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Review

The pathophysiology of cancer-induced bone pain: current understanding

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Cancer-induced bone pain (CIBP) is a common clinical problem. Although treatment has been revolutionised in the past 10 years with the introduction of bisphosphonates, pain arising spontaneously or from movement, remains a leading cause of unresolved pain in many patients. Until recently little was understood about the peripheral and central mechanisms of bone pain. Insight into the mechanisms of osteoblast and osteoclast activation, via receptor activator for nuclear factor κB (RANK) dependent and independent mechanisms and a re-evaluation of primary afferent terminals within bone have led to a suggestion that CIBP may be a mixture of inflammatory and neuropathic stimuli. The recently published animal model of localised but progressive bone destruction has allowed greater insight into the peripheral and dorsal horn pathophysiology, which hitherto was precluded. Immunocytochemical markers of neurotransmitters and receptors indicate that CIBP has unique characteristics, unlike neuropathy or inflammation. Evidence for an increased excitability within the dorsal horn, and especially Lamina I, and possible mechanisms underlying this unique pain state will be discussed. *Palliative Medicine* 2004; **18:** 267–274

Key words: bone; cancer; dorsal horn; pain

Introduction

Bone pain arising from a primary bone sarcoma, or metastatic spread from a carcinoma, is a common sequel in disease progression. Bone metastases are a common cause of cancer-related pain, with metastatic cancer invading bone in 60–84% of cases.[1–3] Cancer-induced bone pain (CIBP) has been shown to correlate with an increased morbidity, reduced performance status, increased anxiety and depression, and a reduced quality of life.[4–6]

CIBP consists of a triad of background pain, spontaneous pain and movement-induced (incident) pain.[7,8] The background pain is described as a constant dull ache and increases in intensity over time.[2] The intermittent episodes of intense pain occurring spontaneously or upon movement/weight-bearing come under the umbrella heading of 'breakthrough pain', but must be differentiated from end-of-dose failure, a new acute pain or a recrudescence of the background pain.[6] Unfortunately in the literature incident pain is often not differentiated from breakthrough pains or cancer pain, making interpretation of data relating to the efficacy of treatment difficult. Portenoy reported that more than 50/90 inpatients with advanced cancer had breakthrough pain,[4] whilst Banning more specifically reported a series of 200 ambulatory cancer patients with more than 90% experiencing pain on moving.[9] Within a hospice population 93% of 242 consecutive admissions reported episodic pain related to weight bearing pain. Two thirds were cancer related (1/3 were due to concurrent noncancer disease) and satisfactory analgesia was achieved in only 54% of cases.[10] The development of a Breakthrough Pain Questionnaire is an attempt to characterise the acute pain flares,[6] thereby allowing subanalyses of movement-induced, spontaneous or end-of-dose failure pains. However this questionnaire, whilst easy to use, is not yet validated.

Current therapeutic options

Current analgesic therapies for CIBP have not altered significantly in over a decade, since the introduction

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of bisphosphonates. Treatment is multimodal and includes systemic analgesics (opioids, nonsteroidal anti-inflammatory drugs (NSAIDs)), bisphosphonates, anti-tumour chemotherapy, radiotherapy, systemic radioisotopes, local surgery, and anaesthetic techniques.[11–14] The use of NSAIDs in CIBP has been questioned due to the lack of evidence. However, there is no published trial of NSAID efficacy specifically in incident pain. The three randomised trials of NSAIDs in cancer pain do not separate out bone metastases, and six nonrandomised trials mention bone metastases but do not record incident pain.[15–23] The newer COX II specific inhibitors may be of greater therapeutic potential, due to their anti-tumour/anti-angiogenesis properties.[24,25] In an animal model of CIBP acute treatment with a highly selective COX II inhibitor attenuated both background and movement-induced pain, whilst chronic treatment in addition reduced tumour burden and osteoclast destruction.[26] Opioid based therapy remains the basis for most analgesia in CIBP. Whilst these regimens often attenuate the background (tonic) pain, the doses are insufficient to ameliorate incident or spontaneous pain. An increased dose sufficient to attenuate movement-induced pain produces unacceptable side effects at rest.

Bisphosphonates and local radiotherapy are both powerful agents in reducing CIBP. Bisphosphonates were primarily used to treat hypercalcaemia (from excess bone resorption) and more recently CIBP.[27] A recent review indicated that regular use of bisphosphonates reduced the number of skeletal related events in numerous cancers.[28] Briefly their mode of action is to inhibit the recruitment and activation of osteoclasts, increase osteoprogeterin levels, induce apoptosis, inhibit cancer cell proliferation, reduce cytokine production and metalloproteinase secretion.[29–32] A Cochrane review of the clinical efficacy of bisphosphonates for pain relief in metastatic bone disease suggested that there was some evidence for their use as analgesics, although the effect was delayed. The number needed to treat to achieve 50% pain relief at 4 weeks was 11, falling to 7 at 12 weeks.[13] External beam radiotherapy, whether single or multiple fractions, produced 50% pain relief in 41% of patients and complete pain relief at one month in 25% of patients.[12]

The current therapeutic regimens leave up to 45% of patients with inadequate and undermanaged pain control.[33,34] The lack of novel therapies is in part due to a lack of understanding of the mechanisms underlying the pathophysiology of CIBP. It was not until 1999, when a novel method of inducing CIBP in an otherwise well animal was published, that the unique peripheral and central pathophysiology could be investigated.

