Topical treatment with Xiaozheng Zhitong Paste alleviates bone cancer pain by inhibiting proteinase-activated receptor 2 signaling pathway

YANJU BAO1, GAIMEI WANG2, YEBO GAO1,3, MAOBO DU4, LIPING YANG5, XIANGYING KONG4, HONGGANG ZHENG1, WEI HOU1 and BAOJIN HUA1

1 Department of Oncology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Xicheng, Beijing 100053; 2 Department of Gynecology, the Third Hospital of Zhengzhou, Zhengzhou, Henan 450000; 3 Beijing University of Chinese Medicine, Chaoyang, Beijing 100029; 4 Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Dongzhimen, Beijing 100700; 5 Department of Nephrology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Xicheng, Beijing 100053, P.R. China

Received April 23, 2015; Accepted June 2, 2015

DOI: 10.3892/or.2015.4073

Abstract. Herbal analgesic Xiaozheng Zhitong Paste (XZP) and related modifications are often used in traditional Chinese medicine to manage cancer pain. However, its underlying mechanism remains unknown. To investigate the effects and mechanism of XZP on bone cancer pain in a rat model of breast cancer-induced bone pain, a bone cancer pain model was established by inoculating Walker 256 cells into Wistar rats. Bone cancer-bearing rats were topically treated with different doses of XZP or injected with 5 mg/kg of osteo-protegerin (OPG) as positive control. Bone destruction, bone mineral content (BMC) and bone mineral density (BMD) were analyzed by radiology. Paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) were examined to determine pain levels. Trypsin, TNF-α and IL-1β serum levels were determined using enzyme-linked immunosorbent assay (ELISA). Central sensitization markers such as c-Fos, GFAP, IBA1 and CGRP, as well as proteinase-activated receptor 2 (PAR2) signaling pathway mediators such as PAR2, PKC-γ, PKA and TRPV1, were determined by quantitative RT-PCR and western blotting assay. XZP treatment significantly mitigated bone cancer-related nociceptive behavior, bone damage, BMC and BMD; and decreased radiological scores in rats. XZP treatment significantly inhibited IBA1, GFAP, c-Fos and CGRP expressions in the spinal cord; and significantly mitigated trypsin, TNF-α and IL-1β serum levels. Furthermore, PAR2, PKC-γ, PKA and TRPV1 relative mRNA levels and protein expression in bone lesions were significantly reduced in rats treated with XZP. XZP significantly alleviates breast cancer-induced bone pain by inhibiting the PAR2 signaling pathway.

Introduction

Primary and metastatic bone cancers are often accompanied by severe pain. Bone cancer pain occurs in 75-90% of patients with metastatic cancer (1) and is often debilitating and intractable, affecting the quality of life of patients (2,3). Bone cancer pain is associated with breakthrough pain, which is defined as ‘a transitory exacerbation of pain experienced by patients who have relatively stable and adequately controlled baseline pain’ (2). Hence, controlling bone cancer pain would be of great significance in the management of patients with bone cancer.

Currently, there are many therapeutic options available for alleviating bone cancer pain including external beam radiotherapy, local surgery, opioid analgesia, non-steroidal anti-inflammatory drugs (NSAIDs), bisphosphonates and other anesthetic techniques (4). However, these therapies either have poor responses or require high dosages that are often associated with severe adverse effects (5-8). For instance, although bisphosphonates are effective in reducing skeletal morbidity from bone metastases and bone cancer pain, their efficacy remains unclear (9,10). Therefore, there is an urgent need for the discovery and development of new therapeutic reagents to control bone cancer pain, particularly for breakthrough pain.

We and other researchers have shown that some Chinese herbal medicines have potent antinociceptive activity, and could effectively control cancer pain including bone cancer pain in clinical practice (11-14). Indeed, it is reported that ~41-62% of cancer patients use herbs as a complementary or alternative medical therapy for cancer pain management (15). Topical treatment is an attractive and effective option, particularly for bone cancer pain management; since oral administration of herbal medicines causes several side-effects.
such as nausea, vomiting and diarrhea, affecting the quality of life of patients (11-13, 16). Xiaozheng Zhitong Paste (XZP) is a herbal analgesic paste that is prepared from six herbs including Xuejie (Dragon's blood), Yanhusuo (Corydalis Rhizoma), Ruxiang (Olibanum), Moyao (Myrrha), Qingdai (Natural Indigo) and Bingpian (Borneolum Syntheticum). Our previous studies showed that XZP significantly alleviated cancer pain including bone cancer pain in patients with middle and late stages of cancer (16, 17). However, its underlying mechanism has not been systemically explored.

