Analgesic effects of minodronate in a rat chronic pain model

Yuji KASUKAWA, Naohisa MIYAKOSHI, Masazumi SUZUKI, Hiroyuki TSUCHIE, Chie SATO, Tetsuya KAWANO, Manabu AKAGAWA, Yuichi ONO, and Yoichi SHIMADA
Department of Orthopedic Surgery, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan
(Received 20 July 2018; and accepted 14 August 2018)

ABSTRACT
We evaluated the analgesic effects of minodronate, alendronate and pregabalin on mechanical and thermal allodynia, as well as changes in bone mineral density and skeletal muscle volume caused by chronic constriction injury (CCI) in an ovariectomized rat. Ovariectomy was performed on four-week-old female Wistar rats. Thereafter, at 8-weeks of age, the left sciatic nerve was ligated to create the chronic pain model (CCI limb), and sham surgery was performed on the right hindlimb. In all rats, either minodronate (0.15 mg/kg/week), alendronate (0.15 mg/kg/week), pregabalin (10 mg/kg/week), or their vehicle was administered for 2 weeks starting on the 0th day of CCI. Behavioral evaluations, with von Frey testing and the hot plate test, were performed on days 0, 7 and 14. After 2 weeks, bilateral femurs and tibialis anterior muscles were harvested for bone mineral density and cross sectional area measurements, respectively. Two weeks treatment with minodronate significantly improved mechanical and thermal allodynia evaluated by the von Frey and hot plate tests in the CCI limb ($P < 0.05$). Minodronate and alendronate treatment for 2 weeks significantly increased total femoral bone mineral density in the CCI limb compared with pregabalin or vehicle treatment ($P < 0.01$). Cross sectional area of the CCI limb in the minodronate group was significantly larger than that of the alendronate group ($P < 0.05$) and pregabalin group ($P < 0.05$). Two-week treatment with minodronate, but not alendronate or pregabalin, improved mechanical and thermal allodynia caused by CCI in ovariectomized rats.

Osteoporosis is characterized by low bone mineral density and impairment of bone quality, resulting in bone fragility and fractures. Elderly people with osteoporosis who develop fragility-related vertebral fractures present with acute back pain. Osteoporotic patients sometimes also complain of chronic back pain, even in the absence of fragility fractures (21). In addition to the chronic osteoporotic pain, some patients complain of severe pain after fragility fractures or during treatment for the fractures. It has been considered to be complex regional pain syndrome (CRPS) type 1, which shows sensory changes including pain, allodynia, and hyperalgesia (24). Osteoporotic chronic pain interferes with the patients’ activities, which further increases their risk of fragility fractures. Thus, decrease in chronic pain is an important goal in the treatment of osteoporosis, in order to prevent fragility fractures.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of pain in osteoporotic patients. However, there are several concerns related to the side effects of long-term use of NSAIDs, particularly in aged patients (23). Bisphosphonates, which are analogs of endogenous pyrophosphate and inhibitors of bone resorption, are the most commonly used drugs for treating osteoporosis. Bisphosphonates are used to prevent osteoporotic fractures by improving bone fragility through increases in bone mineral density (11, 12). Reported-
ly, bisphosphonates have analgesic effects in addition to their actions on bone resorption and bone mineral density. Furthermore, they are recommended to be a first-choice medicine for pain management of CRPS by a recent review (33). We previously reported that minodronate, which is a third-generation bisphosphonate, demonstrated anti-nociceptive effects in a rat formalin-injection acute pain model (25). Several studies have revealed that bisphosphonates exert analgesic effects on chronic pain via inhibition of bone resorption in patients with osteoporosis (1, 29). However, the effect of minodronate on chronic pain has not been elucidated as yet.

