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Effect of an estrogen-deficient state and its therapy on bone loss resulting from an experimental periodontitis in rats

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Objective: The aim of this study was to evaluate the impact of an estrogen-deficient state and its therapies (estrogen and calcitonin administration) upon bone loss resulting from an experimental periodontitis.

Methods: Fifty-eight Wistar rats were divided into four groups: group 1 ($n = 15$): sham operated; group 2 ($n = 15$): ovariectomized; group 3 ($n = 14$): ovariectomized plus calcitonin administration; group 4 ($n = 14$): ovariectomized plus estrogen administration. Twenty-one days after ovariectomy or sham surgeries, the ligature was randomly placed. Sixty days later, the animals were killed and the specimens routinely processed. In addition, serum levels of alkaline phosphatase and calcium were assessed.

Results: Intergroup analysis revealed that an estrogen-deficient state significantly increased bone loss resulting from periodontitis and that such an effect could not be prevented either by estrogen or calcitonin administration (0.34 ± 0.13, 0.65 ± 0.06, 0.63 ± 0.19, 0.67 ± 0.28 for groups 1, 2, 3 and 4, respectively). Furthermore, an estrogen-deficient state presented a direct effect on the alveolar bone regardless of plaque accumulation and this effect may be significantly reduced by estrogen administration ($p < 0.05$). Serum analysis demonstrated a higher bone turnover for the animals with estrogen deficiency, and estrogen therapy restored bone metabolism.

Conclusion: Estrogen administration may prevent the direct effect of an estrogen-deficient state on alveolar bone; however, neither estrogen nor calcitonin administration could prevent this effect when associated with a response to a plaque-related inflammatory process.

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Periodontitis is characterized by inflammation of the supporting tissues of the teeth, resulting in alveolar bone resorption and soft tissue attachment loss (1). The role of systemic factors in the initiation and progression of periodontitis has been proposed (2).

Osteopenia and osteoporosis are age-related metabolic bone diseases that result in low bone mass and susceptibility to fractures (3). Currently,

estrogen hormone replacement remains the single most effective treatment of menopausal symptoms and prevention of osteoporosis (4). However, alternative therapies such as calcitonin have also been proposed (5). Since osteoporotic changes have been observed in the oral bone (6) and loss of alveolar bone is a prominent feature in periodontal disease, osteoporosis could be suspected of being an aggravating factor in periodontal disease.

Therefore, the aim of this study was to evaluate the impact of an estrogen-deficient state and its treatments, estrogen and calcitonin administration, on bone loss resulting from an experimental periodontitis in rats.

Materials and methods

Experimental design

On the day after the ovariectomies, the animals were randomly assigned to one of four groups: group 1 ($n = 15$): sham surgeries (negative control); group 2 ($n = 15$): ovariectomized (positive control); group 3 ($n = 14$): ovariectomized plus 4 days/week subcutaneous injections of calcitonin (Miacalcic®, Sandoz A.G., Fertigung Schützenstrsse, Ravenburg, Germany) at a dose of 16 IU/kg body weight; group 4 ($n = 14$): ovariectomized plus a daily subcutaneous injection of 17β estradiol (Sigma Chemical, St. Louis, MO, USA), dissolved in 100% ethanol and diluted in mineral oil at a dose of 20 μg/kg body weight.

Ligature placement/clinical and biochemical analyses

Twenty-one days after ovariectomy, one of the mandibular first molars of each animal was randomly assigned to receive cotton ligature and the contralateral tooth was left unligated. Ovariectomy success was confirmed by monitoring the estrous cycle and, at autopsy, by the atrophy of uterine horns in rats not given estrogen therapy. Before the sacrifice, blood samples were collected in order to obtain plasma concentration of alkaline phosphatase (Gold Analisa Diagnóstica, Belo Horizonte, MG, Brazil) and calcium (AVL Scientific Corporation, Roswell, GA, USA).

