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THE EFFECTS OF IBANDRONATE SODIUM IN THE CONSOLIDATION OF RAT FEMORAL FRACTURES SUBJECTED TO INTRAMEDULLARY NAILING

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ABSTRACT

Background: Ibandronate sodium is used for the clinical treatment of osteoporosis and in having anti-catabolic action it improves bone structure, bone mineralization, bone fracture healing, mechanical fixation and osseointegration of implants in animal models. The objective of this study was to assess the effect of ibandronate sodium in the consolidation of rat femoral fractures subjected to intramedullary osteosynthesis. **Methods:** Forty-eight male, adult Wistar rats were used in the study, randomly distributed in two groups (ibandronate and control). All animals were submitted to surgical procedure for intramedullary osteosynthesis, and subsequently submitted to a right femoral fracture. Six animals in each group were euthanized on the 7th, 14th, 28th and 42nd day after surgery. The femurs of the animals were resected and submitted to radiological and histological analysis of bone callus. **Results:** Radiographic analysis presented no significant differences between groups. In the ibandronate group, optical density was significantly higher on the 42nd day. In the analysis of collagens, type I collagen was denser and type III collagen was less dense in the ibandronate group (compared to control). **Conclusions:** From the results, it was possible to conclude that ibandronate sodium favorably interferes in the consolidation of femur fractures in rats treated with intramedullary mailing.

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INTRODUCTION

The femur is the strongest and longest bone in the human body and fractures in young people are usually the result of high-energy injuries. Femoral shaft fractures are due to high-energy trauma such as motor vehicle accidents (either as a pedestrian, in cars, on motorcycles or bicycles), gunshot injuries, and falls from heights (Paccola, 2000; Reis & Fernandes, 2004; Whiting *et al*, 2018). Due to the extreme morbidity and mortality associated with conservative treatment (non-surgical), surgical treatment of femoral shaft fractures has become gold standard (Norris &Nowotarski, 2007). Different fixation devices have been used in surgical procedures for the treatment of fractures to guide and support bone healing (Kellam &Audigé, 2007). For the surgical fixation of femoral fractures external plates, compression plates, bridge plates, percutaneous plates and intramedullary nails have been used. Standard fixation for the surgical treatment of femoral shaft fractures is intramedullary nailing (Norris &Nowotarski, 2007; Whiting *et al*, 2018).

Ibandronate sodium is a third-generation nitrogen-containing bisphosphonate (Dooley& Balfour, 1999) used in the management of osteoporosis. As all bisphosphonates, ibandronate sodium has a chemically stable structure, possessing a hydroxyl group at R1 and a tertiary nitrogen group on its R2 side chain, which together confer the most potent antiresorptive effect *in vivo* of all oral bisphosphonates available (Russell, 2006). As far as the review of literature is concerned, we were unable to identify any studies that combined ibandronate sodium in the treatment of fractures fixed with an intramedullary nail. Thus, this study had as objective to identify the effects of ibandronate sodium in the consolidation of rat femoral fractures subjected to intramedullary osteosynthesis.

METHODS

Ethical principles: This study was conducted according to the Ethical Principles on Animal Experimentation adopted by The

Brazilian Laboratory Animal Science Association (COBEA). This study was approved by Ethics Committee in Animal Experimentation at the UniversidadeFederal doCeará.

Sample characterization: Forty-eight male, adult Wistar rats (*Rattus norvegicus albinus*) of mean weight 336.63g (varying between 264 and 386g), from the animal facility at the UniversidadeFederal doCeará (Pici Campus) were used in the study.

Study design: The animals were randomly distributed in two groups, the ibandronate group and the control group. All the animals were submitted to surgical procedure for osteosynthesis with an unlocked reamed intramedullary nail, after the procedure they were submitted to a right femoral fracture. In the ibandronate group (n=24), ibandronate sodium was administered orally, 15mg/kg, by gavage, in a single dose, on the same day that the surgical procedure was performed. Six animals in each group were euthanized with a lethal dose of ketamine chloridrate (148 mg/kg) administered intraperitoneally, on the 7^{th}, 14^{th}, 28^{th} and 42^{nd} day after surgery. After euthanasia, the femurs of the animals were resected in a whole anatomic part, without soft tissue and with the preservation of bone callus. Subsequently, they were submitted to radiological and histological analysis of bone callus on the respective day for bone consolidation (Udupa & Prasad, 1963).

