Alendronate Induces Antinociception in Mice, Not Related With Its Effects in Bone

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ABSTRACT—The antinociceptive effect of alendronate was studied. The bisphosphonate was i.p. administered and two tests were carried out: acetic acid in mice and formalin test in rats. In the acetic acid test, alendronate induced a dose-dependent antinociceptive effect that was statistically significant for the doses of 10, 20 and 40 mg/kg, and could be detected 48 hr after its administration. In the formalin test, however, alendronate, at the doses of 10 and 20 mg/kg, did not modify the pain score nor the number of flinches, when it was administered either 30 or 60 min before the test. However it must be noted that doses inducing analgesic effect are close to those inducing toxicity.

Keywords: Alendronate, Acetic acid test, Formalin test, Pain, Rodent

Bisphosphonates are analogues of inorganic pyrophosphate, a naturally occurring chemical in bone. They bind to hydroxyapatite crystals, making it more difficult for osteoclasts to recognize exposed unmineralized bone surfaces, thereby inhibiting osteoclast-mediated bone resorption (1).

The therapeutic utility of bisphosphonates has focused on mineralization disorders associated with Paget’s disease (2), multiple myeloma of bone (3) and osteoporosis (4).

Although the main reason for the use of bisphosphonates is their ability to increase bone density, several reports reveal that treatment with bisphosphonates is able to reduce the pain associated with different painful diseases (1, 5, 6).

Alendronate is an aminobisphosphonate, a 4-carbon compound with an amino-group side chain. In rats, alendronate has shown up to a 1000-fold greater potency than etidronate in the inhibition of bone resorption (7). The relative potencies are reported to be very similar in humans (7). It has been used in the treatment of Paget’s disease, hypercalcæmia of malignancy and osteoporosis (8), and it is interesting to note that using alendronate, the incidence of gastric erosions did not differ significantly from the placebo group (9).

Alendronate has also been used in the treatment of painful diseases. It has been demonstrated that alendronate, administered intravenously or given orally, is able to reduce bone pain in patients with bone metastases due to prostate carcinoma (5). Alendronate, given intravenously, induced a reduction in the spontaneous pain associated with reflex sympathetic dystrophy syndrome (6). However, no work has been carried out to evaluate the ability of bisphosphonates to reduce pain in pathologies not related to bone diseases. It is well known that other drugs used for the treatment of bone diseases, as calcitonin, have shown an analgesic effect in osteoarticular disorders (10), cancer pain (11) and other pathologies such as phantom limb (12), as well as in experimental pain (13).

In this work, we analyzed if alendronate could induce antinociception not related to its effect on bone. For this purpose, we studied the effect of alendronate in two different tests, the abdominal constriction test, where the administration of acetic acid to mice induces visceral pain, and the formalin test, where the ability of alendronate to reduce pain associated with inflammatory processes can be tested.

MATERIALS AND METHODS

Experiments have been carried out following the Guiding Principles for the Care and Use of Laboratory Animals, approved by The Japanese Pharmacological Society, as well as the Recommendations from the Declaration of Helsinki.
**Abdominal constriction test**

This test was carried out using male CD-1 mice weighing 25–30 g. A modification (13) of the abdominal constriction test described by Hayashi and Takemori was used (14). Mice were injected intraperitoneally (i.p.) with acetic acid solution (2%, 0.3 ml/mouse) to produce the typical reaction, which is characterized by a wave of contractions of the abdominal musculature followed by extension of the hind limbs. The mice were then placed in individual transparent containers and the number of contractions in a 10-min period were counted, starting 5 min after the acetic acid injection.

The doses of alendronate chosen were 10, 20, 40, 60 and 80 mg/kg, i.p. administered; and the test was carried out 30, 60 and 120 min after alendronate administration. In order to analyze the duration of the analgesia, the test was carried out 24, 48 and 96 hr after the administration of alendronate at 10, 20 or 40 mg/kg. The effect of the i.p. administration of morphine (2.5, 5 and 10 mg/kg) given 30 min before the test was evaluated in order to have a positive control of the antinociception.