Histometric procedure

Sixty days after ligature placement, the animals were killed and the specimens routinely processed for decalcified sections in a mesio-distal direction (6 μm). Using an image analysis system (Image-Pro®; Media Cybernetics, Silver Spring, MD, USA), the area of bone loss in the furcation region was histometrically determined. Measurements from 10 sections for each group were averaged to allow intra- and intergroup analysis.

Statistical analysis

Measurements were averaged to allow intergroup and intragroup analysis, using the one-way analysis of variance (ANOVA) ($\alpha = 0.05$). Pairwise multiple comparisons were carried out by Bonferroni test ($\alpha = 0.05$) in the cases where the ANOVA test showed significant differences. In addition, the paired *t*-test ($\alpha = 0.05$) was used for intragroup comparisons between ligated and unligated teeth.

Results

Clinical observations

Macro analysis of the uterine horns and assessment of the estrous cycle of the rats confirmed the success of the ovariectomy surgery. Groups 2 and 3 presented *diestrous* smears and their reproductive organs were atrophied, confirming the reduction of estrogen levels. Conversely, group 1 presented the four stages of the estrous cycle and group 4 remained in the *estrus* stage. Finally, pink and fluid-filled uteri were clearly identified in groups 1 and 4, confirming that the serum estrogen levels were kept normal in these animals.

Biochemical serum analysis

The alkaline phosphatase level (IU/l) was statistically higher in groups 2 and 3 ($p < 0.05$) and therefore confirmed a high bone turnover in the animals in the estrogen-deficient state (29.13 ± 10.93, 80.47 ± 20.16, 98.20 ± 14.27 and 33.29 ± 14.91 for groups 1, 2, 3 and 4, respectively). With respect to calcium serum levels, group 2 presented higher values than the other groups ($p < 0.05$). The mean calcium serum levels (mmol/l) were 1.10 ± 0.07, 1.24 ± 0.08, 1.10 ± 0.14 and 1.07 ± 0.13 for groups 1, 2, 3 and 4, respectively.

Histometric results

Intragroup analysis showed that cotton ligatures placed around the teeth were able to promote periodontitis ($p < 0.05$) (Table 1). In unligated teeth, intergroup analysis showed that an estrogen-deficient state may have a direct effect on the alveolar bone regardless of plaque accumulation, resulting in some bone loss in the furcation region. However, this negative effect was restored by 17β estradiol administration, but not by calcitonin treatment (Table 1). In ligated teeth, an estrogen-deficient state resulted in a significant bone loss when compared to estrogen-sufficient animals ($p < 0.05$). In addition, none of the treatments (groups 3 and 4) were able to protect against the impact of an estrogen-deficient state on the alveolar bone in the ligated teeth ($p < 0.05$) (Table 1). Figures 1(A–D) illustrate the histological findings.

Table 1. Mean and standard deviation (mm^2) of bone loss and periodontal ligament areas around ligated and unligated teeth, according to each group

	Sham	Ovariectomized	Calcitonin	Estradiol
Unligated	0.18 ± 0.03^{aA}	0.32 ± 0.08^{aB}	0.26 ± 0.04^{aB}	0.17 ± 0.03^{aA}
Ligated	0.34 ± 0.13^{bA}	0.65 ± 0.06^{bB}	0.63 ± 0.19^{bB}	0.67 ± 0.28^{bB}

Capital letters should be considered in lines (intergroup analysis: ANOVA, $p < 0.05$) and lower case letters in columns (paired *t*-test, $p < 0.05$). Means followed by different letters differ statistically.