Specific cellular and molecular mechanisms underlying bone cancer pain remain largely obscure (18, 19). While several lines of studies have demonstrated remarkable sensitization of peripheral nociceptors in bone, resulting from the tumor-induced acidosis and cytokine synthesis; emerging evidence indicates that central sensitization exists in the spinal dorsal horn of a rodent model of bone cancer pain such as the activation of astrocytes (20-22) and altered glutamatergic synaptogenesis (23, 24).

Proteinase-activated receptors (PARs) are a family of the G-protein coupled receptors that are activated by proteases, which liberates a tethered ligand by cleaving the N-terminus of the receptors initiating several intracellular signal pathways (25). Among the four PAR subtypes, PAR1, PAR3 and PAR4 are preferentially activated by thrombin, while PAR2 is preferentially activated by trypsin and trypsin-like proteinases (26, 27). All four PARs are expressed throughout the peripheral and central nervous system (26-33). In the spinal dorsal horn, PAR2 receptors are located in the microglia, astrocytes, neurons and terminals of afferent fibers originating from dorsal root ganglia (26). Previous studies have demonstrated the critical involvement of PAR2 in the pathogenesis of several types of inflammatory or neuropathic pain (26). Activation of PAR2 signaling participate in the induction of peripheral nociceptor sensitization in a rodent model of bone cancer pain (34). We have also demonstrated that the activation of PAR2 signaling contributes to the central sensitization in rats with bone cancer pain (26, 27). However, there is no information on the impact of XZP treatment on peripheral and central sensitization, and on the PAR2 signaling pathway. Given that XZP effectively alleviates bone cancer pain, we hypothesized that XZP treatment inhibits the PAR2 signaling pathway; mitigating peripheral and central sensitization. The present study was designed to test this hypothesis, utilizing a rat model of bone cancer nociception to evaluate the therapeutic effect of XZP on bone cancer pain and explore its potential underlying mechanism. We believe that our results have an impact on the development of XZP as an effective topical treatment for bone cancer pain, and future discoveries of novel PAR2-targeted therapy for cancer pain in general.

Materials and methods

XZP preparation. XZP is composed of the six aforementioned traditional Chinese medicines. High performance liquid chromatography (HPLC) analysis has shown that XZP contained tetrahydropalmatine (0.0568%), imperatorin (0.0041%), isoimperatorin (0.0122%), coptisine (0.0358%) and palmatine chloride (0.112%) (16).

Animals. Animal use and care protocols were reviewed and approved by the Animal Care and Use Committee of the China Academy of Chinese Medical Sciences, Beijing, China. All animal studies were carried out in accordance with the guidelines of the International Association for the Study of Pain (35). Female Wistar rats, weighing 150-180 g, were obtained from the Department of Experimental Animal Sciences, Peking University Health Science Center (Beijing, China). Individual rats were housed in specific pathogen-free (SPF) facilities under strict temperature (24±1°C) and humidity (60%) control on a 12/12 h light/dark cycle with free access to standard food and tap water.

Rat bone cancer pain model and XZP treatment. Wister rat Walker 256 breast sarcoma cells were prepared, and a rat bone cancer pain model was established as previously reported (32). Briefly, Wistar rats were intraperitoneally (i.p) injected with Walker 256 cells (2x10^7 in 0.5 ml of Hank's solution). Seven to 14 days later, cells in the produced ascites were collected. After being anesthetized, 50 rats were injected with 10^7 Walker 256 cells in 10 µl of Hank's solution into the left tibia of the hind paw. Then, the injection site was covered by bone wax and the wound was closed. Sham control rats (n=10) received the same surgery and volume of vehicle injection. All rats were subjected to the same post-operative care.

