Alendronate administered at the fracture site. Experimental study in rabbits

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Abstract

Introduction: The aim of this experimental study was to evaluate radiographic and histological results with the use of alendronte locally instilled on fracture lines in rabbits' femoral bones.

Materials and Methods: We used 30 rabbits which underwent fracture in their right femur, and they were divided into three groups of 10 animals each. Rabbits in group 1 were instilled a solution with alendronate on the fracture line; those in group 2 were subject to the same procedure seven days after the fracture, and those in group 3 played the part of the control group. We carried out radiographic evaluation at the time of the fracture and 42 days after the procedure. We evaluated the characteristics of bone calluses by histological analyses, X-rays and CT scan.

Results: We evaluated 24 rabbits (2 rabbits in group 2 and 4 in group 3 passed away). The histological analysis showed moderate bone formation in the three groups with no statistically significant differences (p=0.8336). According to imaging studies, there were no statistically significant differences in the sizes of bone calluses between groups in neither of the two imaging studies (X-rays: p=0.777 and CT scan: p=0.349).

Conclusion: Alendronate instilled locally on fracture line immediately after the fracture and one week later did not change in statistically significant ways the normal process of bone healing as judged by histological analyses and radiologic studies six weeks after the rabbits had undergone femoral bone fracture.

Key words: Alendronate; bone healing; fracture.

Level of evidence: II

Alendronato administrado en el foco de fractura. Estudio experimental en conejos

Resumen

Introducción: El objetivo de este estudio experimental fue evaluar el resultado radiológico e histológico del empleo de alendronato colocado localmente en el foco de fracturas de fémur en conejos.

Materiales y Métodos: Se utilizaron 30 conejos a los cuales se les fracturó el fémur derecho y se los dividió en tres grupos de 10 animales cada uno. A los conejos del grupo 1 se les colocó una solución con alendronato en el foco de fractura; los del grupo 2 fueron sometidos al mismo procedimiento a los siete días de la fractura y el grupo 3 era de control. Se realizó la evaluación radiográfica en el momento de la fractura y a los 42 días del procedimiento. Se evaluaron las características del callo óseo mediante anatomía patológica, radiología y tomografía computarizada.

Conflict of interests: The authors have reported none.

ISSN 1852-7434 (online) • ISSN 1515-1786 (printed) • http://dx.doi.org/10.15417/699

Resultados: Se evaluaron 24 conejos (2 conejos del grupo 2 y 4 del grupo 3 murieron). El análisis histológico reveló moderada formación ósea en los tres grupos, sin diferencias estadísticamente significativas (p = 0,8336). Según los resultados de los estudios por imágenes, no existieron diferencias estadísticamente significativas en el tamaño del callo óseo entre los grupos para los dos estudios (radiografía: p = 0,777 y tomografía: p = 0,349).
Conclusión: El alendronato colocado localmente en el foco de fractura, en la etapa aguda y luego de una semana, no alteró, de manera estadísticamente significativa, el proceso normal de consolidación, determinado por anatomía patológica y radiología, a las seis semanas de la fractura de fémur en conejos.

Palabras clave: Alendronato; consolidación; fractura.
Nivel de Evidencia: II

Introduction

Bisphosphonates are chemical compounds whose main effect is the inhibition of bone resorption. They inhibit the action of osteoclasts and, moreover, they might act on osteblasts and osteocytes.[1] The bisphsphonates used in medical practice share the following characteristics: they are mainly incorporated by the bones, they develop close bonds with hydroxyapatite crystals, they suppress osteoclastic bone resorption, they are kept by the skeleton for a long time, and they are later eliminated via urine route without undergoing metabolization.[2]

Most fractures heal by means of the so called "process of secondary bone healing", which is a three-stage process: inflammation, reparation, and remodeling.[3] As regards the effects of bisphosphonates on fracture healing, there are reports on children with osteogenesis imperfecta treated with IV bisphosphonates, and these drugs did not have any influence on bone healing.[4] In other publications, bisphosphonates were administered subcutaneously immediately after the fracture, and they did not verify a higher percentage of lack of bone healing.[5] Ruchelli et al. reported that alendronate associated locally with allograft may help improve allograft osseointegration, and they are not associated with neither local nor systemic risks, even in high doses.[6]

The aim of this experimental study was to evaluate the radiologic and histological results of the local administration of alendronate in rabbits' femur-fracture lines.