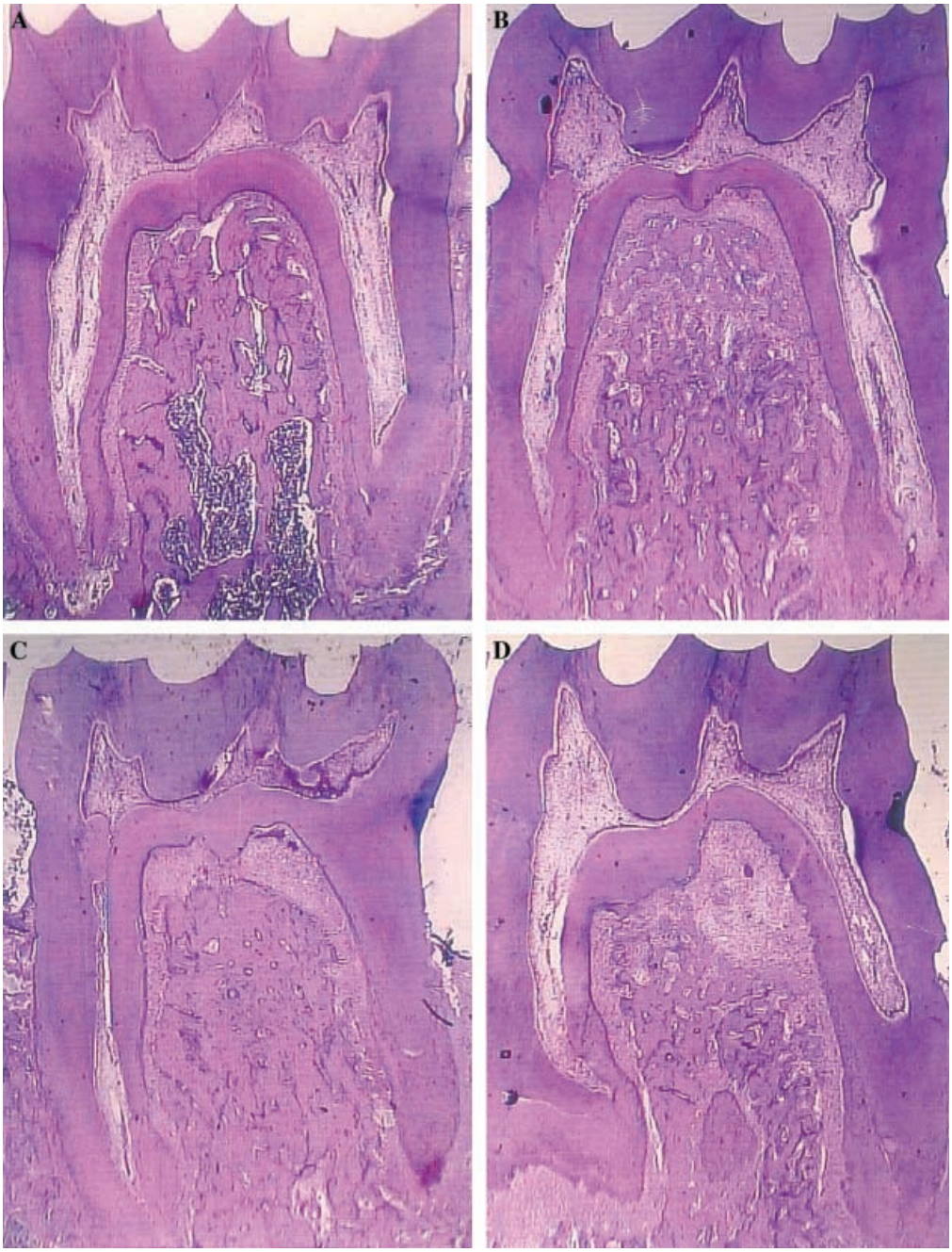

Fig. 1. Photomicrography illustrating periodontal ligament and bone loss areas in the furcation region for unligated and ligated teeth, respectively. (A) Unligated tooth (group 1), (B) ligated tooth (group 1), (C) unligated tooth (group 2) and (D) ligated tooth (group 2). Original magnification 12.5×; hematoxylin and eosin; bar = 0.45 mm.