Rats with bone cancer were randomly divided into five groups (n=10/group): one placebo control group, topically treated with inert paste; three XZP groups, treated with low (15.75 g/kg), medium (31.5 g/kg) and high (63 g/kg) doses of XZP; one OPG group, subcutaneously injected with 5 mg/kg of OPG twice per day. Sham control rats were also topically treated with inert paste. XZP and placebo pastes were evenly spread on the tibia of the hind paw. Then, the injection site was covered with gauze and a layer of plastic film, sealed and fixed with desensitized adhesive plaster. Treatments were performed twice a day at 8:00 am and 20:00 pm for a total of 21 days, beginning at day one after cancer cells were implanted into the tibias (17).

Radiological analysis of bone lesions. Cancer-related osteolytic lesions in rat tibias were examined by X-ray radiology at day 21 after cell inoculation. Rats were anaesthetized with sodium pentobarbital (45 mg/kg) by i.p injection and exposed to X-ray (Emerald 125) at 40 kVp for 1/20 sec. X-ray film (Henry Schein blue sensitive film; Shanghai Han Ruixiang Trade Co., Ltd.) was developed with a film developer (Konica SRX-101; Suzhou Zhongyi Electronic Technology Co., Ltd.). Tibias were scanned and reconstructed with an isotropic voxel size of 8-µm on a micro-CT system (eXplore Locus SP; GE Medical Systems, Zhongtong Shanghai Automation & Electrics Co., Ltd.). Reconstructed 3D images of femurs were analyzed using Microvieweer (GE Medical Systems, Zhongtong Shanghai Automation & Electrics Co., Ltd.) as previously described (36). Bone mineral density (BMD) of left tibias in individual rats were measured by dual energy X-ray absorptiometry using a PIXImus Mouse Densitometer; and bone mineral contents were calculated using small animal analysis software (both from GE Lunar Medical System, Zhongtong Shanghai Automation & Electrics Co., Ltd.) at day 21, after cell inoculation.
**Analysis of trypsin, TNF-α and IL-1β serum by enzyme-linked immunosorbent assay (ELISA).** Orbital venous blood samples from each rat were collected on day 0, 7, 14 and 21 after cell implantation. Inflammatory mediator levels were determined using ELISA kits (Beijing Jia Mei Nuo Si Biotechnology Co., Ltd.; trypsin, ng/ml, catalog no. ABIN1117631, detection range 0.16-10 ng/ml, detection minimum 0.09 ng/ml; interleukin-1β, pg/ml, catalog no. ABIN365189, detection range 62.5-4,000 pg/ml, detection minimum 62.5 pg/ml; tumor necrosis factor-α, pg/ml, catalog no. ABIN365380, detection range 6.25-400 pg/ml, detection minimum 6.25 pg/ml).

**Histological evaluation.** Rats were deeply anesthetized by pentobarbital and transcardially perfused with saline on day 0.2 µM of each paired primer and 8.6 µl of deionized water. Protocols were as follows: initial denaturation for 5 min at 95°C, followed by 35 cycles denaturation for 15 sec at 95°C, and extension for 30 sec at 56°C. Last cycle for dissociation was reverse transcribed to cDNA with a first-strand cDNA synthesis kit (Invitrogen). Quantitative PCR was performed using a LightCycler system (Roche, Beijing Mao Jian united Stars Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. Total RNA (5 µg) was subjected to four tests with 5-min intervals before surgery; and 2, 5, 8, 11, 14, 17 and 20 days after cell inoculation. Mean PWL values were calculated for each time point for individual rats.

**Western blot assay.** To analyze c-Fos, GFAP, IBA1 and CGRP protein levels, total proteins from the L4-L5 spinal cords in various groups were extracted by sonication in RIPA buffer containing protease inhibitors (Roche). Extracted proteins were dialyzed in PBS. After measuring protein content with a BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) for 30 min before the test. Mechanical hyperalgesia was defined as the minimal force (g) required in evoking the cited positive responses. Paw withdrawal threshold (PWT) was defined as the minimal force (g) required in evoking the cited positive responses. Paw withdrawal latency (PWL) was automatically recorded for the nearest 0.1 sec. Stimuli intensity was adjusted to generate an average baseline PWL of ~10.0 sec in naive animals. Maximum stimulation was controlled to <20 sec to prevent potential tissue damage. Paws were alternated randomly to preclude order effects. Individual rats were subjected to four tests with 5-min intervals before surgery; and 2, 5, 8, 11, 14, 17 and 20 days after cell inoculation. Mean PWL values were calculated for each time point for individual rats.

