

The Effect of Ibandronate on Fracture Healing in Rat Tibia Model

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Abstract

Objective: This study aims to investigate the effect of ibandronate, which is a biphosphonate and acts by inhibiting osteoclasts in osteoporosis treatment, on fracture healing in rat tibia model.

Material and Method: 60 Wistar-Albino type male rats were divided into two groups as study (ibandronate) and control. Under general anesthesia standard closed fractures were created on right tibias of all rats using blunt ended needle holder by three point principle and then closed reduction and fixing with intramedullary nail (0.5 mm) were provided. The day of fractures were created, one oral dose of 250 µg ibandronate (Bonviva® tablet 150 mg, Roche, Istanbul, Turkey) was given to each group A, B, C and D. 1 month after 250 µg oral dose administration to groups E and F the day the fractures were created, the second dose of 250 µg ibandronate was administered. The rats were killed at 2nd week in groups A and B, at 4th week in groups C and D, and at 6th week in groups E and F. Oral administrations were performed by the same person with 15 gauge plastic feeding through nasogastric gavage. Fracture healing was evaluated as biomechanically, radiologically and histologically.

Results: No positive or negative radiological, biochemical and histological effect of ibandronate was detected, starting from the first day and reaching to 6 weeks, in healing process of closed tibia fractures created and fixed with IM nails in rats.

Introduction

Bone is an active and dynamic tissue that builds up the skeleton support of the organism and regenerates itself continuously for a life time [1]. Bone tissue is a very important structure that constitutes the frame in human body in order for the organs to work properly, and the disruption of its integrity is defined as fracture. This pathology formation negatively affects neighboring soft tissues and organ systems [2].

One of the frequently seen diseases of this dynamic tissue is osteoporosis which is a systematic skeleton illness characterized with increased fragility of bones as a result of disruption in bone mass, and bone tissue structure and quality [3]. As a consequence of increased possibility of fractures in hips and vertebra in the patients, prophylactic medicines are used to avoid fractures and reduce mortality.

Bisphosphonates is currently the most commonly used medicine group in medical treatment of osteoporosis [4]. Ibandronate prevents bone dissolving and vanishing by mixing in the blood stream gradually, and avoids bone loss manifesting this way. It also effects negatively on osteoclasts by changing the bone formation and decomposition cycle [5].

Whether this group of medicines, which has a common field of usage in orthopedic clinics, has effects on fracture healing, has incited us to study on this subject.

Materials and Method

Necessary permissions were obtained from university's Ethics Committee (Date of the decision: 12.02.2009, Session no: 2009/2, Decision no: 3) Study was carried out in experimental research laboratory. In this study 60 Wistar-Albino type male rats were used. The average age and weight of the rats used in this study was 2, 8 months (2.5-3.1 months) and 195 grams (176-218 grams) respectively. Animals were randomly divided into two as control and study groups, and then these groups were divided into three in themselves (Table 1). They were preoperatively monitored for 48 hours in laboratory environment as 10 animals per cage separated into 6 groups in total. Water and rodent feed were provided throughout the study. Animals were monitored at 22°C, given 12 hours of light and 12 hours of darkness.

For surgical intervention anesthesia, 50 mg/kg Ketamine hydrochloride (Ketalar flakon, Parke Davis, Istanbul) was administered into periton. Anesthesia stat was followed by reaction of the rat to squeezing its skin in every 5 minutes. Rats were covered with green cloth after clean up using



Figure 1: After closed fracture was created on tibia plateau anterior face in accordance with bending three points principle, sending intramedullar to distal fracture fragment by pushing forward the black injector needle with 0.5 mm (22 gauge) width.

Betadine solution. Dermal and subcutaneous tissues were penetrated through approximately 1 cm cut at upper end of right tibia anteriorly and tibia plateau was exposed with the aid of hemostatic forceps. After tibia fracture was created due to bending three points principle, the fracture was examined by hand and fixed by using 0.5 mm (22 gauge) wide black injector needles intramedullary. Black injector needle was cut with the aid of forceps and embedded towards the bone in a way that the skin wouldn't be discomforted. The wound was sutured with 2/0 silk. Created simple fractures were verified by radiologically soon after clinic examination. Fixation was repeated on three rats, and right tibia fracture and intramedullar fixation were confirmed for all rats. The rats with segmental and open fracture were left out of the study (Figure 1).

The study began with a total of 60 rats. Because 1 rat from group A, 1 rat from group B, 1 rat from group D had died and osteomyelitis had been developed in 1 rat from each groups E and F, the study was completed with 55 rats. As these numbers give statistically meaningful results, no rats were added to the study (Table 2).

