Analgesic effects of minodronate on formalin-induced acute inflammatory pain in rats

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ABSTRACT

Minodronate is expected to produce greater analgesic effects than other bisphosphonates. However, there are no studies comparing bisphosphonate analgesic effects on formalin-induced acute inflammatory pain in rats. The purpose of the present study was to evaluate the analgesic effects of minodronate, morphine, and placebo. Four-month-old female Wistar rats were administered minodronate (50 mg/kg), morphine (10 mg/kg), or vehicle (n = 10 each) injections. Thirty minutes later, all rats were injected with formalin (right hind paw) to induce acute inflammatory pain. Paw licking and lifting as indicators of nociceptive pain responses were monitored from 0 to 5 min (phase 1; chemical-stimulation state) and then from 10 to 30 min (phase 2; spinal-sensitized state) after injection. The percentage of limb usage of the formalin-injected and the non-injected sides were measured in phases 1 and 2 by counting foot stamps. Minodronate significantly decreased nociceptive responses and increased limb usage compared with vehicle in phase 2 only (P < 0.05). Morphine significantly decreased nociceptive responses and increased limb usage compared with minodronate and vehicle in both phase 1 and 2 (P < 0.05). In conclusion, minodronate showed significant analgesic effects for formalin-induced acute pain in the spinal-sensitized state.

Osteoporosis is a systemic disease characterized by low bone mass and micro-architectural deterioration of bone structure resulting in bone fragility. Bone pain is one of the most common complications in patients with osteoporosis. This is a frustrating complication that, along with other morbidities such as vertebral fracture, can substantially reduce quality of life (6). Bisphosphonates are analogs of endogenous pyrophosphate, inhibit bone resorption, and are used to prevent osteoporotic fractures by improving bone fragility through increases in bone mineral density (BMD) (3, 8, 14). In addition to successful application in the treatment of osteoporosis, bisphosphonates are also able to reduce bone pain. Recent reports have shown that bisphosphonates exert analgesic effects on bone pain by inhibiting bone resorption in patients with osteoporosis (1, 2, 9, 10, 13, 15, 16).

Reports have shown that among bisphosphonates, only minodronate (a third-generation bisphosphonate) has antagonistic effects on the P2X2/3 receptor (12), and is thereby expected to show greater analgesic effects than the other bisphosphonates. This receptor is specifically expressed on nociceptive sensory neurons and is considered to be involved in various pain states such as inflammatory, neuropathic, and cancer pain (4, 5). Previous reports have shown that minodronate decreases the duration of formalin-induced nociceptive behavior in mice in a dose-dependent manner (12). Acute pain can be divided into an initial acute phase (phase 1) and a prolonged tonic phase (phase 2). Morphine has been shown to completely control formalin-induced acute inflammatory pain in mice in both phases (11). However, there are no studies evaluating the analgesic effects of minodronate specifically in phases 1 and 2.
To the best of our knowledge, this is the first study to analyze the analgesic effects of minodronate compared with morphine as a positive control for analgesia against formalin-induced acute inflammatory pain in rats during each phase.

MATERIALS AND METHODS

Animals. Four-month-old female Wistar rats (Clea Japan, Tokyo, Japan) were housed in a controlled environment at 22°C with a 12-h light/dark cycle. Rats were pair-fed and allowed ad libitum access to water and standard food (CE-2; Clea Japan) containing 1.14% calcium, 1.06% phosphorus, and 250 IU vitamin D$_3$ per 100 g of food, as described previously (21, 22).

Experimental design. Rats were divided into the following three groups (n = 10, each) all injected subcutaneously: 1) control group: vehicle; 2) minodronate group: 50 mg/kg of minodronate (Astellas Pharma, Tokyo, Japan) dissolved in saline solution at a concentration of 5 mg/mL; or 3) morphine group: 10 μL of 5.0% morphine. Thirty minutes after each treatment, the rats were injected with 20 μL of 2.0% formalin (Wako Chemical, Osaka, Japan) into the dorsal surface of the right hind paw. The dose of formalin and injection followed a previously reported method (23). All animal experiments conformed to the Guidelines for Animal Experimentation at Akita University School of Medicine and were therefore performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

Behavioral analysis. The number of nociceptive responses, comprising leg-raising and leg-licking behaviors, was measured during the 30-min period starting just after the injection of formalin. The measurement period was divided as described previously (11, 24, 26). Briefly, 0 to 5 min after formalin injection was defined as the initial acute phase evoked by chemical stimulation of the peripheral nerve (phase 1), and 10 to 30 min after injection was defined as the prolonged tonic phase mediated by the spinal-sensitized state induced by acute inflammation (phase 2). The total number of nociceptive responses occurring in each phase was recorded.

