J Periodont Res 2006; 41; 541–546

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00903.x

Estrogen and alendronate therapies may prevent the influence of estrogen deficiency on the tooth-supporting alveolar bone: a histometric study in rats

P. M. Duarte[1], P. Gonçalves[2], M. Z. Casati[2], S. de Toledo[2], E. A. Sallum[2], F. H. Nociti Jr[2]

[1]Department of Periodontics, Dental Research Division, Guarulhos University, Guarulhos, São Paulo, Brazil and [2]Department of Prosthodontics and Periodontics, Division of Periodontics, School of Dentistry at Piracicaba, UNICAMP, Piracicaba, São Paulo, Brazil

Duarte PM, Gonçalves PF, Casati MZ, de Toledo S, Sallum EA, Nociti Jr FH. Estrogen and alendronate therapies may prevent the influence of estrogen deficiency on the tooth-supporting alveolar bone: a histometric study in rats. J Periodont Res 2006; 41: 541–546. © 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Background and Objective: The aim of this study was to evaluate histometrically the influence of estrogen deficiency, and its therapies, on the quality of the tooth-supporting alveolar bone.

Material and Methods: Seventy-three female rats were randomly assigned to one of the following groups: group 1 ($n = 15$), sham surgery; group 2 ($n = 15$), bilateral ovariectomy (OVX); group 3 ($n = 14$), OVX plus calcitonin (16 IU/kg); group 4 ($n = 14$), OVX plus estrogen (20 μg/kg); and group 5 ($n = 15$), OVX plus alendronate (5 mg/kg). Eighty days after surgery, the animals were killed and their mandibles were removed and processed for histology. Bone density (BD) in the furcation area of the first mandibular molar (i.e. the percentage of demineralized bone tissue in a 1,000 μm zone under the furcation) was histometrically obtained.

Results: Data analysis demonstrated that estrogen deficiency negatively affected the tooth-supporting bone density (79.45% ± 4.22 and 55.23% ± 6.45, for groups 1 and 2, respectively), and that estradiol and alendronate therapies prevented this effect (61.67% ± 6.87, 78.09% ± 3.12 and 81.47% ± 4.58, for groups 3, 4 and 5, respectively).

Conclusion: Within the limits of this study, it can be concluded that the density of tooth-supporting bone is affected by estrogen deficiency, and that estradiol and alendronate therapies, but not calcitonin, provide protection against this effect.

Poliana M. Duarte, Praça Tereza Cristina, 58-07023-070, Guarulhos, São Paulo, Brazil
Tel: +55 11 64641769
Fax: +55 11 64641758
e-mail: pduarte@ung.br/poliduarte@yahoo.com

Key words: alveolar bone; bisphosphonate; calcitonin; osteoporosis

Accepted for publication March 16, 2006

For many years, clinicians have reported observations indicating that systemic bone quality and quantity are reduced in postmenopausal women. Postmenopausal osteoporosis and osteopenia are common skeletal disorders characterized by low bone mass and microarchitectual deterioration of bone tissue, following the cessation of ovarian function, either naturally or surgically induced (1,2). Both diseases have a complex etiology and are considered to be multifactorial diseases in which genetic determinants are modulated by hormonal, environmental and nutritional factors (1,2). It has been suggested that the loss of ovarian function at menopause could be associated with the loss of postcranial and oral bone. Although some studies have reported associations between oral and skeletal bone mass, others have produced conflicting results (3,4), probably because of differences in

measurement methods and confounding risk factors (5).

Estrogen is known to play an important role in regulating bone homeostasis and preventing postmenopausal bone loss (6–9). Based on the protective effect of estrogen on bone tissue, estrogen hormone replacement currently remains among the most frequently used treatments for menopausal symptoms and prevention of osteoporosis (10,11). However, the possibility of clinical contra-indications, and the risk of side-effects, have prompted the search for alternative approaches (12). Thus, calcitonin (CT) and bisphosphonates, such as alendronate (ALD), have been proposed as promising alternative therapies for the treatment of postmenopausal osteoporosis (13–15).