Surgical procedure: The surgical procedure was standard for all animals and the material used in the surgical process was sterilized in an autoclave for each animal. The animals were submitted to 12 hours fasting before surgery; anesthesia was performed using ketamine chloridrate (90mg/kg) and xylazine chloridrate (10mg/kg) and administered intramuscularly to the left thigh (Goss-Sampson&Kriss, 1991). Animals were considered anesthetized when they remained quiet and immobile upon handling with a normal range of breathing. The animals were trichotomized manually in the region of the right knee, placed in supine position on the operating table, after antisepsis of the right pelvic member, right hip, right inguinal and abdominal region with polyvinylpyrrolidone (topical iodine) and placed on the sterile fenestrated surgical field with an opening centralized over the hip.

A 2-cm anterior longitudinal incision was made centered on the flexed right knee joint. Diaeresis of tissue planes was performed until the patella, the patella tendon and the quadriceps muscle came into view. A new incision was made at the level of the medial border of the patella with distal and proximal continuation up to at most 1cm on the medial border of the patellar tendon. A careful subtendinous dissection preserving the extensor mechanism was made, and with the knee in extension in a smooth and continuous movement it was flexed, and the patella was dislocated laterally to visualize the intercondylar region and notch of the right femur. A 1.2 x 64mm intracath needle was positioned 0.5mm proximal to the edge of the intercondylar notch, opening of a tunnel in the medullary canal of the right femur. Then, a 1 x 60mm Kirschner-wire (K-wire) was inserted manually, in a retrograde fashion, into the medullary canal, until it until passed the greater trochanter and came out of the skin. To prevent migration, the proximal extremity of the wire was bent at about 90º and subsequently placed beneath the skin. The distal extremity of the wire exteriorized at the intercondylar notch was firmly attached to provide anchoring of proximal extremity at the greater trochanter. After traction, the wire was cut at the femoral condyles. The wire remained in the medullary canal, being used as an intramedullary nail, supported at the greater trochanter. The patella was reduced to an anatomical position and tissue synthesis by anatomic planes was achieved with surgical wires for 4.0 mononylon type suture. After surgery, the animals from both groups, received tramadol chlorhydrate 10mg/kg diluted in Milli-Q® water (Millipore Corporation, Darmstadt, Germany), for 2 days (Jang *et al*, 2010).

Fracture induction: To induce a fracture in the femur of the rat, a fracture device that reproduces a blunt guillotine was made of steel, weighing 746g, released at a height of 274.3mm (Figure 1). The fractures were induced with the animal under anesthesia immediately after the surgical procedure. The researcher held the anesthetized animal during the whole fracture induction process. After fracture induction the animal was assessed clinically, to check for the existence of signs of mobility in the diaphysis of the right femur to confirm the fracture. The still anesthetized animals were subjected to radiographic assessment using portable x-ray equipment, Vetmax® 80/20 (AstexEquipamentosRadiológicos, São Paulo), with Kodak, Diagnostic Film T-Mat, 240 x 180mm, to confirm the fracture.

Figure 1. Fracture device. A: base; B and C: cylindrical rods; D: platform; D1: transversal groove; D2: longitudinal groove; E: sliding steel bar; F: blunt blade; G: latch; H: steel anti-roll bar; d: height of blunt blade (274.3mm)

Treatment: After full recovery from anesthesia, using the gavage technique, 1.0ml of 0.9% sodium chloride solution was administered to the animals in the control group, and sodium ibandronate (Bonviva® 150mg), also using the gavage technique, diluted in Milli-Q® water at a dose of 15mg/kg, was administered to the ibandronate group (Lalla *et al*, 1998).

The following recommendations for the administration of oral bisphosphonates were observed: in fasting; the drug was diluted in Milli-Q® water; feeding *ad libitum* was allowed 1 hour after the administration of the drug. Up to the day of euthanasia, the animals could exercise.

Radiographic analysis: After euthanasia of the animals in each subgroup, the operated thighs were carefully dissected with the intention of removing soft tissue from the femur, being careful to preserve the bone callus and the K-wire. After the femur was disarticulated from the hip and knee, it was immediately put in 15ml Falcon tubes containing 10% formaldehyde. The anatomical parts were then submitted to radiographic examination on a mammography apparatus (Alpha ST, GE®). After the radiographic images were digitalized, analysis of bone callus in each image was performed using a computerized morphometric analysis system called SAMM (*Sistema de AnáliseMorfométrica* (system of morphometric analysis)) designed at the Universidade Federal do Ceará and modified for this analysis (Jamacaru, 2006). This software provides two types of data: optical density and area of bone callus. Optical density was quantified as the mean gray value, and the size of the area of bone callus was assessed in mm^2. Grey value is a scale using in computer science, where absolute black is 0 and absolute white is 255, and each number in this interval corresponds to a shade of grey. The mean of these grey values was calculated by the program in each area of interest, which corresponds to the optical density of bone callus, the closer this mean is to white (255), the more radiopaque the radiographic image is, in other words, the more consolidated bone callus there is. Radiographic analysis of bone callus began with the selection of the area of interest in the image; then, quantification of the area of bone callus (in mm^2) and mean grey values were obtained. To enable better analysis, callus in upper area of the image was studied first, followed by the callus in the lower area. To determine the optical density of the callus, from the upper and lower regions of the image the mean grey values were calculated; and to determine the area of bone callus, the areas of bone callus in the upper and lower areas of the image were totaled.

