Effects of local administration of clodronate on orthodontic tooth movement and root resorption in rats

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SUMMARY Clodronate, a non-N-containing bisphosphonate, strongly inhibits bone resorption and also has anti-inflammatory properties. The purpose of this study was to examine the effect of the local administration of clodronate on orthodontic tooth movement. Both the right and left upper first molars of 26 7-week-old male Wistar rats were moved buccally with a standardized expansion spring. Clodronate solution was injected into the sub-periosteum area adjacent to the left upper molar every third day during the experimental period. The right first molar served as the control.

Local injection of clodronate caused a significant \( P < 0.001 \) and dose-dependent reduction in tooth movement in the rats. The number of osteoclasts on the clodronate-injected side was significantly less \( P < 0.01 \) than on the control side. Local clodronate also inhibited root resorption incident to tooth movement. These results suggest that localized use of clodronate could be a useful therapeutic adjunct in orthodontic treatment.

Introduction

In orthodontic treatment, mechanically induced bone resorption is an essential step in tooth movement and, therefore, can be a major target for pharmacological intervention to enhance the treatment outcome. If undesirable tooth movement could be prevented with blockers of bone resorption, treatment would be less complex and more secure.

Bisphosphonates selectively inhibit osteoclasts, and have been used to treat various metabolic bone diseases associated with excessive bone resorption (Fleisch et al., 2002). However, studies on the structure–activity relationships of these compounds indicate that their potency and mode of action vary depending on the side-chain on the carbon atom of the P–C–P bond, a common structure of bisphosphonates (Shinoda et al., 1983; Rogers et al., 2000; Dunford et al., 2001). Recent investigations have revealed that bisphosphonates that contain a nitrogen atom in the side-chain (N-containing bisphosphonates) are more potent than those without a nitrogen atom (non-N-containing bisphosphonates), and that they can inhibit the production of isoprenoid compounds (farnesyl pyrophosphate and geranylgeranyl pyrophosphate) in the mevalonate pathway and, hence, prevent protein prenylation in osteoclasts (Dunford et al., 2001). It has also been shown that non-N-containing bisphosphonates can be incorporated into ATP analogues and act by inhibiting protein synthesis and inducing apoptosis in osteoclasts (Rogers et al., 2000; Frith et al., 2001).

Clodronate is a non-N-containing bisphosphonate that contains two chlorine atoms in its side-chain. Due to its close structural similarity to pyrophosphate, this bisphosphonate, like etidronate and tiludronate, is known to inhibit osteoclast function by being incorporated metabolically into a non-hydrolysable ATP analogue (Rogers et al., 2000). In addition to this anti-bone resorbing activity, it has been suggested that this compound also has anti-inflammatory activity (Elomaa et al., 1992; Österman et al., 1995; Richards et al., 2001). The present study was undertaken to examine the effect of the local administration of clodronate on tooth movement in rats as the first step to determine future clinical application of this compound.

Materials and methods

The general condition and weight of each animal used in this study were monitored during the experimental period. The animals were treated in accordance with the guidelines for the use of experimental animals of the Animal Care and Use Committee of Tohoku University Graduate School of Dentistry.

Drug

Clodronate (dichloromethylene bisphosphonate disodium salts) was obtained from Proctor and Gamble Pharmaceuticals (Woods Corners Laboratories, Norwitch, New York, USA).
**Tooth movement in rats**

Twenty-six male Wistar rats, 7 weeks old and weighing an average of 180 g, were used in this study. Both the right and left upper first molars of the animals were moved buccally with a standardized expansion spring, which initially generated an average force of 120 mN on each side. The expansive force was applied without adjustment for 3 weeks. Clodronate solution, 50 µl at a concentration of either 0 (saline), 2.5, 10 or 40 mM, was injected into the sub-periosteum area adjacent to the left upper first molar every third day during the experimental period under light ether anaesthesia. The right first molar served as the control, with an injection of 50 µl of saline into the corresponding area. The clodronate solutions were prepared by dissolving clodronate in distilled water; the pH was adjusted to 7.4 with NaOH and osmolarity was adjusted to 310 mOsm with NaCl. Tooth movement was measured according to a method described previously (Igarashi et al., 1994). Briefly, the occlusal view of a precise plaster model of the right and left upper jaws was magnified ×10 with a profile projector and traced. The contours of the palatal cusps of the second and third molars of the tracings were then superimposed on those of the second and third molars on tracings from a pre-treatment plaster model. The distance between the crests of the mesiopalatal cusps of the first molars before and after tooth movement was measured with sliding callipers. Data were expressed as either millimetres or as a percentage of the paired control value. The latter was calculated as: (tooth movement on the clodronate-treated side ×100)/tooth movement on the control side.