We have previously reported the influence of estrogen deficiency and its therapeutic possibilities (CT, estrogen and ALD) on bone loss resulting from experimental periodontitis in rats. Our data provided evidence that estrogen deficiency promoted increased bone loss resulting from ligature-induced periodontitis, and that during estrogen deficiency, ALD showed a beneficial effect on periodontitis-induced bone loss, while the administration of estrogen or CT was unable to prevent the negative influence of estrogen depletion (16,17). Similarly, other studies have shown that estrogen deficiency may increase oral bone resorption, attachment loss and tooth loss in the presence of dental plaque accumulation (3,18,19).

Based on these considerations and on the role of alveolar bone in dentistry, it also seems relevant to study whether estrogen deficiency and its therapies may affect tooth-supporting alveolar bone under local healthy conditions. Thus, the aim of this study was to evaluate in rats, by histometric analysis, the influence of an estrogen-deficient state, and its treatments, on the density of tooth-supporting alveolar bone.

Materials and methods

Animals

The experimental animals were 73 female Wistar rats (90 d old) that weighed an average of 211 g (208–214 g) at the beginning of the study. During the period of the experiment, the animals were kept in plastic cages with access to food and drinking water *ad libitum*, with the exception of the ovariectomized rats (pair feeding) (20). This protocol was approved by the University of Campinas Institutional Animal Care and Use Committee.

Ovariectomy and sham surgery

At the beginning of the study, the animals were anesthetized by intramuscular administration of ketamine (0.5 ml/kg). Bilateral ovariectomies (OVXs) were performed in 58 rats from a dorsal approach, and sham surgeries (in which the ovaries were lifted up and returned intact to the original position) were performed on the remaining 15 rats. Postoperatively, the animals received antibiotic given as a single intramuscular injection (1 ml/kg) (Pentabiótico®; Wyeth-Whitehall Ltda, São Paulo, SP, Brazil).

Estrous cycle

In order to confirm the success of OVX and estrogen administration, the estrous cycle was monitored 2 wk after the OVX surgeries. Changes in the vaginal smear during 4–5 d of the estrus cycles were observed for each group. At autopsy, success of the OVX was also confirmed by the absence of ovaries and atrophy of uterine horns in ovariectomized rats, and success of estrogen administration was confirmed by the presence of normal uterine horns.

Experimental design

At the beginning of the study, the animals were randomly assigned to one of the following five groups:

- group 1 ($n = 15$): sham surgeries (negative control);
- group 2 ($n = 15$): bilateral OVX (positive control);
- group 3 ($n = 14$): OVX plus 4 d/wk subcutaneous injections of CT (Miacalcic®; Sandoz A.G., Fertigung Schützenstrsse, Ravenburg, Germany) at a dose of 16 IU/kg body weight, for 80 d;
- group 4 ($n = 14$): OVX plus a daily subcutaneous injection of 17β-estradiol (E) (Sigma Chemical Co., St Louis, MO, USA), dissolved in 100% ethanol and diluted in mineral oil at a dose of 20 μg/kg body weight, for 80 d;
- group 5 ($n = 15$): OVX plus 4 d/wk subcutaneous injections of ALD (Teva Pharmaceutical Ltda, Petach Tikva, Israel) at a dose of 5 mg/kg body weight, for 80 d.

Therefore, the animals from groups 3, 4 and 5 began receiving the drugs 1 d after the the OVX was performed until the time that they were killed.

Alkaline phosphatase analysis

Blood samples were collected in the morning to measure serum concentrations of alkaline phosphatase at the time of death (80 d after OVX and sham surgery). Using automated laboratory techniques, total alkaline phosphatase activity was colorimetrically obtained (Gold Analisa Diagnóstica, Belo Horizonte, MG, Brazil).

Histometric procedure

At the end of the experimental period, the animals were killed. The jaws were removed and the specimens were fixed in 4% neutral formalin for 48 h and subsequently demineralized in a solution containing equal parts of 50% formic acid and 20% sodium citrate for 45 d. Paraffin serial sections (6 μm) were obtained in a mesio–distal direction and stained by hematoxylin and eosin. After excluding the first and the last section in which the furcation region was evident, five equally distant sections of each tooth were selected for histometric analysis. Using an image-analysis system (Image-Pro®; Media Cybernetics, Silver Spring MD, USA), bone density (BD) (i.e. the percentage of demineralized bone tissue in a 1,000-μm zone under the furcation) in the inter-radicular area of the first mandibular molar was histometrically determined using the point counting technique by a blinded and calibrated examiner.