In the present study, we examined the effects of minodronate on chronic pain in a rat model of the sciatic nerve as the chronic pain model (2). Several previous studies have demonstrated that ovariectomy causes an osteoporosis and hypersensitivity to mechanical or thermal pain (1, 19). CCI model rats have shown hypersensitivity to nociceptive pain, decreased bone mineral density, and muscle atrophy (15, 28). We speculated that the decrease in bone mineral density and muscle volume might be related to other causes of chronic pain in CCI model rats. Based on this background, the purpose of this study was to evaluate the effects of minodronate on chronic pain, bone mineral density, and skeletal muscle volume in a rat post-menopausal osteoporosis and chronic pain model, which was created using an ovariectomy and CCI model, by comparing the effects of minodronate with those of alendronate, a second-generation bisphosphonate and pregabalin, a drug with anti-nociceptive effects on the neuropathy of herpes and peripheral nerve injury (30).

MATERIALS AND METHODS

Animals. Four-week-old female Wistar rats (Japan SLC, Shizuoka, Japan) were housed in a controlled environment (temperature 23 ± 2°C, humidity 40 ± 20%) with a 12-h light/dark cycle. Rats were allowed ad libitum access to tap water and standard food (CE-2; Clea Japan, Inc., Tokyo, Japan) containing 1.14% calcium, 1.06% phosphorus, and 250 IU vitamin D3 per 100 g.

Experimental design. General anesthesia was induced by intraperitoneal injection of xylazine hydrochloride (Sederac; Nippon Zenyaku Kogyo Co. Ltd., Fukushima, Japan) and ketamine hydrochloride (Ketal; Daiichi Sankyo Propharma, Tokyo, Japan). Rats were ovariectomized under general anesthesia at the age of 4 weeks, as a model of estrogen deficiency. Four weeks after ovariectomy, a CCI model of chronic pain was created by ligating the sciatic nerve of the left hindlimb with a 4-0 silk suture (3). Sham surgery, consisting of only exposure of the sciatic nerve without ligation, was performed on the right hindlimb, to serve as the control limb of the chronic pain model.

Experimental protocol. After these procedures, rats were divided into the following four groups: 1) ALN group (n = 10), that was given weekly 0.15 mg/kg of alendronate (Wako Chemical, Osaka, Japan) subcutaneously; 2) MIN group (n = 10), that received weekly 0.15 mg/kg of minodronate (Astellas Pharma Inc., Tokyo, Japan) subcutaneously; 3) Preg group (n = 9), that was given weekly 10 mg/kg of pregabalin (Sigma-Aldrich Japan K.K., Tokyo, Japan) subcutaneously; and 4) Vehicle group (n = 10), in which 0.2 mL of saline solution was weekly administered subcutaneously. These treatments were continued for 1 or 2 weeks, and the following evaluations were performed on the day of CCI (0 week), 1 and 2 weeks after the CCI. Behavioral evaluations were performed 6 h after CCI under general anesthesia at the day of CCI (0 week). After evaluations for 2 weeks, the rats were sacrificed and bilateral femurs and tibialis anterior muscles were harvested for assessment of bone mineral density and muscle cross-sectional area, respectively. All animal experiments were approved by the “Guidelines for Animal Experiments” of our institute (IACUC number: a-1-2513).

Behavioral analyses. To evaluate the responses to nociceptive stimulation, we performed the von Frey test and hot plate test, as shown below, on the day of CCI (0 week), and at 1 and 2 weeks after CCI.

To assess their sensitivity to a tactile stimulus, as a measure of mechanical allodynia, rat hindlimb withdrawal in response to a tactile stimulus was measured using von Frey filaments (Aesthesio, Dan-Mic Global LLC, CA) with a 0.16 g bending force. We performed and evaluated the tests as previously reported (20). Briefly, the filament was applied to the plantar surface of the hindlimb for 3 s and the response of the hindlimb to the stimulus was recorded. Each of the hindlimbs was tested individually. Hindlimb withdrawal in response to the stimulus was evaluated using the following score: 0: no response, 1: slow and/or slight response to the stimulus, 2: quick withdrawal response away from the
stimulus without flinching or licking, and 3: an intense response to the stimulus with brisk flinching and/or licking. The right and left hindlimbs were evaluated alternatively with an interval of more than 3 min between consecutive measurements. The test was carried out three times on each limb and the mean was calculated as the pain score.

