BJP British Journal of Pharmacology

DOI:10.1111/bph.12589
www.brjpharmacol.org

Themed Section: Opioids: New Pathways to Functional Selectivity

REVIEW

Comparison and analysis of the animal models used to study the effect of morphine on tumour growth and metastasis

B Afsharimani[1], C W Doornebal[2], P J Cabot[1], M W Hollmann[2] and M-O Parat[1]

[1]*School of Pharmacy, University of Queensland, Woolloongabba, Qld, Australia, and*
[2]*Department of Anaesthesiology, Academic Medical Center, Amsterdam, The Netherlands*

Correspondence
M-O Parat, School of Pharmacy, University of Queensland, 20 Cornwall Street, Woolloongabba, Qld, Australia. E-mail: m.parat@uq.edu.au

Received
16 October 2013
Revised
27 November 2013
Accepted
5 December 2013

The effect of opioids on tumour growth and metastasis has been debated for many years, with recent emphasis on the possibility that they might influence the rate of disease-free survival after tumour resection when used in the perioperative pain management of cancer surgery patients. The literature presents conflicting and inconclusive *in vitro* and *in vivo* data about the potential effect of opioids, especially morphine, on tumour growth and metastasis. To inform clinical practice, appropriate animal models are needed to test whether opioids alter the course of tumour growth and metastasis. Here, we review the literature on animal-based studies testing the effect of morphine on cancer so far, and analyse differences between the models used that may explain the discrepancies in published results. Such analysis should elucidate the role of opioids in cancer and help define ideal pre-clinical models to provide definitive answers.

LINKED ARTICLES
This article is part of a themed section on Opioids: New Pathways to Functional Selectivity. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-2

Abbreviations
HPA, hypothalamic–pituitary–adrenal; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; TLR, Toll-like receptors

Introduction

Morphine and other opioid analgesics are potent pain-relieving agents that are essential for pain management in cancer patients (Dalal and Bruera, 2013). Besides being the standard of care for the treatment of cancer-related pain in patients with advanced stage disease, opioids – especially morphine – are also routinely used for anaesthetic procedures in cancer patients undergoing surgery. However, there have been concerns that they may affect the rate of post-operative cancer recurrence and metastasis (Afsharimani *et al.*, 2011). Recent retrospective clinical studies evaluating the effects of anaesthetic technique on relapse-free survival after cancer surgery indicated that cancer patients receiving perioperative morphine-based analgesia had a worse prognosis compared with those receiving loco-regional anaesthesia (Exadaktylos *et al.*, 2006; Biki *et al.*, 2008). Based upon these findings, morphine and other opioid analgesics have been postulated to promote cancer progression and relapse (Heaney and Buggy, 2012). Although still rather controversial, these studies collectively raised the question of whether the anaesthetic technique applied during cancer surgery might affect relapse-free survival after surgery (Sessler, 2008; Singleton and Moss, 2010).

To resolve this controversy, several randomized clinical trials in breast, lung and prostate cancer patients have been initiated. These clinical studies are designed to directly compare relapse-free survival after cancer surgery in patients receiving either loco-regional anaesthesia or perioperative morphine-based analgesia. Yet, given their design, these

studies will not allow assessment of any potential tumour-promoting effects of morphine-based analgesia. To address this question, we need to rely upon *in vivo* studies that evaluate the effects of morphine on tumour progression and metastatic disease in a well-controlled experimental setting. In this review, we summarize the currently available data from pre-clinical studies evaluating the effects of morphine on tumour growth and metastatic disease. Interestingly, results from these studies show discrepant results ranging from deleterious, null to protective effects for morphine. This review critically evaluates the models that have been used, in an attempt to elucidate the parameters that may explain these discrepancies and therefore shed some light on the role of morphine in cancer. To support future research, we further discuss some essential characteristics that should be met by pre-clinical models in order to address this question in a clinically relevant setting.

The tumour models used

To evaluate the effects of morphine on tumour progression and metastatic disease, a wide variety of pre-clinical models have been employed. As shown in Table 1, most studies are performed with cancer cell line-based tumour models. In these models, *in vitro* maintained cancer cell lines are transplanted either orthotopically (in the anatomic location of origin for this specific tumour cell line) or ectopically (in another organ or location), or injected i.v. into hosts. Unfortunately, these models present considerable shortcomings, as they do not faithfully reproduce *de novo* tumourigenesis and metastatic disease in humans. For example, cancer cell lines, maintained *in vitro,* often fail to reflect the original heterogeneity of the parental tumour (Keller *et al.*, 2010; Domcke *et al.*, 2013). As intra-tumour heterogeneity corresponds to a wide phenotypic variety and at least partially determines clinically relevant tumour-related features including the ability to seed and responses to therapy (Marusyk *et al.*, 2012), data from such studies cannot easily be extrapolated to the clinical setting.

Most studies evaluating the impact of morphine on metastatic disease have not used orthotopic tumour models. Most of them utilize s.c. tumour cell inoculation or tail vein injection assays. Tail vein injections of tumour cells have been used in rats (Yeager and Colacchio, 1991; Page *et al.*, 1993; 1994; 1998; Colacchio *et al.*, 1994; Bar-Yosef *et al.*, 2001; Franchi *et al.*, 2007) and mice (Harimaya *et al.*, 2002; Afsharimani *et al.*, 2014) with measurement of the tumour burden in the lungs or liver. These models attempt to mimic homing and outgrowth of circulating tumour cells or cells released during surgery, at distant sites. However, cultured, usually adherent, tumour cells are likely to be different from the circulating cells that are found in increased numbers in patients undergoing surgery, as well as spontaneous circulating tumour cells (Thompson and Haviv, 2011). Furthermore, these models fail to reproduce the biology of *de novo* metastatic disease (Fantozzi and Christofori, 2006; Jonkers and Derksen, 2007; Valastyan and Weinberg, 2011). These defects are further complicated by the fact that most inoculated tumour cells are likely to undergo apoptosis. The massive release of tumour-related antigens may induce acute adaptive anti-tumour immune responses, which are normally absent due to the formation of immuno-suppressive networks driving escape from immune surveillance in spontaneously arising tumours (Willimsky *et al.*, 2008). Consequently, the efficacy of immune surveillance may be overestimated in cancer cell line-based tumour models.