Materials and Methods

We used 30 adult New Zealander rabbits of unspecified sex. The procedure was approved by the Institutional Ethics Committee on Health Research at Sanatorio Allende. We worked on the rabbits' right femur in all cases. The rabbits were randomly distributed among three groups of 10 animals each, and were assessed before the surgery to rule out diseases or malformations. They inhabited individual cages at 22-25º-environmental temperature; they were fed on balanced food for rabbits (approximately 150-200 g/day) and ad libitum water.

All the rabbits received anesthetic induction with intramuscular 1mg/kg-ketamine and 3-5mg/kg-diazepam; before the procedure, the animals were kept in food-and-water fast for 8 and 2 hours, respectively. We performed a fracture in the rabbits' right femoral dyaphisis following a transverse pattern in all cases. To do that we used two davier levers and a blunt chisel, always protecting soft tissues with compresses.

Animals in group 1 were instilled a solution with alendronate on the fracture line, whereas those in group 2 were subject to the same procedure although 7 days after the fracture. Rabbits in group 3 played the part of the control group (they did not receive alendronate solution at fracture level). All the procedures were guided by fluoroscopy (Figure 1).

The alendronate solution was prepared dissolving two 70 mg-alendronate tablets in 20ml-sterile saline solution; rabbits in groups 1 and 2 were instilled 2 cm^3 of the alendronate solution at the level of the fracture line by fluoroscopic guidance. After the procedure, the fractured limb was put in a splint so as to limit mobilization.

Forty-two days after the procedure we made radiographic evaluation and, immediately afterwards, we sacrificed the animals. We evaluated the characteristics of the bone callus by histological analysis, radiographs, and CT scan, establishing the size of the bone callus formed by week 6 by measuring the distance (in mm) between the femoral cortex and the most prominent area in the bone callus in both CT scan slides and standard X-rays—selected by the same operator in all cases.

For the sake of the histological analysis, we got the specimens' slides by severing the pieces at the level of the middle of the defect and, then, we included the material in paraffin.

We got multiple 6-mm-thick slides by microtome and we processed the pieces with H-E stain for conventional light microscope so as to study bone formation and vascularization using a three-category scale: scarce, light, and moderate.

The sizes of the bone calluses determined by imaging studies were the response or dependant variables we estimated the differences among the three analyzed groups for by maximum likelihood estimation. For this

ISSN 1852-7434 (online) • ISSN 1515-1786 (printed)