The foot stamp test was conducted following a previously reported method (18). In this study, both red and black ink-soaked sponges (5 mm × 5 mm) were glued to each heel of the animals’ hind limbs. The red ink-soaked sponge was on the formalin-injected side and the black ink-soaked sponge was on the non-injected side. Rats were allowed to ambulate on white paper in their cages for 2 min in phase 1 (2 to 4 min) and 2 min in phase 2 (28 to 30 min). Limb use was evaluated by counting foot stamps as red or black marks on the paper. Data are expressed as percent use of the formalin-injected-side = (number of red stamps / number of black stamps) × 100.

Statistical analysis. All values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using a one-way analysis of variance (ANOVA). Statistical differences among groups and between time periods in each group were compared using Scheffe’s method or the Kruskal-Wallis rank-sum test for multiple comparisons. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Nociceptive responses

The minodronate group showed significantly lower nociceptive responses than the vehicle group in phase 2 (20–30 min after formalin injection) (Fig. 1). The morphine group showed significantly lower nociceptive responses than the other two groups in both phases 1 and phase 2.

Foot stamp test

Typical results of the foot stamp test in phase 2 are shown in Figure 2. In rats treated with vehicle, the number of red stamps (formalin-injected side) was lower than the number of black stamps (non-injected side) (Fig. 2a). Rats treated with minodronate showed similar numbers of red and black stamps (Fig. 2b). The minodronate group had a significantly higher percentage limb usage than the vehicle group in phase 2. The morphine group had a significantly higher percentage limb usage than the other groups in both phases 1 and 2 (Fig. 3).

DISCUSSION

Morphine is used for the treatment of moderate to severe pain and has been used as a positive control for analgesia against formalin-induced acute inflammatory pain in previous studies (11, 24–26). In the present study, morphine completely controlled formalin-induced acute inflammatory pain in both the initial acute phase (phase 1) and the prolonged tonic phase (phase 2). The strength of this study was in evaluating the analgesic effects of minodronate as
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Based on these findings, minodronate exerts milder analgesic effects than morphine in relation to acute inflammatory pain in the spinal-sensitized state (phase 2).

Recent reports have shown that bisphosphonates exert analgesic effects in patients with osteoporosis by inhibiting bone resorption (1, 2, 9, 13, 15, 16). Furthermore, the acidic microenvironment created by osteoclasts contributes to the induction of hyperalgesia in mammary rat metastasis tumor (MRMT-1)-inoculated rats through the up-regulated expression of acid-sensing ion channels (17). Bisphosphonates exert anti-osteoclastic and analgesic effects against MRMT-1-induced chronic bone pain (18). As a

compared with morphine as a positive control for analgesia against formalin-induced acute inflammatory pain in rats in phases 1 and 2.

In this study, significant analgesic effects of minodronate were seen in the tonic phase (phase 2) of formalin-induced acute inflammation, but not in the initial phase (phase 1) of acute pain. This result supports previous findings that minodronate decreases the duration of formalin-induced nociceptive behavior in mice (12). Minodronate shows antagonistic actions against the P2X2/3 receptor (12) which is specifically expressed on nociceptive sensory neurons and is considered to be involved in various pain states, such as inflammatory, neuropathic, and cancer pain. Based on these findings, minodronate exerts milder analgesic effects than morphine in relation to acute inflammatory pain in the spinal-sensitized state (phase 2).

Recent reports have shown that bisphosphonates exert analgesic effects in patients with osteoporosis by inhibiting bone resorption (1, 2, 9, 13, 15, 16). Furthermore, the acidic microenvironment created by osteoclasts contributes to the induction of hyperalgesia in mammary rat metastasis tumor (MRMT-1)-inoculated rats through the up-regulated expression of acid-sensing ion channels (17). Bisphosphonates exert anti-osteoclastic and analgesic effects against MRMT-1-induced chronic bone pain (18). As a
bisphosphonate, minodronate is also speculated to exert analgesic effects against chronic bone pain, including pain resulting from the acidic environment created by osteoclasts. Furthermore, minodronate may exhibit additional effects on acute inflammatory pain.

Among these additional effects, minodronate has been reported to antagonize the P2X2/3 receptor (12), which is a unique action not possessed by other bisphosphonates used in the clinical setting. A recent study also indicated that P2X2/3 receptors are involved in the pathophysiology of pain and inhibiting the function of these receptors is considered an attractive mechanism for analgesia (19). If minodronate exerts P2X2/3 receptor-antagonistic activity in addition to its known potent inhibition of bone resorption (20), it would have potential as a treatment for the symptoms of not only bone pain, but acute inflammatory pain after osteoporotic fractures as well.

A previous study showed that other bisphosphonates, including zoledronate and pamidronate, did not antagonize the P2X2/3 receptor (12). We speculated that the analgesic effects of minodronate against acute inflammatory pain are exerted via antagonism of the P2X2/3 receptor. However, the analgesic effects of other bisphosphonates compared with minodronate were not evaluated in the present study. This point represents one of the major limitations of this study.

In conclusion, minodronate showed significant analgesic effects against formalin-induced acute inflammatory pain in the tonic phase. Minodronate may exert analgesic effects in osteoporotic patients with acute pain resulting from osteoporotic fractures.

REFERENCES


