A histometric study in rats of the effect of the calcium antagonist amlodipine on bone healing after tooth extraction

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Abstract

The purpose was to investigate whether amlodipine, a second-generation calcium antagonist used for the treatment of hypertension and angina, interferes with healing of rat alveolar bone. A progressive increase in volume density of new bone filling the socket was quantified by a histometric differential point-counting method 7–42 days after tooth extraction. The results showed a 20–30% decrease in bone volume fraction in the alveolus of amlodipine-treated animals from 7 days on, in addition to a higher (7–35%) volume fraction of connective tissue and a tendency toward an increase in the volume fraction of persisting coagulum. If confirmed in humans, the knowledge of a deleterious effect of Ca-channel blockers in hindering alveolar bone healing would be important in planning oral operations involving bone tissue, including those for device implantation. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Osseous healing; Alveolar healing; Calcium antagonist; Amlodipine

1. Introduction

Besides being the main mineral constituent of bone, calcium (Ca) plays a pivotal part in the control of numerous cell activities. Thus Ca antagonists, by blocking Ca transport across the cell membrane, could theoretically affect many metabolic processes. Ca-channel blockers are, however, widely used in the treatment of hypertension and angina, as they block transmembrane influx of Ca in vascular smooth muscle cells and lower blood pressure by decreasing peripheral vascular resistance (Clement et al., 1994).

Although vascular specificity is highly desirable, in vitro experiments have shown that some Ca antagonists (verapamil, diltiazem, nifedipine, lacidipine) can affect hormone release and metabolism. However, these effects have not always been confirmed in vivo, as clinically useful doses of the drugs seem to have no dramatic effects on neuroendocrine regulation (see Schoen et al., 1988, for references). Nevertheless, there is some experimental evidence to that the treatment of male rats with verapamil, a first-generation Ca antagonist, can interfere with bone formation, inhibiting the growth of the tibia, which becomes more densely mineralized (Samnegard and Sjoden, 1992). Those investigators proposed that a possible increase in androgen secretion could explain why opposite results were obtained in female rats.

A second generation of Ca-channel blockers, mostly dihydropyridines, was developed to achieve greater vas-
cular specificity. Amlodipine is a new dihydropyridine Ca antagonist that has been tested for the treatment of hypertension and angina, being considered effective and well tolerated, with minor side-effects (Haria and Wagstaff, 1995). However, a recent study from our laboratory has revealed deleterious effects of amlodipine on the reproductive function of male rats, including a significant decrease in the plasma testosterone (Almeida et al., 2000). Considering the importance of androgens for the control of bone growth (Orwoll, 1996), the present study sought to investigate whether treatment of male rats with amlodipine interferes with new bone formation, using as an experimental model the alveolar bone healing after tooth extraction.

2. Material and methods

Male Wistar rats (170.2 ± 2.3 g initial body wt, n = 55) received oral doses (0.04 mg/0.5 ml per rat per day) of an aqueous solution of amlodipine besylate (Norvask®; Pfizer, Guarulhos, SP, Brazil) starting 12 days before tooth extraction and continuing until death. Control rats received tap water. The animals were housed in a climate-controlled room (12 h light–12 h dark, 23 ± 2°C) with free access to laboratory chow and water. On the 13th day of treatment, the rats were anaesthetized with an intraperitoneal injection (25 mg/100 g body wt) of 2,2,2-tribromoethanol (Aldrich, USA) and the upper right incisors were extracted with forceps, after disconnection of the surrounding gingiva and luxation with an enamel hatchet with a cutting edge. Immediately after surgery, the gingival tissues were sutured and a single intramuscular dose of a polyvalent antibiotic for veterinary use (Pentabiotico Veterinario; Wyeth, Sao Bernardo do Campo, SP, Brazil; 0.2 ml/rat) was administered. All the procedures were conducted in accordance with ethical and humane principles of animal research, as approved by institutional guidelines.