Antibiotic prophylaxis was not applied during or after the surgical interventions.



Figure 2: Radiography of control and test groups at 4th week.

Table 1: Distribution of groups in tibial fracture created rats.

	Group	Number of Rat
Study 1	A	10
Control 1	B	10
Study 2	C	10
Control 2	D	10
Study 3	E	10
Control 3	F	10

Table 2: Lane and Sandhu classification to evaluate radiological data.

0	No callus
1	Callus formation present.
2	Bone union origin
3	Fracture line not observed
4	Fully united bones observed

Ibandronate was administered with the calculation as 250µgr/month to 30 rats in the study group, from the first day of treatment to the day they were sacrificed.

The day of fractures were created, one oral dose of 250 µg ibandronate (Bonviva[®] tablet 150 mg, Roche, Istanbul, Turkey) was given to each group A, B, C and D. One month after 250 µg oral dose administration to groups E and F the day the fractures were created, the second dose of 250 µg ibandronate was administered.

The rats were killed at 2nd week in groups A and B, at 4th week in groups C and D, and at 6th week in groups E and F. Oral administrations were performed by the same person with 15 gauge plastic feeding through nasogastric gavage.

The experiment was administered with the calculation as 250 µgr/month to 30 rats in the study group, from the first day of treatment to the day terminated by sacrificing the rats in medicated groups and control groups by cervical dislocation at 2nd, 4th and 6th weeks in order.

Right tibias of the sacrificed rats were disarticulated at the knee joints. Soft tissue on the tibia was stripped from the bone without damaging the callus by the pathology specialist, in accordance with

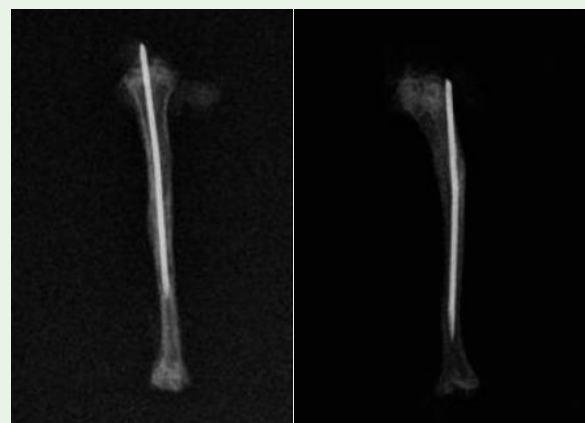


Figure 3: Radiography of control and test groups at 6th week.

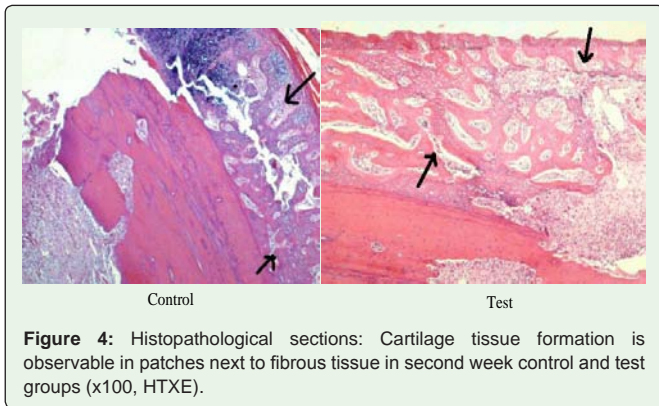


Figure 4: Histopathological sections: Cartilage tissue formation is observable in patches next to fibrous tissue in second week control and test groups (x100, HTXE).

histopathological procedures. All of the right tibias were examined as radiologically, histopathologically and biomechanically.

For radiological assessment, direct graphs of control and test groups were obtained by Siemens branded radiography device at 105 cm and magnified to 100% (Figure 2,3). 10 rat legs were placed according to their groups, on the surface to take graphs at front and the back, and the graphs were taken on a single tape for each group. Graphs were evaluated due to Lane-Sandhu classification by the same orthopedics and traumatology specialists without given the information of which groups they belong to.

Samples from the fractured region were collected for histopathological examinations. Collected bone tissue samples were fixated in 10% neutral formaldehyde and kept in 5% formic acid solution. The materials taken into paraffin-embedded blocks after routine histopathological preparation were sectioned into 5 mm slices with the aid of Leica Rotary microtome. Obtained sections were dyed with Hematoxylin-Eosin and Hematoxylin van Giesson stains and examined. Tissue micrographs were evaluated by pathology specialist with digital camera combined binocular research microscope (Figure 4).

