Disodium Chlodronate Prevents Bone Resorption in Experimental Periodontitis in Rats

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**Background:** Periodontitis is a chronic disease characterized by alveolar bone loss and inflammatory changes. We studied the effect of disodium chlodronate (CD), a bisphosphonate used in metastatic and metabolic bone disease as a bone resorbing drug, in an experimental periodontitis model (EPD) focusing on anti-resorptive and anti-inflammatory parameters.

**Methods:** A nylon thread ligature was placed around the left maxillary molars of 72 male Wistar rats who were sacrificed after 7 or 11 days. Groups were treated daily with CD (1, 5, or 25 mg/kg/sc) starting at day 0 until day 7 (prophylactic CD) or from day 5 until day 11 (curative CD) after periodontitis induction. Non-treated group (NT) consisted of rats subjected to periodontitis that received no pharmacological treatment. Alveolar bone loss (ABL) was measured as the distance between the cuspid tips and the alveolar bone. The right jaw was used as control. The hemiarcades were processed for histopathologic analysis.

**Results:** In NT group there was significant ABL, severe mononuclear cells influx, and increase in osteoclast numbers. Prophylactic CD treatment decreased the ABL 25.8%, 61.6%, and 75.5% as compared to NT for the 1, 5, and 25 mg/kg CD doses, respectively. Curative CD treatment decreased the ABL 20%, 62%, and 69% as compared to NT for the 1, 5 and 25 mg/kg CD doses, respectively. Both prophylactic and curative CD decreased histological changes, as compared to NT rats ($P < 0.01$).

**Conclusions:** CD has both bone sparing and anti-inflammatory activity in EPD in rats when administered as a pretreatment or in an ongoing process. The possibility of using CD as an alternative treatment in human periodontitis should be considered. *J Periodontal* 2002;73:251-256.

**KEY WORDS**
Bone loss/prevention and control; anti-inflammatory agents; animal studies; chlodronate/therapeutic use; bone resorption/prevention and control; dental models; periodontitis/drug therapy.

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accelerated bone resorption, such as Paget’s disease, hypercalcemia malignancy, and osteoporosis. Apart from being able to inhibit bone resorption, disodium chlodronate has been shown to have anti-inflammatory properties. In the adjuvant-arthritis model in rats, chlodronate was more effective than ethane-1-hydroxy-1,1-disphosphonate (EDHP) in reducing both bone resorption and inflammatory parameters. Also, in vitro, disodium chlodronate was able to inhibit both cytokines and nitric oxide release by RAW-264 cells, a macrophage cell line. Our objective was to study the effect of administering disodium chlodronate to rats subjected to an experimental periodontitis model, focusing on bone resorption and inflammatory parameters.

MATERIALS AND METHODS

Animals
Seventy-two male Wistar rats (180 to 200 g body weight) obtained from our own animal facilities were housed in temperature-controlled rooms and received water and food ad libitum. The experimental protocol was in accordance with the guidelines approved by Council of American Psychological Society for the use of animal experiments.

Protocol of Experimental Periodontal Disease (EPD)
We used an experimental periodontitis model in rats as described previously with minor modifications. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg-ip) and a nylon (000) thread ligature was surgically placed around the cervix of the second left maxillary molars. The contralateral maxilla was used as the control. The ligature was knotted on the vestibular side so that it remained subgingivally in the palatal side and supragingivally in the vestibular side. We have previously observed that alveolar bone loss in this model peaks after 7 days and remains unaltered until 11 days after periodontitis induction. After sacrifice by cervical dislocation, under anesthesia (chloral hydrate, 400 mg/kg-ip), maxillae were excised. The left maxillary jaws were used to determine the degree of bone loss (macroscopic analysis or histologic analysis).

Drug Dosage
There were 5 groups of 6 animals each for both the prophylactic and curative disodium chlodronate treatments. The prophylactic disodium chlodronate (CD) treatment group received CD (1, 5, or 25 mg/kg), subcutaneously, 1 hour before the surgical procedure and daily until sacrifice at day 7. The curative CD treatment group received CD (1, 5, or 25 mg/kg), subcutaneously 5 days after periodontitis induction and daily until sacrifice at day 11. This strategy assured that both the prophylactic and curative groups received the same total dose of chlodronate. The non-treated (NT) group consisted of 6 animals subjected to EPD which received a saline solution daily and were sacrificed after 7 or 11 days after periodontitis induction. A control (naive) group (6 animals) did not receive either CD or saline.