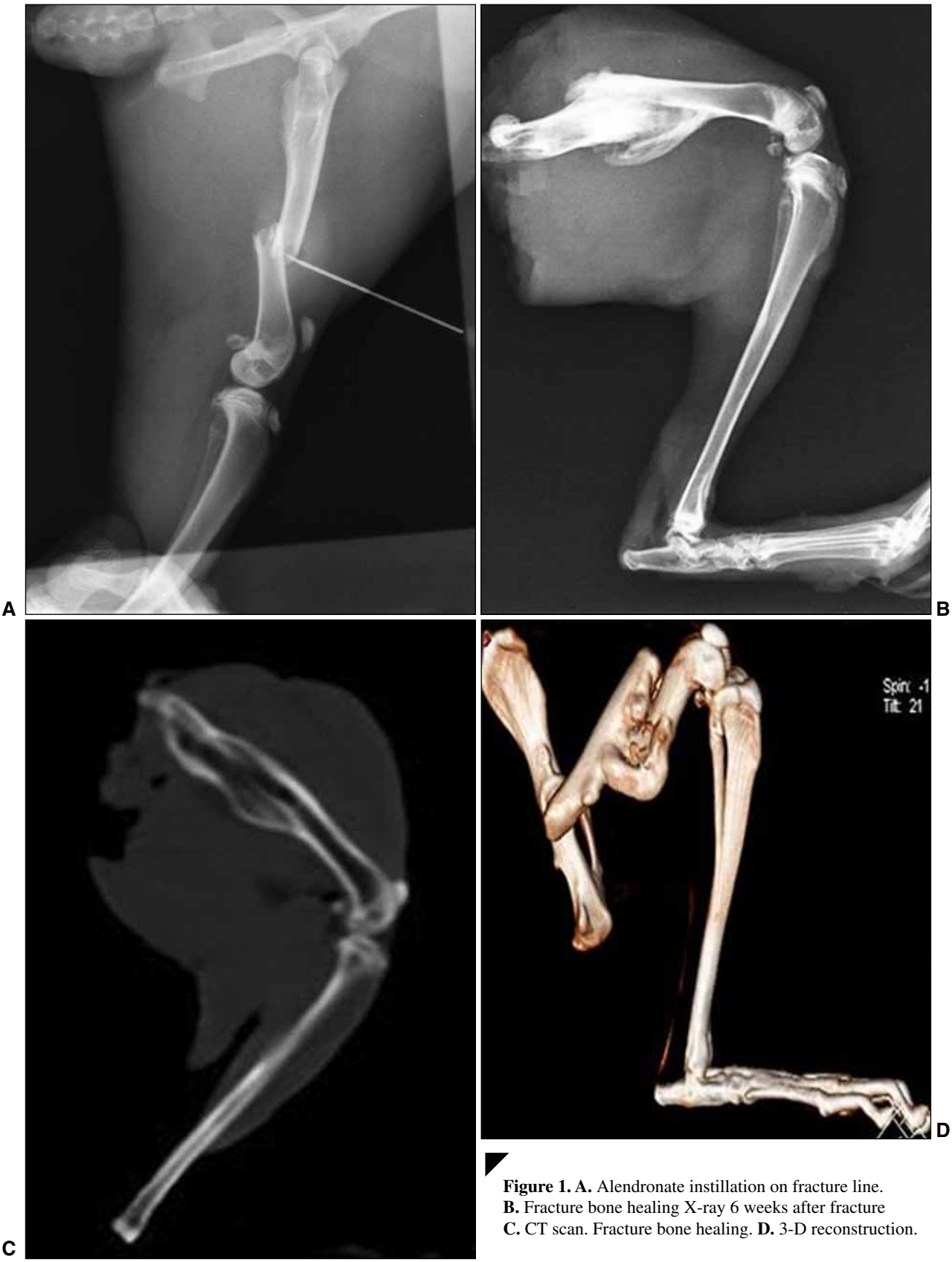

Figure 1. A. Alendronate instillation on fracture line.
B. Fracture bone healing X-ray 6 weeks after fracture
C. CT scan. Fracture bone healing. **D.** 3-D reconstruction.

we adjusted the generalized linear models with gamma distribution (given the asymmetry in the empiric distribution in each group), using a classification variable or treatment factor and considering three levels: alendronate in immediate fracture, alendronate one week after fracture, and control group.

Afterwards, we estimated the association measures (odds ratio) between on the one hand, the healing scales, the vascularization, and the characteristics of bone calluses and, on the other hand, the groups, so as to establish whether or not there are different distributions between them in relationship with the analyzed qualitative characteristics.

We carried out the latter by means of chi square tests. Statistical analyses were performed using the Stata V13 program (StataCorp, Texas, 2013). We considered a significance level of $\alpha = 0.05$.

Results

The rabbits' evaluation was carried out 42 days after the fracture, and immediately afterwards we sacrificed the animals. At final evaluation we included 24 rabbits because two of them in group 2 and four in group 3 (control group) had passed away. Mortality rates were 0.20% and 40%, respectively. The histological study was conducted in these 24 animals: 10 of them were pieces of femoral bone tissue with local alendronate (group 1); eight, of femoral bone tissue with local alendronate instilled one week after the fracture (group 2), and six, of control animals (group 3).