All slides were assessed due fibrous tissue, cartilage, new bone and mature bone ratios according to the scale recommended by Huo *et al.* (Table 3). Radiographic and histopathological scores of control and experimental groups were compared.

Table 3: The scoring system of Huo *et al.* in histological evaluation of fracture healing.

Score	Histological findings of fractured region
Grade 1	Fibrous tissue
Grade 2	Mostly fibrous tissue, little cartilage
Grade 3	Equal amounts of fibrous and cartilage tissue
Grade 4	Mostly cartilage, little fibrous tissue
Grade 5	Cartilage tissue
Grade 6	Mostly cartilage, little immature bone
Grade 7	Equal amounts of cartilage and immature bone tissue
Grade 8	Mostly immature bone, little cartilage tissue
Grade 9	Bone healing with immature bone
Grade 10	Bone healing with mature bone

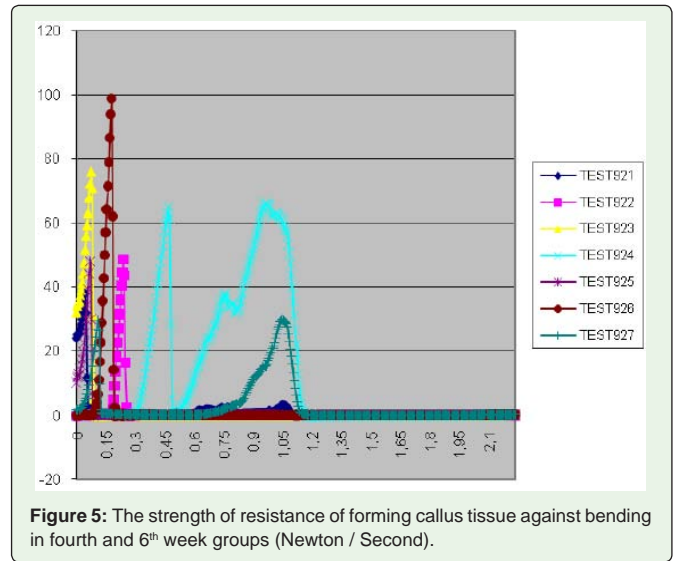


Figure 5: The strength of resistance of forming callus tissue against bending in fourth and 6th week groups (Newton / Second).

Table 4: Radiographical and Histopathological Scores.

	2nd week		4th week		6th week	
	STDY 1	CTRL 1	STDY 2	CTRL 2	STDY 3	CTRL 3
Group(n)	n(9)	n(9)	n(10)	n(9)	n(9)	n(9)
Radio graphical score			3,66±0.32 p= 0,698	3,6±0.84	3 p= 0,587	3
Histopatho logical score	6,36±0.51 p= 0,655	6,7±0.43	8,5±0.54 p= 0,458	9,23±0.40	9,75±0.45 p= 0,387	7

Rat tibias were kept in 10% neutral formaldehyde until biomechanical evaluation. Fine wires, sent for intramedullar fixation, were removed. It has been observed that the reduction was disrupted after removal of intramedullar fixation on tibias of all rats in second week control and study groups. In both groups biomechanical evaluations of 2nd week tibias were not performed. In order to apply bending three point test, after the removal of intramedullar fixations were removed, all rat tibias in fourth and sixth control and test groups placed in ‘The TA-XT2i Texture Analyzer’ (Stable Micro Systems Ltd. Godalming, Surrey, UK) which works extension controlled and can transmit the force, applied by a movement with the velocity of 2mm/second, graphically and quantitatively to the computer screen. The resistance of elements in each group were measured in Newton’s applying force to callus region and compared. The results of control and test groups were statistically compared using Mann-Whitney U test (Table 4) (Figure 5).

Results

Second week groups were excluded from evaluation as no radiological union was observed. The differences between average biomechanical examination values of fourth and 6th week test and study groups were not statistically meaningful (p>0,05). The differences between average histopathological examination values of second, 4th and 6th week test and study groups were not statistically meaningful (p>0,05). No biomechanical assessment could be done on the rat tibias in second week test and study groups. The differences between average biomechanical examination values of fourth and 6th week test and study groups were not statistically meaningful (p>0,05).

Discussion

The most effective medicament group currently used in men and women and on all subgroups - primary or secondary, slow or rapid cycle- of osteoporosis is nitrogen containing bisphosphonates. Bisphosphonates are deposited on the surface of the bone, and thus, inhibit bone resorption. For this reason this group of medicines is used for patients with Paget's disease, hypercalcemia and bone metastasis.