Orthotopic models (Gupta *et al.*, 2002) appear appropriate if the objective of the study is to assess the effect of morphine on the growth of a primary tumour. Moreover, spontaneously metastasizing models have been proposed to present the advantage of allowing the study of the effect of morphine on metastasis. A major factor that needs to be taken into consideration is whether the animals are immunocompetent. Immunocompromised mice must be used when allogeneic tumour cells are implanted (Gupta *et al.*, 2002; Tegeder *et al.*, 2003; Roy *et al.*, 2006), and, while this allows the study of cancer cells of human origin, the effects of opioids on the immune response are underestimated in such models. This is of paramount importance, as accumulating evidence indicates that the immune system plays a crucial role both at the level of the primary tumour and at distant, metastatic sites (de Visser *et al.*, 2006; Joyce and Pollard, 2009).

The presence of pain and surgical stress in the model

In addition to perioperative pain, surgery involves an inflammatory and neuroendocrine response to tissue injury that alters immune competence including (but not limited to) the activity of natural killer cells, which play a crucial role in the metastatic process (Talmadge *et al.*, 1980). The inclusion of pain or surgical stress into an animal model of tumour cell dissemination and growth is thus a major factor that will influence the role of opioids and the experimental outcome. Indeed, with few exceptions (Colacchio *et al.*, 1994; Farooqui *et al.*, 2007), morphine affords protection towards tumour growth or dissemination in the context of pain and surgical stress – elicited intentionally by laparotomy or tumour-induced hyperalgesia, or unintentionally, for example, by surgical insertion of drug-releasing pellets – but not in the absence of pain (Simon and Arbo, 1986; Yeager and Colacchio, 1991; Page *et al.*, 1993; 1994; 1998; Bar-Yosef *et al.*, 2001; Sasamura *et al.*, 2002; Franchi *et al.*, 2007). A model with no pain can specifically reveal the non-analgesic effects of morphine. In contrast, an animal tumour model, which includes pain or stress response to surgery, is better suited to represent the perioperative period in humans but does not allow dissection of the mechanisms (analgesia-mediated or -independent) of morphine's actions.

Designing new animal models to evaluate the effect of morphine on tumour growth and metastasis

Given these considerations, how should models be designed to study the effects of morphine on tumour growth and

Table 1

Studies employing a pre-clinical model to assess the effect of morphine on tumour growth and metastasis

Rodent model	Induced pain or surgical stress	Dose of morphine	Effect of morphine on tumour growth and metastasis	Reference
Walker 256 carcinoma cells cultivated in ascites, then injected in Sprague-Dawley rats Immuno-competent rats Ectopic (injection in the tail vein)	No	5 $mg \cdot kg^{-1}$ single i.p. injection at the time of tumour inoculation	Increased number of lung metastases	Simon and Arbo (1986)
Both syngeneic and allogeneic Immuno-competent mice Ectopic (s.c., i.p.)	No	10 $mg \cdot kg^{-1}$ s.c. once daily for 10 days	Promotion of tumour growth	Ishikawa *et al.* (1993)
Syngeneic Immunocompetent rats Injection of tumour cells in ileocecal vein	Yes (laparotomy)	Morphine sulfate (15 $mg \cdot kg^{-1}$ 4 doses over 24 h perioperatively)	Increased tumour burden	Colacchio *et al.* (1994)
Allogeneic Immuno-compromised mice Orthotopic breast tumour	No	0.714 $mg \cdot kg^{-1} \cdot day^{-1}$ s.c. for 2 weeks, followed by 1.43 $mg \cdot kg^{-1} \cdot day^{-1}$ for 3 weeks	Increased tumour volume after 32 days	Gupta *et al.* (2002)
Syngeneic A/J mice are immuno-competent but present a defect in macrophage function Ectopic (s.c. in right hind thigh)	Pain increases with tumour growth	0.714 $mg \cdot kg^{-1}$ day^{-1} for 7 days followed by 1 $mg \cdot kg^{-1} \cdot day^{-1}$ for 7 days Morphine non-analgesic	Increased tumour weight and presence of metastases	Farooqui *et al.* (2007)
Syngeneic Immuno-competent rats Tail vein injection of tumour cells	Yes (laparotomy)	10 $mg \cdot kg^{-1}$ immediately and 5 h after surgery	Increased lung diffusion of tumour cells in the absence of surgery. Slightly decreased tumour load (non-statistically significant) in the presence of surgery.	Franchi *et al.* (2007)
Syngeneic colon adenocarcinoma Immuno-competent rats Injection of tumour cells in ileocecal vein	Yes (laparotomy)	20 $mg \cdot kg^{-1}$ morphine s.c. 1 day before and 2 days after tumour inoculation	Reduced tumour burden	Yeager and Colacchio (1991)
Syngeneic Immuno-competent rats Tail vein injection of tumour cells	Yes (laparotomy)	5 $mg \cdot kg^{-1}$ i.p. 30 min before surgery, 5 $mg \cdot kg^{-1}$ s.c. in slow-release suspension immediately after surgery 5–10 $mg \cdot kg^{-1}$ s.c. in slow-release suspension 5 h after surgery	Reduced tumour burden in the presence of surgical stress. No effect in the absence of surgical stress.	Page *et al.* (1993)
Syngeneic Immuno-competent rats Tail vein injection of tumour cells	Yes (laparotomy)	10 $mg \cdot kg^{-1}$ i.p. 30 min before surgery and 5 $mg \cdot kg^{-1}$ s.c. in slow-release suspension after surgery	Reduced tumour burden in the presence of surgical stress, No effect in the absence of surgical stress.	Page *et al.* (1994)