Histological analysis: After radiographic analysis, the parts were decalcified in 7.5% nitric acid until the part offered no resistance to perforation with a needle. After this period the K-wires were removed and the parts were first cut transversely, 0.5cm proximal and 0.5cm distal to the facture focus in order to select only the bone callus and the region next to the fracture. Subsequently, a cut was made longitudinally dividing the bone callus and the regions next to the fracture in halves. The halves were embedded in paraffin wax, the inclusion blocks were subjected to longitudinal sections using a microtome, resulting in 5 micrometer-thickness cuts. The sections were colored using the Picrosirius method (EasyPath®, Milan, Italy) to assess collagen fibers. After the slides were mounted, histological examination was performed on a polarized light microscope (N-200T, Coleman, São Paulo, Brazil) to analyze birefringence of type I and III collagen in the bone callus and in the cortex close to the fracture. The slide images from the optical microscope were captured by a normal digital camera and with ordinary due care. The images were transferred to a computer and saved as Windows® Bitmap (.bmp) files, with a resolution of 640x480 pixels, using the IrfanView® program.

For histological analysis, specific areas on each slide, which corresponded to bone callus were selected: the lower and upper regions of the image, the cortex of the femur close to the fracture, one cortex to the right of fracture focus and another cortex to the left of the fracture focus. Under polarized light, it was possible to differentiate collagen fibers: type I collagen fibers presented an intense yellow-red birefringence while type III collagen fibers displayed a green to greenish yellow birefringence (Montes &Junqueira, 1991). Quantitative analysis of collagen was conducted using the image analysis computer program SAMM, designed specifically for this purpose (Jamacaru, 2006). Measurements in pixels were made of the bundles that represented birefringence in the study areas of each slide. These measurements were obtained according to difference in color detected by the program, which was calibrated to identify the color spectrum for type I and type III collagen, which were previously colored using the Picrosirius method. The program automatically segmented the structures of interest in the image and quantified the percentage of these structures in relation to the area of field of study.

Statistical analysis

The quantitative, continuous, and discrete variables were initially analyzed using the Kolmogorov-Smirnov test to check for normal distribution. For descriptive statistics, mean and standard deviation (parametric data) or median, interquartile range and minimum and maximum values (non-parametric data) were calculated. Intergroup comparisons (control *versus* ibandronate) at each time interval were performed with the unpaired t test (parametric data) or the Mann-Whitney test (non-parametric variables). Intragroup comparisons, between the 7^{th}, 14^{th}, 28^{th} and 42^{nd}-day subgroups, were conducted using the analysis of variance (ANOVA) associated to the Tukey's multiple comparison test (parametric data) or the Kruskal-Wallis test complemented by Dunn's multiple comparison test (non-parametric variables). The limit for statistical significance was set at $p=0.05$. The statistical software package GraphPad Prism®, version 5.0 for Windows (GraphPad Software, San Diego, California, USA, 2007) was used for all analyses.

RESULTS

There were no cases of infection or problems with osteosynthesis in either the control or ibandronate groups.

Radiographic analysis

The mean area of bone callus increased in the groups during the period, with higher values on the 42^{nd} day of bone consolidation, with 12.4±6.5mm² in the control group and 17.17±5.74mm² in the ibandronate group. However, these differences were not significant (Table 1). In the same group analysis, for the control group, the area of callus measured on the 28^{th} ($p<0.05$) and 42^{nd} day ($p<0.01$) were significantly greater than on the 7^{th} day. For the ibandronate group, the area of callus was significantly greater on the 14^{th} ($p<0.05$), 28^{th} ($p<0.001$) and the 42^{nd} day ($p<0.001$) when compared to the 7^{th} day, it was also possible to observe that area on the 42^{nd} day was significantly higher than on the 14^{th} day, $p<0.001$ (Table 1). Figure 2 illustrates, in a comparative manner, the evolution of bone callus formation in the digitalized radiographs on a surgical microscope, on the 7^{th}, 14^{th}, 28^{th} and 42^{nd} day, from both groups.

