Evaluation of sodium alendronate activity on bone repair under hormones absence

Avaliação da ação do alendronato sódico sobre a reparação óssea na ausência dos hormônios ovarianos

Ana Lourdes da Silva MACHADO
Claudia Maria de Oliveira Monteiro da SILVA
Mestrando – Programa de Pós-Graduação – Área de Concentração em Biopatologia Bucal – Faculdade de Odontologia de São José dos Campos – UNESP – São José dos Campos – SP – Brazil

Adriana Aigotti H. BRANDÃO
Professora – Departamento de Biopatologia e Diagnóstico – Faculdade de Odontologia de São José dos Campos – UNESP – São José dos Campos – SP – Brazil

Emília Angela Loschiavo ARISAWA
Faculdade de Ciências da Saúde –Universidade do Vale do Paraíba – UNIVAP – São José dos Campos – SP – Brazil

Rosilene Fernandes da ROCHA
Professora – Departamento de Biopatologia e Diagnóstico – Faculdade de Odontologia de São José dos Campos – UNESP – São José dos Campos – SP – Brazil

Abstract

This research proposes to study sodium alendronate effect on bone repair of ovarectomized rats. Thirty-six adult rats were ovariectomized and distributed in four groups, with nine rats in each group. A surgical defect was performed in one tibia of each animal. The control group received only water "ad libitum" and the three experimental groups received sodium alendronate diluted in the drinking water in the doses of 0.25 mg/kg; 0.5 mg/kg e 0.75 mg/kg, respectively. Three animals of each group were sacrificed after three, seven and 14 days. The histhologic analysis of the tibia material showed a more marked bone deposition in the groups treated with higher doses of sodium alendronate. The most mature bone repair process was observed in the 14-days/0.5 mg/kg group, with bone spindles and remodeling in a higher degree of development, showing a dose-dependency regarding sodium alendronate.

Uniterms

Bone regeneration; alendronate; estrogen replacement therapy, ovariectom; rats

Introduction

The bony repair became a great research field, with the development of the Molecular Biology, because the studies about the cytokines participation, growth factors and hormones are fundamental in this process.

Ovarian hormones seem to have the capacity to interfere with the balance between the bony reabsorption and the bony formation, in the women after the menopause, the rate of bony loss can be accentuated, generating a reduced bony mass as it is the case of the osteoporosis, that is a generalized disturbance of the skeleton, and can turn it in a serious fracture.

It is possible to intervene in this process through hormonal therapy and medications that inhibit the bony reabsorption. The bisphosphonates are new medications and considered a the great hope of the therapeutic possibilities in the prevention and treatment of the osteoporosis, mainly the sodic alendronate, a bisphosphonate of a second generation, presenting as an effective inhibit of the bony reabsorption with smaller collateral effects.

The cellular and molecular events responsible for the formation of the bone during the embryogenesis, the bony remodeling and repair of a fracture are virtually identical. Although this perspective seems initially to simplify a group of complex events too much, detailed studies of responsible individual parts for these sequences, they reveal notable referring likeness to its histological, functional and physiologic aspects. Where a common cell would be involved in these events, that denominated cell of mesenchymal origin cell, it would not only participate of the bony embryogenesis, but it would also continually supply

of cells osteoprogenitor, in the repair process and bony remodeling during the adult life[4].

The bone is one of the few tissues of an adult human being that totally regenerates its form and function after an injury. After a fracture, a hematoma is formed and an inflammatory response, where biochemical signals are liberated, as well as the Bone morphogenic protein, a potent inducer of bony formation. Local cellular proliferation begins and continues for approximately three days, until the induction of the mesenchymal cells. For five days, polymorphonuclear leukocytes, histiocytes, lymphocytes and mast cells migrate for the place of the lesion, the granulation tissue is formed while platelets liberate platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), which are chemoattractants and mitogens. The fibroblasts join proliferating capillary bud, allowing that the place of the fracture in repair comes to be colonized by preosteoblasts[16].