Control and treated rats were killed by intravenous overdose of sodium pentobarbital 7, 14, 21 and 42 days after tooth extraction (n = 6–8 per time/treatment group), their mandibles were removed and their heads immersed in 10% formalin for 48 h. After fixation, the maxillae were dissected and divided along the median sagittal plane and the right halves were cut tangentially to the distal surface of the molars, demineralized and processed for paraffin embedding. Semiserial longitudinal 6-μm-thick sections cut at 60-μm intervals were stained with haematoxylin and eosin. An integration eyepiece with 100 equidistant points was used to estimate the volume fraction of healing alveolar components by a differential point-counting method. A total of 1200–1500 points were counted in the apical, middle and cervical thirds of each alveolus (final magnification × 400), the percentage of points lying on connective tissue, bone trabeculae and blood clot being proportional to their volume density.

The results were analysed by two-way analysis of variance, followed by the Tukey test. The data on the volume fraction of blood clot were subjected to cube-root transformation to obtain a normal distribution before analysis (GMC Basic Software).

3. Results

The various phases of alveolar healing were recognized by histological examination in control and treated rats from 7 to 42 days after tooth extraction. At the end of the first week, immature bone trabeculae lined with osteoblasts were found side by side with remnants of coagulum and abundant connective tissue rich in neoformed capillaries (granulation tissue) (Fig. 1A). With progressive bone formation, by the second week the alveolar socket was occupied equally by connective tissue and bone trabeculae (Fig. 1B). By the third week, the socket was occupied by a network of thick trabeculae surrounding medullary spaces filled with loose connective tissue, as also observed by the sixth week (Fig. 1C). A possible delay in the osteogenic process was apparent in the alveolus of treated rats, which presented a smaller bone volume density and persisting remnants of coagulum as late as 42 days after tooth extraction (Fig. 1D), when they were absent in the alveolus of control animals.

Histometric analysis provided quantitative data showing progressive bone formation in parallel to a decrease in the volume fraction of connective tissue up to 42 days after tooth extraction. Beginning at 7 days, a 20–30% decrease in bone volume fraction was observed in the alveolus of amlodipine-treated animals, accompanied by a higher (7–35%) volume fraction of connective tissue (Fig. 2). The volume fraction of coagulum tended to be higher in the middle and apical thirds of the alveolus of treated rats, this increase reaching statistical significance in the apical third 21 days after extraction (Table 1).

4. Discussion

Results from our laboratory have shown that amlodipine has no adverse effect on body weight gain (Almeida et al., 2000) or on the plasma and urine Ca and phosphorus (data not shown). In the present study, as in the previous ones, the animals received daily doses progressively smaller than 0.023 mg/100 g body wt as they continued to grow, a dose compatible with human prescriptions (5–10 mg per day, the lower dose sug-
gested for elderly patients and for those with hepatic insufficiency). Published data on the therapeutic efficacy of amlodipine in rats report daily doses ranging from 0.02 mg/100 g (Kanno et al., 1996) to 3 mg/100 g body wt (Takahashi et al., 1996).

The histological features of intra-alveolar bone healing closely resembled those described previously (Lin et al., 1994), including earlier reports from our laboratory (Brentegani et al., 1996; Lamano Carvalho et al., 1997a,b). However, the histometric analysis confirmed a delay in reparative bone formation in response to amlodipine treatment, somewhat related to impairment of the remission and/or organization of the coagulum. The coagulum filling the alveolar socket immediately after tooth extraction is progressively reabsorbed as it is invaded by fibroblasts derived from the periodontal ligament that actively proliferate and migrate into it, form an immature connective tissue and differentiate into osteoblasts responsible for new bone production during socket healing (Lin et al., 1994).

It is known that each stage of bone healing is enabled and controlled by a number of priming, differentiating, proliferating, migrating and organizing agents such as angiogenic, epidermal and platelet-derived growth factors, bone morphogenetic proteins, prostaglandins and interleukins (Frost, 1989; Bilezikian et al., 1996). In the specific case of alveolar wound healing, some coagulum-derived growth factors such as PDGF and TGF-β appear to be of particular importance. Fibroblasts from the periodontal ligament express PDGF receptors and demonstrate a strong mitogenic and chemotactic response to PDGF in vitro, while TGF-β reportedly inhibits fibroblast proliferation and stimulates the formation of periosteal woven bone in vivo (see Lin et al., 1994 for references). Based on these experimental findings, it was suggested that PDGF from platelets stimulates proliferation of periodontal fibroblasts and their migration towards the coagulum at early stages of socket healing, while TGF-β may play an important part in the final differentiation of fibroblasts into osteoblasts.

