$ .

in the culture medium control group, although this difference was only significant for IBA1 ( $P < 0.05$ ; Fig. 6a and b). Treatment with Emend caused a reduction in the number of GFAP-positive and IBA1-positive cells to levels comparable to the culture medium-injected control group (Fig. 6a and b). Dexamethasone induced a phenomenon similar to Emend, particularly with respect to the number of IBA1-positive cells (Fig. 6a and b). Furthermore, there was a small reduction in the number of astrocytes and microglia present in the peritumoral area of NAT-treated animals (Fig. 6a and b).

## Discussion

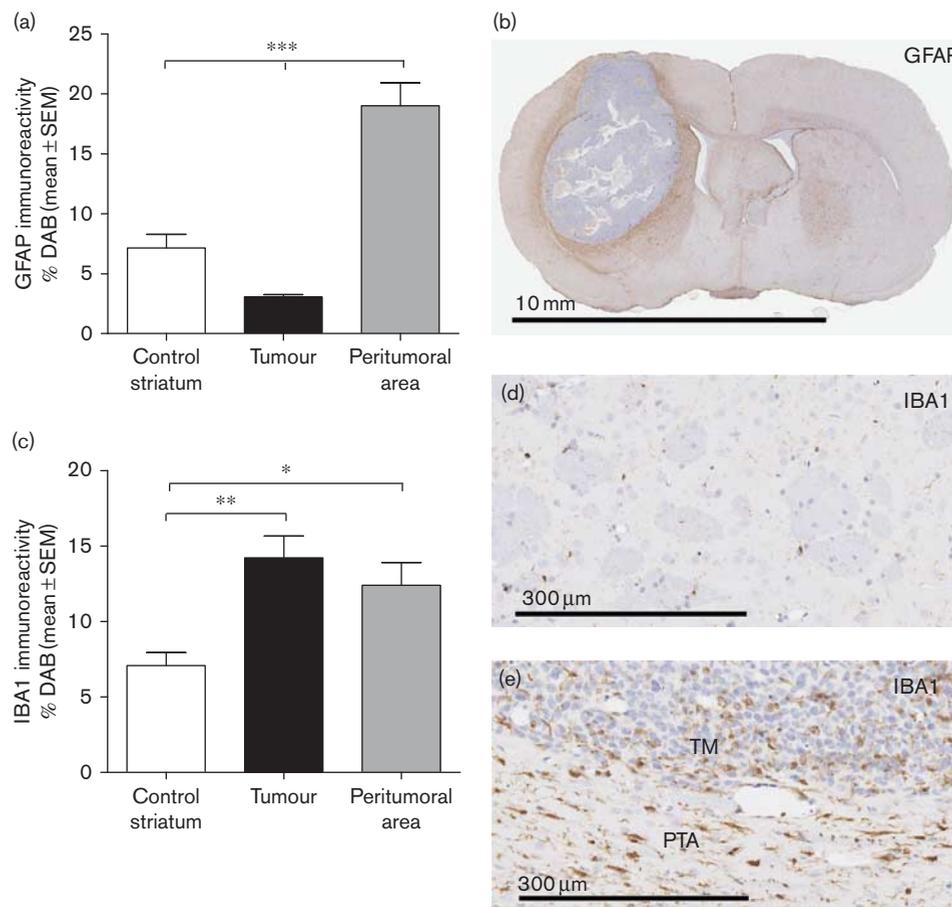
This model of direct stereotaxic inoculation of Walker 256 tumour into the striatum of male albino Wistar rats caused consistent growth of large, spherical-shaped secondary brain tumours. These tumours showed a prominent cystic component and extensive central necrosis and haemorrhage, as described previously in the literature [57]. The model used in the current study is advantageous in that it is used in immune-competent animals, thus allowing the study of the interaction of tumour cells with the host microenvironment and immune system.

SP is an excitatory tachykinin, which acts predominantly on NK1 receptors and has been implicated in many aspects of cancer growth and progression. The Walker 256 rat breast carcinoma cells expressed NK1 receptors *in vivo* when injected into the striatum of male Wistar rats. It has been reported previously that many human tumour specimens and their derived tumour cell lines express NK1 receptors [40,41,43,46,64–66]. Some cell lines have been reported to express up to 40 000 NK1 receptors per cell [67], with the level of NK1 receptor expression being linked to the invasiveness of the cancer cell lines [68]. Moreover, noncancerous epithelial cell lines did not show evidence of NK1 receptor expression [33,69]. However, NK1 receptors are by no means present on all cancer cell types, with one study reporting NK1 expression on SNB-19, DBTRG-05 MG and U373 MG human glioma cell lines, but not on the U138 MG or MOG-G-GCM human glioma cells [70]. Furthermore, the murine neuroblastoma cell line C1300 did not show evidence of NK1 receptor expression [71].