The osteoporosis is a systemic metabolic disorder associated with a decreased bone mass and resistance[10], it means that an individual presents a mass bone inferior than it would be expected from relative norms for the age and sex with increasing risk of fractures. For the women in the menopause, where it happens the loss of the ovarian hormones for natural menopause or induced artificially, it is the most decisive and clearly identifiable factor of the subsequent loss of the bone mass and of fracture risk[17].

Female rats are models adapted for the osteopenia by estrogen deficiency, since the events in the bony levels are similar to those that happen in human beings, after the surgical menopause. It is observed, osteopenia and increased indices of reabsorption and bony formation in tibias of ovariectomized rats in the 14th day after the ovariectomy, through histomorphometry analysis. Those values became progressively pronounced above a hundred days. Therefore its results indicated that there was a great bony loss in the ovariectomized rats, thus the increasing reabsorption overcomes the concomitant increasing bony formation[18].

Estrogen deficiency in mammals increases the bony turnover and it results in an imbalance between a bone reabsorption and formation that takes to a decrease in the bony mass. In mice, the bony mass is reduced in eight weeks after the ovariectomy, while in human beings the bony loss is detected in one year[15].

Bisphosphonates are synthetic drugs with high affinity for the phosphate of calcium. Thus, the sodic alendronate appeared as a new medication with capacity of inhibiting the bony reabsorption without intervening with the bony formation, bringing great hopes in its therapeutic use for not presenting the undesirable effect of inhibiting the bony formation and to present quite reduced, collateral effects when compared with other first generation biphosphonates, but also with other medications used in the treatment of the osteoporosis as the calcitonin, that is an excellent medication but, with many collateral effects[12].

The bisphosphonates have a strong affinity for calcium phosphate and therefore, for the bone. They inhibit the formation and aggregation, as well as the slow down the dissolution of calcium phosphate crystals. Studies in vitro and in vivo demonstrated the inhibition of the bone reabsorption, clinically considered the most important action. Thus, there is a consent that the bisphosphonates have their action in the inhibition of the osteoclasts[5-6], but it still exists indirect action through the osteoblasts. It is not known which is the more important of the two mechanisms. It is known that the alendronate concentration in the osteoclasts has been calculated reaching very high values. Those bisphosphonates induce changes in the morphology of the osteoclasts, which presented more nuclei, changes in the border in brush that seemingly it is shown inactive[6]. Bisphosphonates, aside from their role as inhibitors of osteoclastic bone resorption, are promoters of osteoblast proliferation and maturation[7].

The most favorable dose in the osteoporosis is 10mg daily in women with age from 45 to 80 years. The results still showed an increase around 7-8% in the bony densitometry of treated patients, against a loss around 1% for the patients treated with placebo, allowing to conclude that there was a decreasing in the incidence of fractures in the women treated with the alendronato[5]. Significant reduction in serum and urinary calcium levels and the number of osteoclasts revealed the pronounced suppression of bone resorption in the patients treates with the alendronato[2].

This research proposes to observe the action of the sodic alendronate about the bony repair of surgical defects produced in tibias of ovariectomized female rats.

Material and method

This research was approved by the Committee of Ethics in Research – CEP, under the Protocol no. 08/99-PA/CEP. 36 adult female rats were used, of the race Wistar (Rattus norvegicus, var. albinus) with sixty days of age, weighing around 200g.

The used medication was the sodic alendronate*, a bisphosphonate of second generation with capacity of inhibiting the reabsorption of the bone without inhibiting its mineralization, being presented in tablets of 10 mg, that were diluted and added in the drink water.

The female rats were weight and anesthetized with solution of Ketalar** (cetamina-2-[0-clorofenil]2-[metilamino]cloridrato of ciclohexanona) and Rompun*** (cloridrato of 2-(2,6-xilidino)-5,6-dihidro-4H-1,3-tiazina), in the proportion of 1:0,5mL, in the dose of 0,1mL/100g, through intramuscular.