To quantify the sensitivity to thermal stimulation, as a measure of thermal alldynia, each of the hindlimbs of the rats was tested individually using the hot plate technique (16). Briefly, the rat under observation was placed on a smooth metal surface at 52°C. Hindlimb withdrawal latency, which was the time until the rats withdrew their hindlimb from the plate, was measured. The examination was carried out three times and the mean was calculated as hindlimb withdrawal latency.

Bone mineral density measurement. Bone mineral density of the entire excised femur was measured using dual-energy X-ray absorptiometry (DXA, Hologic QDR-4500; Hologic, MA) in the anterior plane. Bones were scanned in the “small animal” scan mode, with the “regional high-resolution” scan option. The total femoral bone mineral density was measured.

Histological analysis of muscle to assess their cross sectional area. Bilateral tibialis anterior muscles were used for histological analysis. Muscles were rapidly frozen in liquid nitrogen, then stored at −80°C until analysis. Samples were then cut into 10-μm thick serial transverse sections at the thickest part of the muscle belly, with the cryostat maintained at −18°C. Sections were stained using a histochemical method with hematoxylin and eosin (H&E). To measure the cross sectional area of muscle fibers, microscopic images with a magnification of ×200 were captured digitally (BX-50; Olympus, Tokyo, Japan), and individual muscle fibers were traced on-screen using Image J image analysis software (National Institutes of Health, Bethesda, MD). The area of the traced fibers was calculated using the Image J software, based on a calibrated pixel-to-actual size (μm) ratio. Five fields were randomly chosen, and 50 fibers per muscle were measured (10, 17).

Intra-observer variations, as assessed by the coefficient of variation for five corresponding measurements in 10 randomly selected fibers, ranged from 0.5% to 3.3%. Inter-observer variations between three investigators, as assessed by the coefficient of variation of measurements in 10 randomly selected images, ranged from 1.5% to 6.8%.

Statistical analyses. All numerical values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). Statistical differences between groups and between time periods in each group were compared using Scheffe’s or Dunn’s method. Differences between sham and CCI limbs were analyzed using the paired t-test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Behavioral analyses (Tables 1 and 2)
The pain scores of the CCI limb, as an indication of mechanical alldynia, at 0 weeks were significantly higher than those of the sham limb in all except the pregabalin (Preg) group, and in all groups at one week. The pain score of the CCI limb was significantly decreased only in the minodronate (MIN) group at two weeks ($P < 0.05$) (Table 1). MIN improved the mechanical alldynia caused by the CCI in ovariectomized rats.

In all groups, hindlimb withdrawal latencies in the CCI limbs were significantly longer than those in the sham limb at each time point ($P < 0.05$). In addition, withdrawal latency was significantly decreased in a time dependent manner in the sham limbs of the alendronate (ALN) group, MIN group, and Vehicle group ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively) (Table 2).

In the CCI limb, on the other hand, there was a significant time dependent decrease in hindlimb withdrawal latency only in the MIN group ($P < 0.01$). Only the hindlimb withdrawal latency at 1 week in the MIN group was significantly shorter than its corresponding value at 0 weeks ($P < 0.05$) (Table 2). We speculate that minodronate also improved the thermal alldynia caused by the CCI in ovariectomized rats.

Bone mineral density (Fig. 1)
Total femoral bone mineral density in the ALN ($P < 0.01$ and $P < 0.01$, respectively) and MIN ($P < 0.01$ and $P < 0.01$, respectively) groups were significantly higher than those in the Preg and Vehicle groups for both sham (Fig. 1A) and CCI limbs (Fig. 1B). Both minodronate and alendronate improved the bone mineral density decreased by CCI in the postmenopausal osteoporosis model rats.