Measurement of Alveolar Bone Loss
The left and right jaws were defleshed and stained for 30 seconds in 1% aqueous methylene blue. The alveolar bone loss was measured under a stereoscope loupé (4× magnification), as the distance between the cuspid tip and the alveolar bone. In these conditions, the alveolar bone landmark is determined by methylene blue staining that clearly determines the adjacent enamel alveolar bone limits. Recordings (mm) were made in the long axis of buccal surfaces of all molar teeth. There were 3 measurements for the first molar and 2 measurements for the second and third molar roots. Alveolar bone loss was defined as the sum of the differences between the value of the left maxilla and the right maxilla (control).

Histological Analysis
The half jaws were fixed, decalcified, and prepared for hematoxylin and eosin staining. Six µm sections corresponding to the area between the first and second molars where the ligature was placed were analyzed. Histological analysis consisted of a 0 to 3 score based on cell influx, osteoclast numbers, and alveolar process and cementum integrity. The maximum possible score for each sample (animal) was 3 (Table 1).

Table 1. Histopathologic Parameters Analyzed in Rats With Experimental Periodontitis

<table>
<thead>
<tr>
<th>Score</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absent or discrete PMN cell influx</td>
</tr>
<tr>
<td></td>
<td>Rare osteoclasts (OC)/high power field (HPF)</td>
</tr>
<tr>
<td></td>
<td>Well-preserved alveolar process</td>
</tr>
<tr>
<td></td>
<td>Well-preserved cementum</td>
</tr>
<tr>
<td>1</td>
<td>Moderate PMN cell influx</td>
</tr>
<tr>
<td></td>
<td>Less than 6 OC/HPF</td>
</tr>
<tr>
<td></td>
<td>Slight alveolar process resorption</td>
</tr>
<tr>
<td></td>
<td>Well-preserved cementum</td>
</tr>
<tr>
<td>2</td>
<td>Intense PMN cell influx</td>
</tr>
<tr>
<td></td>
<td>More than 6 OC/HPF</td>
</tr>
<tr>
<td></td>
<td>Moderate alveolar process resorption</td>
</tr>
<tr>
<td></td>
<td>Moderate cementum resorption</td>
</tr>
<tr>
<td>3</td>
<td>Intense PMN cell influx</td>
</tr>
<tr>
<td></td>
<td>More than 6 OC/HPF</td>
</tr>
<tr>
<td></td>
<td>Absent alveolar process</td>
</tr>
<tr>
<td></td>
<td>Absent cementum</td>
</tr>
</tbody>
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§ Boehringer, Mannheim, Germany; distributed by Asta Medica do Brasil Ltd., São Paulo, SP, Brazil.
Statistical Analysis
Data were described as either means ± s.e.m or medians, as appropriate. Univariate analysis of variance followed by Tukey’s test was used to compare means. Mann-Whitney was used to compare histological scoring which were described as medians. *P* < 0.05 was considered significant.

RESULTS
Effect of CD on Alveolar Bone Loss
Figure 1 illustrates the macroscopy of the maxilla of a control rat (A), an animal 7 days after periodontitis induction that received no treatment (B), an animal 7 days after periodontitis induction that received 25 mg/kg/d prophylactic CD (C), and an animal 11 days after periodontitis induction that received 25 mg/kg/d curative CD (D). There is clear reduction of the ABL in both the prophylactic and curative CD-treated rats, as compared to the non-treated animals (*P* < 0.05). Figure 2 shows that both prophylactic (A) and curative (B) CD administration reduced alveolar bone loss index (ABL), reaching statistical significance, as compared to non-treated animals (*P* < 0.05). Prophylactic CD reduced ABL by 25.8%, 61.6%, and 75.5% for the 1, 5, and 25 mg/kg/d doses, respectively, compared to non-treated rats. Curative CD reduced ABL by 20%, 62%, and 69% for the 1, 5, and 25 mg/kg/d doses, respectively, compared to non-treated rats. The distance between the dental cuspids and the adjacent alveolar bone (mm) was 3.21 ± 2.13, 1.66 ± 1.69, and 1.06 ± 0.51 for the prophylactic 1, 5, and 25 mg/kg/d CD groups and 3.60 ± 0.59, 1.75 ± 1.32, and 1.28 ± 0.49 for the curative 1, 5, and 25 mg/kg/d CD groups, as compared to 4.33 ± 1.14 for the NT group. Both 5 and 25 mg/kg prophylactic and curative CD treatment groups were significantly different compared to the NT group (*P* < 0.05).