Animal models of CIBP

Until the late twentieth century all animal models of CIBP had relied on the systemic injection of carcinoma cells, which resulted in systemically unwell animals and random and multiple sited bone deposits. In 1999, Schwei *et al.* reported a method of local infusion of cancer (osteosarcoma cells) into a single bone, with no systemic spread of the cancer. The sarcoma cells were injected into the medulla of a mouse femur, with control groups of sham operated (media only injected). Over the subsequent 21 days the animals with sham medulla injection showed no signs of spontaneous or movement-induced pain. However, the animals receiving the intramedullary injection of sarcoma cells displayed progressively severe nocifensive behaviour which correlated with a progressive destruction of the femur.[35] The animals remained well, with good weight gain. The model has been developed to include the use of breast carcinoma cells within the rat tibia and fibrosarcoma, melanoma or adenocarcinoma cells within the mouse humerus or femur.[36,37] The animal model parallels the clinical course of bone metastases, with progressive bone destruction leading to a pathological fracture (Figure 1), accompanying progressive limping, guarding, spontaneous flinching and vocalisation on palpation, reduced movement, secondary hyperalgesia and allodynia (Figure 2). In addition the response to systemic opioids in this mouse model was similar to that found in humans: when compared with inflammation induced pain the attenuation with morphine was reduced.[38]

Why is tumour-induced bone destruction painful?

Primary afferents

In the past it was suggested that tumour-induced bone pain may be due to vascular occlusion, compression of the bone or peripheral nerve or due to mechanical instability. However these failed to consider the basic mechanisms of nociceptor activation, transmission, or primary and secondary sensitisation. Primary afferent nerve fibres transmit non-noxious (Aβ fibres) and noxious stimuli (Aδ and C fibres) to the dorsal horn of the spinal cord. There the signals undergo extensive modulation, both excitatory and inhibitory before being relayed to higher centres in the brain.

Recently it has been demonstrated that the periosteum and the mineralised bone are richly innervated by primary afferents, Aδ expressing neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) and neuropeptidergic C fibres expressing calcitonin gene related peptide (CGRP), vallinoid receptor (VR1) and sympathetic neurones (SNS).[39–44] Neuropeptides such as VIP,

