The Bisphosphonates Clodronate and Etidronate Exert Analgesic Effects by Acting on Glutamate- and/or ATP-Related Pain Transmission Pathways

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Bisphosphonates (BPs) are typical anti-bone-resorptive drugs, with nitrogen-containing BPs (N-BPs) being stronger than non-nitrogen-containing BPs (non-N-BPs). However, N-BPs have inflammatory/necrotic effects, while the non-N-BPs clodronate and etidronate lack such side effects. Pharmacological studies have suggested that clodronate and etidronate can (i) prevent the side effects of N-BPs in mice via inhibition of the phosphate transporter families SLC20 and/or SLC34, through which N-BPs enter soft-tissue cells, and (ii) also inhibit the phosphate transporter family SLC17. Vesicular transporters for the pain transmitters glutamate and ATP belong to the SLC17 family. Here, we examined the hypothesis that clodronate and etidronate may enter neurons through SLC20/34, then inhibit SLC17-mediated transport of glutamate and/or ATP, resulting in their decrease, and thereby produce analgesic effects. We analyzed in mice the effects of various agents [namely, intrathecally injected clodronate, etidronate, phosphonoformic acid (PFA; an inhibitor of SLC20/34), and agonists of glutamate and ATP receptors] on the nociceptive responses to intraplantar injection of capsaicin. Clodronate and etidronate produced analgesic effects, and these effects were abolished by PFA. The analgesic effects were reduced by N-methyl-D-aspartate (agonist of the NMDA receptor, a glutamate receptor) and α,β-methylene ATP (agonist of the P2X-receptor, an ATP receptor). SLC20A1, SLC20A2, and SLC34A1 were detected within the mouse lumbar spinal cord. Although we need direct evidence, these results support the above hypothesis. Clodronate and etidronate may be representatives of a new type of analgesic drug. Such drugs, with both anti-bone-resorptive and unique analgesic effects without the adverse effects associated with N-BPs, might be useful for osteoporosis.

Key words  analgesic effect; clodronate; etidronate; phosphate transporter; ATP; glutamate

Bisphosphonates (BPs) are used against various bone-resorptive diseases, and the nitrogen-containing BPs (N-BPs) have much more powerful anti-bone-resorptive effects than the non-nitrogen-containing BPs (non-N-BPs) (Fig. 1). However, N-BPs have inflammatory and/or necrotic effects, including fever, musculoskeletal pain, direct injuries to esophageal and gastric tissues, and osteonecrosis of the jaw. N-BPs, having entered cells (including osteoclasts), exhibit cytotoxicity due to inhibition of farnesyl pyrophosphate synthase in the mevalonate pathway of cholesterol biosynthesis, reducing the prenylation of various proteins, including small guanosine 5′-triphosphatase (GTPases).

In contrast to N-BPs, the non-N-BPs etidronate and clodronate are almost devoid of inflammatory/necrotic effects. Interestingly, they can reduce such side effects of N-BPs in mice. Concerning the mechanisms by which clodronate (Clo) and etidronate (Eti) reduce the inflammatory/necrotic effects of N-BPs, our pharmacological studies have suggested that: (a) N-BPs may enter soft-tissue cells via phosphate transporters of the SLC20 and SLC34 families, (b) Clo and Eti may inhibit those families, possibly by competitive inhibition, and thereby inhibit the entry of N-BPs into soft-tissue cells, and (c) Clo and Eti may also inhibit phosphate transporters of the SLC17 family.

Pain is a cause of distress in many patients with osteoporosis. Fujita et al. found that in patients with osteoporosis and/or osteoarthritis, Eti displayed an analgesic effect that was greater than those of N-BPs. We recently reported that Clo and Eti exhibit analgesic effects in mice, possibly via direct interaction with neurons. However, the mechanism underlying the analgesic effects of Clo and Eti remains unclear.

Surprisingly, and very interestingly, the SLC17 family of transporters, initially characterized as phosphate carriers, was recently found to include neuronal vesicular transporters of glutamate and ATP. The N-methyl-D-aspartate (NMDA) receptor (NMDA-R), an ionotropic receptor for glutamate, is distributed within the spinal cord and plays an important role in pain transmission. In addition, nucleotides are released or leaked from non-excitable cells, as well as neurons, under both physiological and pathophysiological conditions, and recent studies indicate that ATP plays important roles during pain transmission through the ionotropic purinoceptor P2X-R.