The animals were fixed in lateral position in a cork plate, allowing the observation and easy access to the lateral flank. After shaving the area below the rib the skin was cleaned with iodized alcohol, a 1cm incision was accomplished in the skin and in the musculature, permitting to visualize the ovary. It is pulled out of the abdominal cavity with aid of tongs, the ovarian arteries were tied, and they were taken out. Then, the musculature and the skin were sutured, being that process accomplished bilaterally.

After thirty days of the ovariectomy, all the female rats were anesthetized again; the left posterior paw was shaved. With an incision in the skin, the musculature was pulled, exposing the animal tibia. The periosteum was retracted with a number 7 spatula and with aid of number 6 spherical drill, in low rotation motor, under constant irrigation with physiologic serum (NaCl – 0,9%), the same was perforated. After the lesion, the place was plenty washed with physiologic serum. And it was sutured with number 4.0 nylon thread.

Then four groups were formed of ovariectomized female rats with nine animals in each, three of them were treated and one was not (a control group):

a) group 1 – control animals, treated only with drink water;
b) group 2 – animals treated with the sodic alendronate diluted in the drink water, in a daily dose of 0.25mg/kg, for 3, 7 and 14 days;
c) group 3 – the sodic alendronate daily dose was 0.5mg/kg for 3, 7 and 14 days;
d) group 4 – the sodic alendronate daily dose was 0.75mg/kg for 3, 7 and 14 days.

The female rats were sacrificed in three, seven and 14 days after the bony lesion, being three animals of each group. Then the tibias were taken out, dissected and placed in formol at 10% for fixation, being guided for the histologycal preparation.

The surgical pieces were decalcified with solution of formic acid at 20%, for two months, to undergo later they by the histologycal technical common of impregnation for paraffin.

The sections were cut in microtome in a 5µm thickness and stained by the method HE (Hematoxylin and Eosin) and Mallory's Trichrome. The sections were examined in the light microscopia for analysis of the levels of bony repair.

Results

The sections were analyzed as the form of the trabeculae, its maturity (Table 1), place in the cavity of the bony defect (for example, if the new bone occupied the whole cavity, just the close part to the perforation hole or just the deep of the cavity) and still the amount of new bone (Table 1), filling a third, two thirds or the total of the cavity of the bony defect (Figure 4). The quality of the granulation tissue was also checked through the amount of its cells and morphologic aspect observed in the cavity of the bony defect.

The animals of the control group were ovariectomized, not treated, within three days formed bone trabeculae, occupying two thirds of the cavity, presenting immature aspect with great amount of osteoblasts; within seven days, they still presented slight morphologic difference with immature trabeculae aspect; and at 14 days the specimens presented a new bone formed with a mature aspect, with long, interlaced and thick trabeculae accumulating in the superior portion close to the bony lesion without reaching in the cavity deep. The remaining of the cavity had already begun to be filled by medular tissue.

The ovariectomized animals and treated with 0.25 mg/kg dose for three days, just presented granulation tissue, with plenty cells, organized and adhered to the cavity walls of the bony defect, but without bone formation; within 7 days plenty of bone formation was observed, practically closing the cavity with thin, irregular and immature trabeculae, beside a small amount of granulation tissue and within 14 day poslesion, two superior thirds of the cavity were filled by thick and mature trabeculae and the remaining of the cavity were filled by narrow (Figure 1).