Histological sections of tibialis anterior muscle stained with H&E (Fig. 2)
Cross sectional areas in the sham limbs of the Vehi-
Cross sectional area of the tibialis anterior muscle (Fig. 3)

Cross sectional area of the tibialis anterior muscle of the sham limb was not significantly different between the four groups (Fig. 3A). Cross sectional ar-

Table 1  Pain score with von Frey testing

|          | 0 w     | 1 w     | 2 w     | ANOVA |
|----------|---------|---------|---------|-------|
| Vehicle  | sham    | 0.3 ± 0.4 | 0.2 ± 0.4 | 0.3 ± 0.7 | 0.452 |
|          | CCI     | 0.8 ± 0.4 \(^a\) | 0.6 ± 0.5 \(^b\) | 0.6 ± 0.6 | 0.535 |
| ALN      | sham    | 0.3 ± 0.4 | 0.3 ± 0.5 | 0.1 ± 0.2 | 0.545 |
|          | CCI     | 0.7 ± 0.6 \(^b\) | 0.9 ± 0.6 \(^a\) | 0.4 ± 0.6 | 0.278 |
| MIN      | sham    | 0.2 ± 0.2 | 0.3 ± 0.4 | 0.1 ± 0.2 | 0.127 |
|          | CCI     | 1.1 ± 0.6 \(^b\) | 1.1 ± 0.8 \(^a\) | 0.5 ± 0.6 \(^*\) | 0.048 |
| Preg     | sham    | 0.6 ± 0.7 | 0.5 ± 0.4 | 0.6 ± 0.6 | 0.782 |
|          | CCI     | 0.8 ± 0.6 | 0.9 ± 0.6 \(^b\) | 0.7 ± 0.6 | 0.847 |

Values are expressed as mean ± SD. \(^a\): \(P < 0.05\) vs. CCI of MIN at 1 week by Dunn’s method. \(\text{ANOVA}\): analysis of variance. SD: standard deviation, ALN: alendronate, MIN: minodronate, Preg: pregabalin, CCI: chronic constriction injury

Table 2  Hindlimb withdrawal latency (seconds) in the hot plate test

|          | 0 w     | 1 w     | 2 w     | ANOVA |
|----------|---------|---------|---------|-------|
| Vehicle  | Sham    | 29.1 ± 7.5 | 13.8 ± 2.8 \(^*\) | 11.5 ± 2.2 \(^**\) | < 0.001 |
|          | CCI     | 87.5 ± 14.5 \(^b\) | 79.9 ± 22.1 \(^b\) | 74.5 ± 20.9 \(^b\) | 0.057 |
| ALN      | sham    | 31.6 ± 11.2 | 23.5 ± 5.1 | 18.7 ± 11.9 \(^**\) | 0.015 |
|          | CCI     | 77.3 ± 35.8 \(^b\) | 65.7 ± 45.1 \(^a\) | 68.6 ± 33.8 \(^b\) | 0.543 |
| MIN      | sham    | 37.7 ± 10.1 | 23.8 ± 4.3 \(^*\) | 23.7 ± 7.6 \(^*\) | 0.001 |
|          | CCI     | 74.4 ± 46.7 \(^a\) | 41.0 ± 19.7 \(^*\) | 48.6 ± 32.7 | 0.004 |
| Preg     | sham    | 31.9 ± 19.5 | 27.1 ± 13.9 | 20.7 ± 9.9 | 0.162 |
|          | CCI     | 69.0 ± 27.8 \(^b\) | 75.8 ± 20.3 \(^a\) | 71.1 ± 23.0 \(^b\) | 0.752 |

Values are expressed as mean ± SD. \(^a\): \(P < 0.05\), and \(^*\): \(P < 0.01\) vs. 0 week by Dunn’s method. \(\text{ANOVA}\): analysis of variance. SD: standard deviation, ALN: alendronate, MIN: minodronate, Preg: pregabalin, CCI: chronic constriction injury

**Fig. 1** Bone mineral density (g/cm\(^3\)). Values are expressed as mean ± SD. Total femoral bone mineral density in the sham limb (A) and the CCI limb (B). \(^\ast\): \(P < 0.01\) vs. Preg group, \(^\ast\ast\): \(P < 0.01\) vs. Vehicle group by Scheffe’s method. SD: standard deviation, ALN: alendronate, MIN: minodronate, Preg: pregabalin, CCI: chronic constriction injury