There are as yet no reports describing relationships between BPs and neuronal transmitters. However, on the basis of the findings described above, we hypothesized that Clo and Eti may enter neurons through SLC20 and/or SLC34, and then act on neuronal vesicles to inhibit SLC17 transporter-mediated transport of glutamate and/or ATP (resulting in decrements in the levels of glutamate and ATP within the vesicles) (Fig.

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Here, we pharmacologically examined these hypotheses, employing NMDA as a specific agonist of NMDA-R, and α,β-methylene ATP (mATP) as a specific agonist of P2X-R.

**MATERIALS AND METHODS**

**Animals** ddY and BALB/cA mice were purchased from SLC (Shizuoka, Japan) and CLEA (Tokyo, Japan), respectively. All experiments complied with the Guidelines for Care and Use of Laboratory Animals in Tohoku University and Tohoku Pharmaceutical University.

**Reagents** The following drugs and chemicals were used: acetic acid, capsaicin, Eti, and phosphonoformic acid (PFA) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), Clo, NMDA, and mATP (Sigma-Aldrich, St. Louis, MO, U.S.A.), and substance P (SP) (Peptide Institute, Osaka, Japan). The above drugs (except capsaicin) were dissolved in sterile saline, with the pH of the solutions being adjusted to 7 with NaOH if necessary. Capsaicin was dissolved in dimethyl sulfoxide, and this stock solution (5 µmol/mL) was diluted to 0.25 µmol/mL with sterile saline on the day of the experiment. Experimental protocols are described in the text or in the legend to the figure relating to the relevant experiment. Experimental protocols are described in the text or in the legend to the figure relating to the relevant experiment.

**Injection of Test Samples into Mice**

**Intrathecal (i.t.) Injection**

i.t. injection into the intervertebral space between L5 and
L6 was performed without anesthesia as described by Hylden and Wilcox, using a 28-gauge stainless steel needle attached to a 50-μL Hamilton microsyringe. The volume of each i.t. injection was 5 μL, and the doses of reagents given by this route are expressed as nmol in 5 μL.

Intravenous (i.v.) Injection

I.v. injection was performed by injecting a given test solution into a tail vein. The volume of each i.v. injection was 50 μL/10 g body weight, and the doses of i.v. injected reagents are expressed as mg/kg body weight.

Evaluation of Anti-nociceptive or Analgesic Effects of BPs

Capsaicin Test

To assess antinociception, ddY mice (22–26 g body weight at the time of the test) were subjected to the capsaicin test. To reduce variability, each mouse was acclimatized to an acrylic observation chamber (22.0 × 15.0 × 2.5 cm) for approximately 1 h before the injection of capsaicin. Each mouse was injected with 20 μL of a capsaicin solution (0.25 μmol/mL) (i.e., 5 nmol/paw) beneath the skin of the plantar surface of the right hindpaw using a 26-gauge needle. The injection was completed as quickly as possible, with only minimal animal restraint. Following this capsaicin injection, the animals were immediately placed in the test box for a 5-min observation period. The licking/biting behavior induced by the injection was observed as an indicator of the nociceptive response. The accumulated response-time (in seconds) spent in licking/biting the capsaicin-injected paw was recorded for a period of 5 min immediately after the injection.

Writhing Test

Writhing was induced by intraperitoneal (i.p.) injection of 0.7% (v/v) acetic acid (0.1 mL/10 g body weight) into BALB/cA mice. The number of writhing movements was counted in the period from 5 to 30 min after the acetic acid injection.

Detection of mRNAs of SLC20 and SLC34 Transporters in Spinal Cord

Total RNA was isolated from lumbar dorsal cord (or kidney, as positive control) using TRI Reagent® (Sigma-Aldrich) according to the manufacturer’s protocol. Total RNA was reverse-transcribed using ReverTraAce® (Toyobo, Osaka, Japan) and the oligo(dT) primer (Ambion, Foster City, CA, U.S.A.). Polymerase chain reaction (PCR) products were separated by electrophoresis through an agarose gel and stained with ethidium bromide. An image of each gel was digitally captured using FASIII (Toyobo).

