Brief Communication

Selective Blockade of the Capsaicin Receptor TRPV1 Attenuates Bone Cancer Pain

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Cancer colonization of bone leads to the activation of osteoclasts, thereby producing local tissue acidosis and bone resorption. This process may contribute to the generation of both ongoing and movement-evoked pain, resulting from the activation of sensory neurons that detect noxious stimuli (nociceptors). The capsaicin receptor TRPV1 (transient receptor potential vanilloid subtype 1) is a cation channel expressed by nociceptors that detects multiple pain-producing stimuli, including noxious heat and extracellular protons, raising the possibility that it is an important mediator of bone cancer pain via its capacity to detect osteoclast- and tumor-mediated tissue acidosis. Here, we show that TRPV1 is present on sensory neuron fibers that innervate the mouse femur and that, in an in vivo model of bone cancer pain, acute or chronic administration of a TRPV1 antagonist or disruption of the TRPV1 gene results in a significant attenuation of both ongoing and movement-evoked nocifensive behaviors. Administration of the antagonist had similar efficacy in reducing early, moderate, and severe pain-related responses, suggesting that TRPV1 may be a novel target for pharmacological treatment of chronic pain states associated with bone cancer metastasis.

Key words: tumor; skeletal malignancies; vanilloid receptor; metastasis; nociception; therapy

Introduction

Cancer pain is a significant clinical problem because it is the first symptom of disease in 20–50% of all cancer patients and 75–90% of advanced or terminal cancer patients must cope with chronic pain syndromes related to failed treatment and/or tumor progression (Mercadante and Arcuri, 1998; Portenoy et al., 1999). Malignant bone tumors occur in patients with primary bone cancer but far more commonly occur as distant metastases of non-bone primary tumors, notably those in breast, prostate, and lung. As such, bone is the most common site of origin of chronic pain in patients with metastatic lung, prostate, and breast cancers or myeloma (Coleman, 1997, 2001). Osteoclasts, the body’s principal bone-resorbing cells, have been implicated recently in bone cancer pain (Adami, 1997). The mechanisms underlying attenuation of bone cancer pain after inhibition of osteoclast activity may include reduction of osteoclast-induced mechanical deformation of bone and/or local tissue acidosis. Osteoclast-mediated bone remodeling is also accompanied by the robust production of extracellular protons, which are known to be potent activators of primary afferent neurons (Krishtal and Pidoplichko, 1980; Bevan and Geppetti, 1994; Reeh and Steen, 1996), raising the possibility that the acidic microenvironment produced by osteoclasts contributes significantly to bone cancer-associated pain via activation of acid-sensitive nociceptors that innervate the marrow and mineralized bone.

Tissue acidosis may activate nociceptors via multiple molecular mechanisms, but one important site of proton action is the capsaicin receptor TRPV1 (transient receptor potential vanilloid subtype 1), a heat- and proton-activated ion channel that is located on primary afferent sensory neurons (Caterina et al., 1997; Tominaga et al., 1998). Acid (pH 5)-evoked excitatory responses are greatly reduced in sensory neurons from TRPV1-deficient mice (Caterina et al., 2000; Davis et al., 2000), substantiating a role for this ion channel in the detection of extracellular protons in vivo. Moreover, TRPV1−/− animals show impaired thermal hypersensitivity in response to tissue inflammation (Caterina et al., 2000; Davis et al., 2000), supporting the idea that TRPV1 serves as a detector of injury-induced chemical stimuli that excite primary afferent nociceptors or sensitize their response to heat (Tominaga et al., 1998). In light of the clinical significance and severity of bone cancer pain and the potential relevance of tissue...
acidosis to this process, we examined the involvement of TRPV1 and the effectiveness of a TRPV1 antagonist in attenuating bone cancer-induced hyperalgesia in a mouse model that closely mirrors the human condition.

Materials and Methods

Induction of bone cancer. Experiments were performed on adult (25–30 g) male C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) and TRPV1 wild-type (+/+) or mutant (+/−, −/−) mice, which were maintained at 22°C with a 12 h light/dark cycle and given food and water ad libitum. The mice were housed in accordance with National Institutes of Health guidelines, and all procedures were approved by the Animal Care and Use Committee of the University of Minnesota. Injection of 2472 osteolytic sarcoma cells was performed as described previously (Luger et al., 2001).