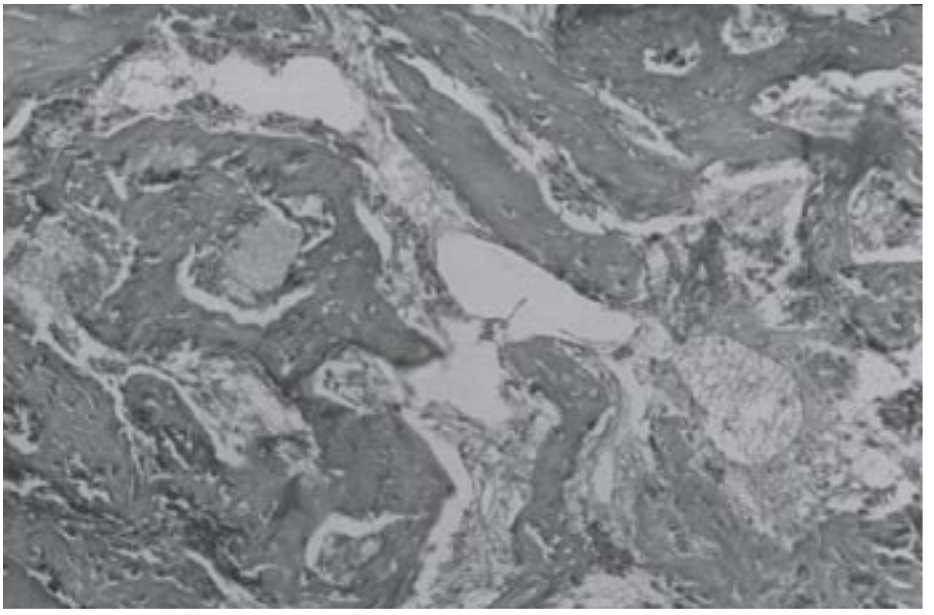

FIGURE 1 – Thin, irregular and immature trabeculae bony –.0.25mg/kg/7days.

The treated animals with the 0.5 mg/kg dose for three days, presented granulation tissue close to the hole well provided in cells and organized while the deep of the bony cavity defect had slack aspect, with several edema areas and no bony formation.

The animals sacrificed in the seventh day showed a great amount of bone formation of endosteal origin. The trabeculae were of immature aspect, and the remaining of the cavity presented granulation tissue plenty of cells (Figure 2).

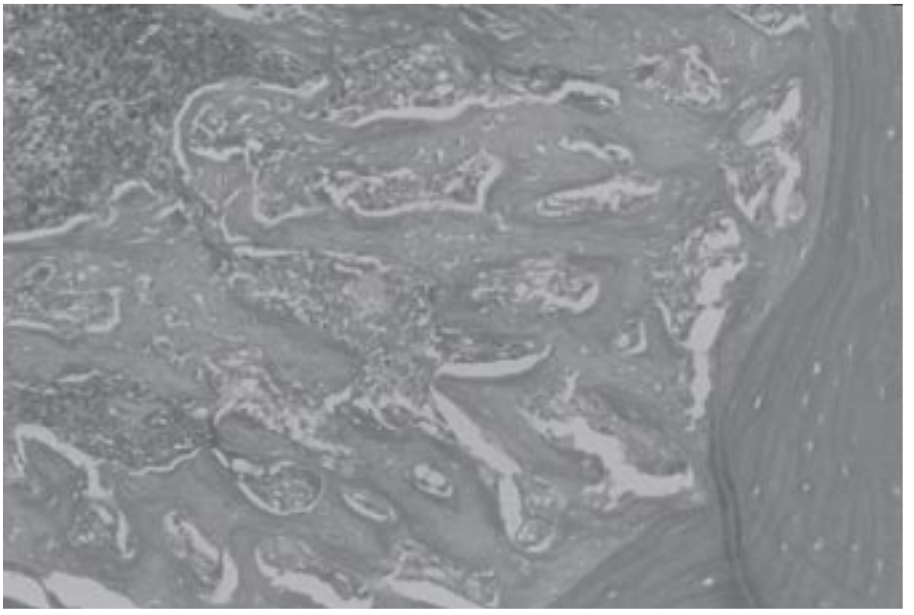

FIGURE 2 – Immature trabeculae bony with great amount of osteoblasts 0.5 mg/kg/7days.

In the 14th day sacrificed animals many part of the cavity was filled by many thick, mature and fused trabeculae and the remaining of the cavity with marrow tissue. The new bone tissue presented closer to the perforation hole and showed cortical remodeling.