**Fig. 2** Cross sectional area of the tibialis anterior muscle (Fig. 3)

Cross sectional area of the tibialis anterior muscle (Fig. 3)

Cross sectional area of the tibialis anterior muscle of the sham limb was not significantly different between the four groups (Fig. 3A). Cross sectional ar-

**Fig. 3** Cross sectional area of the tibialis anterior muscle (Fig. 3)

Cross sectional area of the tibialis anterior muscle of the sham limb was not significantly different between the four groups (Fig. 3A). Cross sectional ar-
Minodronate effects on chronic pain

Minodronate is a potent antagonist of purinergic P2X2/3 receptors, with an inhibition constant (IC50) of 62.7 μM (4). Furthermore, it has been reported that minodronate inhibits the P2X2/3 receptors around osteoclasts resulting in its analgesic effects (31). It has been suggested that TRPV1 and ASICs are also targets of amino-containing bisphosphonates, which could be other mechanisms of the analgesic effects of minodronate (18). Recently, it was reported that minodronate prevents the tibialis anterior muscle atrophy compared with the alendronate or pregabalin, however the cross sectional area ratio of CCI and sham limb was not statistically different among the four groups. Thus, preventive effects of minodronate on muscle atrophy need a further investigation.

DISCUSSION

Chronic low back pain is sometimes a complaint in postmenopausal osteoporosis patients without vertebral fractures (21). Several causative factors for this pain have been reported. Estrogen deficiency causes an increase in nociceptive sensitivity (14). A high bone turnover, such as with postmenopausal osteoporosis, might augment skeletal pain via activation of P2X2/3 receptors due to increased extracellular ATP levels on the bone surface (31). Contributions of transient receptor potential V1 (TRPV1) and acid-sensing ion channel (ASIC) signals in bone pain have been shown in several studies (5, 6), while physical association and crosstalk of TRPV1 and P2X3 receptors reportedly occur in nociceptive neurons (27). In addition, an acidic extracellular pH enhances the activity of ATP via P2X receptors (9, 13), and facilitates ATP-mediated mechanical allodynia (26). These data indicate that activation of P2X2/3 receptors in sensory neurons might augment skeletal pain via TRPV1 and ASIC signals in postmenopausal osteoporotic model rats.

Fig. 2  Histological sections of the tibialis anterior muscle. Histological sections of the tibialis anterior muscle stained with hematoxylin and eosin (H&E). Sections were obtained from the sham limb of the Vehicle (A), alendronate (ALN) (B), minodronate (MIN) (C), and pregabalin (Preg) (D) groups, and the chronic constriction injury (CCI) limb of the Vehicle (E), ALN (F), MIN (G), and Preg (H). Bar: 100 μm.
have demonstrated that pregabalin (10 ~ 40 mg/kg) improves the mechanical allodynia in the von Frey filament test caused by CCI (8, 35). The difference between ours and previous reports could be because the weekly dose of pregabalin (10 mg/kg) in the present study may not have been enough to improve mechanical allodynia in our ovariectomized and CCI model. Lack of significant effect of pregabalin on the pain scores evaluated by the von Frey test in the ovariectomized and CCI model rats in this study might also be explained by the fact that the analgesic effects of pregabalin appear to be weaker and shorter acting than that of clodronate (7). Further investigations are needed to confirm the effects of pregabalin on mechanical allodynia in ovariectomized and CCI model rats.