**Formalin test**

Wistar male rats (180–200 g) were randomly assigned to two groups, saline- and alendronate-treated groups, comprising 12 rats per group. All rats received a single subcutaneous injection of 5 μl of formalin (2.5%) into the plantar surface of either the left or right hind paw. The formalin tail was carried out in a clear plastic chamber with a mirror placed under the floor to allow an unobstructed view of the paws. To habituate the rats to the test environment, they were placed in test chambers for 1 hr for 4 days prior to the test. Rats were observed during 45 min by an observer who was unaware of the treatment. During observations, the experimenter recorded the amount of time spent in each of 4 behavioral categories, listed with their category weights: 0 = normal weight bearing on the injected paw, 1 = limping during locomotion or resting the paw lightly on the floor, 2 = elevation of the injected paw so that at most the nails touch the floor, 3 = licking, biting or grooming the injected paw. The ‘flinching’ response (15) was counted simultaneously. An average pain intensity score was calculated, according to the weighted-scores technique of Dubuisson and Dennis (16). This score was calculated using a BASIC program, kindly furnished by Dr. K.B.J. Frankling.

The doses of alendronate used were 10 and 20 mg/kg, i.p. administered, and the bisphosphonate effect was tested 30 and 60 min after its administration.

As in the abdominal constriction test, the effect of morphine was tested to assess the antinociceptive sensitivity under our experimental conditions. For this, a group of mice was i.p. injected with morphine (10 mg/kg) 30 min before the test.

Alendronate was a gift from Merck Sharp & Dohme, Madrid, Spain. Morphine was obtained from Alcaliber, S.A., Madrid, Spain. All drugs were dissolved in saline solution.

**Data analyses**

The mean response±S.E.M. was calculated, and comparisons between experimental groups were made using the one or two-way ANOVA test followed by Sheffe's test as post-hoc comparison. Differences were considered statistically significant when P<0.05.

**RESULTS**

In the acetic acid test, saline administration did not affect the number of stretches induced by i.p. administration of acetic acid compared with the control values, and as expected, morphine (2.5, 5 and 10 mg/kg, i.p.) induced a dose-dependent antinociceptive effect (Fig. 1). The administration of alendronate at doses of 10–80 mg/kg, i.p. induced a dose-dependent antinociceptive effect, which was statistically significant at all the doses tested (Fig. 2A). Animals treated with the highest doses of alendronate (60 and 80 mg/kg, i.p.) showed abdominal inflammation, weight loss, tremor and motor impairment; these data were a direct observation of the experimenter, and it was quantified as only the presence or absence of disturbances. These signs were evident in mice treated with alendronate 60 mg/kg (in 16% of animals 24 hr after administration and 100% after 96 hr) and 80

![Fig. 1. Effect of i.p. morphine in the abdominal constriction test. Bars represent the mean number of stretches±S.E.M. induced by acetic acid administration in control animals (C) and in animals i.p. treated with saline solution (SS) or different doses of morphine (n≥12). Morphine-treated groups were compared with salinetreatment groups using the one-way ANOVA test followed by Sheffe's test; **P<0.01, ***P<0.001.](image-url)
mg/kg (75% after 24 hr, 100% after 96 hr). Due to this toxicity, the experiments to determine the duration of the antinociception were carried out only using the lowest doses (10, 20 and 40 mg/kg). It is interesting to note that a residual antinociceptive effect of alendronate could be detected 48 hr after its administration at the dose of 40 mg/kg, i.p. (Fig. 2B).

In the formalin test, morphine (10 mg/kg, i.p.) reduced

the pain score as well as the number of flinches (Fig. 3). The administration of alendronate (10 and 20 mg/kg, i.p.) slightly reduced the pain score compared with the control, although the difference did not reach statistical significance when it was analyzed using the one-way ANOVA test, either administered 30 or 60 min before the test (Figs. 3A and 4A). With regard to the number of flinches, another parameter used to evaluate nociception, the bisphosphonate was again unable to modify the shape of the curve compared with the control (Figs. 3B and 4B). The administration of alendronate at 40 mg/kg induced signs similar to those that appeared in mice, that is, abdominal inflammation, weight loss and motor impairment, in 25% of rats 24 hr after its administration. For this reason, this dose was not included in the experimental protocol.