Fig. 1. Rat alveolar socket: (A) Control rat 7 days after tooth extraction, showing immature bone trabeculae lined with osteoblasts (→) immersed in abundant connective tissue rich in neoformed capillaries; control rats 14; (B) and 42 days; (C) after tooth extraction, exhibiting a progressive increase in new bone formation; (D) amlodipine-treated rat 42 days after tooth extraction, showing a smaller volume density of reparative bone and a coagulum remnant (↑) × 56; haematoxylin and eosin; bar = 100 μm.
Fig. 2. Volume fraction (%) of bone and connective tissues in the apical, middle and cervical alveolar thirds of control (CON) and amlodipine-treated (AMLO) rats 7–42 days after tooth extraction (mean ± SEM). For each healing component/alveolar third, different letters indicate statistically significant differences between means (A ≠ B ≠ C ≠ D; analysis of variance; α = 0.05).

Table 1
Volume fraction (%) of coagulum in the apical, middle and cervical alveolar thirds of control and amlodipine (0.04 mg per rat per day)-treated rats 7–42 days after tooth extraction (Mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>42 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Apical</td>
<td>15.0 ± 4.0</td>
<td>22.8 ± 6.3</td>
<td>5.3 ± 1.8</td>
<td>13.2 ± 7.1</td>
</tr>
<tr>
<td>Middle</td>
<td>14.7 ± 5.5</td>
<td>21.2 ± 9.0</td>
<td>5.3 ± 1.8</td>
<td>9.6 ± 5.2</td>
</tr>
<tr>
<td>Cervical</td>
<td>16.9 ± 5.8</td>
<td>9.0 ± 2.5</td>
<td>6.5 ± 3.2</td>
<td>1.8 ± 1.8</td>
</tr>
</tbody>
</table>

* Statistically significant difference between control and treated groups (analysis of variance, α = 0.05).
Although the mechanism(s) by which amlodipine caused a delay in alveolar bone healing was not determined, by blocking Ca transport into cells other than vascular smooth muscle (the desirable effect) the drug could theoretically affect this complex local regulation of bone metabolism. This hypothesis is validated by reports that certain Ca-channel blockers, including amlodipine, can modulate the inflammatory process by enhancing/reducing local mediators of angiogenesis and of cell proliferation, migration and differentiation (Roth et al., 1992; Rodler et al., 1994).

Besides paracrine/autocrine control, bone metabolism is regulated by a variety of hormones, including androgens. Androgen receptors and specific binding sites were identified in osteoblastic cells of human and animal origin, in which testosterone and dihydrotestosterone appear to have similar binding affinities. The binding affinity of androgen receptor sites, as well as the number and specificity of androgen-binding sites, the character of androgen receptor mRNA and the size of androgen receptor protein are consistent with the precept that androgen effects in osteoblasts are typical of those found in other classically androgen-responsive tissues (see Orwoll, 1996 for references).

A recent study from our laboratory showed a significant reduction in the plasma testosterone and follicle-stimulating hormone inducing hypogonadotropism, in male rats treated with amlodipine (Almeida et al., 2000). Although most of the effects of androgens on bone formation have been assessed in orchidectomized animals, which exhibit a significant reduction in trabecular mass and whole mineral density (Orwoll, 1996), it could be that the decreased testosterone observed in amlodipine-treated rats may have contributed to a delay in alveolar bone healing.

To the best of our knowledge, this is the first report on the deleterious effect of a Ca-channel blocker on reparative bone formation. If confirmed in humans, these data would be useful for planning oral surgery involving bone tissue, including procedures for device implantation.

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References


