The effect of zoledronate on bone remodeling during the healing process[1]

Os efeitos do zoledronato na remodelação óssea durante o processo de reparação

Marcos Almeida Matos[2], Francisco Pereira Araújo[3], Fábio Brasileiro Paixão[3]

1. Study performed at Center of Operative Technique and Experimental Surgery, Bahia School of Medicine and Public Health. Salvador, Brazil.
2. PhD, Division of Orthopedics and Traumatology, Department of Surgery, Bahia School of Medicine and Public Health. Salvador, Brazil.
3. Fellow, Department of Surgery, Bahia School of Medicine and Public Health. Salvador, Brazil.

ABSTRACT

Purpose: To check the effect of zoledronate in bone remodeling during bone healing. **Methods**: Thirty rabbits were divided into two groups of fifteen animals each (control and experimental group respectively). Shaft osteotomy was performed on the cranial portion of the fibula of each animal. In the experimental group, a single dose of 0.04mg/kg of zoledronate was administered. In the control group, the same volume of bi-distilled water was administered. After one, two and four weeks, animals of both groups were killed and histological sections of the fibular metaphyseal area were examined histomorphometrically. The parameters analyzed were tissue volume (TV), fractional trabecular bone volume (BV/TV) and fractional medullary fibrous volume (FbV/TV). **Results**: Tissue volume increased in the experimental group ($237.2mm^2.10^{-2}$) compared to the control ($166.62mm^2.10^{-2}$). Trabecular bone volume was significantly larger in the experimental (60.2%) than in the control group (34.8%).The amount of fibrosis volume decreased in the experimental group (22%) compared to the control (49.4%). **Conclusion**: The effect of zoledronate is characterized by accentuated stimulus of bone formation in the metaphyseal area, resulting in a larger amount of trabecular bone volume and little fibrosis volume.

Key words: Diphosphonates. Bone and bones. Metabolism. Osteotomy.

RESUMO

Objetivo: Verificar os efeitos do zoledronato na remodelação óssea durante a reparação óssea. **Métodos**: Trinta coelhos foram divididos em dois grupos de quinze animais cada (grupos controle e experimento, respectivamente). Realizou-se osteotomia na diáfise proximal da fíbula de cada animal. No grupo experimento, aplicou-se dose única de 0,04mg/Kg de zoledronato. No grupo controle, a mesma quantidade de água bi-destilada foi administrada. Após uma, duas e quatro semanas, os animais de ambos os grupos foram sacrificados e cortes histológicos da metáfise fibular foram examinados histomorfometricamente. Os parâmetros analisados foram volume tecidual (TV), fração do volume ósseo trabecular (BV/TV) e fração do volume fibroso medular (FbV/TV). **Resultados**: O volume tecidual aumentou no grupo experimento ($237.2mm^2.10^{-2}$) em comparação com o controle ($166.62mm^2.10^{-2}$). O volume ósseo Trabecular teve aumento significativo no grupo experimento (60.2%) em relação ao grupo controle (34.8%). A quantidade de fibrose medular diminuiu no grupo experimento (22%) em comparação com o controle (49.4%). **Conclusão**: O efeito do zoledronato é caracterizado por acentuado estímulo da formação óssea na área metafisária, resultando em grande quantidade de volume ósseo trabecular e pouca fibrose medular.

Descritores: Difosfonatos. Osso e ossos. Metabolismo. Osteotomia.

Introduction

Bisphosphonates are used in the clinical treatment of several diseases that run their course along with pathological fractures and alteration of bone remodeling, such as osteoporosis, bone metastases, malignant hypercalcemia, Paget's disease, hyperparathyroidism and osteogenesis imperfecta[1,2,3]. Bisphosphonates inhibits osteoclasts recruitment[4,5]. On the other hand, fracture increases osteoblasts recruitment significantly in the healing site and produces rapid bone loss in the metaphyseal area following immobilization[6]. The altered relationship between osteoclasts and osteoblasts population may result in derangement of bone remodeling. It is believed that bisphosphonates do not modify the reparative pattern of the callus[6,7,8], but there are not consistents reports about the effects of these drugs on bone remodeling (formation and resorption) process during fracture healing. It has been observed decrease in bone mineral density of the tibial (44%) and femoral (61%) metaphysis after osteotomy for limb lengthening[9]. Stress-shielding or immobilizatiom create osteopenic bone adjacent to the lesion (fracture) site that is more susceptible to refracture. This loss was only partially recovered at two years after osteotomy[6,9]. The purpose of this study is to check the effect of zoledronate, the most powerful bisphophonate in clinical use, in the histology of the metaphyseal trabecular bone, by means of a controlled experimental model in rabbit, submitted to fibular osteotomy in accordance with the method described by Matos in 2001[10].