Immunostaining of SLC20 in Spinal Cord

Mice were anesthetized and perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C overnight. After being dehydrated using a graded series of ethanol solutions and infiltrated with xylene, specimens were embedded in paraffin. Serial sections of 5-μm thickness were cut and immunostaining was performed as described previously. After deparaffinization, antigen retrieval was performed using proteinase K solution (20 μg/mL in TE buffer, pH 8.0) (Wako Pure Chemical Industries, Ltd.) for 5 min at 37°C. Then, tissue sections were blocked for endogenous peroxidase activity with 0.3% hydrogen peroxide in methanol for 15 min at room temperature. Sections were incubated at 4°C overnight with the primary antibody for SLC20a1 (dilution 1:1000; Abcam, Cambridge, MA, U.S.A., Cat. #: ab104687) or SLC20a2 (dilution 1:100) (Bioss USA, Woburn, MA, U.S.A., Cat. # bs-8610R) diluted with 5% normal goat serum in phosphate-buffered saline containing 0.05% Triton-X and 5% bovine albumin. Next, the sections were incubated with biotinylated goat anti-rabbit antibody (1:1000; Vector Laboratories, Funakoshi Co., Tokyo, Japan) for 1 h at room temperature, and then visualized using a Vectastain ABC kit (Vector Laboratories). Sections were counterstained with methyl green solution. Unfortunately, we could not obtain antibodies against SLC34 family members that could be used for immunostaining.

Fluorescent double-label immunohistochemistry was carried out using the primary antibody for a neuronal nuclear antigen (NeuN) (1:100 dilution; EMD Millipore, Billerica, MA, U.S.A., #MAB377, clone A60), and sections were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) to stain the nuclei of all cells. The secondary antibodies used were Alexa®-conjugated goat-anti rabbit and Alexa®-conjugated goat anti-mouse (1:750 dilution; Molecular Probes, Eugene, OR, U.S.A.). The slides were examined under a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan). The specificity of the immune reactions was verified by replacing the primary antibody with rabbit immunoglobulin G (IgG) (Abcam, #ab27472) as an isotype control, and additional blocking for endogenous mouse IgG was performed using mouse-to-mouse blocking reagent (Seytek, Logan, UT, U.S.A.).

Data Analysis

Experimental data for the pain analysis are given as the mean±standard deviation (S.D.). Statistical differences between groups were evaluated by Fisher’s protected least significant difference (PLSD) post hoc test for multiple comparisons, following an ANOVA conducted using StatView-J5.0 software (SAS Institute, Inc., U.S.A.). p Values less than 0.05 were considered significant.

Table 1. Primer Sequences for Phosphate Transporters

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Sense primer</th>
<th>Antisense primer</th>
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<tbody>
<tr>
<td>Slc20a1</td>
<td>5'-GTG GGA GAC TGC ATG GGA GAT TC-3'</td>
<td>5'-TAT GGG TGT TGC CGC TTT TGT AGA-3'</td>
</tr>
<tr>
<td>Slc20a2</td>
<td>5'-AGC GGA CCG GAC GAC CTC-3'</td>
<td>5'-GCC CCC AGC AGC ACA GA-3'</td>
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<tr>
<td>Slc34a1</td>
<td>5'-GCA GGC AGG GAC CAG GAC-3'</td>
<td>5'-GAG CCC AGC AGC ATG-3'</td>
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<tr>
<td>Slc34a2</td>
<td>5'-TCA GGC GCC CAG AAC AAG AG-3'</td>
<td>5'-GAT GGG CAG AGG GGT GAA TG-3'</td>
</tr>
<tr>
<td>Slc34a3</td>
<td>5'-TGG CGG GCT TGG TCA TTG-3'</td>
<td>5'-CTT CCC TGG GGC GTC TCC-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-ACC CAG AAG ACT GTG GAT GG-3'</td>
<td>5'-CCC TGT TGC TGT AGC CGT AT-3'</td>
</tr>
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</table>
RESULTS

I.t. Injected Clo and Eti Displayed Analgesic Effects, and PFA Abolished Them  In the capsaicin test, i.t. injected Clo and Eti displayed analgesic effects with the potencies Clo > Eti (Fig. 3A). PFA is an inhibitor of SLC34 (although at higher concentrations it also inhibits SLC20).\(^{28}\) PFA, when injected i.t. by itself, did not affect the test response (Fig. 3B). However, PFA abolished the analgesic effects of Clo and Eti (Fig. 3C). Incidentally, 7.5 nmol Til (a cyclic non-N-BP) (Fig. 1), did not exhibit any analgesic effect. Although i.t. injected N-BPs (Ale, Ris, Zol, and Min) (Fig. 1) were toxic at 1 nmol or more (producing abnormal behaviors), they did not exhibit analgesic effects at 0.5 nmol (data not shown). In the present study, Clo, Eti, and PFA, by themselves, had no detectable effect on the behavior of the mice.