In the present study, both minodronate and alendronate resulted in similar increases in femoral bone mineral density in the CCI limb. The ratio of cross sectional area of the tibialis anterior muscle between CCI and sham limbs (C). *: P < 0.01 vs. the sham limb in each group by the paired t-test. **: P < 0.05 vs. ALN group, and ***: P < 0.05 vs. Preg group by Scheffe’s method. SD: standard deviation, ALN: alendronate, MIN: minodronate, Preg: pregabalin, CCI: chronic constriction injury.

clodronate, a non-nitrogen-containing bisphosphonate, selectively inhibits vesicular nucleotide transporter (VNUT) in vivo; the study also revealed the analgesic effects on pathological neuropathic and inflammatory pain (7). In that study, minodronate, but not alendronate or risedronate, showed inhibitory effects on VNUT (7). Based on these findings, we speculated that minodronate exerts analgesic effects via antagonistic binding to the P2X2/3 receptor. In a clinical study, minodronate also demonstrated lowering of back pain in postmenopausal osteoporotic patients (22, 34).

On the other hand, in the current study, alendronate did not show any analgesic effects as evaluated in terms of pain scores by von Frey testing. There is no report of the antagonistic effect of alendronate on P2X2/3 receptors. Alendronate and risedronate also did not have antagonistic actions for VNUT in the previous report (7). These differences between minodronate and alendronate for the nociceptive pathway may relate to the significant analgesic effects of minodronate in the CCI limb in this study. Furthermore, pregabalin also did not result in significant pain reduction in this study. Several previous studies have demonstrated that pregabalin (10 ~ 40 mg/kg) improves the mechanical allodynia in the von Frey filament test caused by CCI (8, 35). The difference between ours and previous reports could be because the weekly dose of pregabalin (10 mg/kg) in the present study may not have been enough to improve mechanical allodynia in our ovariectomized and CCI model. Lack of significant effect of pregabalin on the pain scores evaluated by the von Frey test in the ovariectomized and CCI model rats in this study might also be explained by the fact that the analgesic effects of pregabalin appear to be weaker and shorter acting than that of clodronate (7). Further investigations are needed to confirm the effects of pregabalin on mechanical allodynia in ovariectomized and CCI model rats.
have resulted in improvement of activity and, consequently, higher femoral bone mineral density and cross sectional area of tibialis anterior muscle in the minodronate group as compared to the alendronate group. However, we observed no significant difference in femoral bone mineral density or the muscle cross sectional area between the two groups. This could have been due to the fact that the treatment period with bisphosphonates was only two weeks, which may not have been enough to exert significant effects. Several recent studies have reported that high doses of bisphosphonate increase muscle volume (32) and that first generation bisphosphonates exert significant nociceptive effects compared with second or third generation ones (7). Further investigations are, thus, needed to elucidate the different effects of each generation of bisphosphonates on bone mineral density, skeletal muscle volume, and nociceptive pain.

There are several limitations to the present study. First, the dose and duration of treatment with alendronate, minodronate, or pregabalain were single; the higher dose or longer duration of treatment with alendronate, minodronate, or pregabalain may produce different results. Second, pain was evaluated only by behavioral observation. Histopathological or immunohistochemical examinations of the spinal cord are required to elucidate the detailed effects of these agents on nociceptive pain.

In conclusion, in our study, 2-weeks treatment with minodronate improved mechanical and thermal allodynia caused by CCI in ovariectomized rats.