Methods

This study was approved by the Research Ethics Committee at Bahian School of Medicine and Public Health and follows the Council for International Organization of Medical Sciences (CIOMS) ethical code for animal experimentation (WHO Chronicle 1985; 39(2):51-6).

Animals – experimental groups

Sample size was calculated for a standard deviation of 10%, difference between proportions of 5% and significance of 0,05. Thirty immature male albino New Zealand rabbits were divided into two groups of fifteen animals each, being assigned to the control and experimental group respectively. The animals were aged about a month and a half when the experiment began in both groups. Initial weight was 918g in the control group and 875 in the experiment group (no significant difference by t-test). The rabbits were acclimatized in the animal care facility for several days, and were housed in individual cages during the entire study period with water and chow diet *ad libitum*.

Experimental design

Food was suspended eight to ten hours prior to administrating anesthesia. To decrease the vagal tonus, each animal received 0.2 mg/kg dose of atropine sulphate by intramuscular injection. Animals were anesthetized by intraperitoneal injection of ketamine (25.0 - 30.0 mg/kg of body weight) and intramuscular injection of diazepam (5.0 to 10.0 mg/kg of body weight)[10].

This experimental osteotomy model in rabbits was reported in 2001[10]. Under aseptic technique conditions, the fibula of each animal was accessed by a lateral incision of approximately 5 mm on the right pelvic limb. After division of the skin and subcutaneous tissue, the fascia of fibular muscles and periosteum were opened and dissected from the cranial portion of the fibula. Shaft osteotomy was performed on the cranial portion of the exposed fibula, using an electric saw with a standardized blade (10.0 mm wide and 0.5 mm thick). The incision was closed in layers, using absorbable 5-0 polyvicryl sutures for the fascia and 5-0 mononylon sutures for the skin.

In the experimental group (Group 2), a single dose of 0.04mg/kg of zoledronate was administered via intraperitoneal injection immediately before the surgical procedure. In the control group (Group 1), the same volume of bi-distilled water was administered under similar conditions. Both groups were submitted to the same surgical procedure. After that, groups 1 and 2 were divided into subgroups A, B, and C, containing five animals each and were numbered 1A, 1B, 1C for control; and 2A, 2B, 2C for experiment. After one (subgroups A), two (for subgroups B) and four weeks (subgroups C), animals of both groups were anesthetized and killed with a 2 ml intracardiac injection of potassium chloride. The fibula of each animal was removed, dissected from the surrounding soft tissue, and fixed in 10% formalin for microscopic evaluation. Formalin-fixed bones were decalcified with 7.5% nitric acid, embedded in paraffin and longitudinally sectioned. Histological sections (7µm thick) were stained with hematoxylin and eosin stain prior to optical microscope examination.

Histomorphometric evaluation of metaphysis

Histomorphometric evaluation was performed in a blinded analysis by all authors and results were an average of three counts. Three histological sections were analyzed for each animal. After the cuts had been chosen, a preliminary analysis was performed at 100X magnification in order to define the area of the methaphysis. Histomorphometric evaluations of all microscopic fields were performed using a test eyepiece reticule with 10 parallel lines and 100 points containing a grid with a total area of 10,500µm^2 (Zeiss 23-9901) at a magnification of 200X. The associated parameters analyzed were tissue volume (TV), fractionary trabecular

bone volume (BV/TV) and fractional medullary fibrous volume (FbV/TV), taken according to previous reports by Prafitt et al[11].