The treated animals with a 0.75 mg/kg dose, sacrificed in the third day, presented two thirds of the cavity of the bony defect filled by slack granulation tissue, poor in cells, with edema areas and a third filled with granulation tissue richer in cells and organized, without bone formation.

In the seven day group was observed a lot of bone formation of endosteal origin which filled two thirds of the cavity of the bony defect and the granulation tissue was rich in cells and were organized occupying the remaining of the cavity (Figure 3).

The cavity of the bony defect of the 14 days group, showed near the perforation hole, a third filled by thick bony trabeculae of mature aspect, showing a further repair process, besides presenting a formation of marrow tissue, that occupied the deep of cavity with cortical remodeling.

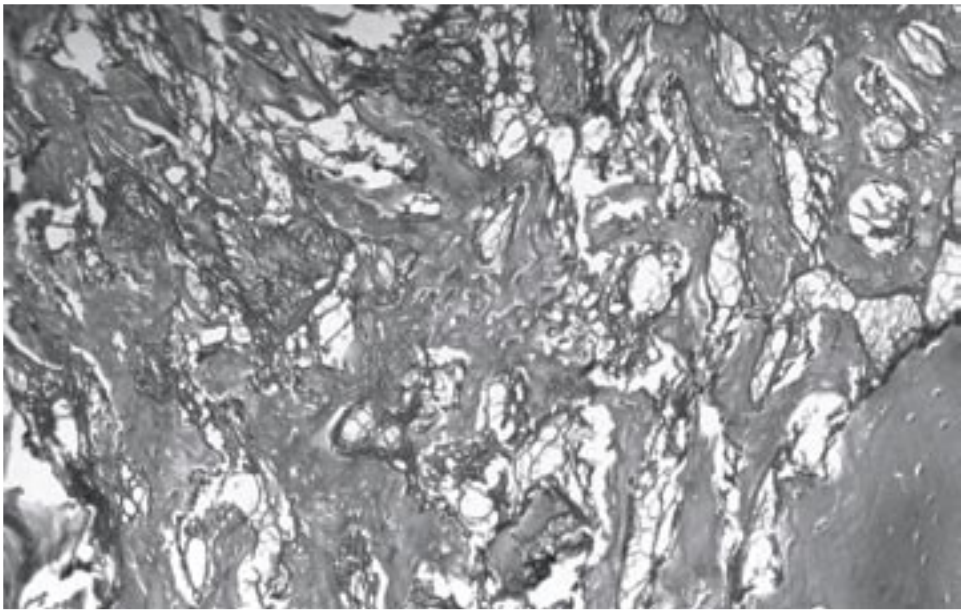

FIGURE 3 – Great bone formation but of a more immature aspect 0.75 mg/kg/7days.

Table 1 – Bone new formed quantify and bone trabeculae maturity, in the several groups

Groups	**3 days**		**7 days**		**14 days**	
	amount	maturity	amount	maturity	amount	maturity
Control	2/3	immature	1/3	immature	2/3	mature
0.25mg/kg	0	--	3/3	immature	2/3	mature
0.5mg/kg	0	--	2/3	immature	2/3	mature
0.75mg/kg	1/3	immature	2/3	immature	1/3	mature