They are successfully used for reversal of increased bone resorption due to malignancy and treatment of hypercalcemia [6,7].

Ibandronate is a bisphosphonate that prevents bone dissolving and vanishing by mixing in the blood stream gradually, and avoids bone loss manifesting this way, and changes the cycle of bone formation and decomposition in the body. It inhibits osteoclasts. It slows down the loss in bones [8].

It has been shown in the literature that in many experimental studies on the effects of methods used in fracture healing, various animal types were used, such as rats, rabbits, dogs, sheep and pigs [9-12]. As bone models, tibia and femur were used on account of the ease of manipulation and exposure, and the ease of histopathologic slide preparation [13,14]. Female mammals have unstable metabolisms due to seasonal hormonal differences. By taking all these reasons into consideration, in this study, we took adult male rat tibia as a model.

Open or closed fracturing techniques can be used for creating a fracture model [15]. As in fractures created by open osteotomy the union happens late and there can be no union, we did not prefer this technique. Accordingly, we applied fracturing technique based on bending three points principle in our study [16].

The bone faces the on-loading in the fractures in which the cortical contact is supplied, and this enhances the bone healing. Fracture callus stimulates remodeling when loaded on progressively. From the biomechanical angle, as the intramedullary nails have a structure that they more likely share the load than carrying, intramedullary nailing technique was preferred for fixation [17,18].

This technique was easy in terms of application and supply of necessary materials but non-rigid fixation was present. We adopted this disadvantage as an advantage as fracture healing was observed more in fixations with non-rigid callus tissue.

In literature, it has been seen that many different time periods were guiding. It has been seen that there are studies that the sacrifice was performed in 3rd day and in 6th, 7th, 10th, 14th, 21th, 24th, 28th and 38th days postoperatively, and there are also studies the sacrifice period was elongated to 10th even 12th weeks. However, it is remarkable that most frequently the healing at the 6th week was examined [6,12,14,15].

We aimed to observe the inflammation and repair phases by performing sacrifice at 2nd and 4th weeks. For this reason, in line with the mentioned literature, postoperative 2nd, 4th and 6th weeks were preferred.

We realized that there are many different methods for radiological evaluation. In these studies, bridge situation between fractured ends were evaluated by direct graphies. Callus sizes were measured with several measurement techniques and various scoring systems were used. We applied the Lane and Sandhu's radiological scoring which Şener *et al.* used in their study [6,10].

However, as the assessments may not be objective enough in fracture healing evaluation, we took the averages of the scores given by two different surgeons that were uninformed about each other during radiological evaluation. It was thought that the results could support histological evaluation [18,19]. In literature for histological evaluations, the evaluation scale of Huo *et al.* was being used. This scale is an evaluation method that shows at what stage is the healing tissue and it is based on scoring between numbers 0 and 4 [8]. As the healing criteria in the scale, the fibrous tissue, cartilage, immature and mature bone ratios in the healing region at proximal and distal sides of fracture line were scored. We also used this scale in our study for histological evaluation.

During the biomechanical evaluation of our study, the forces required for fracturing the rats' tibias were measured. In order to reach the right results, first of all, it has been attentive to fracture all rats' tibias at the same position. After the experiment, before measurement, soft tissues on the bones were fully stripped. Thus, the possibility of the soft tissue affecting the breaking force was prevented.

In a study on strontium renelate by Cebesoy *et al.* parallel to our study, the effects on rat tibia fractures were examined, but they were not able to find the meaningful difference between groups [9]. In a study of Manabe *et al.* after osteotomy, parathyroid hormones were administered and fracture healing was observed in simian femurs [11]. Despite the fact that PTH accelerates the fracture healing process by increasing the mineralization, no meaningful difference was found between groups.

In the study of Paavolainen *et al.* on fracture healing with calcitonin, even though the calcitonin enhances mineralization just like PTH, no difference could be found between groups [20].

It can be considered as a disadvantage of our study that the histopathologic evaluation was performed only one pathologist. As the biomechanical study was performed fully by computer, the disadvantages of the radiological and histopathological evaluations were at the minimum level. During biomechanical evaluation, as there was not sufficient callus in 2nd week, the biochemical evaluation couldn't be performed. There were similarities in 4th and 6th weeks again. The average of fracture forces was increasing by time, and this showed that the strength of callus tissue was improving day by day.