Table 1

Continued

Rodent model	Induced pain or surgical stress	Dose of morphine	Effect of morphine on tumour growth and metastasis	Reference
Syngeneic Immuno-competent rats Tail vein injection of tumour cells	Yes (laparotomy)	8 mg·kg^{-1} i.p. 30 min before surgery and/or 4 mg·kg^{-1} s.c. immediately after surgery in a slow-release suspension and/or 2 mg·kg^{-1} s.c. in a slow-release suspension 5 h after surgery	Reduced lung tumour burden in the presence of surgery in all treatment groups	Page *et al.* (1998)
Syngeneic Immuno-competent rats Tail vein injection of tumour cells	Yes (laparotomy)	10 mg·kg^{-1} i.p. at induction of anaesthesia	Reduced lung retention of tumour cells in the presence of surgical stress, but no statistical significance	Bar-Yosef *et al.* (2001)
Syngeneic Immuno-competent mice i.v. injection of tumour cells	no	10 mg·kg^{-1} i.p. for 6 days	Decreased lung metastases	Harimaya *et al.* (2002)
Syngeneic Immuno-competent mice Ectopic (melanoma cells s.c. in hind paw) even though the authors claim orthotopic	Yes (tumour-induced hyperalgesia)	5 and 10 mg·kg^{-1} s.c. daily for 6 days (days 16–21 post-inoculation) Analgesia was demonstrated	Reduced tumour growth and metastasis	Sasamura *et al.* (2002)
Allogeneic Immuno-compromised mice Ectopic (tumour cells inoculated s.c. in dorsal flank)	No	10, 20 and 30 mg·kg^{-1}·day^{-1} i.p. during first, second and third weeks after inoculation respectively Morphine concentrations checked in plasma after injection, 50–60 μM at 10–25 min, 0.9–3.4 μM at 1–2 h	Decreased tumour volume for breast cancer cell lines MCF7 and MDA-MB231, no effect for colon cancer HT-29	Tegeder *et al.* (2003)
Allogeneic Immuno-compromised (nude) mice Ectopic (LLC implanted in the right flank s.c.)	Not intentionally (but surgical insertion of the pellets)	Day of tumour inoculation: 75 mg morphine pellets implanted days 7–14 20 mg·kg^{-1}·day^{-1} i.p. Days 15–21, 30 mg·kg^{-1}·day^{-1} i.p.	Decreased tumour volume and wet weight	Koodie *et al.* (2010)
Syngeneic Immuno-competent mice Tail vein injection of tumour cells	No	10 mg·kg^{-1} i.p. every day for 3 days	Decreased number of tumour nodules	Afsharimani *et al.* (2014)

Table rows highlighted in blue denote a protective effect of morphine, in red a tumour-promoting effect of morphine.

metastasis? To address this question, pre-clinical tumour models that most closely mimic the clinical setting must be carefully designed. To study the effect of morphine on metastasis independent of the surgery, one approach may be to evaluate the effects of morphine on genetically engineered mouse models of *de novo* tumourigenesis, which have been used successfully to study many aspects of tumour biology (Frese and Tuveson, 2007). These models are generated by tissue-specific manipulation of genes known to be relevant in a certain subtype of human cancer and allow the study of spontaneously arising tumours that closely mimic their human counterparts in an orthotopic, immuno-competent setting. However, with some exceptions (Muller *et al.*, 1988; Boggio *et al.*, 1998; Paez-Ribes *et al.*, 2009), employing geneti-

cally engineered mouse models to study metastatic disease is complicated by asynchroneously arising, rapidly growing, primary tumours that do not allow sufficient time for the establishment of (advanced) metastatic disease (Francia *et al.*, 2011). As a consequence, these models generally show a relatively low incidence of metastatic disease and do not allow the effects of morphine on advanced metastatic disease to be analysed. Another condition is required to better mimic the perioperative setting, which is that the animal model should include a surgical intervention, either primary tumour resection or a more artificial event, inducing surgical stress, tissue damage and pain.

To circumvent these limitations, and to provide information relevant to the context of cancer surgery patients, we have recently developed a pre-clinical mouse model of *de novo* breast cancer metastasis formation (Doornebal *et al.*, 2013). In this model, small tumour fragments of a *de novo* mouse mammary tumour (Derksen *et al.*, 2006) are orthotopically transplanted into wild-type recipients. Once mammary tumours are established, a mastectomy is performed and the mammary tumour is surgically resected. Following surgery, these mice spontaneously develop clinically overt metastatic disease in lungs, liver, spleen and lymph nodes. Using a similar approach to exploit other genetically engineered mouse models provides a unique opportunity to create models that not only reproduce the biology of *de novo* metastatic disease but also allows the evaluation of the effects of morphine using clinically defined outcomes – that is, metastasis-specific survival – in a context that closely mimics the perioperative setting.

The dose and mode of administration of morphine used

A wide range of morphine doses have been used in the pre-clinical experiments testing its effect on tumour growth and metastasis (Table 1), which may contribute to the differences in outcome of these studies. It has been proposed that low, sub-analgesic doses of morphine have mitogenic and angiogenic properties (Tegeder and Geisslinger, 2004). Most studies employ doses of morphine of 5–20 mg·kg^{-1} daily (Table 1) and very few (Tegeder *et al.*, 2003) verify the resulting circulating morphine concentrations. Affinities of the μ-opioid receptors are not critically different between humans and mice (K_i database at http://pdsp.med.unc.edu/). However, as previously noted (Parat, 2013), rodents metabolize morphine differently from humans and produce mostly morphine-3-glucuronide (M3G) (Kuo *et al.*, 1991), which is not analgesic (Shimomura *et al.*, 1971). In contrast, humans produce not only M3G but also morphine-6-glucuronide (M6G), which is a more potent analgesic than morphine (Shimomura *et al.*, 1971; Osborne *et al.*, 1988; 1990). To achieve analgesia, doses of morphine (in mg·kg^{-1}) are therefore much higher in mice than humans. The effect of morphine *per se* can only be compared between rodents and humans, if the circulating (and presumably tissue) concentrations of morphine are similar. Furthermore, given that pain influences tumour growth and metastasis (Page *et al.*, 2001), it is important to note whether the dose of morphine employed in rodent models is actually producing analgesia, especially if the model includes pain. In addition, the metabolite M3G, predominantly produced in rodents, might have non-opioid receptor-mediated activities (see below).