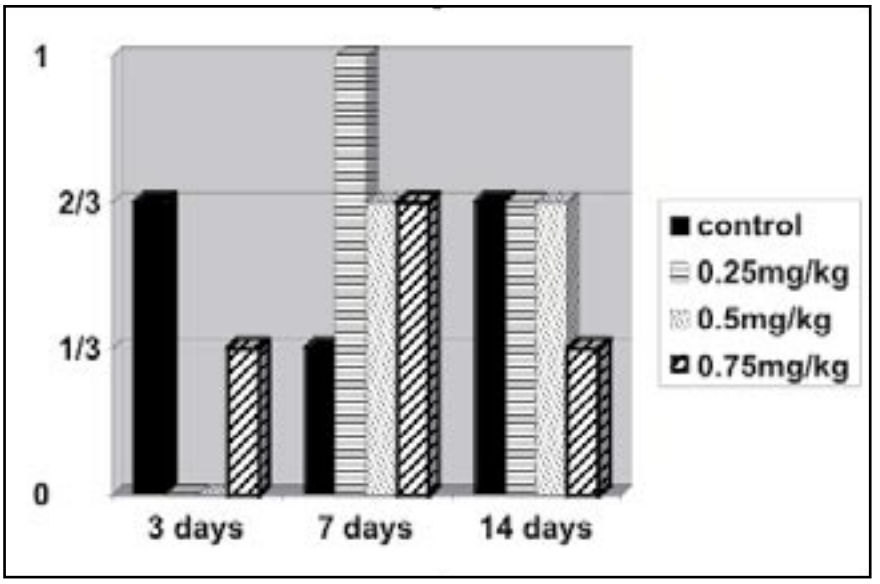

FIGURE 4 – Amount of the bone new formed, in the several groups.

Discussion

Our results are in agreement with the literature, because we have found an important bony deposition in the treated group for 14 days with a 0.5mg/kg dose. That group presented a greater number of mature trabeculae already in remodeling process, further than the group of 14 days of 0.75mg/kg dose, what is in agreement with Seedor et al.[15], 1991, Müller et al.[9], 1998 & Sama et al.[14], 2004 that affirmed to be the sodic alendronate action dose-dependent. Thus, group 3 with a 0.5mg/kg dose presented better results, in spite of having begun with a slacker granulation tissue than the other groups.

In the experimental time of seven days, it was observed that as larger the dose was, the best was the quality and the amount of the formed bone, because the treated group with 0.25 mg/kg dose, formed more bony tissue than the group control; but the treated group with 0.5 mg/kg dose presented a larger bony formation with mature trabeculae, while the group of 0.75mg/kg dose, also presented a great bone formation, but of a more immature aspect. Those results are in agreement with Lin et al.[7], (1991), Seedor et al.[15] (1991), Sahni et al.[13] (1993), Rodan & Fleisch[11] (1996), Fleisch[5], (1997), Azuma et al.[3], 1998, Müller et al.[9], 1998; McClung et al.[8], (2004).

The granulation tissue is much well organized in the smaller doses of alendronate groups, richer in cells in the treated groups with 0.25 and 0.5 mg/kg dose, and very slack in the group of 0.75mg/kg dose. Thus, in the experimental time of three days, the control seems to develop better and as larger the medication dose, is slacker the granulation tissue is, what seems to be due to an inhibition of the inflammatory answer[1].

Conclusions

The histologic evaluation of the treated defects and control determined the following conclusions:

a) Sodic alendronate acts in the bony repair, forming a greater bone amount in a seven and 14 days periods.
b) The sodic alendronate actions in bony repair is dose-dependent.
c) Better results were obtained with the 0.5mg/kg dose.
d) Probably there was interference in the initial inflammatory process, by delaying the formation and organization of the granulation tissue.

RESUMO

Objetivou-se avaliar a ação do alendronato sódico na reparação óssea de ratas ovariectomizadas. Foram utilizados 36 ratos fêmeas adultas ovariectomizadas sendo formados quatro grupos, com nove ratas em cada grupo, onde foi realizada uma lesão na tíbia. O grupo controle não recebeu medicamento, enquanto os outros três foram tratados com doses de 0,25 mg/kg, 0,5mg/kg e 0,75 mg/kg na água de beber, diariamente. Foram sacrificadas três ratas de cada grupo nos períodos de três, sete e 14 dias. A análise histológica das tíbias desses animais revelou uma deposição óssea mais marcante nos grupos tratados com as doses maiores de alendronato. O grupo de 14 dias com dose de 0,5 mg/kg, apresentou a deposição de trabéculas mais maduras e organizadas, sugerindo um processo de remodelação já bem desenvolvido, mostrando assim ser a ação do alendronato sódico dose-dependente.