As a result of our study, the data obtained from radiological, histopathological and biomechanical tests exhibit that ibandronate has no possible effect on fracture healing. We think that the study will be more powerful with larger number of test subjects and more than one pathologist's evaluation. Besides, another result of this study is that it has no negative effect on fracture healing.

References

1. Einhorn TA. The cell and molecular biology of fracture healing. *Clin Orthop Relat Res.* 1998; S7-21.
2. Huo MH, Troiano NW, Pelker RR, Gundberg CM, Friedlaender GE. The influence of ibuprofen on fracture repair: biomechanical, biochemical, histologic, and histomorphometric parameters in rats. *J Orthop Res.* 1991; 9: 383-390.
3. Einhorn TA. Enhancement of fracture-healing. *J Bone Joint Surg Am.* 1995; 77: 940-956.
4. Rasubala L, Yoshikawa H, Nagata K, Lijima T, Ohishi M. Platelet-derived growth factor and bone morphogenetic protein in the healing of mandibular fractures in rats. *Br J Oral Maxillofac Surg.* 2003; 41: 173-178.

5. Göktürk E, Turgut A, Bayçu C, Günel I, Seber S, Gülbas Z. Oxygen-free radicals impair fracture healing in rats. *Acta Orthop Scand*. 1995; 66: 473-475.
6. Sevimli R, Uzel M, Sayar H, Kalender AM, Dökmeci O. The effect of dexketoprofen trometamol on the healing of diaphysis fractures of rat tibia. *Acta Orthop Traumatol Turc*. 2013; 47: 423-429.
7. Cao Y, Mori S, Mashiba T, Kaji Y, Manabe T, Iwata K, et al. 1alpha, 25-dihydroxy-2beta (3-hydroxypropoxy) vitamin D3 (ED-71) suppressed callus remodeling but did not interfere with fracture healing in rat femora. *Bone*. 2007; 40: 132-139.
8. Eckardt H, Christensen KS, Lind M, Hansen ES, Hall DW, Hvid I. Recombinant human bone morphogenetic protein 2 enhances bone healing in an experimental model of fractures at risk of non-union. *Injury*. 2005; 36: 489-494.
9. Cebesoy O, Tutar E, Kose KC, Baltaci Y, Bagci C. Effect of strontium ranelate on fracture healing in rat tibia. *Joint Bone Spine*. 2007; 74: 590-593.
10. Lane JM, Sandhu HS. Current approaches to experimental bone grafting. *Orthop Clin North Am*. 1987; 18: 213-225.
11. Manabe T, Mori S, Mashiba T, Kaji Y, Iwata K, Komatsubara S, et al. Human parathyroid hormone (1-34) accelerates natural fracture healing process in the femoral osteotomy model of cynomolgus monkeys. *Bone*. 2007; 40: 1475-1482.
12. Ho AM, Philips NW, Friedlaenden GE. The effect of ketorolac on fracture repair: biomechanical, histologic, and histomorphometric parameters in rats. *J Orthop Res*. 1995; 3: 461-472.
13. Giordano V, Giordano M, Knackfuss IG, Apfel MI, Gomes RD. Effect of tenoxicam on fracture healing in rat tibiae. *Injury*. 2003; 34: 85-94.
14. Turk C, Halici M, Guney A, Akgun H, Sahin V, Muhtaroglu S. Promotion of fracture healing by vitamin E in rats. *J Int Med Res*. 2004; 32: 507-512.
15. Miranda HF, Puig MM, Dursteler C, Prieto JC, Pinardi G. Dexketoprofen-induced antinociception in animal models of acute pain: Synergy with morphine and paracetamol. *Neuropharmacology*. 2007; 52: 291-296.
16. Moore RA, Barden J. Systematic review of dexketoprofen in acute and chronic pain. *BMC Clin Pharmacol*. 2008; 8: 11.
17. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. *J Bone Miner Res*. 2002; 17: 963-976.
18. Lieberman Jay R. Role of fracture hematoma and periosteum during healing in rats. *Bone Regeneration and Repair Review of Orthopaedics*. 2007; 221: 254-263.
19. Cabré F, Fernández MF, Calvo L, Ferrer X, García ML, Mauleón D. Analgesic, antiinflammatory, and antipyretic effects of S(+)-ketoprofen in vivo. *J Clin Pharmacol*. 1998; 38: 3S-10S.
20. Paaivolainen P, Taivainen T, Michelsson JE, Lalla M, Penttinen R. Calcitonin and fracture healing. An experimental study on rats. *J Orthop Res*. 1989; 7: 100-106.