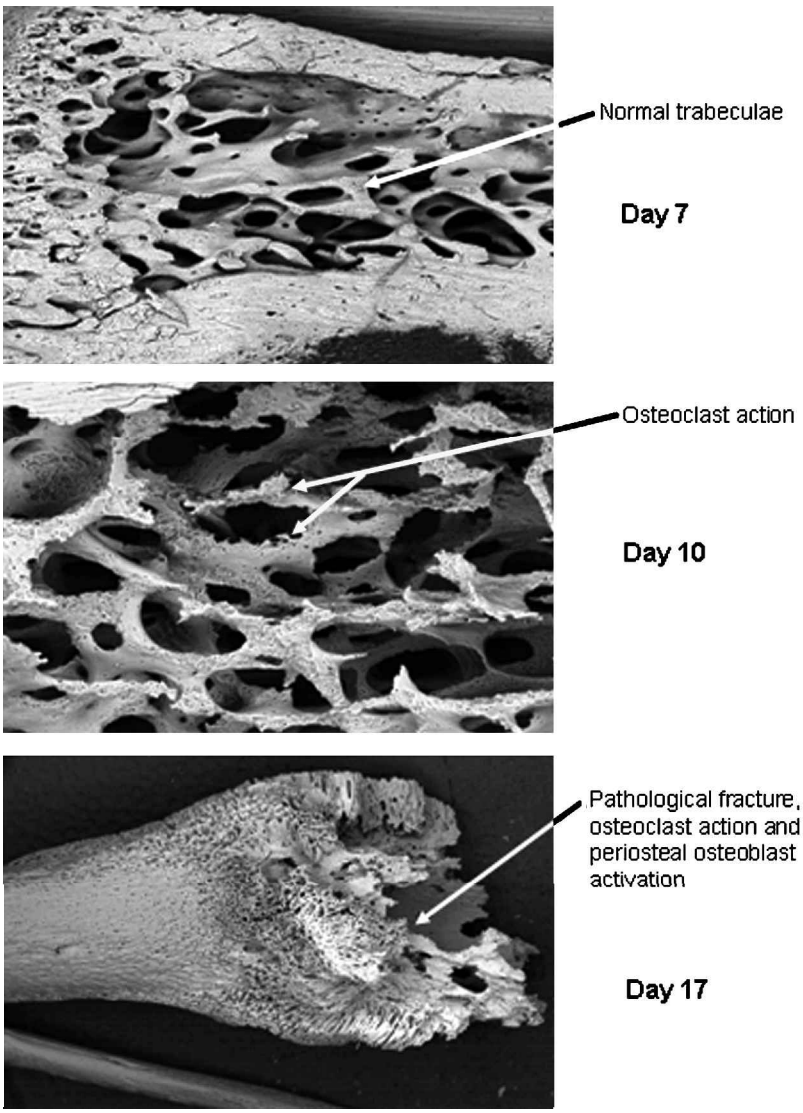

Figure 1 Three scanning electron micrographs taken at day 7 (× 33 magnification), 10 (× 66 magnification) and 17 (× 7 magnification) post-MRMT-1 cancer injection. By day 7 there is no sign of osteoclast action (arrow). New bone formation, osteoblast activation, was present at the site of the injection for both cancer and sham injection. By day 10, the trabeculae appear ragged, indicating osteoclast activation (arrow). By day 17 profound osteoclast activation is evident, resulting in fracture of the bone. Extensive periosteal osteoblast activation is evident.

CGRP and substance P (SP) and glutamate have been implicated in bone metabolism, and neonatal abolition of C fibres and SNS resulted in a 21% and 45% reduction in bone surface osteoclasts.[41,45–47]

Proexcitatory factors released within the bone

Tumours invading and growing within the medullary space of the bone interact and activate primary afferent fibres, alter osteoblast/osteoclast balance and induce a pronounced inflammatory infiltrate. Tumour cells have been shown to release a host of growth factors (i.e., nerve growth factor, NGF), cytokines (i.e., tumour necrosis factor (TNF), interleukin (IL)-1, IL-6), chemokines, prostanoids, endothelins, reduce the pH to below 5 in their vicinity and directly deform primary afferents.[48–53] The paracrine interactions between tumours and peripheral nerves are crucial to understand the mechanisms of peripheral nociceptor activation.

Prostanoids are proinflammatory derivatives of arachidonic acid, are formed by the action of cyclooxygenase (COX) and are secreted by both cancer cells and invading immune cells. Prostanoids have been demonstrated to activate prostanoid receptors on primary afferents and induce pain behaviours.[1,54,55] The inhibitory actions of NSAIDs and selective COX II inhibitors at the COX isoforms, underlie the theoretical basis for their use as analgesics in CIBP.[26]

Cytokines are small soluble proteins that activate receptors in autocrine and paracrine actions to produce numerous reactions and are divided into families: such as

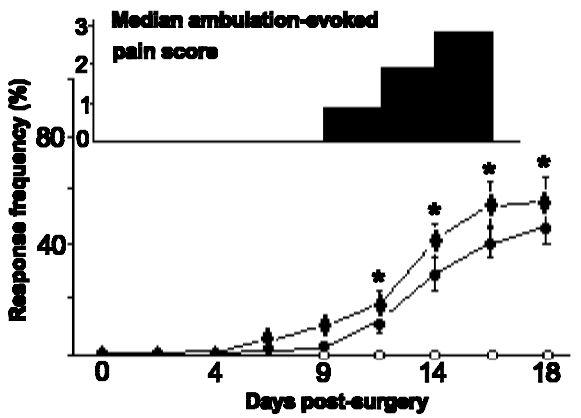

Figure 2 The temporal development of mechanical allodynia/hyperalgesia of the hindpaw of rats receiving intratibial injection of MRMT-1. Rats show brisk withdrawal responses of the ipsilateral hindpaw to stimulation with von Frey 1 g (closed circle) and 5 g (closed diamond) significantly different (*$P < 0.05$) to responses of the contralateral hindpaw (1 g – open circle, 5 g – open diamond) from day 11 onwards.[85] **Inlay graph**, animals exhibit an increase in ambulation-evoked pain scores, developing from day 9 onwards and correlating closely with the time course for development of mechanical allodynia seen with von Frey 1 g and 5 g (these are the median values for 16 rats). Each data point represents the mean ± SEM for between 6 and 26 animals.[85]

the TNF-receptor family or chemokine receptor family. Cytokines are known to be involved in the generation and maintenance of inflammatory and neuropathic pain and have been shown to be released in cancer cell assays.[52,56–60]

In addition to their vasoconstrictor actions, endothelins (ET) promote pain behaviours when applied to the sciatic nerve, induce nociception via the endothelin receptors in inflammatory pain and are involved in tumour cell signal transduction, mitogenesis, endothelial cell growth and angiogenesis and are a potential therapeutic target.[61–63] In a mouse model in which osteolytic connective tissue carcinoma was injected into and onto the calcaneous bone, the mouse demonstrated a peak in pain on day 10 which correlated with an increase in ET-1 secretion. Further a blockade of the ET_A receptor reduced nociceptive behaviour.[64] In humans prostate carcinoma has been shown to express high levels of endothelins, the plasma level of which correlates with the severity of pain.[65,66]

