The effect of zoledronate on bone remodeling during the healing process

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

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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 (237.2mm².10⁻²) compared to the control (166.62mm².10⁻²). 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 fibula 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 (237.2mm².10⁻²) em comparação com o controle (166.62mm².10⁻²). 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 metáfisária, resultando em grande quantidade de volume ósseo trabecular e pouca fibrose medular.

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 inhibit osteoclasts recruitment\(^4\,\,^5\). On the other hand, fracture by t-test). The rabbits were acclimatized in the animal both groups. Initial weight was 918g in the control group about a month and a half when the experiment began in experimental group respectively. The animals were aged animals each, being assigned to the control and Zealand rabbits were divided into two groups of fifteen significance of 0.05. Thirty immature male albino New of 10%, difference between proportions of 5% and

Experimental design

Food was suspended eight to ten hours prior to administering 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 metaphysis. Histomorphometric evaluations of all microscopic fields were performed using a test eyepiece reticle 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
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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-Wallis 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>
<thead>
<tr>
<th>TABLE 1 - Methaphyseal tissue area (mm²)</th>
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<td>Group</td>
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<td>Control</td>
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<td>Experimental</td>
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(#{}) p<0,05 for Kruskal-Wallis e Dunns tests intragroup
(*{}) p<0,05 for Mann-Whitney test intergroup

<table>
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<th>TABELA 2 - Bone volume in the methaphyseal area</th>
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<tr>
<td>Group</td>
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<td>Control</td>
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<td>Experimental</td>
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(#{}) p<0,05 for Kruskal-Wallis e Dunns tests intragroup
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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)
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 disease of one system cannot be used as a model to study a disease of another. Thus a healing model alone (growth and modeling) should not be used to study a disease of remodeling (postmenopausal 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 prominently expressed. This process is carried out by the osteoclasts and osteoblasts on bone surfaces which, together with their precursor cells, make up the remodeling system12.

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 medullary area of the metaphysis and produces a larger amount of trabecular bone volume in this region. It suggests that this drug has a stimulatory effect on osteoblastic activity and an inhibitory effect on osteoclastic reabsorption and remodeling, shown by the increase of trabecular (lamella) bone. These findings confirm other published experimental studies17,13,14,15,16, 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 15,3mm². 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 prominence of the metaphyseal area in the fractured 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 al14. examined distal femoral metaphysis of rabbits with inflammatory 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 problematic6. 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 removed1,6. On the other hand, these drugs may have a stimulatory effect on the osteoblastic formation and contribute to more bone being laid down1,6.

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 treating 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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References


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Conflict of interest: none
Financial source: none

Received: November 24, 2006
Review: December 19, 2006
Accepted: January 16, 2007

How to cite this article:

* Color figures available from www.scielo.br/acb