Statistical analysis

Mean values of alveolar BD were obtained for each group and compared statistically by the Kruskal–Wallis test ($\alpha = 0.05$). In order to test the hypothesis that estrogen deficiency and its therapies did not influence alkaline phosphatase level, a one-way analysis of variance (ANOVA) ($\alpha = 0.05$) was performed. If statistical difference was detected, a pairwise multiple comparison procedure was used (Bonferroni *t*-test).

Results

Clinical observations

Clinical appearance of uterine horns and assessment of the estrous cycle confirmed the success of OVX and estrogen replacement. Groups 2, 3 and 5 (OVX, CT and ALD, respectively) presented *diestrus* smear, characterized by a predominance of leucocytes, and their reproductive organs atrophied, confirming the reduction of serum estrogen levels in these groups. In contrast, the animals submitted to SHAM surgery (group 1) presented the four regular stages of estrous cycle *(estrus*, *diestrus*, *proestrus and metestrus),* and a pink and fluid-filled uterus. Finally, animals that were ovariectomized and administered with estradiol (group 4) remained in the *estrus* stage (enucleated cornified cells). These animals also presented normal uteri, demonstrating that the serum estrogen levels had been maintained as normal.

Alkaline phosphatase analysis

Alkaline phosphatase serum concentrations (± standard deviation), determined at the time of death, were 29.13 IU/l ± 10.93; 80.47 IU/l ± 20.16; 98.20 IU/l ± 14.27; 33.29 IU/l ± 14.91; 40.93 U/l ± 11.05, for groups 1–5, respectively. Alkaline phosphatase levels were statistically higher for groups 2 (OVX) and 3 (CT) ($p < 0.05$) and, as expected, confirmed a high bone turnover in these groups. Figure 1 graphically illustrates the results observed for alkaline phosphatase levels.

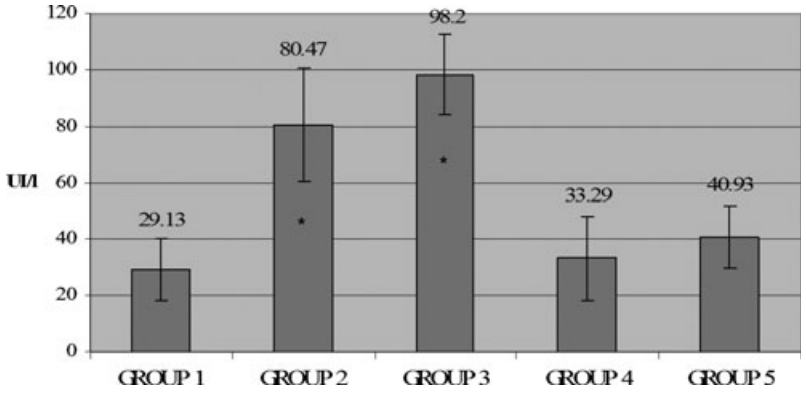

Fig. 1. Mean and standard deviation (IU/l) of alkaline phosphatase serum levels for all experimental groups [SHAM, ovariectomy (OVX), calcitonin (CT), 17β-estradiol (E) and alendronate (ALD)]. *Significant differences among the groups ($p < 0.05$) [one-way analysis of variance (ANOVA)].

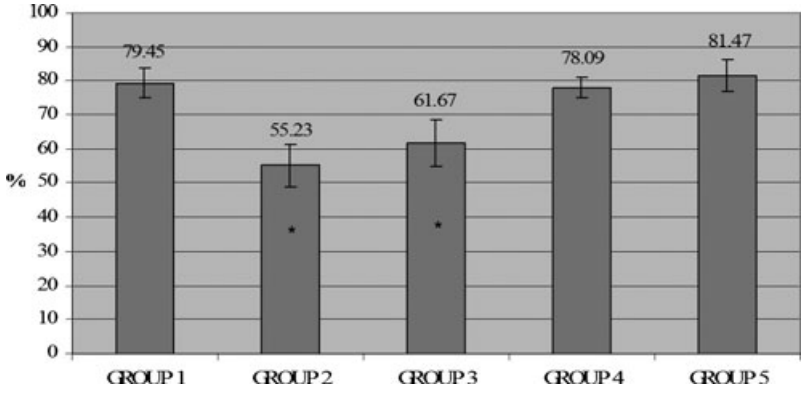

Fig. 2. Means and standard deviation (%) of bone density (BD) for all the experimental groups [SHAM, ovariectomy (OVX), calcitonin (CT), 17β-estradiol (E) and alendronate (ALD)]. *Significant differences among the groups ($p < 0.05$) (Kruskal–Wallis and Dunn's tests).