Lastly, the continuity of delivery (i.e. osmotic pumps or morphine-releasing pellets vs. injections at time intervals) and the duration of morphine treatment both differ between studies. This may be important if the effect of morphine on tumours is mediated by mechanisms subject to tolerance and withdrawal, such as the immune function (West *et al.*, 1998; Eisenstein *et al.*, 2006). Indeed, in contrast to continuous administration by constant infusion or slow-release pellets, intermittent administration of morphine (every 12 h for 4 days) to rats was characterized as a chronic stressor, inducing withdrawal-like conditions in each interval and increasing the hypothalamic–pituitary–adrenal (HPA) axis response to novel stimuli (Houshyar *et al.*, 2003; 2004). Activation of the HPA axis is known to facilitate cancer progression and metastasis (Li *et al.*, 2013), via many mechanisms, including suppression of cell-mediated immunity (Benish *et al.*, 2008), promotion of angiogenesis (Yang *et al.*, 2009) and direct action on cancer cells (Bernabe *et al.*, 2011). Only a few studies have tested the effect of continuous administration of morphine on tumour growth and metastasis. Koodie *et al.* used morphine-releasing pellets, and the studies by Page *et al.* mention s.c. injection of morphine in a slow-release suspension. They all resulted in anti-tumour ,rather than pro-tumour effects (Page *et al.*, 1993; 1994; 1998; Koodie *et al.*, 2010). Implantation of a 75 mg morphine-releasing pellet in mice provided serum morphine concentrations of 7 μM at 6–24 h and 2 μM at equilibrium (Bryant *et al.*, 1988). Patient-controlled analgesia with morphine, often used in post-operative pain management, was suggested, using pharmacokinetic simulation, to result in relatively stable effect-site concentrations of morphine and its metabolites M3G and M6G in patients (Sam *et al.*, 2011), and animal models should therefore mimic this continuity. Of the animal studies on tumour growth and metastasis that employed doses of morphine escalating over the course of the treatment to account for the development of tolerance (Gupta *et al.*, 2002; Tegeder *et al.*, 2003; Farooqui *et al.*, 2007; Koodie *et al.*, 2010), only those using high doses (Tegeder *et al.*, 2003; Koodie *et al.*, 2010) demonstrated anti-tumour effects of morphine. Taken together, these observations indicate that continuous administration of high doses of morphine that produce analgesia is more likely to result in prevention of tumour growth and metastasis, in rodent models.

The receptors involved

We have limited inclusion in Table 1 to studies measuring tumour growth and metastasis in animals treated with morphine, but it should be noted that further studies using genetic or pharmacological manipulation of the μ-opioid receptor (nomenclature follows Alexander *et al.*, 2013) have been carried out. Overexpression and down-regulation of the μ-opioid receptor in cancer cells before injecting them into mice were shown to increase and decrease, respectively, primary tumour growth and metastasis in mice expressing

μ-opioid receptors (Biji *et al.*, 2011; Lennon *et al.*, 2012). In addition, infusion of the μ-opioid receptor antagonist methylnaltrexone reduced tumour growth and metastasis in wild-type mice (Biji *et al.*, 2011). Growth of cancer cells expressing μ-opioid receptors in mice lacking μ-opioid receptors (knockout mice) was also reduced compared with that in wild-type mice. This indicates that μ-opioid receptor activation on both tumour cells and cells of the host may promote tumour growth and metastasis. However, neither of these studies have included evidence that morphine increases tumour growth and metastasis *in vivo* (Biji *et al.*, 2011; Lennon *et al.*, 2012).

Very little is known about the possible consequences of μ-opioid receptor dimerization on cancer. A role for μ- and δ-opioid receptor heterodimerization has been suggested in natural killer cells, in terms of their cytolytic function, with reciprocal regulation of each receptor homodimerization and potential consequences on tumour growth (Sarkar *et al.*, 2012). In addition, activation of opioid receptors other than μ-receptors may contribute to the role of morphine in cancer, depending upon the doses of morphine involved. Expression of μ-, δ- and κ-opioid receptors has been detected in cancer cell lines and in tumour tissue (Nylund *et al.*, 2008; Tang *et al.*, 2013; Zhang *et al.*, 2013; Zylla *et al.*, 2013), and some studies suggest that opioid receptors in tumours are up-regulated, compared with control tissue (Madar *et al.*, 2007; Biji *et al.*, 2011; Tang *et al.*, 2013; Zhang *et al.*, 2013). *In situ* detection of opioid receptor expression in tumour stroma is lacking, although endothelial, immune and fibroblast cells are known to express opioid receptors in non-tumour contexts (Stefano *et al.*, 1995; Sharp, 2006; Cheng *et al.*, 2008). Similarly, endogenous opioids can be produced by cancer cells and are detected in some tumours (Bostwick *et al.*, 1987; Krajnik *et al.*, 2010) where they modulate cancer progression (Boehncke *et al.*, 2011) presumably via regulation of tumour-associated immune cells (Ohmori *et al.*, 2009; Boehncke *et al.*, 2011). Lastly, whether μ-opioid receptor alternative splicing, which results in multiple variants in both humans and mice, modulates tumour growth is underexplored.