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**Fig. 2.** Effect of i.p. alendronate in the abdominal constriction test. Bars represent the mean number of stretches ± S.E.M. induced by acetic acid administration in control animals (C) and in animals i.p. treated with saline solution (SS) or different doses of alendronate (n≥12). A: Bars represent the effect of saline solution or alendronate administered 30 (open bars), 60 (hatched bars) and 120 min (crosshatched bars) before the test. B: Bars represent the effect of saline solution or alendronate administered 24 (filled bars), 48 (vertical bars) and 96 hr (dotted bars) before the test. Alendronate-treated groups were compared with saline-solution-treated groups using the two-way (dose and time) ANOVA test followed by Sheffe's test; *P < 0.05, **P < 0.01.

**Fig. 3.** Effect of alendronate and morphine in the formalin test when i.p. administered 30 min before the test. A: Lines represent the mean pain score ± S.E.M. in control animals (circles) and in rats treated with alendronate at 10 mg/kg (crosses) or 20 mg/kg (triangles) and morphine at 10 mg/kg (squares) (n≥12). B: Lines represent the mean number of flinches ± S.E.M. in control animals (circles) and in rats treated with alendronate at 10 mg/kg (crosses) or 20 mg/kg (triangles) and morphine at 10 mg/kg (squares) (n≥12). Alendronate- and morphine-treated groups were compared with the saline-solution-treated group using the one-way ANOVA test followed by Sheffe’s test; **P < 0.01.
A

Pain score

2.5
2
1.5
1
0.5
0
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 40-45
Time (min)

B

No. of flinches

120
100
80
60
40
20
0
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 40-45
Time (min)

Fig. 4. Effect of alendronate in the formalin test when i.p. administered 60 min before the test. A: Lines represent the mean pain score ± S.E.M. in control animals (circles) and in rats treated with alendronate at 10 mg/kg (crosses) and 20 mg/kg (triangles) (n ≥ 12). B: Lines represent the mean number of flinches ± S.E.M. in control animals (circles) and in rats treated with alendronate 10 mg/kg (crosses) and 20 mg/kg (triangles) (n ≥ 12). Alendronate-treated groups were compared with the saline-solution-treated group using the one ANOVA test followed by Sheffe’s test; no significant differences were found.

DISCUSSION

Alendronate has been used in painful pathologies such as prostate carcinoma (5), where skeletal metastases induce pain in patients, or reflex sympathetic dystrophy syndrome (6), a disease associated with increased bone resorption and patchy osteoporosis. In those pathologies, the reduction in pain has been proposed to be related to its effect on bone. Nevertheless, no work has been carried out to determine if this analgesic effect also exists in painful situations not related to bone pathologies.

From our results, it could be concluded that alendronate is able to induce antinociception, independently of its effects on bone, but we found that the two tests gave different results. Whereas in the abdominal constriction test alendronate produced an analgesic effect that was dose- and time-dependent, in the formalin test, alendronate failed to induce significant antinociception at the tested doses. This difference could be attributed to the different nociceptive stimulus that is used in each test: in the abdominal constriction test, acetic acid administration induces a visceral pain (14), whereas the formalin test is a test more suitable for the study of inflammatory pain (15) and, on the other hand, the abdominal constriction test is one of the most sensitive tests for all types of antinociceptive drugs (14). From these data, it could be suggested that the ability of alendronate to produce analgesia should not be related with inflammatory processes.

At the doses required to reach an antinociceptive effect, several animals showed toxic signs such as abdominal inflammation weight loss, tremor or motor impairment, and some died. According to a Technical Summary furnished by Merck Sharp & Dohme, the LD₅₀ for alendronate administered intravenously (i.v.) varies from 85 mg/kg to 128 mg/kg in mice and from 35 mg/kg to 55 mg/kg in rats. Those doses, considering the difference in the route of administration, are close to the highest used in this work, and this agrees with the incidence of toxic signs that appeared during our experiments. It should be noted, however, that those doses are clearly higher than those recommended for humans.

It can be concluded that, although alendronate is able to induce antinociception, not related to bone pathology, this analgesic effect would not have a therapeutic application, since the doses needed are too close to the toxic ones.

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