Histometric results

Data analysis demonstrated a significant difference in BD in the furcation area ($p < 0.05$) among the groups (Fig. 2). Estrogen deficiency (group 2) resulted in a lower BD in the inter-radicular area of the first mandibular molar, and this negative effect was prevented in the OVX animals that were administered with E (group 4) and ALD (group 5). On the other hand, CT administration (group3) did not prevent the influence of estrogen deficiency on the tooth-supporting alveolar bone ($p > 0.05$) (Fig. 3). Figure 3A–E illustrates the histological aspects of the inter-radicular BD.

Discussion

In addition to anchoring the teeth, the maxillary and mandibular alveolar bone allows dental rehabilitation, such as dental-supported implants and removable prostheses. It has been recognized that the success of some dentistry treatments may be influenced by the quality and quantity of this oral bone. Changes in microtrabecular alveolar bone following estrogen deficiency and its therapies have rarely been reported in oral bone under periodontally healthy conditions. Therefore, the present investigations aimed to evaluate histometrically structural changes in the tooth-supporting alveolar BD of ovariectomized rats

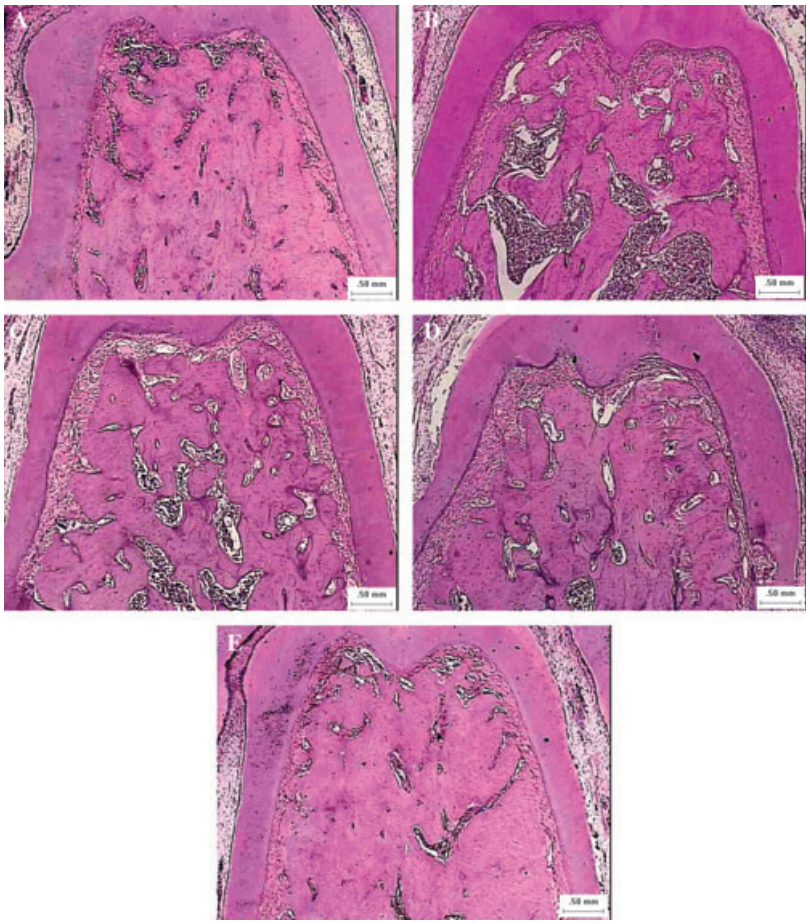

Fig. 3. Photomicrographs (A) to (E) illustrate the histological aspects observed within the limits of the inter-radicular bone for groups 1–5, respectively. Original magnification ×12.5. Hematoxylin and eosin.

under CT, estrogen or ALD therapies. Data analysis showed that an estrogen-deficient state may negatively affect the tooth-supporting alveolar bone, resulting in a lower density of bone tissue than observed in estrogen-sufficient animals. Furthermore, it has been clearly demonstrated, in the present investigation, that E and ALD, but not CT, given immediately after OVX, are able to prevent the influence of the estrogen deficiency on the alveolar bone. These findings are in line with previous reports which showed that estrogen deficiency promoted an increased bone loss resulting from ligature-induced periodontitis (16,17), decreased bone healing and density around titanium implants (21), and changes in the alveolar bone of ovariectomized rats (22).