Table 1. Mean values and standard deviation of the area of bone callus according to measurements taken at the four-time intervals studied (on the 7th, 14th, 28th and 42nd day) of the animals in the control and ibandronate groups

Consolidation time (days)	Control		Ibandronate		p-value (intergroup)
	Mean	Standard deviation	Mean	Standard deviation	
7th	0.65	0.27	0.55	0.18	0.4743
14th	8.77	6.43	6.82	1.91*	0.4924
28th	10.36#	5.01	11.51	4.71‡	0.6922
42nd	12.94†	6.85	17.17	5.74§	0.2731
p-value (intragroup)	F=5.9691	0.0045	F=20.3180	<0.0001	

28th day *versus* 7th day, p<0.05 (control group); † 42nd day *versus* 7th day, p<0.01 (control group); * 14th day *versus* 7th day, p<0.05 (ibandronate group); ‡ 28th day *versus* 7th day, p<0.001 (ibandronate group); § 42nd day *versus* 14th day, p<0.001 (ibandronate group), calculated using Tukey's test.

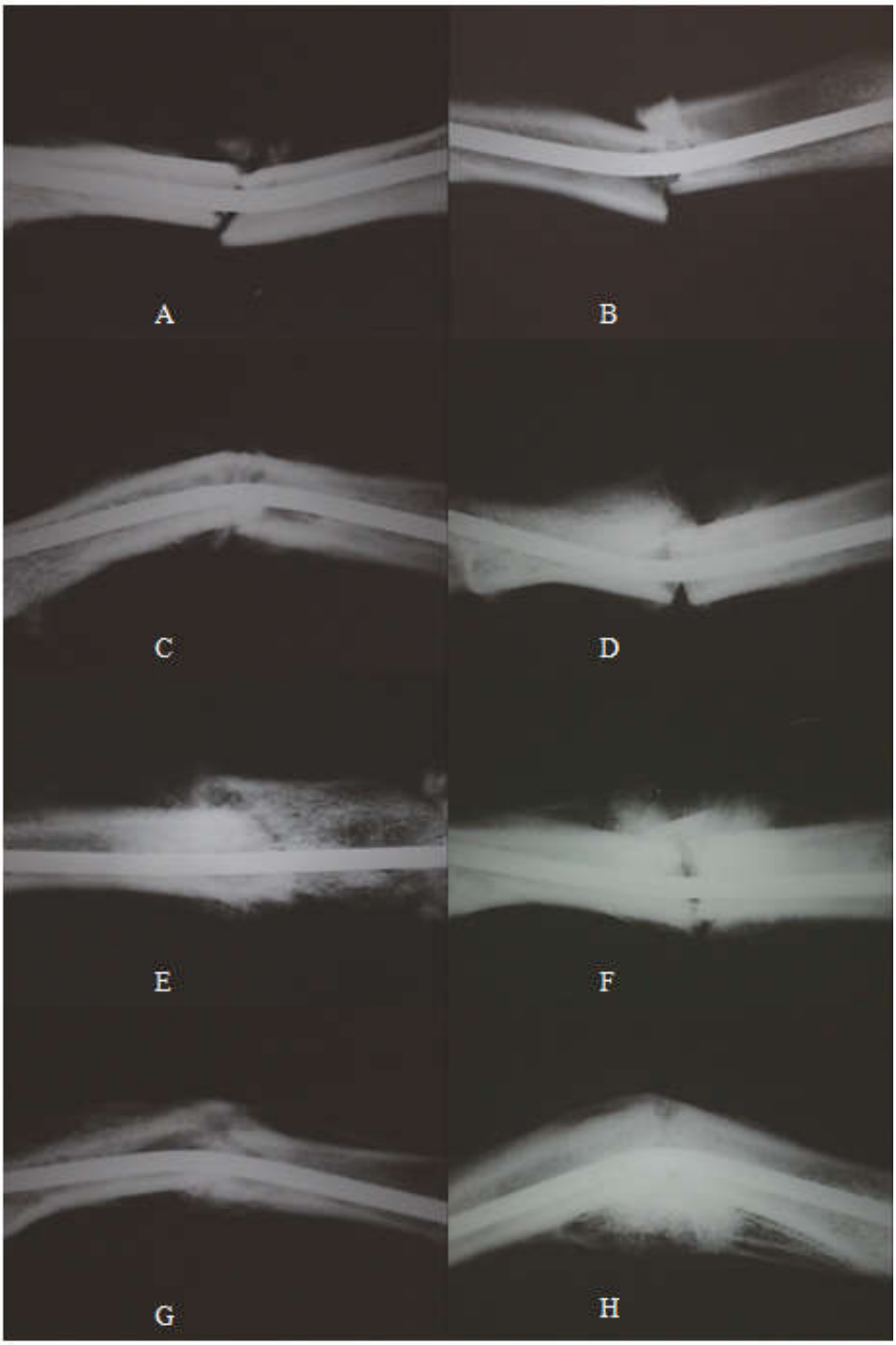

Figure 2. Digitalized radiographic images. Magnification: 10X
Control group - A, C, E, G; Ibandronate group - B, D, F H; A and B - 7th day; C and D - 14th day; E and F - 28th day; F and G - 42nd day of bone consolidation

Optical density of bone callus: In the analysis between the groups, it was possible to observe that in the ibandronate group, on the 42nd day, optical density was statistically greater than in the control group, p=0.0056. There were no other significant differences within or between the groups (Table 2).

Histological analysis

Density of type I collagen in the bone callus: At all bone consolidation time intervals, deposition of type I collagen predominated in the ibandronate group, which presented an

Table 2. Mean values and standard deviation of optical density of bone callus according to measurements taken at the four-time intervals studied (on the 7th, 14th, 28th and 42nd day) of the animals in the control and ibandronate groups

Consolidation time (days)	Control		Ibandronate		p-value (intergroup)
	Mean	Standard deviation	Mean	Standard deviation	
7th	60.40	5.98	67.37	8.32	0.1264
14th	68.15	12.61	79.10	13.60	0.1789
28th	70.25	8.77	74.32	6.49	0.3820
42nd	63.59	7.63	81.62	9.99	0.0056
p-value (intragroup)	F=1.4359	0.2620	F=2.3776	0.1002	