A growing amount of studies looking for non-GPCR-mediated actions of opioids on immune pathways have identified that the Toll-like receptor 4 (TLR4), which is activated by LPS produced by bacteria, may respond to opioids. One group has proposed that opioid receptor ligands produce a slight but significant activation (morphine) or antagonism (naloxone) of the TLR4, in a non-stereospecific fashion, i.e. the (+) enantiomers were active at TLR4 receptors but not at opioid receptors (Wang *et al.*, 2012). In contrast, others have suggested that morphine produced, by itself, a slight activation of TLR4, but inhibited TLR4 activation by LPS in a non-competitive fashion, as did naloxone (Stevens *et al.*, 2013). Interestingly, M3G, which has limited opioid receptor activity (Ulens *et al.*, 2001), induced activation of TLR4 (Lewis *et al.*, 2010; Due *et al.*, 2012). This might be of considerable importance if TLR4 mediates some of the effects of opioids on cancer growth and metastasis, as rodent models employ high doses of morphine that result in high doses of M3G in the circulation (Zelcer *et al.*, 2005) and, presumably, at tissue level.

Lastly, a variety of mouse strains are used in experiments testing the effect of morphine on tumour growth and metastasis, and they may respond differently to the drug since it is known that different mouse strains exhibit polymorphisms in the 5′ flanking region and 3′ untranslated region of the μ-opioid receptor gene that are associated with differences in opioid sensitivity (measured as locomotor hyperactivity and antinociception) (Shigeta *et al.*, 2008). The immunosuppressive effects of morphine are also likely to vary between mouse strains. This was clearly shown for the direct effect of morphine on mouse spleen cells (Eisenstein *et al.*, 1995).

The cells targeted by morphine

A major question remains whether the putative effects of morphine on tumour growth and metastasis might be mediated by direct activation of cellular receptors or indirectly mediated by morphine-initiated effects that lead to the release of secondary factors. The cells on which morphine can act directly to modulate the growth and metastasis of tumours include the cancer cells as well as other cell types such as immune cells, and cells of the tumour microenvironment such as tumour-associated macrophages and endothelial cells. Experiments employing disruption of the μ-opioid receptor show that opioid receptor activation on the cancer cells injected into the mice as well as the cells of the tumour-bearing animal can interfere with tumour growth and metastasis (Biji *et al.*, 2011; Lennon *et al.*, 2012).

Much of the literature on the effect of morphine on the immune response has assessed the functions of immune cells collected from mice or humans after they were given morphine, thereby testing potential indirect and direct effects of morphine on those cells. However, morphine added to immune cells *ex vivo* also showed some direct effects (Eisenstein *et al.*, 1995; Condevaux *et al.*, 2001; Malik *et al.*, 2002; Fuggetta *et al.*, 2005). Macrophage phagocytic ability was inhibited by acute, but not chronic, direct exposure to morphine *in vitro* (Casellas *et al.*, 1991; Tomei and Renaud, 1997). This phenomenon occurred via activation of opioid receptors (Tomassini *et al.*, 2003) and was subject to '*in vitro* withdrawal' (Tomei and Renaud, 1997). In co-cultures of tumour cells with macrophages, morphine prevented paracrine communication through which macrophages could promote the production of matrix-degrading enzymes by the tumour cells (Afsharimani *et al.*, 2014). A direct effect of morphine on endothelial cells has also been proposed (Gupta *et al.*, 2002; Singleton *et al.*, 2006; Leo *et al.*, 2009) and suggests pro-angiogenic properties for low concentrations of morphine. All these reports suggest that some of the effects of morphine *in vivo* might be mediated by direct action on the immune or endothelial cells.

In line with *in vivo* data showing that the dose and mode of administration influenced the effect of morphine on tumours, at the cellular level, responses that may be involved in tumour progression, such as proliferation or apoptosis, or immune cell responses, have also been shown to depend upon the concentration of morphine applied, with low doses promoting cell proliferation and high doses promoting apoptosis, and to be susceptible to development of tolerance and receptor desensitization (see Tegeder and Geisslinger, 2004; Eisenstein *et al.*, 2006).

Conclusion and perspectives

To extrapolate animal experimental data to human patients, mouse models used to study the effects of morphine on tumour growth and metastasis should adhere to the following criteria. The mice should spontaneously develop orthotopic primary tumours in an immuno-competent setting. In addition, the tumour models should reproduce the biology of *de novo* metastatic disease. To relate the animal data to perioperative use of morphine in cancer surgery patients, surgical resection of the primary tumour is desirable as part of the model. The doses of morphine used should be analgesic in mice and the duration of morphine exposure should match post-operative analgesia regimens, avoiding unnecessary withdrawal as much as possible.

Overall, the current literature does not provide definitive evidence for a modulation of tumour growth and metastasis by morphine. Morphine might modulate tumour growth and metastasis though a combination of direct (on cells) and indirect (neuroendocrine) responses, central and peripheral mechanisms and modulation of physiopathological functions key to tumour development, such as inflammation, stress and pain. It is further likely that the effects of morphine are in addition to the effects of endogenous opioids and are regulated by tolerance and withdrawal responses. The discrepancies found in the literature are thus not surprising, and refining the animal models that we use, on the basis of all these criteria, will hopefully provide, in the future, definitive answers than can be taken into consideration for patient care.

Acknowledgements

M.-O. P. and P. J. C acknowledge the financial support of the Australian and New Zealand College of Anaesthetists.

Conflict of interest

The authors declare no conflict of interest.

References

Afsharimani B, Cabot PJ, Parat MO (2011). Morphine use in cancer surgery. Front Pharmacol 2: 46.

Afsharimani B, Baran J, Watanabe S, Lindner D, Cabot P, Parat MO (2014). Morphine and breast tumor metastasis: the role of matrix-degrading enzymes. Clin Exp Metastasis 31: 149–158.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013). The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. Br J Pharmacol 170: 1459–1581.