Discussion

Osteoporosis and periodontal disease are major health problems in older populations (7). Determination of the correlation between these two diseases may be critical for the prevention of morbidity and mortality related to these disorders in elders.

Thus, the present study aimed to investigate the impact of an estrogen-deficient state and two therapies on the alveolar bone loss resulting from experimental periodontitis in rats. The results of the present study showed that an estrogen-deficient state may directly affect alveolar bone regardless of plaque accumulation and may also significantly increase bone loss resulting from ligature-induced periodontitis. Other studies have shown that an estrogen-deficient state and osteopenia/osteoporosis may increase oral bone resorption, attachment loss and tooth loss (6, 8, 9); however, this is the first study to show a direct correlation between periodontitis-related

bone loss and lower levels of estrogen.

On a molecular basis, bone resorption has been characterized by two key molecules, receptor activator of nuclear factor kappaB ligand (RANKL) and osteoprotegerin (10). Inhibition of RANKL function via the decoy receptor, osteoprotegerin, has been shown to significantly reduce alveolar bone destruction, as reported by Teng *et al.* (2000) (11). It has been shown that estrogen presents an important role in controlling bone resorption through its action on osteoprotegerin (12). Therefore, in the present study, the synergistic effect observed between an estrogen-deficient state and plaque accumulation may be explained by an increased level of RANKL and a decreased level of osteoprotegerin resulting from lipopolysaccharide stimulation and estrogen deficiency, respectively. However, the mechanisms involved remain to be investigated.

In the present study, estrogen therapy immediately after ovariectomy provided protection against the negative effects of an estrogen-deficient state on the alveolar bone around unligated teeth. These findings are in agreement with previous reports showing that an estrogen-deficient state induces osteoclastogenesis and osteoporotic changes in the interradicular septum of rat first molars, and estrogen administration would prevent this effect (13). On the other hand, estrogen therapy was not able to protect the alveolar bone against the negative influence of an estrogen-deficient state associated with plaque accumulation. To date, to the authors' knowledge, no information is available regarding the effects of estrogen administered to estrogen-deficient individuals upon bone loss resulting from periodontitis. The protective mechanism of estrogen on bone tissue apparently involves suppression of bone turnover as a direct effect on bone cells and an indirect effect on the regulation of cytokine expression (14). Since the effects of estrogen on bone metabolism have also been attributed to its effect on osteoprotegerin levels (12), estrogen replacement (in the present study) may have not reproduced osteoprotegerin levels capable of counteracting lipopolysaccharide-stimulated RANKL levels. Caution, however, should be taken when drawing conclusions from these results and further studies should be considered.

Wronski *et al.* (1991) (5), reported that calcitonin therapy for 41 days depresses bone turnover and prevents the development of osteopenia in ovariectomized rats. Shen *et al.* (1996) (15), however, demonstrated that calcitonin, when administered to ovariectomized rats for 90 days, only partially prevented bone loss. Similarly, our results demonstrated that calcitonin partially affected bone loss around unligated teeth, although this effect was not noted in the presence of dental plaque. As previously reported (16), this phenomenon suggests that the skeletal response to calcitonin may decline in a time-dependent manner, probably due to a down-regulation of bone binding receptors (17).

In conclusion, the present study clearly demonstrated, in rats, a synergistic effect of an estrogen-deficient state and plaque accumulation. In addition, the administration of estrogen or calcitonin could not protect against the effect of an estrogen-deficient state on the bone loss resulting from the experimental periodontitis. According to the data presented, a negative influence of an estrogen-deficient state on the alveolar bone can also be expected regardless of plaque accumulation, but estrogen administration was able to prevent such an effect. Therefore, in addition to the importance of estrogen deficiency in the general health status, it may also constitute a critical state with respect to the periodontium, and controlled clinical studies should be considered in order to provide information as to the best approach to deal with this condition.

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