I.t. Injected NMDA and mATP Abolished, or Tended to Reduce, the Analgesic Effects of Clo and Eti  If Clo and Eti inhibit the transport of Glu and/or ATP, their content within neuronal vesicles may be decreased (see the legend to Fig. 2), and there may be a reduction in their release from such vesicles. This effect might underlie the observed Clo- or

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**Fig. 3. Analgesic Effects of Clodronate (Clo) and Etidronate (Eti), and the Modulating Effect of Phosphonoformate (PFA)**

(A) Clo or Eti was i.t. injected at the indicated doses 1 h before the capsaicin test. (B) Saline or PFA was i.t. injected at the indicated doses 1 h before the capsaicin test. (C) Clo, Eti, or a mixture of Clo or Eti with PFA, was i.t. injected at the indicated doses 1 h before the capsaicin test. One-way ANOVA: (A-upper) \(F_{3,36}=14.38, p<0.001\); (A-lower) \(F_{4,46}=6.06, p<0.001\); (B) \(F_{2,27}=1.35, p=0.28\); (C) \(F_{4,45}=7.38, p<0.001\). Post hoc test: *\(p<0.05\), **\(p<0.01\).

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**Fig. 4. Modulating Effects of N-Methyl-D-aspartate (NMDA) and \(\alpha,\beta\)-Methylene ATP (mATP) on the Analgesic Effects of Clodronate (Clo) and Etidronate (Eti)**

At the indicated doses, NMDA or mATP was i.t. injected 55 min after i.t. injection of Clo or Eti, and the capsaicin test was performed 5 min after the 2nd injection. One-way ANOVA: (A-upper) \(F_{3,36}=17.15, p<0.001\); (A-lower) \(F_{5,52}=6.98, p<0.01\); (B-upper) \(F_{3,35}=11.79, p<0.001\); (B-lower) \(F_{3,34}=4.69, p<0.01\). Post hoc test: *\(p<0.05\), **\(p<0.01\).
Eti-induced reduction in the nociceptive responses to capsaicin (i.e., the analgesic effects of Clo and Eti). If that is correct, it might be possible to weaken or abolish these analgesic effects by giving a dose of NMDA or mATP that is too low, by itself, to induce a nociceptive response (since such a non-nociceptive dose of NMDA or mATP might become nociceptive when it is acting in conjunction with the reduced level of capsaicin-induced Glu and/or ATP release from nerve endings that is presumed to occur under Clo or Eti). To test this idea, we first determined the maximum doses of NMDA and mATP that could be given i.t. injection without nociceptive responses being induced. Based on the results from these experiments, including the timing of the i.t. injection, we used 0.2 nmol/5 µL of NMDA and 0.3 nmol/5 µL of mATP (injected 5 min before the capsaicin test) in the following experiments. NMDA, at that dose, abolished the analgesic effect of Clo (Fig. 4A, upper) in the capsaicin test, although the analgesic effect of Eti only tended to be weakened by NMDA (Fig. 4A, lower). On the other hand, mATP abolished the analgesic effects of both Clo and Eti (Fig. 4B). These results suggest that Clo can inhibit both glutamate and ATP transporters in neuronal vesicles, while the inhibitory effect of Eti might be somewhat stronger on ATP transporters than on glutamate transporters.

I.t. Injected SP Did Not Reduce the Analgesic Effects of Clo and Eti

We examined whether SP might be involved as a target for Clo and Eti. The maximum i.t. dose of SP that did not induce nociceptive responses was 50 pmol/5 µL (injected 5 min before the capsaicin test). At this dose, SP, unlike NMDA and mATP, did not alter the analgesic effects of either Clo or Eti (Fig. 5), supporting the idea that SP is not involved in the analgesic effects of Clo and Eti.