With respect to the results evaluated by imaging studies, we did not find statistically significant group differences in the sizes of bone calluses in neither of the two studies (X-rays: p=0.007 and CT scan: p=0.349) (Table, Figure 1).

Table. Estimations of the bone callus sizes (mm) formed in the three groups at week 6.

Imaging studies	Group 1 Alendronate immediately after the fracture	Group 2 Alendronate one week after the fracture	Group 3 Control
Radiographic image (mm)	6.23 4.01 9.02 6.50 3.25 11.47 3.50 2.65 6.40 4.10	8.10 2.82 3.89 4.94 7.79 7.41 6.87 1.94	3.86 5.60 3.87 3.67 5.78 6.27
Average (standard deviation) (mm)	5.7 (2.39)	5.47 (2.89)	4.84 (1.16)
CT scan	6.36 4.26 4.30 4.58 3.15 3.34 2.23 3.89 4.37 2.54	5.33 3.54 4.59 7.01 3.36 2.23 4.87 2.98	4.01 2.19 3.25 4.32 2.23 3.45
Average (standard deviation) (mm)	3.90 (1.18)	4.2 (1.82)	3.24 (0.88)

ISSN 1852-7434 (online) • ISSN 1515-1786 (printed)

From the histological point of view, we verified moderate bone formation in the three groups with no statistically significant differences between them (p=0.8336) (Figure 2). Figure 3 shows the histological patterns observed according to a three-category scale (scarce, light, and moderate) with respect to bone formation and vascularization; group 2 showed the best results in terms of vascularization (p=0,3976) and had more bone formation than the other two (although without statistical significance).

Discussion

The time fractures take to heal and the stimulation of bone callus formation represent areas of constant evaluation and research. So far, the attempts to develop drugs for accelerating and stimulating bone formation have not been successful. The studies on the effects of bisphosphonates on fracture healing that have been published are extremely varied, because they use different bisphophonates at different doses with different administration methods in varied experimental fracture models, and the evaluation methods they use are different too. In this experimental group, which was based on X-rays, CT scans and histological analyses, it was possible to determine that the use of alendronate (a second-generation bisphosphonate) instilled locally on the fracture area did not affect significantly the initial process of healing (first 6 weeks) in rabbits' femoral dyaphiseal fractures. It is worth highlighting that, in our study, we estimated the size of bone calluses, but from the histological point of view we observed a consolidation pattern only subjectively, with neither measurements (histomorphometry) nor immunohistochemistry, i.e. we could not estimate accurately the number of osteoclasts and osteoblasts in each sample. We also highlight as a weakness of this study the lack of estimation of the variables as time went by.

There are reports on diverse experimental studies carried out in animals to observe the influence of bisphosphonates on fracture calluses; there are publications on the effects of subcutaneous ibandronate[7-9] and clodronate[10-13] on fracture healing in rats. Diverse authors have studied the effects of per os raloxifene, estrogens and alendronate on bone healing in ovariectomized rats;[14] there are also reports on dogs to study the effects on bone healing of subcutaneous tiludronate and etidronate,[15,16] and also those of subcutaneous ibandronate[17] and alendronate [18]. Adolphson studied the effects on fracture healing of clodronate subcutaneously administered in women who had suffered Colles fractures and were treated non-invasively with reduction under local anesthesia and immobilization in short arm splint. The author evaluated bone mineral density in bone calluses and verified an increase in callus mineralization in the group treated with clodronate, detecting no case of non-union.[5] In other studies on the effects of