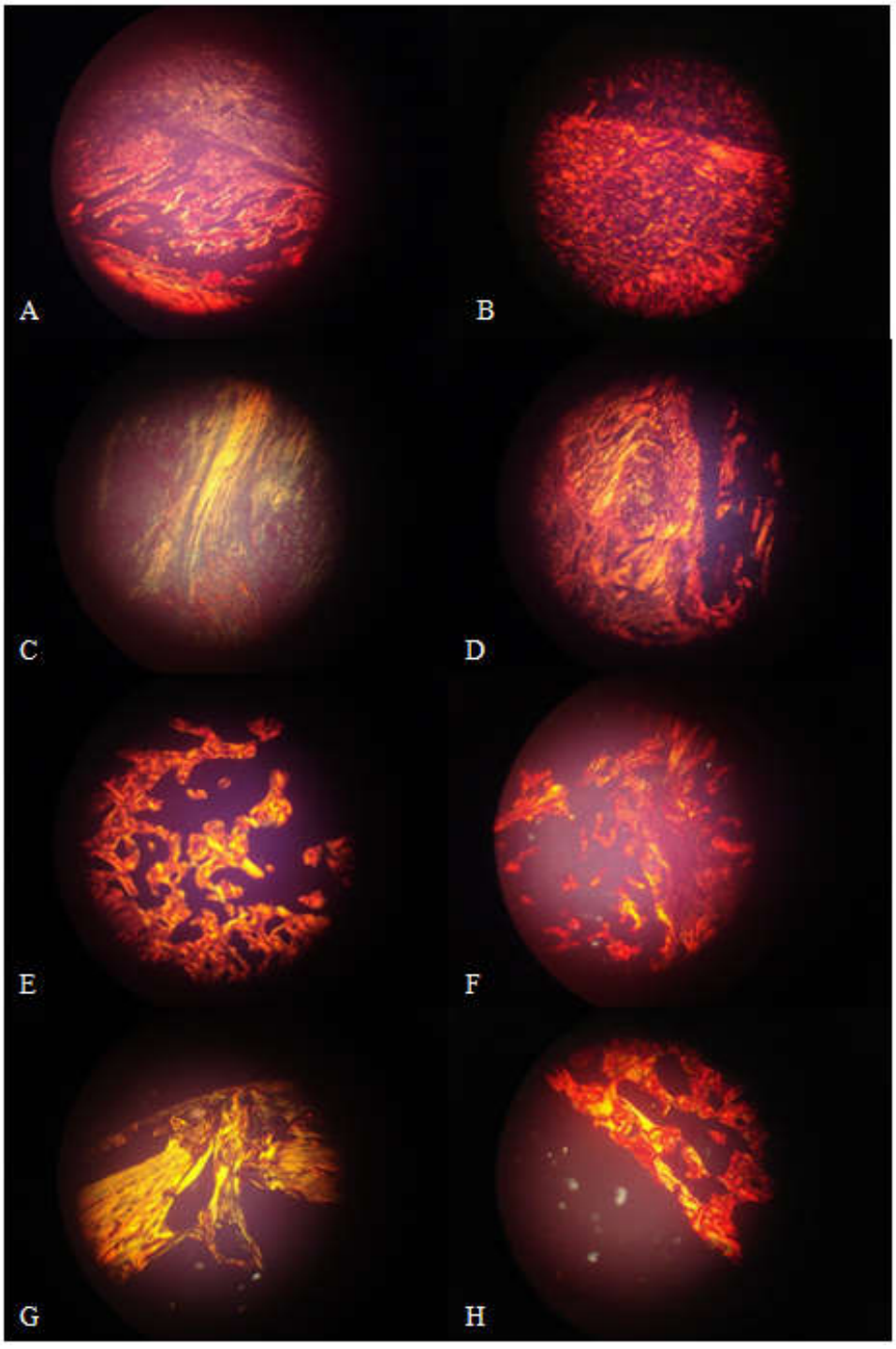

Figure 3. Digitalized histological images. Magnification: 100X.
Control group - A, C, E, G; Ibandronate group - B, D, F, H; A and B - 7th day; C and D - 14th day; E and F - 28th day; F and G - 42nd day of bone consolidation

intense orange color under polarized light (Figure 3). Additionally, deposition was significantly higher on the 7th and 14th day of consolidation in the ibandronate group, p=0.0292 and p=0.0008, respectively. Density measurements of type I collagen in the bone callus were higher in the control group on the 28th (37.32±22.28) and 42nd day (36.22±14.29). No significant differences were identified within the groups (Table 3).

observed in the control group, p=0.0022. Nevertheless, in the intragroup comparison, no significant differences were identified (Table 5).

Density of type III collagen in the cortex: In the intergroup analysis, on the 7th and 14th day the density of type III collagen was significantly lower when compared to the control group, p=0.0022 and p=0.0152, respectively.

Table 3. Mean values and standard deviation of type I collagen in the bone callus region according to measurements taken at the four time intervals studied (on the 7th, 14th, 28th and 42nd day) of the animals in the control and ibandronate groups

Consolidation time (days)	Control		Ibandronate		p-value (intergroup)
	Mean	Standard deviation	Mean	Standard deviation	
7th	17.89	13.03	35.96	11.54	0.0292
14th	17.83	13.21	48.13	3.52	0.0008