Osteoclast activation: acidosis and RANK

Osteoclasts descend from the monocyte lineage and are terminally differentiated multinucleated cells, which act to resorb bone from the bone surface. Osteoclast formation and activation requires macrophage colony stimulating factor (M-CSF) and the interaction between the receptor activator for nuclear factor κB (RANK) expressed on osteoclast precursors with RANK ligand (RANK-L) expressed on several cell types including osteoblasts and an acid environment ($pH < 6$).[67,68]

The RANK–RANK-L interaction is crucial for normal balanced activation of osteoclasts secondary to osteoblasts. To limit the forward feeding cycle, osteoblasts also secrete a cytokine, osteoprotegerin (OPG), which binds and sequesters RANK-L,[67,69,70] preventing RANK and therefore osteoclast activation.[71] Following the invasion and growth of tumour cells within the bone medulla, an imbalance in osteoclast–osteoblast activation is induced. Invading activated T cells and cancer cells secrete RANK-L and may also sequester OPG.[72,73] In one study, 225 patients with myeloma were shown to have serum OPG levels 18% lower than normal controls, compared to raised OPG levels in Hodgkin's/non-Hodgkin's lymphoma; the former having extensive painful metastases in contrast with the latter.[74,75]

OPG is a potentially interesting target for analgesia in CIBP. In the mouse model of CIBP, OPG given subcutaneously from day 5 to 17 postsarcoma infusion, produced a significant attenuation of bone-cancer induced pain and virtually eliminated bone destruction.[76] Several other authors also report the efficacy of OPG, OPG-Fc fusion protein or inhibitory RANK antibodies to treat osteolytic metastases and reduce tumour-associated bone pain.[72,77–79] In addition OPG-Fc fusion protein has been administered to humans, postmenopausal women or patients with myeloma, with minimal side-effects and a significant reduction in markers of bone turnover equivalent in its effect to intravenous pamidronate.[72]

Cancers generate an acid environment both intra- and extracellularly. An accumulation of acid metabolites, ischaemia, apoptosis and phagocytosis induces the local acidosis, which in turn can directly activate nociceptors such as acid sensing ion channels (ASIC) such as VR1 (senses $pH < 6$), and the ASIC 3.[80–82] ASICs have been demonstrated to colocalise with CGRP, in small diameter primary afferents, such as those that innervate bone.[83] The enhanced low pH allows resorption of bone by osteoclasts which in turn contribute to the bone destruction (as seen in Figure 1) and direct destruction of primary afferent fibres.[35]

Dorsal horn changes

Information from animal models of CIBP suggests that the dorsal horn of the spinal cord undergoes significant alterations. Immunocytochemical results in the mouse osteosarcoma model indicated that the alterations in the dorsal horn were unique and different from that seen in inflammatory and neuropathic pain. These changes included astrocyte hypertrophy and upregulation of

dynorphin.[77,84] Evidence for central sensitisation (alterations within the dorsal horn leading to a proexcitatory state) was found in the increased expression of *c-fos* and internalisation of SP-NK1 receptor complex after noxious stimuli in advanced CIBP.[84]

In addition, *in vivo* electrophysiology of individual dorsal horn neurones have indicated a profound change and increased excitation within Lamina I and V.[85] Whilst deep wide-dynamic range neurones had significantly increased C fibre and postdischarge responses to electrical and mechanical stimuli, Lamina I neurones exhibited a greater alteration which has not been reported in either inflammation or neuropathy. Lamina I neurones can be divided into those that respond to only noxious stimuli (nociceptive specific, NS) or those that respond to both innocuous and noxious stimuli (wide-dynamic range, WDR). In normal dorsal horn the ratio of NS:WDR is 75%:25%, however in the rat breast cancer model of CIBP, the ratio alters to 53% NS:47% WDR. In addition the WDR neurones show an increased response to electrical and mechanical stimuli (Figure 3).[85] This alteration in Lamina I responses has in addition been shown to parallel the behavioural development of hyperalgesia and allodynia.[86] Inputs into the dorsal horn are relayed to higher centres in the brain via numerous pathways; in turn descending pathways both excitatory and inhibitory modify further the dorsal horn.[87]

Conclusion

After decades of being ignored, CIBP is coming to the forefront of pain research. The development of an animal model whereby the animals remained well, with localised, reproducible pain development which closely parallels the human situation, has allowed elucidation of some of the basic mechanisms that may underlie this pain state. Clinicians managing CIBP will not be surprised by the above results, suggesting that this is a unique pain state. This type of pain, severe with rapid onset often with movement, has been difficult to effectively treat with common analgesics.