Statistical analysis

The difference among mean continuous values was verified, using the t-Student test (0.05) for parametric data. When verifying the hypotheses of non-parametric data comparing more than two distributions, the Kruskal-Wallace test was used, followed by the Dunn's post-test (when the probability of the former was less than 0.05). In the comparison of two independent non-parametric distributions, the Mann-Whitley test was used at a level of significance of 0.05. Histomorphometric analysis was performed, quantifying the parameters mentioned in Methods, and verification of the difference in relation to time (in the lines of the tables) was checked by the Kruskal-Wallis test; verification of the differences among the control and experimental groups (in the columns of tables) was checked by the Mann-Whitney test.

Results

All animals survived to the end of the study. Neither wound infection nor dehiscence was observed in the animals of either group. The histomorphometric parameters for measuring metaphyseal tissue area, bone volume and fibrous volume in control and experimental groups are presented in Tables 1, 2 and 3. Figures 1 and 2 show the aspects of metaphyseal zone after zoledronate treatment (fourth week) in the control group compared to the experimental group.

TABLE 1 - Methaphyseal tissue area ($mm^2.10^{-2}$)

Group	First week Mean (± standard desviation)	Second week Mean (± standard desviation)	Fourth week Mean (± standard desviation)
Control	166,2 (±37,4)	158 (±50)	166,6 (±29,4)*
Experimental	173,2 (±17,8)#	190,4 (±15,5)	237,2 (±36,8)*#

(#) p<0,05 for Kruskal-Wallis e Dunns tests intragroup
(*) p<0,05 for Mann-Whitney test intergroup

TABELA 2 - Bone volume in the methaphyseal area

Group	First week Mean (± standard desviation)	Second week Mean (± standard desviation)	Fourth week Mean (± standard desviation)
Control	33,4 (±9,68)	26,4 (±4,8)*	34,8 (±7,5)*
Experimental	27 (±6,6)#	55,4 (±10,2)*#	60,2 (±9,4)*#

(#) p<0,05 for Kruskal-Wallis e Dunns tests intragroup
(*) p<0,05 for Mann-Whitney test intergroup

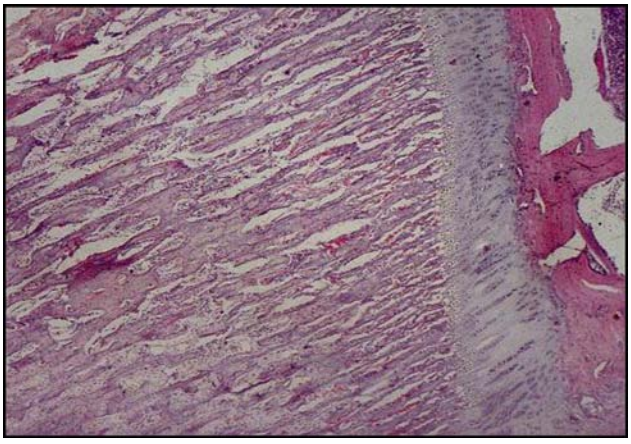

FIGURE 1 - Aspect of bone metaphyseal area from the experimental group displaying a large amount of trabecular bone volume in the fourth week (HE, 200X)

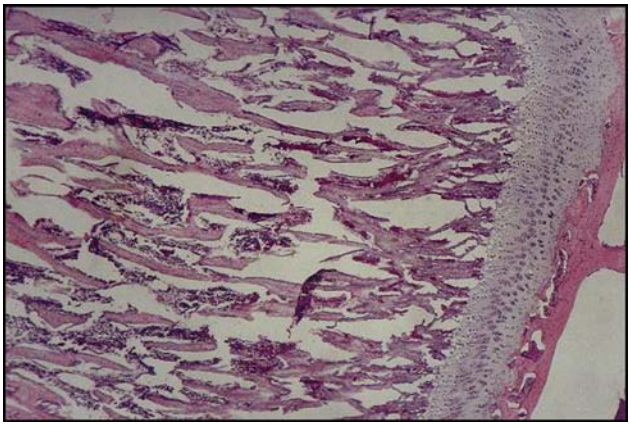

FIGURE 2 - Aspect of bone metaphyseal area from the control group in the fourth week (HE, 200X)

TABELA 3 - Fibrous volume in the methaphyseal area

Group	First week Mean (± standard desviation)	Second week Mean (± standard desviation)	Fourth week Mean (± standard desviation)
Control	55,2 (±14,3)	59,8 (±10,3)	49,4 (±6,9)*
Experimental	55,4 (±10,2)#	41,4 (±9,1)	22 (±5,4)*#