Clinical studies that assessed osteoporosis in the jaws have reported that the reduction in total skeletal mass is directly related to the mandibular BD in estrogen-deficient women (23,24). There have been some reports on the mechanisms involved in the estrogen regulation of bone metabolism. It is known that interleukin-6, interleukin-1 (8,9) and two key bone molecules, receptor activator of nuclear factor-kappa β ligand (RANKL) and osteoprotegerin (OPG) (7) co-operatively control osteoclastic bone resorption during estrogen deficiency. In brief, bone resorption is significantly reduced by RANKL function inhibition via its decoy receptor, OPG. As estrogen has been reported to control bone resorption acting on OPG, it may be suggested that estrogen deficiency induces an imbalance in the RANKL/OPG system, favoring osteoclast differentiation. In addition to this indirect effect of estrogen upon osteoclasts (7–9), there is increasing evidence to suggest that estrogen can also directly modulate osteoclasts (6). Although one can assume that the balance between pro- and antiresorptive molecules is responsible for the present findings, further studies are required to elucidate the predominant molecular mechanism of estrogen deficiency on the alveolar bone.

The skeletal benefits of the antiresorptive medications have been reported in several clinical trials involving all types of treatments for postmenopausal osteoporosis (13,25,26). To the best of the authors' knowledge, the effect of antiresorptive drugs, associated with an estrogen-deficient state, on the density of tooth-supporting bone has not yet been reported. Therefore, this is the first study to compare the impact of three antiresorptive agents on the tooth-supporting alveolar bone around periodontally healthy teeth in estrogen-deficient animals. The findings presented herein are in agreement with previous reports showing that estrogen and ALD, but not CT, protect against the development of osteopenia in OVX rats (16,17). Moreover, the results of the present study support animal and clinical studies suggesting that CT may not be as effective as estrogen and ALD for the prevention of skeletal bone loss resulting from an estrogen-deficient state (15,27).

CT is a polypeptide hormone that inhibits bone resorption by acutely blocking osteoclast activity, and is used in the treatment of postmenopausal and senile osteoporosis (25). In the present investigation, the administration of CT did not protect alveolar bone against the effect of estrogen deficiency. As previously reported, this phenomenon may be explained on the basis of an important drawback to CT therapy (28–30). It has been demonstrated that bone response to CT may decline with the length of treatment, probably as a result of CT-induced loss of CT receptors, resulting in hormone-induced resistance (28,29) and, finally, skeletal resistance to CT may develop as a consequence of antibody formation (30).

In contrast, several studies have reported the benefits of estrogen-

replacement therapy on skeletal bone (26), oral bone, periodontal tissue and tooth loss (31). The present study demonstrated that E therapy provides protection against the development of osteopenia in the alveolar bone to an extent similar to that provided by ALD. The mechanism by which exogenous estrogen inhibits bone resorption is likely to be the same as the mechanism of endogenous estrogen, that is, by controlling the production and stimulation of cytokines (i.e. interleukin-1 and interleukin-6) (8) and molecules (i.e. RANKL and OPG) (9) that promote osteoclast differentiation. In agreement with the results of the present study, in general, ALD has been shown to prevent alveolar bone resorption and to preserve oral bone mass in animals (32) and clinically to increase bone mineral density and to prevent bone loss (13). At an ultrastructural level, studies have shown that ALD exerts a positive influence on mesenchymal progenitor-cell metabolism, influencing the modulation of the osteoblastic cell cycle and enhancing the transition towards greater osteoblast differentiation (33). In addition, ALD acts by attaching itself to the osteoclast surface, preventing the degradation of the calcified tissue and acting as an osteoblast kinase (33). The data of the present study demonstrate that ALD induced a positive balance in the alveolar bone, supporting the hypothesis of this metabolically positive action of the bisphosphonates on bone cells.