CIBP is a complex pain state, arising via activation and ultimately destruction of the primary afferents within bones. These can be stimulated directly by prostaglandins, cytokines, protons, endothelins, growth factors, nitrous oxide, ATP and many more mediators secreted by cancer cells and the invading immune infiltrate. Increased activation of osteoclasts via RANK:RANK-L and acidosis, contributes to the gross destruction of the bones, leading ultimately to pathological fractures. The

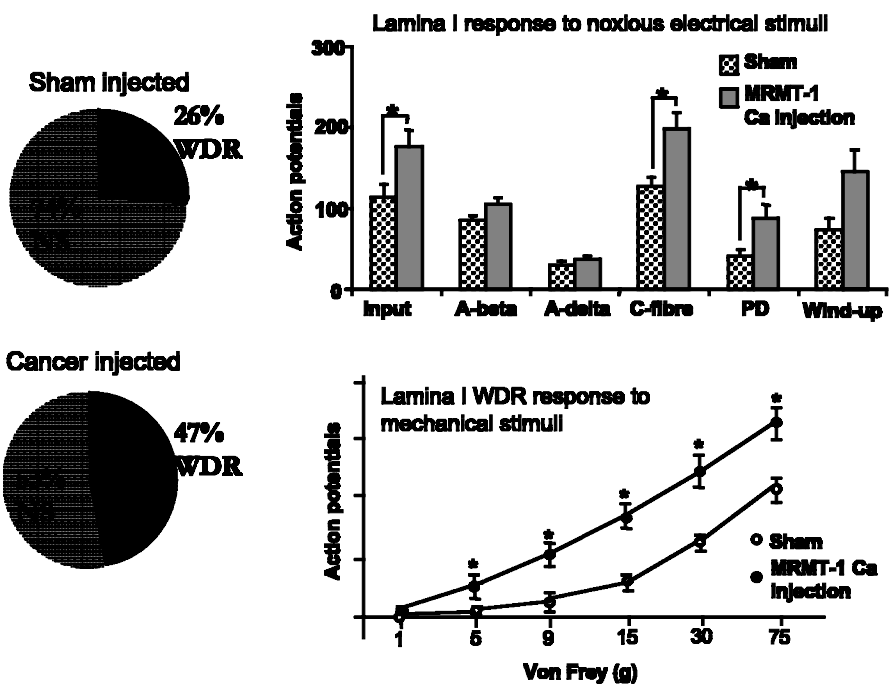

Figure 3 The pie charts show the change in percentage of NS and WDR neurones in Lamina I in response to the injection of MRMT-1 cancer cells. WDR cells comprise 26% in normal and sham injected animals, however this increases to 47% post MRMT-1 injection. The top graph shows the response of lamina I cells to noxious electrical stimuli. The input (response to the first stimuli), the C fibre evoked response (to a train of 16 stimuli) and the postdischarge evoked response are all significantly increased in the MRMT-1 injected group as compared to the sham (*$P < 0.05$, ANOVA). The lower graph summarises the responses of the Lamina I WDR neuronal response to increasing mechanical stimulation with Von Frey filaments. The mean in the MRMT-1-injected rats (closed circle) was significantly greater (*$P < 0.05$, ANOVA) than the neuronal responses of sham operated animals (open circle) between von Frey 5 g and 75 g.[85]

unique features of the clinical pain triad (tonic, incident and spontaneous pain) in CIBP are paralleled in the animal models, which in turn are mirrored in the unique alterations of neurones and astrocytes within the dorsal horn of the spinal cord.

The animal models have also been used to evaluate novel analgesics to treat CIBP pain, such as OPG and endothelin-1 antagonists. In addition, drugs which target cells within the dorsal horn, such as astrocyte inhibitors and drugs used to treat neuropathic pain such as gabapentin may yield therapeutic benefit. The future treatments of incident pain will no doubt be as unique as the pain pathophysiology itself.