Previous studies aimed at determining the effects of NK1 antagonist treatment on tumour cells *in vitro* have reported an inhibition of tumour growth, initiation of apoptosis, decreased migration and reduced cytokine secretion by tumour cells [31,38,41,42,46,47,66,70,72]. The predominant effects of exogenous SP on tumour cells *in vitro* are mitogenesis, migration, cytokine secretion and chemotaxis [40,43,73–78]. These effects are believed to be NK1 receptor dependent [70].

Alternatively, several studies have shown evidence of inhibitory effects for SP on the growth and migration of cancer cells [51,79,80]. However, the NK1 receptor expression status of these cell lines was not determined. In contrast, the Walker 256 cells used in the current study are positive for NK1 receptor expression, but only responded to Emend treatment and not to NAT treatment *in vitro*. Besides Emend having a much greater binding capacity for the NK1 receptor because of its lipophilicity, a plausible explanation for this may be that this cell line does not secrete SP into the culture medium *in vitro* and is thus unable to exert a stimulatory autocrine effect on the NK1-expressing cells. It has been reported

Fig. 5



(a) GFAP immunoreactivity in the striatum of culture medium control animals compared with the tumour mass and the peritumoral area of the right hemisphere of tumour inoculated brains 7 days following surgery. (b) Coronal section of GFAP-immunostained brain 7 days following tumour inoculation showing increased immunoreactivity in the peritumoral area when compared with the similar location in the contralateral hemisphere. (c) IBA1 immunoreactivity in the striatum of the culture medium control group compared with the tumour mass and in the peritumoral area of the right hemisphere of tumour inoculated brains 7 days following surgery. (d) Section from the right striatum of a culture medium control brain stained for IBA1 antibody, showing minimal microglial immunoreactivity. (e) IBA1-immunostained brain 7 days following tumour inoculation showing increased immunoreactivity in the peritumoral area (PTA) and tumour mass (TM). DAB, 3,3'-diaminobenzidine; GFAP, glial fibrillary acidic protein; IBA1, ionized calcium-binding adapter molecule 1. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

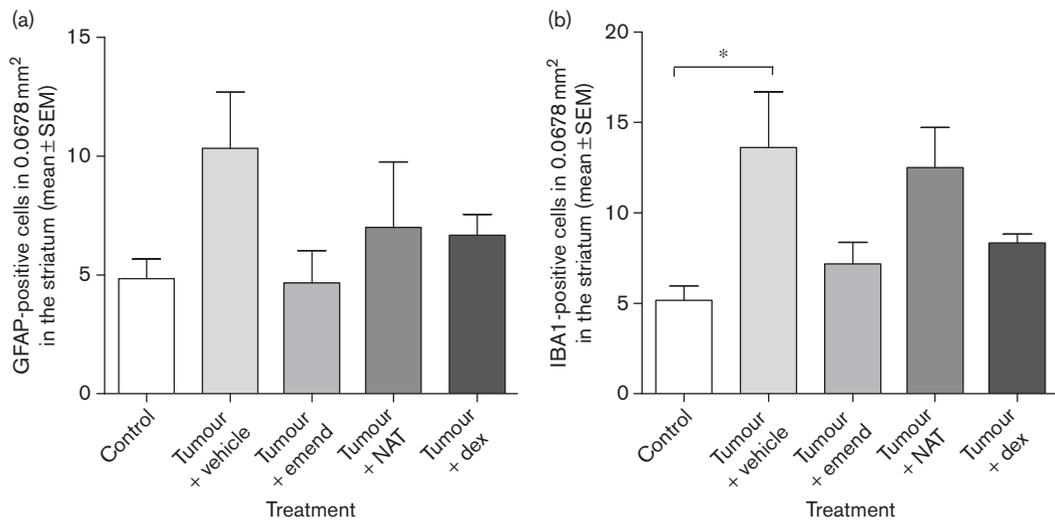
previously that Walker 256 breast carcinoma cells do not express SP in this model when injected directly into the brain [57]. However, tumours induced from this cell line do show increased SP immunoreactivity in the peritumoral neuropil in two different models of metastatic brain tumours [57,81]. Thus, it is plausible that exogenous SP from the brain microenvironment may exert a stimulatory effect on these tumour cells *in vivo*.

NK1 antagonist treatment of metastatic brain tumours grown in this study did not alter tumour cell volume or the volume of necrotic haemorrhagic tissue within the tumour mass. Furthermore, the levels of tumour cell replication and apoptosis remained unaffected by both NK1 antagonist treatments used. There have been few previous studies examining the effects of NK1 antagonism on cancer growth in animal models, despite the fact

that Emend is already approved for use in cancer patients for nausea associated with chemotherapy. The limited number of experiments that have been conducted *in vivo* have been inconsistent in their findings. One published study reported a beneficial effect of the NK1 antagonist MEN 11467 on subcutaneously inoculated human U373 MG astrocytoma grade III cells by arresting tumour growth, but not when used on human A2780 ovarian carcinoma lacking NK1 receptors, also inoculated into the right flank of female nude mice [49].