Bar-Yosef S, Melamed R, Page GG, Shakhar G, Shakhar K, Ben-Eliyahu S (2001). Attenuation of the tumor-promoting effect of surgery by spinal blockade in rats. Anesthesiology 94: 1066–1073.

Benish M, Bartal I, Goldfarb Y, Levi B, Avraham R, Raz A *et al.* (2008). Perioperative use of beta-blockers and COX-2 inhibitors may improve immune competence and reduce the risk of tumor metastasis. Ann Surg Oncol 15: 2042–2052.

Bernabe DG, Tamae AC, Biasoli ER, Oliveira SHP (2011). Stress hormones increase cell proliferation and regulates interleukin-6 secretion in human oral squamous cell carcinoma cells. Brain Behav Immun 25: 574–583.

Biji M, Lennon F, Siegler J, Mirzapoiazova T, Mambetsariev N, Sammani S *et al.* (2011). The novel role of the mu opioid receptor in lung cancer progression: a laboratory investigation. Anesth Analg 112: 558–567.

Biki B, Mascha E, Moriarty DC, Fitzpatrick JM, Sessler DI, Buggy DJ (2008). Anesthetic technique for radical prostatectomy surgery affects cancer recurrence: a retrospective analysis. Anesthesiology 109: 180–187.

Boehncke S, Hardt K, Schadendorf D, Henschler R, Boehncke WH, Duthey B (2011). Endogenous μ-opioid peptides modulate immune response towards malignant melanoma. Exp Dermatol 20: 24–28.

Boggio K, Nicoletti G, Di CE, Cavallo F, Landuzzi L, Melani C *et al.* (1998). Interleukin 12-mediated prevention of spontaneous mammary adenocarcinomas in two lines of Her-2/neu transgenic mice. J Exp Med 188: 589–596.

Bostwick DG, Null WE, Holmes D, Weber E, Barchas JD, Bensch K (1987). Expression of opioid peptides in tumors. N Engl J Med 317: 1439–1443.

Bryant HU, Yoburn BC, Inturrisi CE, Bernton EW, Holaday JW (1988). Morphine-induced immunomodulation is not related to serum morphine concentrations. Eur J Pharmacol 149: 165–169.

Casellas AM, Guardiola H, Renaud FL (1991). Inhibition by opioids of phagocytosis in peritoneal macrophages. Neuropeptides 18: 35–40.

Cheng B, Liu HW, Fu XB, Sheng ZY, Li JF (2008). Coexistence and upregulation of three types of opioid receptors, mu, delta and kappa, in human hypertrophic scars. Br J Dermatol 158: 713–720.

Colacchio TA, Yeager MP, Hildebrandt LW (1994). Perioperative immunomodulation in cancer surgery. Am J Surg 167: 174–179.

Condevaux F, Guichard J, Forichon A, Aujoulat M, Descotes J (2001). Compared effects of morphine and nickel chloride on NK cell activity *in vitro* in rats and monkeys. J Appl Toxicol 21: 431–434.

Dalal S, Bruera E (2013). Access to opioid analgesics and pain relief for patients with cancer. Nat Rev Clin Oncol 10: 108–116.

Derksen PW, Liu X, Saridin F, van der Gulden H, Zevenhoven J, Evers B *et al.* (2006). Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell 10: 437–449.

Domcke S, Sinha R, Levine DA, Sander C, Schultz N (2013). Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun 4: 2126.

Doornebal CW, Klarenbeek S, Braumuller TM, Klijn CN, Ciampricotti M, Hau CS *et al.* (2013). A preclinical mouse model of invasive lobular breast cancer metastasis. Cancer Res 73: 353–363.

Due M, Piekarz A, Wilson N, Feldman P, Ripsch M, Chavez S *et al.* (2012). Neuroexcitatory effects of morphine-3-glucuronide are dependent on toll-like receptor 4 signaling. J Neuroinflammation 9: 200.

Eisenstein T, Rahim R, Feng P, Thingalaya N, Meissler J (2006). Effects of opioid tolerance and withdrawal on the immune system. J Neuroimmune Pharmacol 1: 237–249.

Eisenstein TK, Meissler JJ Jr, Rogers TJ, Geller EB, Adler MW (1995). Mouse strain differences in immunosuppression by opioids *in vitro*. J Pharmacol Exp Ther 275: 1484–1489.

Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI (2006). Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? Anesthesiology 105: 660–664.

Fantozzi A, Christofori G (2006). Mouse models of breast cancer metastasis. Breast Cancer Res 8: 212.

Farooqui M, Li Y, Rogers T, Poonawala T, Griffin RJ, Song CW *et al.* (2007). COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia. Br J Cancer 97: 1523–1531.

Franchi S, Panerai AE, Sacerdote P (2007). Buprenorphine ameliorates the effect of surgery on hypothalamus-pituitary-adrenal axis, natural killer cell activity and metastatic colonization in rats in comparison with morphine or fentanyl treatment. Brain Behav Immun 21: 767–774.

Francia G, Cruz-Munoz W, Man S, Xu P, Kerbel RS (2011). Mouse models of advanced spontaneous metastasis for experimental therapeutics. Nat Rev Cancer 11: 135–141.

Frese KK, Tuveson DA (2007). Maximizing mouse cancer models. Nat Rev Cancer 7: 645–658.

Fuggetta MP, Di FP, Falchetti R, Cottarelli A, Rossi L, Tricarico M *et al.* (2005). Effect of morphine on cell-mediated immune responses of human lymphocytes against allogeneic malignant cells. J Exp Clin Cancer Res 24: 255–263.

Gupta K, Kshirsagar S, Chang L, Schwartz R, Law PY, Yee D *et al.* (2002). Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. Cancer Res 62: 4491–4498.

Harimaya Y, Koizumi K, Andoh T, Nojima H, Kuraishi Y, Saiki I (2002). Potential ability of morphine to inhibit the adhesion, invasion and metastasis of metastatic colon 26-L5 carcinoma cells. Cancer Lett 187: 121–127.