In conclusion, the results of the present investigation provide important evidence to suggest that estrogen deficiency may negatively influence the tooth-supporting BD, and reinforce the hypothesis that, like skeletal bone, oral bone may be affected. Furthermore, E and ALD, but not CT, protected against this negative effect. Despite the benefits of E and ALD observed in the current study, caution should be used to interpret these findings. Estrogen therapies may present relative risks for cardiovascular disease, for breast, colorectal and ovarian cancer, and for pulmonary embolism (12,34). Similarly, recent reports have focused on osteonecrosis of the jaws, gastrointestinal and ocular side-effects as potential complications of bisphosphonate therapy (35–37). Because each osteoporosis treatment has a unique benefit–risk profile, deciding whether estrogen, bisphosphonates or another treatment is appropriate for an individual requires assessing the risks and benefits of each treatment. Therefore, information exchange between doctors and dentists is sometimes essential to determine the patient's risk factors (age, race/ethnicity, antecedent risk status and prior disease), current medications and personal preferences.

Finally, in addition to the evidence of the importance of estrogen in the oral bone status, the findings of the present study may also constitute an exciting approach for future clinical explorations.

Acknowledgements

Dr Duarte was supported by FAPESP (São Paulo State Foundation for Research), process # 02/08555–7. Dr Nociti Jr was supported by the National Council of Research (304464/03–1, CNPq, Brazil).

References

1. Harrison JE. Osteoporosis. In: Tam CS, Heersche JNM, Murray TM, eds. *Metabolic Bone Diseases: Cellular and Tissue Mechanisms*. Boca Raton: CRC Press, 1989: 240–267.
2. Heaney RP, Recker RR, Saville PD. Menopausal changes in bone remodeling. *J Lab Clin Med* 1978;**92:**964–970.
3. Kribbs PJ. Comparison of mandibular bone in normal and osteoporotic women. *J Prosthet Dent* 1990;**63:**218–222.
4. Chesnut CH, 3rd. The relationship between skeletal and oral bone mineral density: an overview. *Ann Periodontol* 2001;**6:**193–196.
5. Bodic F, Hamel L, Lerouxel E, Basle MF, Chappard D. Bone loss and teeth. *Joint Bone Spine* 2005;**72:**215–221.
6. Eriksen EF, Colvard DS, Berg NJ *et al.* Evidence of estrogen receptors in normal human osteoblast-like cells. *Science* 1988;**241:**84–86.
7. Lindberg MK, Erlandsson M, Alatalo SL *et al.* Estrogen receptor alpha, but not estrogen receptor beta, is involved in the regulation of the OPG/RANKL (osteoprotegerin/receptor activator of NF-kappa B ligand) ratio and serum interleukin-6 in male mice. *J Endocrinol* 2001;**171:**425–433.
8. Pacifici R. Is there a causal role for IL-1 in postmenopausal bone loss? *Calcif Tissue Int* 1992;**50:**295–299.
9. Girasole G, Passeri G, Pedrazzoni M, Giuliani N, Passeri M. Interleukin-6: a pathogenetic role in the postmenopausal osteoporosis? *Acta Biomed Ateneo Parmense* 1995;**66:**125–138.
10. Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. *Science* 2000;**289:**1508–1514.
11. Burg MA, Fraser K, Gui S *et al.* Treatment of menopausal symptoms in family medicine settings following the Women's Health Initiative Findings. *J Am Board Fam Med* 2006;**19:**122–131.
12. Regan MM, Emond SK, Attardo MJ, Parker RA, Greenspan SL. Why do older women discontinue hormone replacement therapy? *J Womens Health Gend Based Med* 2001;**10:**343–350.
13. Buffo L, Rossini M, Buoncristiano A *et al.* Diphosphonates: an alternative to estrogen therapy in postmenopausal osteoporosis. Experience with alendronate. *Minerva Ginecol* 1996;**48:**263–272.
14. Wronski TJ, Yen C-F, Burton KW *et al.* Skeletal effects of calcitonin in ovariectomized rats. *Endocrinology* 1991;**129:**2246–2250.
15. Hejdova M, Palicka V, Kucera Z, Vlcek J. Effects of alendronate and calcitonin on bone mineral density in postmenopausal osteoporotic women. An observational study. *Pharm World Sci* 2005;**27:**149–153.
16. Duarte PM, de Assis DR, Casati MZ, Sallum AW, Sallum EA, Nociti FH Jr. Alendronate may protect against increased periodontitis-related bone loss in estrogen-deficient rats. *J Periodontol* 2004;**75:**1196–1202.
17. Duarte PM, Goncalves PF, Sallum AW, Sallum EA, Casati MZ, Humberto Nociti F Jr. Effect of an estrogen-deficient state and its therapy on bone loss resulting from an experimental periodontitis in rats. *J Periodont Res* 2004;**39:**107–110.
18. Klemetti E, Collin H-L, Forss H, Markkanen H, Lassila V. Mineral status of skeleton and advanced periodontal disease. *J Clin Periodontol* 1994;**21:**184–188.
19. Johnson RB, Gilbert JA, Cooper RC *et al.* Alveolar bone loss one year following ovariectomy in sheep. *J Periodontol* 1997;**68:**864–871.
20. Wronski TJ, Schenck PA, Cintron M, Walsh CC. Effect of body weight on osteopenia in ovariectomized rats. *Calcif Tissue Int* 1987;**40:**155–159.
21. Nociti FH Jr, Sallum AW, Sallum EA, Duarte PM. Effect of estrogen replacement and calcitonin therapies on bone around titanium implants placed in ovariectomized rats: a histometric study. *Int J*