28th	37.32	22.28	43.62	5.36	0.5160
42nd	36.22	14.29	39.22	8.74	0.6696
p-value (intragroup)	F=2.7431	0.0701	F=2.3554	0.1042	

Table 4. Mean values and standard deviation of type I collagen in the cortical close to the fracture focus according to measurements taken at the four time intervals studied (on the 7th, 14th, 28th and 42nd day) of the animals in the control and ibandronate groups

Consolidation time (days)	Control		Ibandronate		p-value (intergroup)
	Mean	Standard deviation	Mean	Standard deviation	
7th	18.30	23.64	55.91	5.72	0.0036
14th	48.22	25.11	60.69	15.79	0.3274
28th	55.36*	14.54	52.52	14.79	0.7444
42nd	50.53	18.07	58.63	9.66	0.3555
p-value (intragroup)	F=3.9209	0.0237	F=0.5050	0.6832	

* 28th day *versus* 7th day, p<0.05 (control group), calculated using Tukey's test.

Table 5. Median values and interquartile range (25th percentile and 75th percentile) of the density of type III collagen in the region of bone callus according to measurements taken at the four time intervals studied (on the 7th, 14th, 28th and 42nd day) of the animals in the control and ibandronate groups

Consolidation time (days)	Control		Ibandronate		p-value (intergroup)
	Median	Interquartile range	Median	Interquartile range	
7th	4.40	1.44, 27.43	0.00	0.00, 0.01	**0.0022**
14th	8.35	3.51, 17.19	0.06	0.04, 0.42	0.0649
28th	0.50	0.01, 6.25	0.20	0.00, 1.44	0.5725
42nd	3.71	1.18, 1.44	0.51	0.00, 2.22	0.0649
p-value (intragroup)	KW=3.1400	0.3705	KW=6.5942	0.0860	

KW: Kruskal-Wallis test.