Generation of TRPV1 null mice. TRPV1 heterozygotes (+/−) on a C57BL/6 background (Caterina et al., 2000) were mated with C3H/HeJ mice and backcrossed until the fourth generation was reached, at which time 2472 cells grew and induced bone destruction and pain-related behaviors to a similar extent as that seen in C3H/HeJ mice. All of the animals used in the study were from the fourth generation or later. There were no statistically significant behavioral differences between the TRPV1+/− and the TRPV1−/− animals; therefore, TRPV1+/− animals were used as controls for all studies.

Treatment with the TRPV1 antagonist. To assess the effect of a TRPV1 receptor antagonist (JNI-17203212) on bone cancer-related behaviors, tumor growth, and bone destruction, animals were chronically treated with 30 mg/kg JNI-17203212 dissolved in 120 μl of Soluvert and 680 μl of 5% dextrose twice daily subcutaneously or an equivalent volume of vehicle alone. Previous studies have shown that JNI-17203212 is a selective, potent antagonist of both rodent and human TRPV1 (Dax et al., 2002) exhibiting an IC50 value of 38 ± 10 μM (mean ± SEM; n = 4), comparable with its Kᵢ value (27 ± 3 μM) for inhibiting capsaicin-evoked responses in transplanted human embryonic kidney 293 cells. Treatment was initiated 6 d after injection, when observable bone destruction was evident, and was terminated at 14 or 18 d after injection, when there was significant bone destruction and hyperalgesia. The doses used in the current study caused no observable adverse effects in the animals in rotarod performance, general activity, and body weight. C3H/HeJ animals were given 30 mg/kg JNI-17203212 subcutaneously twice daily for chronic studies, and, to assess the specificity of acute administration of JNI-17203212, both TRPV1+/− and TRPV1−/− animals were given 30 mg/kg JNI-17203212 subcutaneously once 1 h before behavioral testing (see Fig. 3 E, F).

Behavioral analysis. C3H/HeJ and TRPV1 wild-type (+/+) and TRPV1 mutant (+/−, −/−) mice were tested for pain-related behaviors both 10 and 14 d after sarcoma or sham injections, when pain-related behaviors are significantly evident. C3H/HeJ animals were tested 9, 11, 15, and 18 d after tumor or sham injections to assess efficacy of the TRPV1 antagonist JNI-17203212 over the progression of the disease, and ongoing and movement-evoked pain-related behaviors were analyzed as described previously (Luger et al., 2001). Ongoing nociceptive behaviors were evaluated by measuring spontaneous guarding and spontaneous flinching over a 2 min observation period, and movement-evoked alldynia was assessed by measuring the time spent guarding and flinching over a 2 min observation period after normally non-noxious palpation of the distal femur. The number of flinches was defined as lifting the tumor-injected limb while stationary, and time spent guarding was defined as the length of time the tumor-injected limb was held aloft. All of the behavioral data were obtained using at least eight animals for each time point or group, with the exception of the sarcoma-injected C3H/HeJ group (n = 4), which received the TRPV1 antagonist JNI-17203212.

Euthanasia, processing of tissue, and immunohistochemical analysis of neural and bone tissues. Fourteen days after sarcoma injection, mice were killed, and serum and tissues were collected for analysis. To induce c-Fos expression, animals received a normally non-noxious palpation of the ipsilateral knee 90 min before being killed (Hunt et al., 1987; Honore et al., 2000a) and were subsequently processed for immunohistochemical analysis (Luger et al., 2001) using the following primary antibodies: guinea pig anti-mouse TRPV1 (1:5000; from the laboratory of D. Julius), rabbit anti-human activated transcription factor-3 (ATF-3) (1:500; Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal rabbit anti-c-Fos (1:15,000; Oncogene Research, San Diego, CA), and polyclonal rabbit anti-calcitonin gene-related protein (CGRP) (1:45,000; Sigma, St. Louis, MO). Spinal cords and DRGs were analyzed by conventional fluorescent and confocal microscopy, and quantification was performed as described previously (Schwe et al., 1999; Honore et al., 2000b). Tumor burden, bone destruction, and bone immunohistochemistry were processed and analyzed as described previously (Mach et al., 2002; Sabino et al., 2002). The anti-TRPV1 antibody was tested in TRPV1−/− mice, and no specific labeling was observed in DRG or femur.