SLC20 and/or SLC34 Were Detected in the Lumbar Spinal Cord

The SLC20 and 34 families include two and three members, respectively. SLC20 (a1 and a2) and SLC34 (a1, but not a2 and a3) mRNAs were detected in mouse dorsal lumbar spinal cord (Fig. 6A), and SLC20a1 and SLC20a2 proteins were detected in the dorsal lumbar spinal cord by

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*A figure*:

**Fig. 5.** Lack of Modulating Effects of Substance P (SP) on the Analgesic Effects of Clodronate (Clo) and Etidronate (Eti)

Saline or SP (at the indicated dose), was i.t. injected 55 min after i.t. injection of Clo or Eti, and the capsaicin test was performed 5 min after the 2nd injection. One-way ANOVA: \(F_{5,53}=11.69, p<0.001\). Post hoc test: **p<0.01.

**Fig. 6.** Detection of SLC20 and/or SLC34 in the Lumbar Spinal Cord

(A) Two samples of dorsal lumbar spinal cord, prepared separately, were analyzed. Kidney was used as a positive control (p.c.). (B) (a) Hematoxylin–eosin (HE) staining, (b) staining without antibody, (c) staining of SLC20a1, and (d) staining of SLC20a2. Immunostaining was positive (brown spots) for both SLC20a1 and SLC20a2 (see Materials and Methods) throughout the spinal cord gray matter. The positive brown spots for both SLC20a1 and SLC20a2 were present in greater numbers in the dorsal horn than in other regions of the cord (see enlarged views). Examples of positive spots are indicated by arrowheads. Bar: 500 µm for a–d and 200 µm for enlarged views.
immunostaining (Fig. 6B). The positive spots (brown) for both SLC20a1 and SLC20a2 were present in greater numbers in the dorsal horn (i.e., sensory region) than in other regions of the cord (see enlarged views in Fig. 6B). As shown in Fig. 7, SLC20a1 and SLC20a2 were detected widely within the lumbar spinal cord, and NeuN-positive cells were also positive for SLC20a1 and SLC20a2, indicating that SLC20a1 and SLC20a2 are expressed in various cell-types, including neurons. We could not perform immunostaining for the detection of SLC34A1 protein, because we could not obtain an antibody appropriate for such immunostaining.

**I.v. PFA Also Abolished the Analgesic Effects of I.v. Clodronate (Clo) and/or Etidronate (Eti)**

In our previous study, Eti and Clo exhibited analgesic effects when given by various administration routes in the writhing test, and i.v. injection was effective for both of these non-N-BPs at around 10 mg/kg, a dose lower than those required by other routes. Thus, we tested (i) whether i.v. Eti and Clo, at 10 mg/kg, might exhibit analgesic effects in the capsacin test, too, and (ii) whether the analgesic effects of i.v. Eti and Clo could be reversed by i.v. PFA. I.v. PFA by itself did not affect the writhing-test response (Fig. 8A). Like i.t. injected PFA, i.v. PFA abolished the analgesic effect of i.v. Clo in the capsacin test (Fig. 8B), although no significant effect of PFA could be detected on the analgesic effect of i.v. Eti (data not shown). I.v. PFA abolished the analgesic effects of i.v. Clo and Eti in the writhing test, too (Fig. 8C).

**DISCUSSION**

**Summary of the Results** Here, we found the following: (i) i.t. injected Clo and Eti produced analgesic effects, and these effects were abolished by i.t. injected PFA, an SLC20/34 inhibitor, (ii) the analgesic effects of Clo and Eti were reduced by NMDA (agonist of NMDA-receptor) or mATP (agonist of
P2X-receptor), but not by SP, (iii) SLC20a1, SLC20a2, and SLC34a1 were detected within the mouse lumbar spinal cord, and (iv) i.v. PFA also abolished the analgesic effects of i.v. Clo and/or Eti. We discuss these findings in the following paragraphs.

Among BPs, Only Clo and Eti Exhibit Analgesic Effects and These Are Independent of Their Anti-bone-Resorptive Effects  In a previous report, among the various BPs only the non-N-BPs Clo and Eti exhibited analgesic effects at non-toxic doses. In those experiments, BPs were administered subcutaneously, i.e., or orally, and the analgesic potency was Clo > Eti. In the present study, i.v. injected N-BPs did not exhibit analgesic effects, either, at their maximum non-toxic dose, whereas the non-N-BPs Clo and Eti were analgesic, again with the potency Clo > Eti. The anti-bone-resorptive effects of N-BPs tested in the present study (Ale, Ris, Zol, and Min) are roughly 300- to 3000-fold stronger than that of clodronate. In addition, N-BPs reportedly cause musculoskeletal pain in patients. These results suggest that the analgesic effects of Clo and Eti are completely independent of the molecular mechanism underlying their anti-bone-resorptive effects (i.e., inhibition of farnesyl pyrophosphate synthase in the mevalonate pathway of cholesterol biosynthesis). Til, despite being a non-N-BP (Fig. 1), is not analgesic either, suggesting that possession of an analgesic effect that is independent of an anti-bone-resorptive effect-independent may be a characteristic only of Clo and Eti. We also speculate that the smaller molecular sizes of Eti and Clo might be important for their ability to exhibit analgesic effects.