Table 6. Median values and interquartile range (25th percentile and 75th percentile) of the density of type III collagen in the cortical close to the fracture focus according to measurements taken at the four time intervals studied (on the 7th, 14th, 28th and 42nd day) of the animals in the control and ibandronate groups

Consolidation time (days)	Control		Ibandronate		p-value (intergroup)
	Median	Interquartile range	Median	Interquartile range	
7th	37.86	7.57, 45.58	0.00	0.00, 0.04	0.0022
14th	59.77	27.78, 65.17	2.41†	0.66, 13.18	0.0152
28th	1.18‡	0.00, 16.63	3.05	0.59, 9.38	0.8726
42nd	11.89	4.71, 30.86	3.19†	2.34, 10.86	0.2403
p-value (intragroup)	KW=10.2667	0.0164	KW=11.8588	0.0079	

‡ 28th day *versus* 14th day, p<0.05 (control group); † 14th day and 28th day versus 7th day, p=0.05 (ibandronate group), calculated using Dunn's test; KW: Kruskal-Wallis test.

Density of type I collagen in the cortex: In the analysis between the groups, in the cortex close to the fracture focus, the mean density of type I collagen in the ibandronate group on the 7th day was statistically greater than that in the control group, p=0.0036. In the control group the mean density of type I collagen was higher on the 14th (48.22±25.11), 28th (55.36±14.54) and 42nd day (50.53±18.07) than on the 7th day (18.30±23.64). However, this difference was significant only in the comparison between the 7th and 28th day, p<0.05 (Table 4).

Density of type III collagen in the bone callus: In the intergroup analysis, it was found that, on the 7th day the density of type III collagen was significantly lower than that

In the intragroup analysis, in the control group, it was identified that the density of type III collagen measured on the 28th day was significantly lower than that observed on the 14th day, p<0.05. In contrast, in the ibandronate group, the density of type III collagen measured on the 14th and 42nd day was significantly higher than that observed on the 7th day, p<0.05, for both comparisons (Table 6).

DISCUSSION

Performing surgical procedures to enable osteosynthesis prior to the induction of fractures and has already been well-documented (Udupa & Prasad, 1963; Azuma *et al*, 2001; Mussi Filho*et al*, 2006). In our study there was no loss of bone

fixation following fracture or K-wire migration. Manual reaming of the medullary canal with *intracath*, instead of using an electric drill, enabled better control in the confection of the tunnel in the medullary canal, minimizing the possibility of generating a false passage and without damage to fracture consolidation. Grundnes*et al* (1994) concluded that moderate medullary reaming enables the restoration of total bone and cortical blood flow in 24 hours. In the immediate postoperative period and up to 48 hours after, the animals were offered an analgesic, tramadol chlorhydrate 10mg/kg diluted in Milli-Q® water (Jang *et al*, 2010). The animals could exercise on the first postoperative day, without restrictions. To produce the fracture, a fracture device was designed in which the operating mechanism was similar to those already documented by Bonnarens& Einhorn (1984) and Vialle*et al* (2004) in which a blunt blade strikes the area to be fractured after free-falling from a specific height and by means of a three-point flexion the fracture is caused. The devices proposed by Bonnarens& Einhorn (1984) and Vialle*et al* (2004) used a 500g blunt steel bar and in our we study we used a bar which weighed 746g. Furthermore, the studies by Bonnarens& Einhorn (1984) and Vialle*et al* (2004)placed the animals in supine position with the hip abducted and the impact of the blunt guillotine on the medial side of the thigh. The correct positioning of the animal in the transversal groove of the platform was crucial to position the fracture in the femur. After dissecting rat thighs we opted for the lateral impact from the blunt blade on the animal's thigh, as large nerves and veins run on the medial side of the thigh and in a situation of trauma major devascularization may occur and thus compromise fracture consolidation.

Ibandronate sodium was administered using the gavage technique on the same day that the surgical procedure was performed, with the animal awake. The drug was diluted in Milli-Q® water in order not to hinder its absorption and feeding *ad libitum* was only permitted 60 minutes later (Barrett *et al*,2004). The dose was 15mg/kg (Lalla *et al*, 1998), in a convenient single dose which is as effective as daily treatment (Reginster *et al*, 2006). The femurs were radiographed using a mammography apparatus, since mammogram has higher definition and greater detail (Mendes *et al*, 2001). Different adjustments for exposition to radiation have been presented (Mendes *et al*, 2001; Guarniero*et al*, 2003; Guarniero*et al*, 2007), and the best adjustment in this study was based on the best image of bone callus from the control group on the 14th day of bone consolidation, due to the fact that in this phase bone callus is more exuberant and thus better for comparative assessment for the adjustment of radiographic exposition. The presence of bone callus is observed in a single analysis, through the increase in bone density in the external region of the lateral and medial cortex close to the fracture focus, that is, the periosteal callus (Greenspan & Beltran, 2014). The density of this callus is viewed in intensities of grey; these different tones enabled the use of SAMM.