**Table I. Primer sequences.**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primers sequences</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Fos</td>
<td>F 5'-GGGCTAGCGGGAATGCTGC-3'</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td>R 5'-GGGCTCTGTCAAC-3'</td>
<td></td>
</tr>
<tr>
<td>GFAP</td>
<td>F 5'-GAGAGGAAAGGTGAGCTGC-3'</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>R 5'-CGTCTGTAGGTGCCTACAA-3'</td>
<td></td>
</tr>
<tr>
<td>IBA1</td>
<td>F 5'-ATGCTGAGAAACCTGGGGT-3'</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>R 5'-CAGTTGCTCGTTGTTCTC-3'</td>
<td></td>
</tr>
<tr>
<td>CGRP</td>
<td>F 5'-ACTGCAATCTGAATACGTGC-3'</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>R 5'-CAGTTGTCAAAGGAGTACCTT-3'</td>
<td></td>
</tr>
<tr>
<td>PAR2</td>
<td>F 5'-TGGGAAAGCTGAGCTAT-3'</td>
<td>497</td>
</tr>
<tr>
<td></td>
<td>R 5'-TGGGAGCAAGCAGTGAAG-3'</td>
<td></td>
</tr>
<tr>
<td>PKC-γ</td>
<td>F 5'-TCTTCCCCAAAGAACCACACCTC-3'</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>R 5'-AGAACATGGAAAGGAGTTG-3'</td>
<td></td>
</tr>
<tr>
<td>PKA</td>
<td>F 5'-GGCTTCAAAACCTCAACGAT-3'</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTGTTGCTCGATCTGTTCA-3'</td>
<td></td>
</tr>
<tr>
<td>TRPV1</td>
<td>F 5'-TGCAACAATGGCGCAATAGAC-3'</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>R 5'-GTCACTGTCGCCGTCTC-3'</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>F 5'-CCCCCAATTACTCGTTG-3'</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>R 5'-TAGCCCCAGAGTTAGCTTATG-3'</td>
<td></td>
</tr>
</tbody>
</table>

F, forward; R, reverse; PAR2, proteinase-activated receptor 2.
USA), protein samples with equal amounts of total protein were separated on SDS-PAGE gels and transferred to nitrocellulose (NC) membranes (Millipore). Western blot assay was performed, as previously reported (16,27,28,31). Primary antibodies used in the present study were obtained from Abcam (Cambridge, MA, USA) including anti-c-Fos (phospho T325) (ab27793), anti-GFAP (ab4674), anti-IBA1 (ab107159) and anti-CGRP [EPR9670(B)] (ab139264) antibodies. Target protein bands were detected using horseradish peroxidase-conjugated secondary antibodies and visualized by an enhanced chemiluminescence detection system (Beijing Mao Jian United Stars Technology Co., Ltd.). Relative protein expression levels were quantified by ImageQuant™ LAS 4000 (GE Healthcare) and normalized to GAPDH.

To analyze PAR2, PKC-γ, PKA and TRPV1 protein levels in rat bones with tumor invasion, total proteins were extracted, quantified and analyzed by western blotting as previously described. Primary antibodies used in experiments were also obtained from Abcam including anti-PKC γ (phospho T514) (ab109539), anti-PAR2 (ab180953), anti-PKA α + β (catalytic subunits) (phospho T197) (ab5815) and anti-VR1 (ab63083) antibodies.

Statistical analysis. Behavioral data are expressed as mean ± standard deviation (SD) and analyzed with repeated measures ANOVA, while ELISA data were analyzed by one-way ANOVA followed by Newman-Keuls test. SPSS 13.0 statistical software was used in the present study. P<0.05 was considered to indicate a statistically significant result.