Heaney A, Buggy DJ (2012). Can anaesthetic and analgesic techniques affect cancer recurrence or metastasis? Br J Anaesth 109 (Suppl. 1): i17–i28.

Houshyar H, Gomez F, Manalo S, Bhargava A, Dallman MF (2003). Intermittent morphine administration induces dependence and is a chronic stressor in rats. Neuropsychopharmacology 28: 1960–1972.

Houshyar H, Manalo S, Dallman MF (2004). Time-dependent alterations in mRNA expression of brain neuropeptides regulating energy balance and hypothalamo-pituitary-adrenal activity after withdrawal from intermittent morphine treatment. J Neurosci 24: 9414–9424.

Ishikawa M, Tanno K, Kamo A, Takayanagi Y, Sasaki K (1993). Enhancement of tumor growth by morphine and its possible mechanism in mice. Biol Pharm Bull 16: 762–766.

Jonkers J, Derksen PW (2007). Modeling metastatic breast cancer in mice. J Mammary Gland Biol Neoplasia 12: 191–203.

Joyce JA, Pollard JW (2009). Microenvironmental regulation of metastasis. Nat Rev Cancer 9: 239–252.

Keller PJ, Lin AF, Arendt LM, Klebba I, Jones AD, Rudnick JA *et al.* (2010). Mapping the cellular and molecular heterogeneity of normal and malignant breast tissues and cultured cell lines. Breast Cancer Res 12: R87.

Koodie L, Ramakrishnan S, Roy S (2010). Morphine suppresses tumor angiogenesis through a HIF-1alpha/p38MAPK pathway. Am J Pathol 177: 984–997.

Krajnik M, Sch+ñfer M, Sobanski P, Kowalewski J, Bloch-Boguslawska E, Zylicz Z *et al.* (2010). Enkephalin, its precursor, processing enzymes, and receptor as part of a local opioid network throughout the respiratory system of lung cancer patients. Hum Pathol 41: 632–642.

Kuo CK, Hanioka N, Hoshikawa Y, Oguri K, Yoshimura H (1991). Species difference of site-selective glucuronidation of morphine. J Pharmacobiodyn 14: 187–193.

Lennon FE, Mirzapoiazova T, Mambetsariev B, Salgia R, Moss J, Singleton PA (2012). Overexpression of the mu-opioid receptor in human non-small cell lung cancer promotes Akt and mTOR activation, tumor growth, and metastasis. Anesthesiology 116: 857–867.

Leo S, Nuydens R, Meert TF (2009). Opioid-induced proliferation of vascular endothelial cells. J Pain Res 2: 59–66.

Lewis SS, Hutchinson MR, Rezvani N, Loram LC, Zhang Y, Maier SF *et al.* (2010). Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via toll-like receptor 4/MD-2 and interleukin-1β. Neuroscience 165: 569–583.

Li S, Sun Y, Gao D (2013). Role of the nervous system in cancer metastasis. Oncol Lett 5: 1101–1111.

Madar I, Bencherif B, Lever J, Heitmiller RF, Yang SC, Brock M *et al.* (2007). Imaging δ- and μ-opioid receptors by PET in lung carcinoma patients. J Nucl Med 48: 207–213.

Malik AA, Radhakrishnan N, Reddy K, Smith AD, Singhal PC (2002). Morphine-induced macrophage apoptosis modulates migration of macrophages: use of *in vitro* model of urinary tract infection. J Endourol 16: 605–610.

Marusyk A, Almendro V, Polyak K (2012). Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 12: 323–334.

Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P (1988). Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell 54: 105–115.

Nylund G, Pettersson A, Bengtsson C, Khorram-Manesh A, Nordgren S, Delbro DS (2008). Functional expression of mu-opioid receptors in the human colon cancer cell line, HT-29, and their localization in human colon. Dig Dis Sci 53: 461–466.

Ohmori H, Fujii K, Sasahira T, Luo Y, Isobe M, Tatsumoto N *et al.* (2009). Methionine-enkephalin secreted by human colorectal cancer cells suppresses T lymphocytes. Cancer Sci 100: 497–502.

Osborne R, Joel S, Trew D, Slevin M (1988). Analgesic activity of morphine-6-glucuronide. Lancet 331: 828.

Osborne R, Joel S, Trew D, Slevin M (1990). Morphine and metabolite behavior after different routes of morphine administration: demonstration of the importance of the active metabolite morphine-6-glucuronide. Clin Pharmacol Ther 47: 12–19.

Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F *et al.* (2009). Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 15: 220–231.

Page GG, Ben-Eliyahu S, Yirmiya R, Liebeskind JC (1993). Morphine attenuates surgery-induced enhancement of metastatic colonization in rats. Pain 54: 21–28.

Page GG, Ben-Eliyahu S, Liebeskind JC (1994). The role of LGL/NK cells in surgery-induced promotion of metastasis and its attenuation by morphine. Brain Behav Immun 8: 241–250.

Page GG, McDonald JS, Ben-Eliyahu S (1998). Pre-operative versus postoperative administration of morphine: impact on the neuroendocrine, behavioural, and metastatic-enhancing effects of surgery. Br J Anaesth 81: 216–223.

Page GG, Blakely WP, Ben-Eliyahu S (2001). Evidence that postoperative pain is a mediator of the tumor-promoting effects of surgery in rats. Pain 90: 191–199.

Parat MO (2013) Morphine and metastasis: from bench to bedside. In: Parat MO (ed.). Morphine and Metastasis. Springer: Dordrecht, pp. 1–13.

Roy S, Wang J, Kelschenbach J, Koodie L, Martin J (2006). Modulation of immune function by morphine: implications for susceptibility to infection. J Neuroimmune Pharmacol 1: 77–89.

Sam WJ, MacKey SC, Lotsch J, Drover DR (2011). Morphine and its metabolites after patient-controlled analgesia: considerations for respiratory depression. J Clin Anesth 23: 102–106.