Statistical analysis. The StatView statistics package (SAS Institute, Cary, NC) was used to perform statistical tests. One-way ANOVA was used to analyze behavioral results, bone histology results, and immunohistochemical measures among the experimental groups at each time point. Because of the increased risk of type I error associated with multiple comparisons (Fisher’s PLSD), with significance level set at p < 0.05. The individual investigators responsible for bone-destruction scoring, behavioral testing, and immunohistochemical quantification were blinded to the experimental situation, including the genotype of each animal.

Results

TRPV1 expression by sensory neurons that innervate the femur

TRPV1-expressing sensory fibers were present in mineralized bone and bone marrow, in which they were generally seen in close association with blood vessels and had a similar appearance to other sensory fibers, such as those that express CGRP and innervate the bone (Fig. 1 A). At the leading edge of the tumor, sensory
fibers continued to exhibit TRPV1 immunoreactivity (Fig. 1B). As the tumor cells filled the intramedullary space of the tumor, 20–25% of the sensory neurons in the L2 DRG expressed ATF-3, and ~30% of these ATF-3-expressing neurons also exhibited TRPV1 immunoreactivity (Fig. 2E,F). These results suggest that a significant percentage of sensory neurons that innervate the tumor-bearing bone express TRPV1 and that TRPV1 expression is maintained even as the distal processes of these sensory fibers are injured by the invading tumor cells and induce ATF-3 expression.

TRPV1 antagonist-treated and TRPV1 null animals show reduced ongoing and movement-evoked pain-related behaviors and attenuation of spinal c-Fos expression

Chronic treatment of tumor-bearing animals with JNJ-17203212 resulted in decreased ongoing and movement-evoked nocifensive behaviors at days 10 and 14 after tumor injection (Fig. 3A–D, day 14) without any observable behavioral side effects, such as ataxia or hypoactivity. Similar results were observed when the same animals were analyzed 9, 11, 15, and 18 d after tumor injection. In both the acute and chronic administration protocols, there was no loss of efficacy of JNJ-17203212 in reducing ongoing and movement-evoked nocifensive behaviors with disease progression (Fig. 3E,F).

Blockade of TRPV1 also prevented immediate-early gene activation attributable to a painful stimulus. Normally nonnoxious palpation induces significant ipsilateral c-Fos expression in laminae I–II of the L3/L4 spinal cord segment 14 d after tumor injection into the femur when compared with naive or sham animals (Schwei et al., 1999). This increase in laminae I–II c-Fos expression in vehicle-treated animals [17.5 ± 4.0 c-Fos-immunoreactive (IR) neurons/L4 section] was significantly attenuated by chronic TRPV1 antagonist treatment [7.5 ± 3.2 c-Fos-IR neurons/(L3/L4) section; n = 6; data not shown]. TRPV1 expression was not detected in the sarcoma cells, and JNJ-17203212 did not significantly affect tumor growth, as quantitatively determined by hematoxylin and eosin staining and radiographic analysis of total tumor burden within the intramedullary space of the sarcoma-bearing femur.

The TRPV1 +/+ and TRPV1 +/− animals showed the normal development of pain-related behaviors, whereas TRPV1 −/− mice showed a significant reduction in both ongoing and movement-evoked nocifensive behaviors similar to that observed in C3H/HeJ animals treated with JNJ-17203212. Moreover, no additional reduction in bone cancer pain-related behaviors was observed in TRPV1 −/− animals receiving JNJ-17203212, suggesting that the major target for the analgesic action of JNJ-17203212 in this model of bone cancer pain is indeed the TRPV1 channel (Fig. 4A,B).

Discussion

In the present study, bone cancer-induced ongoing and movement-evoked nocifensive behaviors were reduced after the pharmacologic blockade or genetic deletion of TRPV1. In humans with bone cancer, ongoing pain usually occurs in the area of the tumor and is described as dull, constant, and increasing in intensity over time, with intermittent episodes of extreme pain that occur spontaneously or after weight bearing and/or movement of the affected bone (Coyle et al., 1990; Mercadante and Arcuri, 1998). What is in some ways unique about bone cancer pain is that the inflammation, tumor-released products, and tumor-induced injury to primary afferent neurons may simultaneously drive this chronic pain state (Luger et al., 2001). In the present murine model of bone cancer, mice guarded the affected limb when at rest, when applying weight to the affected bone or during normally non-noxious palpation. All such behaviors were significantly attenuated by TRPV1 blockade or deletion.