Acknowledgments

The authors would like to thank Astellas Pharma Inc. for providing minodronate and Ms. Matsuzawa for her support in performing the experiments.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Abe Y, Iba K, Sasaki K, Chiba H, Kanaya K, et al. (2015) Inhibitory effect of bisphosphonate on osteoclast function contributes to improved skeletal pain in ovariectomized mice. J Bone Miner Metab 33, 125–134.
2. Bnnet GJ and Xie YK (1988) A peripheral mononeuropathy in rat that reduces disorders of pain sensation like those seen in man. Pain 33, 87–107.
3. Challa SR (2016) Surgical animal models of neuropathic pain: Pros and Cons. J Pain 17, 889–903.
4. Kakimoto S, Nagakura Y, Tamura S, Watabiki T, Shibasaki K, et al. (2008) Minodronic acid, a third-generation bisphosphonate, antagonizes purinergic P2X2/3 receptor function and exerts an analgesic effect in pain models. Eur J Pharmacol 589, 98–101.
5. Kanaya K, Iba K, Abe Y, Dohke T, Okazaki S, et al. (2016) Acid-sensing ion channel 3 or P2X2/3 is involved in the pain-like behavior under a high bone turnover state in ovariectomized mice. J Orthop Res 34, 566–573.
6. Kanaya K, Iba K, Dohke T, Okazaki S and Yamashita T (2016) TRPV1, ASICs and P2X2/3 expressed in bone cells simultaneously regulate bone metabolic markers in ovariectomized mice. J Musculoskeletal Neuronal Interact 16, 145–151.
7. Kato Y, Hiasa M, Ichikawa R, Hasuzawa N, Kodawaki A, et al. (2017) Identification of a vesicular ATP release inhibitor for the treatment of neuropathic and inflammatory pain. Proc Natl Acad Sci USA 18, E6297–6305.
8. Kaulaskar S, Bhutada P, Rahigude A, Jain D and Harle U (2012) Effects of naringenin on allodynia and hyperalgesia in rats with chronic constriction injury-induced neuropathic pain. Zhong Xi Yi Jie He Xue Bao 10, 1482–1489.
9. King BF, Wildman SS, Ziganshina LE, Pintor J and Burnstock G (1997) Effects of extracellular pH on agonism and antagonism at a recombinant P2X2 receptor. Br J Pharmacol 121, 1445–1453.
10. Kinoshita H, Miyakoshi N, Kasukawa Y, Sakai S, Shiraiishi A, et al. (2016) Effects of edecacitol on bone and skeletal muscles in glucocorticoid-treated rats. J Bone Miner Metab 34, 171–178.
11. Kishimoto H, Fukunaga M, Kushida K, Shiraki M, Itabashi A, et al. (2006) Risedronate Phase III Research Group. Efficacy and tolerability of once-weekly administration of 17.5 mg risedronate in Japanese patients with involutional osteoporosis: a comparison with 2.5-mg once-daily dosage regimen. J Bone Miner Metab 24, 405–413.
12. Kushida K, Shiraki M and Nakamura T (2004) Alendronate reduced vertebral fracture risk in postmenopausal Japanese women with osteoporosis: 3-year follow-up study. J Bone Miner Metab 22, 462–468.
13. Li C, Peoples RW and Weight FF (1996) Acid pH augments excitatory action of ATP on a dissociated mammalian sensory neuron. Neuroreport 7, 2151–2154.
14. Li LH, Wang ZC, Yu J and Zhang YQ (2014) Ovariectomy results in variable changes in nociception, mood, and depression in adult female rats. PLOS One 9, e94312.
15. Liao P, Zhou J, Ji LL and Zhang Y (2010) Eccentric contraction induces inflammatory responses in rat skeletal muscle: role of tumor necrosis factor-alpha. Am J Physiol Regul Integr Comp Physiol 298, 599–607.
16. Macdonald AD, Woolfe G, Bergel F, Morrison AL and Rinderknecht H (1946) Analogic action of pethidine derivatives and related compounds. British J Pharmacol 1, 4–14.
17. Miyakoshi N, Sasaki H, Kasukawa Y, Kamo K and Shimada Y (2010) Effects of a vitamin D analog, alfalcacidol, on bone and skeletal muscle in glucocorticoid-treated rats. Biomed Res (Tokyo) 31, 329–336.
18. Nagae M, Hiraga T, Wakabayashi H, Wang L, Iwata K and Yoneda T (2006) Osteoclasts play a part in pain die to the inflammation adjacent to bone. Bone 39, 1107–1115.