In contrast, studies that have been carried out in immune-competent animals have suggested that SP may be beneficial in the treatment of cancer patients through its immune-stimulating properties. For example, aerosolized SP caused a reduction in the incidence of lung carcinoma in C57BL mice exposed to side stream

Fig. 6



(a) The effect of treatment on the number of GFAP-immunolabelled cells in the striatum of culture medium control animals and the striatum surrounding the tumours in inoculated animals 7 days following surgery. (b) The effect of treatment on the number of IBA1-immunolabelled cells within the striatum of culture medium control animals and the striatum surrounding the tumours in inoculated animals 7 days following surgery. NAT, *N*-acetyl-L-tryptophan; Dex, dexamethasone; GFAP, glial fibrillary acidic protein; IBA1, ionized calcium-binding adapter molecule 1. \* $P < 0.05$ .

cigarette smoke [52]. Furthermore, K1735 melanoma cells injected subcutaneously into nude mice responded to exogenous SP with a delay in tumour growth, but only with the addition of natural killer and T cells, normally absent in immune compromised animals [53]. Therefore, the role of SP in cancer growth *in vivo* remains controversial, with more research required to elucidate the factors that determine whether NK1 antagonist treatment is effective in the inhibition of tumour growth in animal models.

Unlike previous experiments showing cytotoxic effects of dexamethasone on tumour cells [24–26], the current study did not show any evidence of dexamethasone toxicity on Walker 256 breast carcinoma cells as measured by the trypan blue exclusion cell viability assay. The effects of dexamethasone on Walker 256 cell viability have not been investigated previously in culture, although it has been shown to decrease parathyroid hormone secretion in a dose-dependent manner [82]. Previous *in vitro* studies have shown dexamethasone to inhibit mitogenesis and cell viability of C6 glioma cells along with decreasing migration and invasive properties of U373 MG human glioblastoma cells [25,26]. However, if dexamethasone is toxic to tumour cells *in vivo*, then it is also likely that non-neoplastic brain cells would also become apoptotic [24].

Dexamethasone treatment resulted in decreased brain tumour volume compared with the vehicle-treated group. This is likely to be because of impaired accumulation of fluid, as found in previous studies [57], rather than a decrease in the growth of the tumour cells. Evidence

of this is the absence of change in tumour cell replication and apoptosis with dexamethasone treatment in the current study. Moreover, this is supported by the significantly increased tumour cell density, evident in the dexamethasone-treated group when compared with vehicle-treated animals. Thus, the dexamethasone-treated group may have the same number of tumour cells, packed more closely together, reducing the tumour volume. Also, the decreased necrotic tissue within the dexamethasone-treated tumours indicates that although the tumour volume is reduced in this group, it is possible that the tumours had viable tumour cells comparable to the number of cells in the vehicle-treated tumours.

A reduction in tumour volume has been repeatedly reported with dexamethasone treatment in the literature [20–23]. These data have been used with dexamethasone's toxicity *in vitro* to suggest an anticancer effect for dexamethasone. However, dexamethasone has also consistently been shown to reduce BBB permeability and oedema [21–23,59,83–86], which may account for the volume decreases observed. Another proposed mechanism of dexamethasone inhibition of tumour growth is through manipulation of the host microenvironment. Dexamethasone causes a blockade of classical inflammation, normally favourable for tumour invasion and growth [57,87–89].

In the current study, tumour growth caused an increase in both astrocytes and microglia, particularly in the peritumoral environment. This phenomenon has been well documented in many metastatic brain tumour models [90,91]. Emend treatment decreased this glial

response to tumour growth, showing that the dose used in this study was sufficient to elicit a central effect. NAT was less effective than Emend in this capacity, possibly because of its inability to cross the BBB [92]. Emend is a BBB-penetrating drug able to occupy cerebral NK1 receptors [93]; therefore, it is logical that it would exert a greater effect on the brain microenvironment. Dexamethasone also reduced glial numbers to a level similar to Emend treatment, although it is unclear whether this effect was because of inhibition of tumour initiated glial recruitment, activation and proliferation, or because of toxic effects on astrocytes and microglia. It has been postulated that microglia secrete growth-stimulatory substances that aid metastatic brain tumour growth, as evidenced by the increase in MDA-MB-231 human breast cancer cells when cocultured with microglia *in vitro* [94]. Other tumour cells have been shown to differentially activate microglia so that subpopulations express inducible nitric oxide synthase and tumour necrosis factor- $\alpha$  [95]. Thus, with more investigation, glial regulation may provide a possible target for the manipulation of metastatic brain tumour growth.

### Conclusion

Neither of the NK1 antagonists acted as an anticancer agent in this animal model of metastatic brain tumours, despite the expression of NK1 receptors on Walker 256 tumour cells and the fact that Emend reduced cell viability *in vitro*. In conjunction, dexamethasone treatment also did not affect tumour growth, notwithstanding the evident reduction of tumour volume. Therefore, neither Emend nor dexamethasone is effective as an anticancer agent in this model of brain metastatic breast cancer. However, further research is required using different models and cancer cells to definitively determine the exact effect of NK1 antagonists and dexamethasone treatments on tumour growth.

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### Conflicts of interest

There are no conflicts of interest.

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