UNITERMOS

Reparação óssea; alendronato; terapia de reprodução de estrogênios; estrogênios; ovariectomia; ratos

REFERENCES

1. Adami S, Bhalla AK, Dorizzi R, Montesanti F, Rosini S, Salvagno G et al. The acute-phase response after bisphosphonate administration. Calcif Tissue Int. 1987; 41(6): 326-31.
2. Altundal H, Güvener O. The effect of alendronate on resorption of the alveolar bone following tooth extraction. Int J Oral Maxillofac Surg. 2004 Apr.; 33(3): 286-93.
3. Azuma Y, Ove Y, Katani H, Ohta T, Kiyoki E, Komoriya K. Effects of continuos alendronate treatment on bone mass and mechanical properties in ovariectomized rats: comparison with and alendronate in growing rats. J Pharmacol Exp Ther. 1998 July.; 286(1): 128-35.
4. Bruder SP, Fink D J, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J Cell Biochem. 1994; 56: 283-94.
5. Fleisch HA. Bisphosphonates: preclinical aspects and use in osteoporosis. Ann Med. 1997 Feb.; 29: 55-62.
6. Im GI, Qureshi AS, Kenney J, Rubash HE, Shanbhag AS. Osteoblast proliferation and maturation by bisphosphonates. Biomaterials. 2004 Aug.; 25(18): 4105-15.
7. Lin JH, Duggan DE, Chen IW, Ellsworth RL. Physiological disposition of alendronate, a potent anti-osteolytic biphosphonate, in laboratory animals. Drug Metab Dispos. 1991; 19(5):926-32.
8. McClung MR, Wasnich RD, Hosking DJ, Christiansen C, Ravn P, Wu M et al. Prevention of postmenopausal bone loss: six-year results from Early Postmenopausal Intervention Cohort Study. J Clin Endocrinol Metab 2004 Oct.; 89(10): 4879-85.
9. Müller K, Wiesenberg I, Jaeggi K, Green JR. Effects of the bisphosphonate zoledronate on bone loss in the ovariectomized and in the adjuvant arthritic rat. Arzneimittel-forschung. 1998; 48(1): 81-6.
10. Pérez-Lopez FR. Postmenopausal osteoporosis and alendronate. Maturitas. 2004 July; 48(3): 179-92.
11. Rodan GA, Fleisch HA. Bisphosphonates: mechanisms of action. J Clin Invest 1996 June.; 97(12): 2692-6.
12. Russell RG, Rogers MJ. Bisphosphonates: from the laboratory to the clinic and back again. Bone. 1999 July; 25(1): 97-106.
13. Sahni M, Guenther HL, Fleisch H, Collin P, Martin TJ. Bisphosphonates act on rat bone resorption through the mediation of osteoblasts. J Clin Invest. 1993 May; 91(5): 2004-11.
14. Sama AA, Khan SN, Myers ER, Huang RC, Cammisa FP, Samdhu HHS et al. High-dose alendronate uncouples osteoclast and osteoblast function: a study in a rat spine pseudarthrosis model. Clin Orthop. 2004 Aug.; 425: 135-42.
15. Seedor JG, Quartuccio HA, Thompson DD. The biphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. J Bone Miner Res. 1991; 6(4):339-46.
16. Szachowicz EH. Facial bone wound healing: an overview. Otolaryngol Clin North Am. 1995 Oct.; 28(5): 865-80.
17. Woolf AD, Dixon AStJ. Osteoporose um guia clínico. Londres: Martin Ddeinitz; 1988.
18. Wronski TJ, Cintrón M, Dann LM. Temporal relationship between bone low and increased bone turnover in ovariectomized rats. Calcif Tissue Int 1988; 43: 179-83.

Recebido em: 21/07/05
Aprovado em: 25/04/06

Claudia Maria de Oliveira Monteiro da SILVA
(12) 3947-1014 Fax: (12) 3947
cmdmoliveira@uol.com.br
Avenida Shishima Hifumi – 2911 –Urbanova
São José dos Campos – SP
12244-000