For histological analysis, we used the Picrosirius method for the coloring of bone (Junqueira*et al*, 1979), which colors the collagen fibers. Collagen fibers represent 95% of the organic component of bone matrix. Collagen is produced by the osteoblasts, and the fibers in being associated to hydroxyapatite provide strength to bone tissue. Under polarized light and colored using the Picrosirius method, collagen fibers present birefringence, or they become luminous against a dark background. In a study on type of collagen fibers, Montes &Junqueira (1991) observed that collagen types I, II and III presented different colors and intensities of birefringence when colored with Picrosirius. Type I collagen presents intense birefringence and a color that varies from yellow to red; type II collagen presents weak birefringence and a variable color; type III collagen presents weak birefringence and a greenish color. This possibility of individualizing the types of collagen according to color enabled us to use the SAMM program for quantification.

From studies with canine models, in which hip arthroplasties were performed and bisphosphonates were used as therapy to inhibit bone resorption, a hypothesis on the anabolic effect of bisphosphonates was raised (alendronate and risedronate); and, subsequently, supporting this hypothesis, a study *in vitro* with human osteoblasts was conducted in which these drugs improved the proliferation and maturation of these cells, as well as the gene expression of the morphogenetic bone protein (BMP-2), type I collagen and osteocalcin protein (Im*et al*, 20004). Yet, the study *in vitro* by Koch *et al* (2010) with human osteoblasts, suggested that bisphosphonates (zoledronate, ibandronate and clodronate) have limited anti-proliferative effects (with the exception of zoledronate) on these cells, and that an increase in gene expression of type I collagen in the extracellular matrix through the probable stimulus of osteoblast differentiation, supports the results found in our study. Type I, II and III collagen fibers, in fracture consolidation, have the important function of providing mechanical strength and tissue support. During the consolidation process, type I and III form scaffolds for the adhesion and migration of cells. Secretion and distribution of collagen in fracture repair is reflected in these functions. The fibrous tissue mainly consists of type III collagen which serves as a substrate for the migration of osteoprogenitors and capillary neoformation. In the early stages of fracture consolidation in rats, type III collagen predominates. With the presence of trabecular bone, a large amount of type I collagen is secreted (Ashhurst, 1990).

In our study, ibandronate sodium caused greater type I collagen density in the first two weeks of bone consolidation, as much in the bone callus as in the cortex close to the fracture focus, and a reduction in the density of type III collagen in the first week of bone consolidation when compared with the control group. A study by Rocha (2011) with a more powerful bisphosphonate: zoledronic acid, using the same methods to assess bone consolidation, concluded that zoledronic acid significantly increased the percentage of type I collagen in bone callus in the fourth week of bone consolidation. These are studies conducted with bisphosphonates with different anti-resorption potencies, which promote an increase in the density of type I collagen in bone repair at different stages of bone consolidation. In the study of periosteal callus, it was possible to determine that in the ibandronate group the areas of callus in the fourth and sixth week of bone consolidation were significantly larger than at the end of the first week; however, it did not provide a statistically larger area of periosteal callus when compared to control group. In the optical density analysis, the area in ibandronate group was statistically greater on the 42nd day than the control group, that is, the mean levels of grey confirmed that ibandronate sodium promoted an increase in the mineralization (opacity) of bone callus in the final stage of bone consolidation. Studying fracture consolidation in animal models is a complex task. Many

factors may positively or negatively influence the study and alter results. In our study we used a modern quantification technique for collagen and for the area of periosteal bone callus. However, the images, which need to be analyzed by the program SAMM need to be complete, of good quality; if the images to not meet these standards analysis cannot be conducted. Consequently, it is important to consider improving the quality of radiographic and histological images. In this study, radiographies were taken only once, this may have hindered better assessment of bone callus. Differences in the standards of slide coloring could also interfere in the quantification of type III collagen which presented atypical results in the control group. The study of only one segment of bone callus close the fracture focus could underestimate size, density, and constitution of total bone callus. The animals in the ibandronate group only received one dose of the drug, which may not have been sufficient to assess such complex events; and furthermore, the control group did not receive the same vehicle that was used to dilute the ibandronate sodium dose. Despite the results found in this study, more detailed studies are necessary to assess the effects of ibandronate sodium more accurately in the consolidation of fractures. From the results of this study, we conclude that the oral administration of ibandronate sodium favorably interferes in the consolidation of femoral fractures in rats treated with intramedullary mailing, as it proportions an increase in the deposition of type I collagen in the early stages of bone repair.

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