Results

XZP treatment reduces nociceptive responses in rats with bone cancer. We first determined the effects of XZP treatments on bone cancer-related nociceptive behavior in bone cancer bearing Wister rats. While constant levels of mechanical thresholds were observed in sham control rats, levels of mechanical thresholds were gradually reduced throughout the observation period in rats in the placebo group (Fig. 1A). In contrast, treatment with different doses of XZP significantly mitigated mechanical allodynia in a dose- and time-dependent manner; although mechanical threshold values were lower than OPG-treated positive controls. Similar PWL patterns were observed in different groups of rats (Fig. 1B). These data indicated that XZP treatment reduced mechanical and thermal nociceptive behavior in rats with bone cancer.

XZP treatment mitigates bone damage in rats with bone cancer. Next, we examined the effects of XZP treatment on tibia cancer-induced bone structural damage. X-ray images indicated less severe loss of medullary bone and cortical bone erosion were observed in positive control (OPG) rats and XZP-treated rats at various doses at day 21 of post cell inoculation, while full-thickness bicortical bone loss was accompanied by displaced fractures in rats with bone cancer in the placebo group (Fig. 2A). Quantitative analysis revealed that radiological scores in rats that received different doses of XZP significantly mitigated mechanical alldynia in a dose- and time-dependent manner; although mechanical threshold values were lower than OPG-treated positive controls (A). Similar paw withdrawal latency (PWL) patterns were observed in different groups of rats (B). Different groups of rats were longitudinally tested for mechanical allodynia and PWL at indicated time points in a blinded manner. Data are expressed as means ± SD of individual groups of rats (n=10/group). *P<0.05, **P<0.01 and ***P<0.001 vs. control group. XZP, Xiaozheng Zhitong Paste.

XZP treatment, similar to OPG, significantly preserved BMC at day 7, 14 and 21 post cell inoculation (P<0.05, P<0.01, Fig. 2C). Furthermore, medium- or high-dose XZP treatment, similar to OPG treatment, significantly mitigated BMD loss at day 7, 14 and 21 post cell inoculation (Fig. 2D). Histological examinations revealed that very few cancer cells invaded into bone tissues in positive controls and in rats treated with different doses of XZP, while a significant number of cancer cells invaded bone tissues and destroyed bone structure in the placebo group (Fig. 2E). Collectively, XZP treatment mitigated cancer invasion-mediated bone damage and structural changes.

XZP treatment inhibits activation of astrocytes and microglial cells in rats with bone cancer. To determine potential mechanisms underlying the action of XZP, we analyzed microglial
IBA1 and astrocyte GFAP markers, and neurotransmitters c-Fos and CGRP, in the spinal cord of rats (Fig. 3). Significantly increased levels of c-Fos, GFAP, IBA1 and CGRP mRNA transcripts were detected in rats with bone cancer, compared with the sham controls (Fig. 3A); while relative levels of their gene mRNA transcripts were significantly reduced in rats...
treated with XZP, similar to rats that received OPG treatment. A similar pattern was detected when protein expressions in the spinal cord were analyzed (Fig. 3B and C). Thus, XZP treatment inhibited the activation of astrocytes and microglial cells, contributing to its therapeutic effects in rats with bone cancer.
XZP treatment inhibits the PAR2 signaling pathway in rats with bone cancer. ELISA analysis indicated that upstream levels of the PAR2 signaling pathway including trypsin, TNF-α and IL-1β serum in rats treated with XZP were significantly reduced at day 7, 14 or 21 post-inoculation, compared with placebo controls (Fig. 4); and these effects appeared to be dose-dependent. Furthermore, relative downstream levels of PAR2 signaling pathway mediators including PAR2, PKC-γ, PKA and TRPV1 mRNA transcripts in bone lesions were significantly increased in the rats with bone cancer, compared with the sham controls (Fig. 5A); while relative levels of these gene mRNA transcripts were significantly reduced in the rats that received XZP treatment, similar to rats with OPG treatment. A similar pattern was detected when protein expression was analyzed in bone lesions in the different groups (Fig. 5B and C). Collectively, these data indicate that XZP treatment inhibited the PAR2 signaling pathway in rats with bone cancer, which contributes to the observed antinociceptive effects.