(#) p<0,05 for Kruskal-Wallis e Dunns tests intragroup
(*) p<0,05 for Mann-Whitney test intergroup

Discussion

Growth, modeling and remodeling are three fundamentally different dynamic systems all performed by bone cells (osteoclasts and osteoblasts), but under different control and for different biological reasons. Defects in each produce unique clinical consequences and desease of one system cannot be used as a model to study a desease of another. Thus a healing model alone (growth and modeling) should not be used to study a desease of remodeling (postmnopausal osteoporosis).[1,4,12]

The remodeling process is a surface phenomenon and follows a biologically programmed sequence. Activation of osteoclasts is followed by resorption of mineralized bone matrix and in turn is followed by activation of osteoblasts which replace the resorbed bone. Bone remodeling is a dynamic process that takes place more in trabecular bone than in cortical bone. Metaphysis of long bones contains large amounts of trabecular bone, thus histomorphometric evaluation of metaphyseal area represents bone remodeling process.[1,4,5,12]

Metaphysis contains largely trabecular bone and in this site metabolic turnover (remodeling or bone disease) is proeminently expressed. This process is carried out by the osteoclasts and osteoblasts on bone surfaces which, together with their precussor cells, make up the remodeling system[12].

Tissue volume in the metaphyseal area increased in the experimental group during the experiment period and was significantly larger than control group. Metaphysis was characterized by increase in trabecular bone volume in the fourth week (significantly smaller in the control group). The amount of fibrosis volume decreased significantly in the experimental group and was also significantly in the experimental group. The histomorphometric study confirmed that zoledronate promote increase in madullary area of the metaphysis and produces a larger amount of trabecular bone volume in this region. It suggests that this drug has a stimulative effect on osteoblastic activity and an inhibitory effect on osteoclastic reabsorption and remodeling, shown by the increase of trabecular (lamela) bone. These findings confirm other published experimental studies[3,7,13,14,15,16]. Plotkin et al.[3] conclude that pamidronate increased trabecular bone volume of patients with osteogenesis imperfecta in 6,3%; bone formation rate under the effect of pamidronate increased from 6,6 to 15,3mm^2. Hyvonen et al.[16] investigated the influence of clodronate on bone healing. Clodronate did not alter the histology of the callus nor delayed the healing of the fracture, but it caused mild to moderate proeminence of the metaphyseal area in the fractred bone in a dose- and time-dependent manner. Kaastad et al.[13] conclude that clodronate increased medullary area in ovariectomized rats and preserved both trabecular and cortical bone volume completely. Pyskywec et al[14]. examined distal femoral methaphysis of rabbits with inflamatory arthritis under the effect of zoledronate and found that there was a significantly lower percentage of bone area with defects. These authors conclude that zoledronate was effective in preventing intracortical defects in these cases. Bone loss and osteopenia after osteotomy, limb lengthening or fracture remain problematic[6]. Considerable research effort has been directed to optimize conditions for bone formation and remodeling during fracture healing. We have clearly shown that zoledronate produces increase in trabecular bone volume during healing. Inhibited osteoclastic resorption by bisphosphonates would slow the rate at which bony trabeculae is removed[1,6]. On the other hand, these drugs may have a stimulatory effect on the osteoblastic formation and contribute to more bone being laid down[1,6].

This stimulatory effect of zoledronate on bone formation producing increase in trabecular bone volume would be of great importance and extended the potential applications of the findings of our study. We suppose that bisphosphonates may be a safe and effective strategy to improve bone formation and prevent bone loss and osteopenia during fracture healing. The present study makes some new contributions to scientific literature about the effects of bisphosphonates on remodeling during bone healing process. The authors believe that these drugs may prevent bone loss and osteopenia when trating patients during bone fracture healing or associated conditions.

Conclusion

Under the effect of zoledronate, there was accentuated stimulus of bone formation in the metaphyseal area, resulting in a larger amount of trabecular bone volume and little fibrosis volume.

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Correspondence:
Marcos Almeida Matos
Rua da Ilha, 378, casa 21
41620-620 Salvador – Bahia Brazil
malmeidamatos@ig.com.br

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