19. Naito Y, Wakabayashi H, Kato S, Nakagawa T, Iino T and Sudo A (2017) Alendronate inhibits hyperalgesia and suppresses neuropeptide markers of pain in a mouse model of osteoporosis. J Orthop Sci 22, 771–777.
20. Narita M, Usui A, Narita M, Niikura K, Nozaki H, et al.
(2005) Protease-activated receptor-1 and platelet-derived growth factor in spinal cord neurons are implicated in neuropathic pain after nerve injury. J Neurosci 25, 10000–10009.
21. Ohtori S, Akazawa T, Murata Y, Kinoshita T, Yamashita M, et al. (2010) Risedronate decreases bone resorption and improves low back pain in postmenopausal osteoporosis patients without vertebral fractures. J Clin Neurosci 17, 209–213.
22. Sakai A, Ikeda S, Okimoto N, Matsumoto H, Teshima K, et al. (2014) Clinical efficacy and treatment persistence of monthly minodronate for osteoporotic patients unsatisfied with, and shifted from, daily or weekly bisphosphonates: the BP-MUSASHI study. Osteoporos Int 25, 2245–2253.
23. Scheiman JM (2016) NSAID-induced gastrointestinal injury: A focused update for clinicians. J Clin Gastroenterol 50, 5–10.
24. Schwartzman RJ, Erwin KL and Alexander GM (2009) The natural history of complex regional pain syndrome. Clin J Pain 25, 273–280.
25. Segawa T, Miyakoshi N, Kasukawa Y, Aonuma H, Tsuchie H and Shimada Y (2013) Analgesic effects of minodronate on formalin-induced acute inflammatory pain in rats. Biomed Res (Tokyo) 34, 137–141.
26. Seo HS, Roh DH, Kwon SG, Yoon SY, Kang SY, et al. (2011) Acidic pH facilitates peripheral αβmeATP-mediated nociception in rats: differential roles of P2X, P2Y, ASIC and TRPV1 receptors in ATP-induced mechanical allodynia and thermal hyperalgesia. Neuropharmacology 60, 580–586.
27. Stanchev D, Blosa M, Milius D, Gerevich Z, Rubini P, et al. (2009) Cross-inhibition between native and recombinant TRPV1 and P2X receptors. Pain 143, 26–36.
28. Suyama H, Moriwaki K, Niida S, Maehara Y, Kawamoto M and Yuge O (2002) Osteoporosis following chronic constriction injury of sciatic nerve in rats. J Bone Miner Metab 20, 91–97.
29. Suzuki M, Millecamps M, Naso L, Ohtori S, Mori C and Stone LS (2017) Chronic osteoporotic pain in mice: Cutaneous and deep musculoskeletal pain are partially independent of bone resorption and differentially sensitive to pharmacological interventions. J Osteoporos. doi:10.1155/2017/7582716. Epub Feb 19.
30. Tan T, Barry P, Reken S and Baker M (2010) Pharmacological management of neuropathic pain in non-specialist settings; summary of NICE guidance. BMJ 340:c1079.
31. Tanaka M, Hosoya A, Mori H, Kayasuga R, Nakamura H and Ozawa H (2018) Minodronic acid induces morphological changes in osteoclasts at bone resorption sites and reaches a level required for antagonism of purinergic P2X2/3 receptors. J Bone Miner Metab 36, 54–63.
32. Watanabe R, Fujita N, Takeda S, Sato Y, Kobayashi T, et al. (2016) Ibandronate concomitantly blocks immobilization-induced bone and muscle atrophy. Biochem Biophys Res Commun 480, 662–668.
33. Wertli MM, Kessels AG, Perez RS, Bachmann LM and Brunner F (2014) Rational pain management in complex regional pain syndrome 1 (CRPS 1). A network meta-analysis. Pain Med 15, 1575–1589.
34. Yoshioka T, Okimoto N, Okamoto K and Sakai A (2013) A comparative study of the effects of daily minodronate and weekly alendronate on upper gastrointestinal symptoms, bone resorption, and back pain in postmenopausal osteoporosis patients. J Bone Miner Metab 31, 153–160.
35. Zhang MT, Wang B, Jia YN, Liu N, Ma PS, et al. (2017) Neuroprotective effect of liquiritin against neuropathic pain induced by chronic constriction injury of the sciatic nerve in mice. Biomed Pharmacother 95, 186–198.