There are at least three mechanisms in bone cancer that may contribute to the activation/sensitization of TRPV-1 expressed by sensory fibers that innervate the tumor-bearing bone. In both
osteolytic (bone-destroying) and osteoblastic (bone-forming) cancers there is a significant proliferation and hypertrophy of osteoclasts (Adami, 1997; Clohisy and Ramnaraine, 1998). Osteoclasts are terminally differentiated, multinucleated, monocyte lineage cells that are uniquely designed to resorb bone by maintaining an extracellular microenvironment of acidic pH (4.0 – 5.0) at the osteoclast-mineralized bone interface (Delaisse and Vaes, 1992). Recent observations in both preclinical models (Yoneda et al., 2000) and humans (Major et al., 2000; Berenson et al., 2001) have demonstrated that osteoclasts play an essential role in cancer-induced bone loss and contribute to the etiology of bone cancer pain. Therapies such as osteoprotegerin or the bisphosphonates, both of which reduce the number of activated osteoclasts (Luger et al., 2001; Sevcik et al., 2004), owe their efficacy in reducing bone cancer pain, at least in part, to the attenuation of osteoclast-mediated acidosis, thereby potentially decreasing proton-induced stimulation of TRPV1 channels on sensory nerve fibers that innervate bone.

Another source of protons that may contribute to TRPV1 stimulation is lysis of tumor cells themselves. Tumor cells have a lower intracellular pH than normal cells (Griffiths, 1991), and, as a solid tumor outgrows its vascular supply, it becomes necrotic, which may also generate an acidic environment (Reeh and Steen, 1996).

In addition to agents that can directly activate TRPV1, other factors such as bradykinin, ATP, and NGF can modulate TRPV1 function indirectly via activation of second-messenger signaling pathways (Woolf and Salter, 2000; Julius and Basbaum, 2001), resulting in TRPV1 sensitization. Previous studies have shown that these factors can be released from a variety of tumor cells (Drube and Liebmann, 2000; Marchetti et al., 2003; Mujoomdar et al., 2004; Ghilardi et al., 2005).
et al., 2003). For example, NGF has been shown to be expressed by many tumor cells and tumor-associated macrophages, including the 2472 sarcoma cells used in the present study (P. W. Mantyh and M. A. Sevick, unpublished observations). As the bone receives a rich sensory innervation by fibers that express TRPV1, production of these proaggressive agents may also sensitize TRPV1 channels, thereby generating a state of hyperalgesia and/or allodynia (Ji et al., 2002).

Like many chronic pain states, bone cancer pain becomes more severe with disease progression, requiring higher doses of analogies to control the pain (de Wit et al., 2001). In the present report, we show that administration of a TRPV1 antagonist reduces both bone cancer-induced ongoing and movement-evoked pain-related behaviors and retains this efficacy at early, middle, and late stages of tumor growth. One reason that the antagonism or inactivation of TRPV1 attenuates but does not completely block bone cancer-induced pain behaviors is that other receptors (such as bradykinin, P2X3, and prostaglandin receptors, or acid-sensing ion channel 3 and voltage-gated sodium channels) may also be involved in the generation and maintenance of this pain state (Julius and Basbaum, 2001; Mantyh et al., 2002). The ability of a TRPV1 antagonist to maintain its analgesic potency with disease progression is probably influenced by the fact that sensory nerve fibers innervating the tumor-bearing mouse femur maintain their expression of TRPV1 even as tumor growth and tumor-induced bone destruction progresses. Previous studies suggest that sensory neuronal expression of TRPV1 is upregulated during inflammation (Ji et al., 2002) and downregulated after peripheral nerve injury (Michael and Priestley, 1999). Interestingly, this downregulation of TRPV1 can be blocked by NGF (Priestley et al., 2002). In bone cancer, the peripheral nerve terminals that innervate the bone are injured, indicated by their upregulated expression of ATH-3, a marker of nerve injury (Tsujiino et al., 2000; Tsuzuki et al., 2001), but many tumor cells, including the 2472 sarcoma cells used in this model, express NGF (P. W. Mantyh and M. A. Sevick, unpublished observations), which may help maintain expression of TRPV1 with disease progression. The present results suggest that the TRPV1 channel plays a role in the integration of nociceptive signaling in a severe pain state and that antagonists of TRPV1 may be effective in attenuating difficult-to-treat mixed chronic pain states, such as those encountered in patients with bone cancer pain.

References