Discussion

The present study aimed to demonstrate the therapeutic effects of topical XZP treatment in bone cancer pain by establishing a rat model and exploring its underlying mechanism. Our major findings from the present study revealed that various doses of XZP treatment significantly alleviated bone cancer-related nociception; and inhibition of the PAR2 signaling pathway in a dose-dependent manner was most likely the underlying mechanism of action. We anticipate that these results may be beneficial to the development of this TCM for treating bone cancer pain in clinical practice.

Cancer bone metastasis is common and has a devastating impact on the quality of life of patients (3,37,38). Cancer-related bone metastasis cause bone damage and pain, which is particularly difficult to treat (3,37,38). Our previous study has shown that XZP treatment alleviates cancer pain in clinical practice (17). Our results from the present study indicate that XZP treatment alleviated cancer-related bone pain through different mechanisms (39).

Central nervous system (CNS) glia act as immune effector cells in both normal and pathological conditions, playing a vital role in initiating processes of persistent pain states (40-42). Previous studies have shown that early CNS glial response to peripheral nerve injury is predominantly due to the activation of spinal microglia, and that astrocytes subsequently undergo activation and proliferation (43,44). The activation of astrocytes and microglial cells leads to the robust release of proinflammatory cytokines such as IL-1β and TNF-α (45,46). Cytokines are important factors that contribute to the establishment of central sensitization (47). For instance, IL-1β increases NMDAR phosphorylation and NMDAR-mediated intracellular calcium release in sensory neurons (48). TNF-α increased neuronal excitability by stimulating neuronal ion channels (49,50). These proinflammatory cytokines may also activate glial cells, resulting in the amplification of glia-mediated pain-related cascades. The activation of astrocytes and microglial cells, as well as increased activity of proinflammatory cytokines in the spinal cord, are common mechanisms underlying pathological pain in a number of pain syndromes with widely different etiologies, such as peripheral nerve injury, spinal inflammation and bone cancer neuropathy (40,41,45,51).

In the present study, tumor cell implantation caused an increase in microglial IBA1 and astrocyte GFAP markers, and neurotransmitters c-Fos and CGRP; indicating a central sensitization effect. XZP treatment significantly mitigated IBA1, GFAP, c-Fos and CGRP expressions in the spinal cord; and treatment effects appear to be dose-dependent. Our data further supported previous findings; wherein, targeting
the PAR2 signaling pathway inhibits cancer-induced bone pain (34,52,53).

Proteolytic activity is critical to carcinogenesis and cancer microenvironment is associated with various proteases. Cancer-associated serine proteases, such as trypsin, are released during the early stages of tissue invasion and may directly activate PAR2 on nociceptive afferents; resulting in acute pain that is spontaneous and exacerbated during function. PAR2 activation may also sensitize other nociceptive receptors such as PKA, PKC-γ and TRPV1 (26-33,54,55).
With continued cancer cell proliferation and mediator release, nociceptive afferents may be persistently activated or sensitized and consequently maintain a persistent pain state. It has been well demonstrated that PAR2 is upregulated following various inflammatory and ischemic insults (56,57). Mediators, such as trypsin, TNF-α and IL-1β upregulated PAR2 (58). PAR2 upregulation in dorsal root ganglia has been associated with thermal hyperalgesia following chronic pancreatitis (59) and cAMP-dependent neuronal hyperexcitability following chronic nerve compression (60). Additionally, cancer-induced PAR2 upregulation in trigeminal ganglia similarly alters pain processing and mediate the progression to chronic cancer pain (34). The complete absence of cancer-induced functional allodynia in mice lacking PAR2 clearly demonstrates the critical involvement of PAR2 in acute and chronic cancer pain (34).

In the present study, we found that XZP treatment significantly mitigated trypsin, TNF-α and IL-1β serum levels in a dose-dependent manner. Furthermore, relative downstream levels of PAR2 signaling pathway mediators including PAR2, PKC-γ, PKA and TRPV1 in bone lesions were significantly reduced after XZP treatment. PAR2, proteinase-activated receptor 2.

Acknowledgements
This study was partially supported by the National Natural Science Foundation of China (grant nos. 81302961, 81273718 and 81202931).

References