References

1 Coleman RE. Skeletal complications of malignancy. *Cancer* 1997; **80**: 1588–94.
2 Mercadante S. Malignant bone pain: pathophysiology and treatment. *Pain* 1997; **69**: 1–18.
3 Neville-Webbe H Coleman RE. The use of zoledronic acid in the management of metastatic bone disease and hypercalcaemia. *Palliat Med* 2003; **17**: 539–53.
4 Portenoy RK Hagen NA. Breakthrough pain: definition, prevalence and characteristics. *Pain* 1990; **41**: 273–81.
5 Bruera E, Schoeller T, Wenk R, MacEachern T, Marcelino S, Hanson J, Suarez-Almozor M. A prospective multi-center assessment of the Edmonton staging system for cancer pain. *J Pain Symptom Manage* 1995; **10**: 348–55.
6 Portenoy RK, Payne D, Jacobsen P. Breakthrough pain: characteristics and impact in patients with cancer pain. *Pain* 1999; **81**: 129–34.
7 Mercadante S, Arcuri E. Breakthrough pain in cancer patients: pathophysiology and treatment. *Cancer Treat Rev* 1998; **24**: 425–32.
8 Portenoy RK, Frager G. Pain management: pharmacological approaches. *Cancer Treat Res* 1999; **29**: 1001–29.
9 Banning A, Sjogren P, Henriksen H. Pain causes in 200 patients referred to a multidisciplinary cancer pain clinic. *Pain* 1991; **45**: 45–48.
10 Swanwick M, Haworth M, Lennard RF. The prevalence of episodic pain in cancer: a survey of hospice patients on admission. *Palliat Med* 2001; **15**: 9–18.
11 Serafini AN. Therapy of metastatic bone pain. *J Nucl Med* 2001; **42**: 895–906.
12 McQuay HJ, Collins SL, Carroll D, Moore RA. Radiotherapy for the palliation of painful bone metastases. *Cochrane Database Syst Rev* 2000: CD001793.
13 Wong R, Wiffen PJ. Bisphosphonates for the relief of pain secondary to bone metastases. *Cochrane Database Syst Rev* 2002: CD002068.
14 Mercadante S, Radbruch L, Caraceni A, *et al*. Episodic (breakthrough) pain: consensus conference of an expert working group of the European Association for Palliative Care. *Cancer* 2002; **94**: 832–39.
15 Fucella L, Conti F, Corvi G, Mandelli V, Randelli M, Stefanelli G. Double-blind study of the analgesic effect of indoprofen (K 4277). *Clin Pharmacol Therap* 1974; **3**: 277–83.
16 Ventafridda V, Martino G, Mandelli V, Emanueli A. Idoprofen, a new analgesic and anti-inflammatory drug in cancer pain. *Clin Pharmacol Therap* 1974; **2**: 284–89.
17 Stambaugh J, Drew J. A double-blind parallel evaluation of the efficacy and safety of a single dose of ketoprofen in cancer pain. *J Clin Pharmacol* 1988; **28**: S34–39.
18 Sunshine A, Olson NZ. Analgesic efficacy of ketoprofen in postpartum, general surgery, and chronic cancer pain. *J Clin Pharmacol* 1988; **28**: S47–54.
19 Minotti V, Patoia L, Roila F, *et al*. Double-blind evaluation of analgesic efficacy of orally administered diclofenac, nefopam, and acetylsalicylic acid (ASA) plus codeine in chronic cancer pain. *Pain* 1989; **36**: 177–83.
20 Staquet MJ. A double-blind study with placebo control of intramuscular ketorolac tromethamine in the treatment of cancer pain. *J Clin Pharmacol* 1989; **29**: 1031–36.
21 Estape J, Vinolas N, Gonzalez B, *et al*. Ketorolac, a new non-opioid analgesic: a double-blind trial versus pentazocine in cancer pain. *J Int Med Res* 1990; **18**: 298–304.
22 Minotti V, Betti M, Ciccarese G, Fumi G, Tonato M, Del Favero A. A double-blind study comparing two single-dose regimens of ketorolac with diclofenac in pain due to cancer. *Pharmacotherapy* 1998; **18**: 504–508.
23 Mercadante S, Fulfaro F, Casuccio A. A randomised controlled study on the use of anti-inflammatory drugs in patients with cancer pain on morphine therapy: effects on dose-escalation and a pharmacoeconomic analysis. *Eur J Cancer* 2002; **38**: 1358–63.
24 Sheng H, Shao J, Kirkland SC, *et al*. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 1997; **99**: 2254–59.
25 Sumitani K, Kamijo R, Toyoshima T, *et al*. Specific inhibition of cyclooxygenase-2 results in inhibition of proliferation of oral cancer cell lines via suppression of prostaglandin E2 production. *J Oral Pathol Med* 2001; **30**: 41–47.
26 Sabino MA, Ghilardi JR, Jongen JL, *et al*. Simultaneous reduction in cancer pain, bone destruction, and tumor growth by selective inhibition of cyclooxygenase-2. *Cancer Res* 2002; **62**: 7343–49.
27 Body JJ. Current and future directions in medical therapy: hypercalcemia. *Cancer* 2000; **88**: 3054–58.
28 Ross JR, Saunders Y, Edmonds PM, Patel S, Broadley KE, Johnston SR. Systematic review of role of bisphosphonates on skeletal morbidity in metastatic cancer. *BMJ* 2003; **327**: 469.
29 Rogers MJ, Watts DJ, Russell RG. Overview of bisphosphonates. *Cancer* 1997; **80**: 1652–60.
30 Viereck V, Emons G, Lauck V, *et al*. Bisphosphonates pamidronate and zoledronic acid stimulate osteoprotegerin production by primary human osteoblasts. *Biochem Biophys Res Commun* 2002; **291**: 680–86.
31 Derenne S, Amiot M, Barille S, *et al*. Zoledronate is a potent inhibitor of myeloma cell growth and secretion of IL-6 and MMP-1 by the tumoral environment. *J Bone Miner Res* 1999; **14**: 2048–56.

32 Senaratne SG, Pirianov G, Mansi JL, Arnett TR, Colston KW. Bisphosphonates induce apoptosis in human breast cancer cell lines. *Br J Cancer* 2000; **82**: 1459–68.

33 de Wit R, van Dam F, Loonstra S, *et al*. The Amsterdam Pain Management Index compared to eight frequently used outcome measures to evaluate the adequacy of pain treatment in cancer patients with chronic pain. *Pain* 2001; **91**: 339–49.

34 Meuser T, Pietruck C, Radbruch L, Stute P, Lehmann KA, Grond S. Symptoms during cancer pain treatment following WHO-guidelines: a longitudinal follow-up study of symptom prevalence, severity and etiology. *Pain* 2001; **93**: 247–57.

35 Schwei MJ, Honore P, Rogers SD, *et al*. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *J Neurosci* 1999; **19**: 10886–97.