Clo and Eti May Enter Neurons via SLC20/34 Phosphate Transporters Based on pharmacological experiments, we previously suggested that N-BPs might translocate into cells via SLC20/34 phosphate transporters, and that the N-BPs might then be able to cause inflammatory and necrotic effects. Clo and Eti can prevent or reduce the inflammatory and necrotic effects of N-BPs by competitive inhibition at these transporters. The mRNAs of SLC20 family members are detected ubiquitously among various human tissues, including brain and spinal cord. Here, we did indeed detect SLC20a1 and SLC20a2 proteins widely within the mouse lumbar spinal cord, and SLC20a1 and SLC20a2 were found to be expressed in various cell-types, including neurons. We detected SLC34a1 mRNA, too, but not SLC34a2 and SLC34a3 mRNAs. These results suggest that these members of the SLC20 and SLC34 families of transporters are involved in the translocation of Clo and Eti into sensory neurons, although we will need further studies to test this hypothesis.

The Analgesic Effects of Clo and Eti Involves Inhibition of SLC17 The SLC17 transporters, initially characterized as phosphate carriers, include neuronal vesicular transporters of glutamate (SLC17a7, SLC17a6, and SLC17a8) and ATP (SLC17a9). The present pharmacological data suggest that inhibition of these SLC17 transporters is involved in mediating the analgesic effects of Clo and Eti after their entry into neurons. Moreover, we suggest that Eti might act on SLC17a9 more strongly than Clo, because the analgesic effect of Eti was reduced by mATP, but not by NMDA (Fig. 4). However, we need direct confirmation of the inhibition of SLC17 transporters by in vitro experiments.

Clinical Implications of the Analgesic Effects of Clo and Eti Many patients with osteoporosis experience pain. Since N-BPs have powerful anti-bone-resorptive activities, they are currently the first-choice drugs for the treatment of osteoporosis. However, as described in Introduction, N-BPs sometimes have serious inflammatory and necrotic side effects. In contrast, the anti-bone-resorptive effects of the non-N-BPs Clo and Eti are weak, but they lack such side effects. Because of their weak anti-bone-resorptive effects (Fig. 1), Clo and Eti need to be given at much higher doses than N-BPs; indeed, the doses of N-BPs per patient are less than 10 mg, while those of non-N-BPs are several hundred milligrams. It should be noted that even at such high doses, Clo and Eti lack the inflammatory and necrotic effects seen with N-BPs. At such high doses, we should expect Clo and Eti to exhibit analgesic effects that are independent of their anti-bone-resorptive effects, although they would also exhibit anti-bone-resorptive effects at those doses.

CONCLUSION

Clo and Eti may be representatives of a new type of analgesic, a type that enters neurons, and then inhibits the SLC17-mediated vesicular transport of the pain transmitters ATP and/or glutamate, and in this way produces analgesia. If humans respond in the same way as the mice studied here, Clo and Eti may prove to be useful, safe drugs for osteoporosis since they will have both anti-bone-resorptive and unique analgesic effects without the adverse side effects associated with N-BPs.

Perspectives In the present study, we suggest for the first time the possibility that Clo and Eti may exert analgesic effects by acting on glutamate- and/or ATP-related pain transmission pathways. However, there were two questions we did not address, for the indicated reasons. (i) Do clodronate and etidronate indeed enter neurons? This was not addressed because we cannot at present find commercial sources of isotope-labeled clodronate and etidronate. (ii) Do clodronate and etidronate indeed inhibit glutamate and/or ATP transporter(s)? This was not addressed because specialized techniques and materials are needed for direct measurement of the transporation of glutamate and ATP via the SLC17 family of transporters. However, our collaborators are able to perform the experiments needed to answer question (ii), and are currently doing so successfully. Moreover, we hope to be able to answer question (i) in the future.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

(1996).