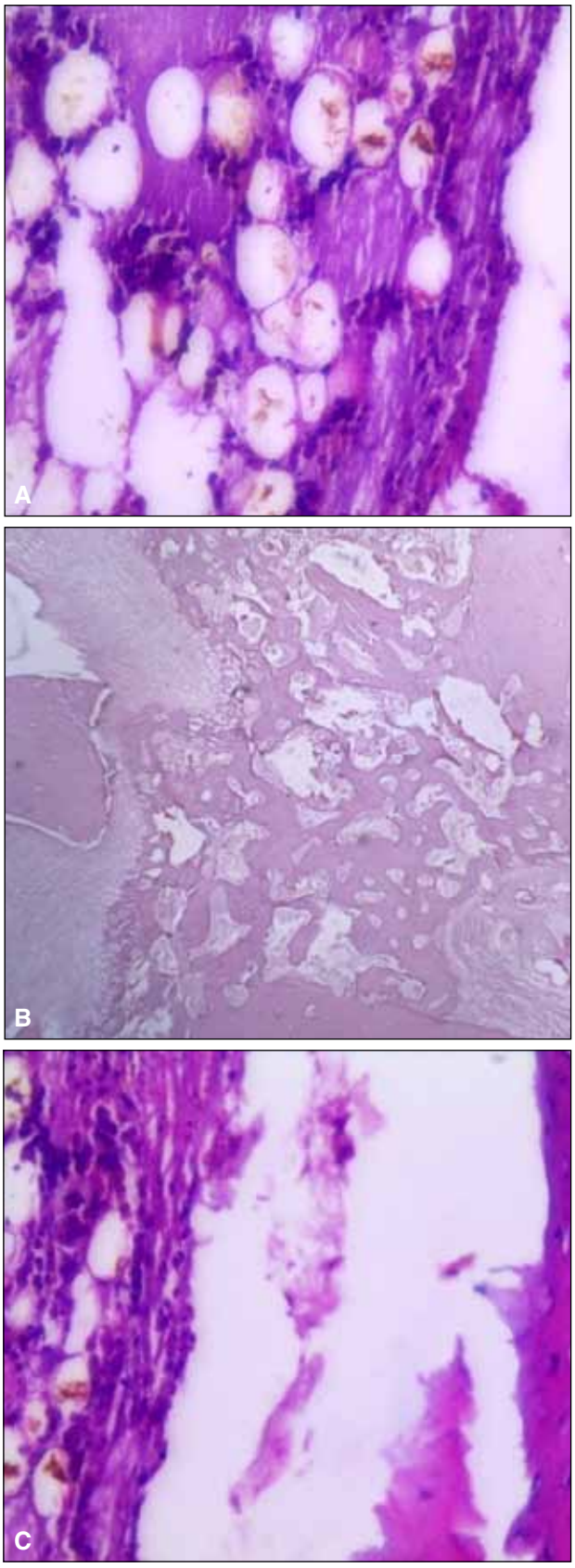

Figure 2. A. Alendronate crystals. **B.** Bone and cartilaginous tissues. **C.** Alendronate crystals and osteocytes.