36 Medhurst SJ, Walker K, Bowes M, *et al*. A rat model of bone cancer pain. *Pain* 2002; **96**: 129–40.

37 Wacnik PW, Kehl LJ, Trempe TM, Ramnaraine ML, Beitz AJ, Wilcox GL. Tumor implantation in mouse humerus evokes movement-related hyperalgesia exceeding that evoked by intramuscular carrageenan. *Pain* 2003; **101**: 175–86.

38 Luger NM, Sabino MA, Schwei MJ, *et al*. Efficacy of systemic morphine suggests a fundamental difference in the mechanisms that generate bone cancer versus inflammatory pain. *Pain* 2002; **99**: 397–406.

39 Hohmann EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL. Innervation of periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve fibers. *Science* 1986; **232**: 868–71.

40 Bjurholm A, Kreicbergs A, Terenius L, Goldstein M, Schultzberg M. Neuropeptide Y-, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. *J Auton Nerv Syst* 1988; **25**: 119–25.

41 Hill EL, Elde R. Distribution of CGRP, VIP, D beta H, SP, and NPY immunoreactive nerves in the periosteum of the rat. *Cell Tissue Res* 1991; **264**: 469–80.

42 Hukkanen M, Konttinen YT, Rees RG, Gibson SJ, Santavirta S, Polak JM. Innervation of bone from healthy and arthritic rats by substance P and calcitonin gene related peptide containing sensory fibers. *J Rheumatol* 1992; **19**: 1252–59.

43 Goto T, Yamaza T, Kido MA Tanaka T. Light- and electron-microscopic study of the distribution of axons containing substance P and the localization of neurokinin-1 receptor in bone. *Cell Tissue Res* 1998; **293**: 87–93.

44 Mach DB, Rogers SD, Sabino MC, *et al*. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience* 2002; **113**: 155–66.

45 Hill EL, Turner R, Elde R. Effects of neonatal sympathectomy and capsaicin treatment on bone remodeling in rats. *Neuroscience* 1991; **44**: 747–55.

46 Chenu C, Serre CM, Raynal C, Burt-Pichat B, Delmas PD. Glutamate receptors are expressed by bone cells and are involved in bone resorption. *Bone* 1998; **22**: 295–99.

47 Serre CM, Farlay D, Delmas PD, Chenu C. Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. *Bone* 1999; **25**: 623–29.

48 Griffiths JR. Are cancer cells acidic? *Br J Cancer* 1991; **64**: 425–27.

49 Suzuki K, Yamada S. Ascites sarcoma 180, a tumor associated with hypercalcemia, secretes potent bone resorbing factors including transforming growth factor alpha, interleukin 1 alpha and interleukin 6. *Bone Miner* 1994; **27**: 219–33.

50 Watkins LR, Wiertelak EP, Furness LE, Maier SF. Illness-induced hyperalgesia is mediated by spinal neuropeptides and excitatory amino acids. *Brain Res* 1994; **664**: 17–24.

51 Safieh-Garabedian B, Poole S, Allchorne A, Winter J, Woolf CJ. Contribution of interleukin 1 beta to the inflammation induced increase in nerve growth factor levels and inflammatory hyperalgesia. *Br J Pharmacol* 1995; **115**: 1265–75.

52 Sorkin LS, Xiao WH, Wagner R, Myers RR. Tumour necrosis factor alpha induces ectopic activity in nociceptive primary afferent fibres. *Neuroscience* 1997; **81**: 255–62.

53 Woolf CJ, Allchorne A, Safieh-Garabedian B, Poole S. Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha. *Br J Pharmacol* 1997; **121**: 417–24.

54 Murata T, Ushikubi F, Matsuoka T, *et al*. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 1997; **388**: 678–82.

55 Bley KR, Hunter JC, Eglen RM, Smith JA. The role of IP prostanoid receptors in inflammatory pain. *Trends Pharmacol Sci* 1998; **19**: 141–47.

56 Basolo F, Calvo S, Fiore L, *et al*. Production of cytokines and response to them in normal and transformed human mammary epithelial cells. *Ann NY Acad Sci* 1993; **698**: 126–30.

57 Fonsatti E, Altomonte M, Coral S, *et al*. Tumour-derived interleukin 1alpha (IL-1alpha) up-regulates the release of soluble intercellular adhesion molecule-1 (sICAM-1) by endothelial cells. *Br J Cancer* 1997; **76**: 1255–61.

58 Arruda JL, Colburn RW, Rickman AJ, Rutkowski MD, DeLeo JA. Increase of interleukin-6 mRNA in the spinal cord following peripheral nerve injury in the rat: potential role of IL-6 in neuropathic pain. *Brain Res Mol Brain Res* 1998; **62**: 228–35.

59 Nasu K, Matsui N, Narahara H, Tanaka Y, Miyakawa I. Effects of interferon-gamma on cytokine production by endometrial stromal cells. *Hum Reprod* 1998; **13**: 2598–601.