Effect of CD on Histological Analysis
Figure 3 shows the microscopical appearance of the region between the first and second molar roots of a normal rat (A), a rat 11 days after periodontitis induc-
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DISCUSSION

In the present study, we reproduced a previously reported model demonstrating that placement of a nylon thread ligature around the molar teeth produces periodontitis in rats characterized by progressive local edema and cell infiltration. The latter consists mainly of neutrophils until the fourth day, whereas mononuclear cells (macrophages) predominate after 5 days, persisting until 7 days of periodontitis. We have recently shown that the alveolar bone loss (ABL) in this experimental periodontitis model (EPD) peaks at 7 days and persists unaltered until 11 days after periodontitis induction, coupled to systemic alterations such as neutrophilia and lymphomonoctyosis in the peripheral blood.

This ABL is coupled to local lymphomonoctye infiltration and increase in the number of osteoclasts, together with severe cementum and alveolar bone resorption.

Periodontitis is a multimediated disease in which eicosanoids, mainly prostaglandins, appear to play a role, apparently via activation of the inducible isoform of cyclooxygenase. Inflammatory cytokines also have been implicated in the pathogenesis of periodontitis. Interleukin-1 and TNF-\(\alpha\) have been shown to be released in periodontitis. Also, inhibition of ABL and inflammatory parameters in EPD after administration of chlorpromazine, a drug known to block TNF-\(\alpha\) activity, either by inhibiting TNF-\(\alpha\) synthesis or its coupling to specific cell receptors, add support for a role of TNF-\(\alpha\) in periodontitis.

In the present study, we aimed to demonstrate that disodium chlodronate, a bisphosphonate known to inhibit bone resorption, could alter the evolution of an EPD in rats. Our data show that CD decreased cell infiltration and osteoclast numbers in the periodontal tissue and reduced ABL both at the macroscopic, measured as the distance between the dental cuspids and the alveolar bone, and microscopic (histopathology) levels. This was a dose-dependent effect that could be observed either when the drug was administered as a prophylactic treatment or in already established periodontitis. The
reduction in the cell infiltration observed at the histopathologic level suggests that down-regulation of local resident or migrating inflammatory cells provoked by CD administration could be involved in the protective effect of this drug in EPD. Although a comprehensive study on potential toxicities related to CD administration was beyond the scope of the present work, we did not observe any adverse effects. In fact, the observation that experimental periodontitis leads to weight loss, was not observed in the CD treated rats, suggesting a beneficial systemic effect of CD (data not shown).

The mechanism of action of CD, as well as of the other bisphosphonates, is far from elucidated. Although these drugs seem to be unable to inhibit acute inflammatory changes, it was reported that bisphosphonates decreased chronic inflammation in mice subjected to either a delayed-type hypersensitivity granuloma or to an antigen-induced arthritis model as well as in rats subjected to adjuvant-induced polyarthritis. Similar data were obtained previously. Sodium alendronate retarded the progression of periodontitis in monkeys, measured by bone densitometry. Also, administration of YM175 prevented bone resorption in dogs subjected to periodontitis.

Apparently, inhibition of both differentiation and activation of mononuclear inflammatory cells could be at least partially responsible for the anti-inflammatory activity of bisphosphonates (BP). In this regard, it was demonstrated that BP specifically inhibits monocyte formation from marrow stem-cell precursor. Administration of liposome-encapsulated dichloromethylene BP was shown to greatly reduce macrophage numbers in lymphoid tissues of rats. Also, direct cytotoxic activity of BP upon macrophages has been suggested after the demonstration that chlodronate was able to induce apoptosis of cultured macrophages and osteoclasts. Additionally, it was reported that chloldronate inhibited in vitro nitric oxide release from RAW 264 cells and that BP were also able to inhibit matrix metalloproteinase-1 activity in vitro, an enzyme linked to tissue degradation during inflammation. Hence, BP probably has a multifunctional activity that leads to its bone-sparing effects.

The mechanisms of reduction of inflammatory parameters and bone resorption in the present EPD model are not straightforward. Although we might speculate that CD acted through inhibition of the release of inflammatory mediators and tissue degrading enzymes, the possibility that CD could induce local mononuclear cell apoptosis thereby decreasing osteoclast numbers is an alternative explanation of CD amelioration of EPD.

In summary, our data provide evidence that disodium chloldronate reduces inflammatory changes and bone resorption in a periodontitis model in rats. More important, CD was able to alter even existing periodontal disease. Since this compound is currently used for other clinical purposes in humans and CD doses used in the present study are comparable to those used in metastatic bone diseases, the possibility of using CD as an alternative treatment for inhibiting inflammatory bone resorption in other pathologies should be considered.

ACKNOWLEDGMENTS
The authors thank Maria Silvandira Freire de França and José Ivan Rodrigues de Souza for technical assistance.

REFERENCES
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Accepted for publication August 24, 2001.