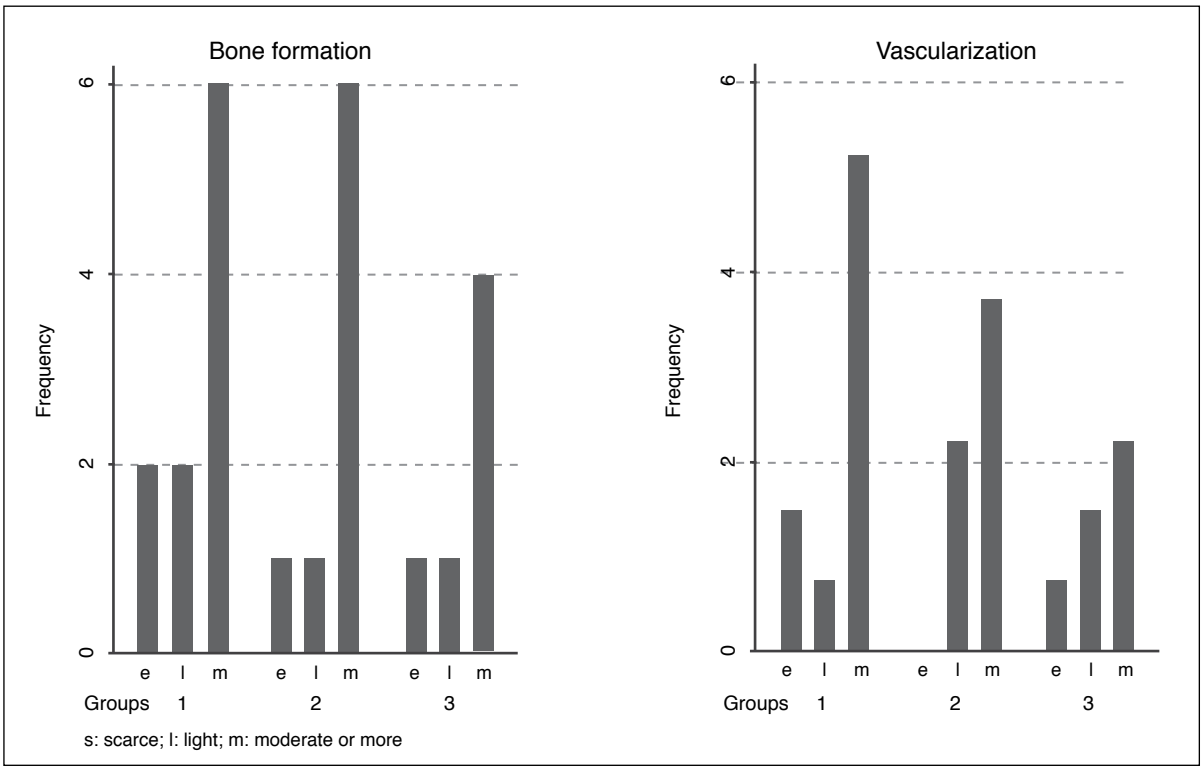

Figure 3. Bar chart showing the characteristics verified in bone formation and vascularization after week 6.

bisphosphonates on bone density following immobilization for the treatment of specific fractures, bisphosphonates were administered immediately after the fracture, and they observed no increase in bone healing delay.[19,20] Different authors studied first-generation bisphosphonates (eidronate, tiludronate) and verified delay and even inhibition of bone healing in fractures produced in dogs in a dose-dependent and reversible way, since fractures healed when the treatment was interrupted.[15,16,21]

Goodship et al. proved that pamidronate is not associated with adverse effects on the restoration of the mechanical condition of long bones after fracture.[22] In a recent study which compared radiographic bone healing between patients with distal radius fracture treated with bisphosphonates and a control group that did not receive this type of treatment, they reported that the treatment with bisphosphonates is associated with an increase in the time necessary to get fracture bone healing, although this difference is less than a week and, therefore, is not clinically relevant.[23]

Li et al. reported that, when subjects are treated with alendronate, bone callus remodeling is suppressed extensively, with production of a callus with greater contents of immature bone, persistence of the fracture line and less contents of cortical bone as compared to the other groups.[7,8,24] Peter et al. carried out studies in dogs, where one group received per os alendronate during 16 weeks after the fracture and they got calluses two or threefold greater than those in dogs not treated throughout this period, maybe due to delay in bone callus remodeling. These authors thus show that chronic administration of alendronate is not associated with adverse effects on bone healing, resistance or mineralization of intentionally caused fractures in dogs' radial bones.[18] As for our study, it evidenced that the use of alendronate locally instilled on the fracture line does not bring about negative consequences for the initial process of bone healing in rabbits.

Conclusions

The results of this experimental study show that the instillation of local alendronate on fracture line, immediately after the fracture and one week later, did not change in statistically significant ways the normal process of bone healing as judged by histological analyses and radiologic studies six weeks after the rabbits had undergone femoral bone fracture.

ISSN 1852-7434 (online) • ISSN 1515-1786 (printed)

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