60 Laughlin TM, Bethea JR, Yezierski RP, Wilcox GL. Cytokine involvement in dynorphin-induced allodynia. *Pain* 2000; **84**: 159–67.

61 Asham EH, Loizidou M, Taylor I. Endothelin-1 and tumour development. *Eur J Surg Oncol* 1998; **24**: 57–60.

62 Davar G, Hans G, Fareed MU, Sinnott C, Strichartz G. Behavioral signs of acute pain produced by application

of endothelin-1 to rat sciatic nerve. *Neuroreport* 1998; **9**: 2279–83.
63 De-Melo JD, Tonussi CR, D'Orleans-Juste P, Rae GA. Articular nociception induced by endothelin-1, carrageenan and LPS in naive and previously inflamed knee-joints in the rat: inhibition by endothelin receptor antagonists. *Pain* 1998; **77**: 261–69.
64 Cain DM, Wacnik PW, Turner M, Wendelschafer-Crabb G, Kennedy WR, Wilcox GL, Simone DA. Functional interactions between tumor and peripheral nerve: changes in excitability and morphology of primary afferent fibers in a murine model of cancer pain. *J Neurosci* 2001; **21**: 9367–76.
65 Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. *Nat Med* 1995; **1**: 944–49.
66 Nelson JB, Carducci MA. The role of endothelin-1 and endothelin receptor antagonists in prostate cancer. *BJU Int* 2000; **85**: 45–48.
67 Lacey DL, Timms E, Tan HL, *et al*. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; **93**: 165–76.
68 Yasuda H, Shima N, Nakagawa N, *et al*. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology* 1998; **139**: 1329–37.
69 Kong YY, Yoshida H, Sarosi I, *et al*. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; **397**: 315–23.
70 Simmonds MA. Management of breakthrough pain due to cancer. *Oncology (Huntingt)* 1999; **13**: 1103–108.
71 Bucay N, Sarosi I, Dunstan CR, *et al*. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260–68.
72 Sezer O, Heider U, Zavrski I, Kuehne CA, Hofbauer LC. RANK ligand and osteoprotegerin in myeloma bone disease. *Blood* 2002; **7**: 7.
73 Standal T, Seidel C, Hjertner O, *et al*. Osteoprotegerin is bound, internalized, and degraded by multiple myeloma cells. *Blood* 2002; **100**: 3002–3007.
74 Seidel C, Hjertner O, Abildgaard N, *et al*. Serum osteoprotegerin levels are reduced in patients with multiple myeloma with lytic bone disease. *Blood* 2001; **98**: 2269–71.
75 Lipton A, Ali SM, Leitzel K, *et al*. Serum osteoprotegerin levels in healthy controls and cancer patients. *Clin Cancer Res* 2002; **8**: 2306–10.
76 Honore P, Luger NM, Sabino MA, *et al*. Osteoprotegerin blocks bone cancer induced skeletal destruction, skeletal pain and pain related neurochemical reorganization of the spinal cord. *Nat Med* 2000; **6**: 521–28.
77 Luger NM, Honore P, Sabino MA, *et al*. Osteoprotegerin diminishes advanced bone cancer pain. *Cancer Res* 2001; **61**: 4038–47.
78 Morony S, Capparelli C, Sarosi I, Lacey DL, Dunstan CR, Kostenuik PJ. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. *Cancer Res* 2001; **61**: 4432–36.
79 Zhang J, Dai J, Lin DL, *et al*. Osteoprotegerin abrogates chronic alcohol ingestion-induced bone loss in mice. *J Bone Miner Res* 2002; **17**: 1256–63.
80 Sutherland SP, Cook SP, McCleskey EW. Chemical mediators of pain due to tissue damage and ischemia. *Prog Brain Res* 2000; **129**: 21–38.
81 Reeh PW, Steen KH. Tissue acidosis in nociception and pain. *Prog Brain Res* 1996; **113**: 143–51.
82 Lingueglia E, de Weille JR, Bassilana F, Heurteaux C, Sakai H, Waldmann R, Lazdunski M. A modulatory subunit of acid sensing ion channels in brain and dorsal root ganglion cells. *J Biol Chem* 1997; **272**: 29778–83.
83 Olson TH, Riedl MS, Vulchanova L, Ortiz-Gonzalez XR, Elde R. An acid sensing ion channel (ASIC) localizes to small primary afferent neurons in rats. *Neuroreport* 1998; **9**: 1109–13.
84 Honore P, Schwei J, Rogers SD, *et al*. Cellular and neurochemical remodeling of the spinal cord in bone cancer pain. *Prog Brain Res* 2000; **129**: 389–97.
85 Urch CE, Donovan-Rodriguez T, Dickenson AH. Alterations in dorsal horn neurones in a rat model of cancer induced bone pain. *Pain* 2003; **106**: 347–56.
86 Donovan-Rodriguez T, Dickenson A, Urch CE. Correlation between superficial dorsal horn neuronal responses and the emergence of behavioural hyperalgesia in a rat model of cancer-induced bone pain. *Neurosci Lett*; in press.
87 Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 2002; **5**: 1319–26.