**Histological examinations**

After 3 weeks of force application, the animals were killed under diethyl ether anaesthesia, the upper jaws were dissected, fixed in 4 per cent paraformaldehyde for 5 days, and then decalcified with 10 per cent EDTA solution (0.01 M phosphate-buffered saline, pH 7.4) for 5 weeks. The samples were dehydrated and embedded in paraffin wax. Periodontal tissues of the mesiobuccal root of the upper first molar were examined with a light microscope in transverse serial sections of the molars. Sections (5 µm thick) were stained for tartrate-resistant acid phosphatase (TRAP) with 0.16 mg/ml naphthol AS-MX phosphate and 1.4 mg/ml fast red violet LB salt in 0.1 M sodium acetate buffer (pH 5.0) containing 50 mM sodium tartrate, and counter-stained with toluidine blue. The mesiobuccal root area was divided into a pressure and a tension side, based on the mesiodistal axis of the root. The osteoclasts on the alveolar bone surface of the pressure side were counted. Osteoclasts were defined as multinucleated TRAP-positive cells on the bone surface or in bone-resorptive lacunae. The root-resorptive area was measured by image analysis (WinROOF, Mitani Shoji Co., Fukui, Japan) of the microscopic image that was fed directly to a television monitor with a CCD video camera at a magnification of ×850, according to a method described previously (Igarashi et al., 1996). The data were expressed as square micrometres. For both the number of osteoclasts and the root-resorptive area, the values for 12 sections, selected at five-section intervals, were averaged for each animal.

**Statistical analysis**

All of the data were first subjected to one- or two-way analysis of variance (ANOVA). The dose–response data were then subjected to the Scheffe F multiple comparison test to identify differences between groups. The paired t-test was used to evaluate the significance of differences in tooth movement, osteoclast count and root resorption between the treated side and the contralateral control side. Statistical calculations were carried out with Microsoft Excel 98.

**Results**

The local administration of clodronate did not cause any appreciable inflammatory reactions at the injection site or any systemic side-effects, such as loss of appetite or change in body weight.

As shown in Figure 1a, tooth movement on the clodronate-injected side was significantly less than on the control side after 3 weeks of orthodontic force application. The percentage of the control value was 81, 65 and 56 at concentrations of 2.5, 10 and 40 mM, respectively. Thus, the inhibitory effect was dose-dependent. Figure 1b shows the time course of tooth movement in animals injected with clodronate (50 µl of 10 mM). There was a significant reduction in tooth movement on the treated side compared with the control side on days 14, 17 and 21.

Figure 2 shows the number of osteoclasts on the pressure side of the mesiobuccal roots of upper first molars on day 21. The number of osteoclasts on the clodronate (10 mM)-injected side was significantly less than on the control side. Figure 3 shows the area of root resorption on the pressure side of the mesiobuccal roots of upper first molars on day 21. The root-resorptive area on the clodronate (10 mM)-injected side was significantly smaller than on the control side.

**Discussion**

The present results demonstrate that clodronate strongly inhibits orthodontic tooth movement. Tooth movement on the control side exhibited three typical...
phases, i.e. a phase of rapid movement, a lag phase, and a phase of progressive movement (Roberts, 1994). The local administration of clodronate caused a significant reduction in tooth movement in the third phase, which is considered to be due to bone resorption by osteoclasts. This indicates that clodronate inhibits bone resorption induced by orthodontic mechanical stress. The results are comparable with those obtained in animals injected with N-containing bisphosphonates such as alendronate (Igarashi et al., 1994) and risendronate (Adachi et al., 1994).

As the number of osteoclasts decreased at the clodronate-injected side, clodronate may have either inhibited the recruitment of osteoclasts or promoted osteoclast apoptosis, or both (Rogers et al., 2000). Osteoclast apoptosis is considered to be a major mechanism of action for the inhibition of bone resorption by this bisphosphonate (Halasy-Nagy et al., 2001). However, as other mechanisms cannot be excluded, an in vitro study is currently being undertaken.

Clodronate is an anti-resorptive agent, and is widely used in the treatment of metabolic bone disease...
Although clodronate is not part of the new class of bisphosphonates and is less potent than N-containing bisphosphonates (Fleisch, 2000), it can be considered a potential anti-inflammatory drug. Clodronate has been shown to suppress signs of inflammation in arthritic rats (Österman et al., 1995; Richards et al., 2001; Iwasaki et al., 2001; Alhashimi et al., 2001; Shirazi et al., 2002; Hayashi et al., 2002), including adverse reactions such as pain (Ngan et al., 1994) and root resorption (Brudvik and Rygh, 1991), clodronate could be a more desirable drug for the control of orthodontic tooth movement than other N-containing bisphosphonates, some of which are rather pro-inflammatory (Mönkkönen et al., 1998; Makkönen et al., 1999; Pecherstorfer et al., 2000). Although the present results show that local clodronate inhibits root resorption incident to tooth movement, further studies are necessary to determine whether clodronate is also useful for preventing inflammatory reactions to orthodontic treatment.

When the clinical application of clodronate is considered, it would be desirable to minimize possible systemic effects of the local administration of this drug. Further studies are also necessary in this respect.

Conclusions

The results of the present study suggest that the localized use of clodronate could be a beneficial therapeutic adjunct for orthodontic treatment. Bisphosphonates such as clodronate that have anti-inflammatory properties may also be helpful in the treatment of increased bone resorption associated with inflammatory diseases such as rheumatoid arthritis and periodontitis.

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