Sarkar DK, Sengupta A, Zhang C, Boyadjieva N, Murugan S (2012). Opiate antagonist prevents μ- and δ-opiate receptor dimerization to facilitate ability of agonist to control ethanol-altered natural killer cell functions and mammary tumor growth. J Biol Chem 287: 16734–16747.

Sasamura T, Nakamura S, Iida Y, Fujii H, Murata J, Saiki I *et al.* (2002). Morphine analgesia suppresses tumor growth and metastasis in a mouse model of cancer pain produced by orthotopic tumor inoculation. Eur J Pharmacol 441: 185–191.

Sessler DI (2008). Does regional analgesia reduce the risk of cancer recurrence? A hypothesis. Eur J Cancer Prev 17: 269–272.

Sharp BM (2006). Multiple opioid receptors on immune cells modulate intracellular signaling. Brain Behav Immun 20: 9–14.

Shigeta Y, Kasai S, Han W, Hata H, Nishi A, Takamatsu Y *et al.* (2008). Association of morphine-induced antinociception with variations in the 5′ flanking and 3′ untranslated regions of the mu opioid receptor gene in 10 inbred mouse strains. Pharmacogenet Genomics 18: 927–936.

Shimomura K, Kamata O, Ueki S, Ida S, Oguri K, Yoshimura H *et al.* (1971). Analgesic effect of morphine glucuronides. Tohoku J Exp Med 105: 45–52.

Simon RH, Arbo TE (1986). Morphine increases metastatic tumor growth. Brain Res Bull 16: 363–367.

Singleton PA, Moss J (2010). Effect of perioperative opioids on cancer recurrence: a hypothesis. Future Oncol 6: 1237–1242.

Singleton PA, Lingen MW, Fekete MJ, Garcia JGN, Moss J (2006). Methylnaltrexone inhibits opiate and VEGF-induced angiogenesis: role of receptor transactivation. Microvasc Res 72: 3–11.

Stefano GB, Hartman A, Bilfinger TV, Magazine HI, Liu Y, Casares F *et al.* (1995). Presence of the mu3 opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. J Biol Chem 270: 30290–30293.

Stevens CW, Aravind S, Das S, Davis RL (2013). Pharmacological characterization of LPS and opioid interactions at the toll-like receptor 4. Br J Pharmacol 168: 1421–1429.

Talmadge JE, Meyers KM, Prieur DJ, Starkey JR (1980). Role of NK cells in tumour growth and metastasis in beige mice. Nature 284: 622–624.

Tang B, Li Y, Yuan S, Tomlinson S, He S (2013). Upregulation of the delta opioid receptor in liver cancer promotes liver cancer progression both *in vitro* and *in vivo*. Int J Oncol 43: 1281–1290.

Tegeder I, Geisslinger G (2004). Opioids as modulators of cell death and survival – unraveling mechanisms and revealing new indications. Pharmacol Rev 56: 351–369.

Tegeder I, Grosch S, Schmidtko A, Haussler A, Schmidt H, Niederberger E *et al.* (2003). G protein-independent G1 cell cycle block and apoptosis with morphine in adenocarcinoma cells: involvement of p53 phosphorylation. Cancer Res 63: 1846–1852.

Thompson EW, Haviv I (2011). The social aspects of EMT-MET plasticity. Nat Med 17: 1048–1049.

Tomassini N, Renaud FL, Roy S, Loh HH (2003). Mu and delta receptors mediate morphine effects on phagocytosis by murine peritoneal macrophages. J Neuroimmunol 136: 9–16.

Tomei EZ, Renaud FL (1997). Effect of morphine on Fc-mediated phagocytosis by murine macrophages *in vitro*. J Neuroimmunol 74: 111–116.

Ulens C, Baker L, Ratka A, Waumans D, Tytgat J (2001). Morphine-6beta-glucuronide and morphine-3-glucuronide, opioid receptor agonists with different potencies. Biochem Pharmacol 62: 1273–1282.

Valastyan S, Weinberg RA (2011). Tumor metastasis: molecular insights and evolving paradigms. Cell 147: 275–292.

de Visser KE, Eichten A, Coussens LM (2006). Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6: 24–37.

Wang X, Loram LC, Ramos K, de Jesus AJ, Thomas J, Cheng K *et al.* (2012). Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc Natl Acad Sci U S A 109: 6325–6330.

West JP, Dykstra LA, Lysle DT (1998). Differential tolerance to morphine's immunomodulatory effects following continuous administration. Drug Alcohol Depend 53: 31–38.

Willimsky G, Czeh M, Loddenkemper C, Gellermann J, Schmidt K, Wust P *et al.* (2008). Immunogenicity of premalignant lesions is the primary cause of general cytotoxic T lymphocyte unresponsiveness. J Exp Med 205: 1687–1700.

Yang EV, Kim SJ, Donovan EL, Chen M, Gross AC, Webster Marketon JI *et al.* (2009). Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. Brain Behav Immun 23: 267–275.

Yeager MP, Colacchio TA (1991). Effect of morphine on growth of metastatic colon cancer in vivo. Arch Surg 126: 454–456.

Zelcer N, van de Wetering K, Hillebrand M, Sarton E, Kuil A, Wielinga PR *et al.* (2005). Mice lacking multidrug resistance protein 3 show altered morphine pharmacokinetics and morphine-6-glucuronide antinociception. Proc Natl Acad Sci U S A 102: 7274–7279.

Zhang YF, Xu QX, Liao LD, Xu XE, Wu JY, Shen J *et al.* (2013). κ-Opioid receptor in the nucleus is a novel prognostic factor of esophageal squamous cell carcinoma. Hum Pathol 44: 1756–1765.

Zylla D, Gourley BL, Vang D, Jackson S, Boatman S, Lindgren B *et al.* (2013). Opioid requirement, opioid receptor expression, and clinical outcomes in patients with advanced prostate cancer. Cancer 119: 4103–4110.