Oral Maxillofac Implants 2002;**17:** 786–792.
22. Tanaka M, Ejiri S, Toyoka E, Kohno S, Ozawa H. Effects of ovariectomy on trabecular structures of rats alveolar bone. *J Periodont Res* 2002;**37:**161–165.
23. von Wowern N, Klausen B, Kollerup G. Osteoporosis: a risk factor in periodontal disease. *J Periodont* 1994;**65:**1134–1138.
24. von Wowern N. Bone mineral content of mandibles: normal reference values – rate of age-related bone loss. *Calcif Tissue Int* 1988;**43:**193–198.
25. González D, Ghiringhell G, Mautalen C. Acute antiosteoclastic effect of salmon calcitonin in osteoporotic women. *Calcif Tissue Int* 1986;**38:**71–75.
26. Weinstein RS, Parfitt AM, Marcus R, Greenwald M, Crans G, Muchmore DB. Effects of raloxifene, hormone replacement therapy, and placebo on bone turnover in postmenopausal women. *Osteoporos Int* 2003;**14:**814–822.
27. Shen Y, Li M, Wronski TJ. Skeletal effects of calcitonin treatment and withdrawal in ovariectomized rats. *Calcif Tissue Int* 1996;**58:**263–267.
28. Gruber HE, Ivey JL, Baylink DJ *et al.* Long-term calcitonin therapy in postmenopausal osteoporosis. *Metabolism* 1984;**33:**295–303.
29. Messer HH, Copp DH. Changes in response to calcitonin following prolonged administration to intact rats. *Proc Soc Exp Biol Med* 1974;**146:**643–647.
30. Singer FR, Aldred JP, Neer RM, Krane SM, Potts JT, Bloch KJ. An evaluation of antibodies and clinical resistance to salmon calcitonin. *J Clin Invest* 1972;**52:**2331–2338.
31. Paganini-Hill A. The benefits of estrogen replacement therapy on oral health. *Arch Intern Med* 1995;**155:**2325–2329.
32. Yaffe A, Iztkovich M, Earon Y, Alt I, Lilov R, Binderman I. Local delivery of an amino bisphosphonates prevents the resorptive phase of alveolar bone following mucoperiosteal flap surgery in rats. *J Periodontol* 1997;**68:**884–889.
33. Rodan GA. Mechanisms of action of bisphosphonates. *Annu Rev Pharmacol Toxicol* 1998;**38:**375–388.
34. La Croix AZ. Estrogen with and without progestin: benefits and risks of short-term use. *Am J Med* 2005;**118:**79–87.
35. Hansen T, Kunkel M, Weber A, James Kirkpatrick C. Osteonecrosis of the jaws in patients treated with bisphosphonates – histomorphologic analysis in comparison with infected osteoradionecrosis. *J Oral Pathol Med* 2006;**35:**155–160.
36. Pires FR, Miranda A, Cardoso ES *et al.* Oral avascular bone necrosis associated with chemotherapy and biphosphonate therapy. *Oral Dis* 2005;**11:**365–369.
37. Aki S, Eskiyurt N, Akarirmak U *et al.* Gastrointestinal side effect profile due to the use of alendronate in the treatment of osteoporosis. *Yonsei Med J* 2003;**44:** 961–967.