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Original Article

The effect of vitamin D and bisphosphonate on fracture healing: An experimental study

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ABSTRACT

Background: The aim of the study was to evaluate the effects of the using bisphosphonate, vitamin D, and a combination of bisphosphonate and vitamin D on fracture healing, by comparison of radiological and histological findings of the study groups and a control group. **Methods:** A total of 24 rats were randomly divided into 4 groups. A mid-third fracture was created in the femur of all rats. Saline was administered to Group A, bisphosphonate (Alendronate) to Group B, bisphosphonate (Alendronate) + vitamin D (Calcitriol) to Group C and vitamin D (Calcitriol) to Group D. All preparations were administered orally for 28 days. **Results:** No statistically significant difference was determined between the groups in respect of the effect on fracture healing according to radiological findings. The histological findings of fracture healing showed Groups B and C to be significantly more advanced than Group A ($p = 0.017$, $p = 0.009$). However no significant difference was found in Group D comparison with Group A ($p = 0.224$).

Conclusion: According to the histological findings, advanced fracture healing was seen in the groups administered with bisphosphonate or combined bisphosphonate and vitamin D compared to the use of vitamin D alone and the control group. It was concluded that bisphosphonate treatment combined with vitamin D can be used safely without any negative effect on fracture healing.

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Bisphosphonates are currently widely used in the treatment of postmenopausal osteoporosis and many disorders related to bone metabolism.¹ Bisphosphonate has been shown to decrease osteoclast activities, which provide direct bone resorption and increase apoptosis.² Bone is the only tissue, which can store bisphosphonate because of the high affinity.³ As soon as it is filtered from plasma, 50% of bisphosphonate is

stored in the bones, and the remainder is discarded in the urine without being metabolized.^{3,4} Clinical studies of this selective tissue uptake have shown that bisphosphonate drugs reduce the incidence of bone loss and fracture.^{5,6}

Vitamin D deficiency is a common condition in postmenopausal women⁷ with 57–82% of untreated patients with postmenopausal osteoporosis reported to have low serum

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25 hydroxyvitamin D (25(OH)D) levels. These low levels have been associated with low levels of bone mass.⁸ Some studies have stated that the vitamin D deficiency was associated with the severity of the osteoporosis.⁹ In addition, vitamin D deficiency has been held responsible for 18–35% of cases treated with bisphosphonate, which have resulted in insufficient treatment of osteoporosis.¹⁰ Intravenous use of bisphosphonate may lead to severe hypocalcemia,⁶ which also adversely affects the bone construction–destruction cycle. Therefore, vitamin D should be added initially to the treatment of bisphosphonate for prophylactic or therapeutic purposes.¹¹

Although there are many studies in literature about the effects of bisphosphonate and vitamin D on fracture healing and bone cells, there are few, which compare the effects of the two treatments. In this study, it was aimed to show the effect of bisphosphonate, vitamin D, and a combination of bisphosphonate and vitamin D on fracture healing by radiological and histological comparison with each other and with the control group.

1. Materials and methods

The experimental protocols were approved by the Animal Care Committee of Gazi University. A total of 24 adult male Wistar albino rats weighing 325 ± 25 g were used. The rats were caged individually in a controlled environment. The room temperature was 23 ± 2 °C and humidity was 40–70%. Rats were fed ad libitum with 12 h light/dark cycles. The device used for fracturing the rat femur was produced by Bonnarens ve Einhorn (Fig. 1A).¹² Preoperative antibiotic prophylaxis was applied prior to anesthesia, Cefamezin 40 mg/kg (Iespor[®] im/iv, I.E. Ulagay, Turkey).

The 24 rats were randomly divided into four groups of 6 as A, B, C, and D. The rats were taken into the operating room after the necessary monitoring and preparation. The dose of anesthesia was adjusted for each rat according to the body

weight measured electronically. A combination of Ketamine (Ketalar[®], Pfizer, Turkey) 50 mg/kg and Xylazine (Rompun[®], Bayer, Turkey) 10 mg/kg was used for anesthesia.¹³ The surgical procedures were applied by the same surgeon. The left knee of each rat was stained with povidone-iodine (Isosol[®], Central Lab., Turkey) and covered with a sterile drape. The joint capsule was opened with an anteromedial longitudinal incision passing through the skin and subcutaneous tissue. The knee was held in flexion with the patella removed laterally to reveal the femoral condyles (Fig. 2A). A K-wire of 0.8 mm was placed through the intercondylar notch (Fig. 2B). Then the K-wire was cut so as not to form an external bulge, the patella was replaced with the knee in extension, and the layers were closed in the anatomical plane. The K-wire position was corrected by radiography examination (Fig. 3A), and then the closed fracture was made in the middle third of the femur with the device, which was identified by Bonnarens and Einhorn on the basis of a three-point index (Fig. 1A). The fractures were corrected by radiography (Fig. 3B). In the postoperative period, the rats were allowed free weight bearing. Medication was applied after the fracture was applied to the rat femur. Daily oral administration of saline solution was applied to Group A (control group), bisphosphonate (Alendronate) 10 mg/day to Group B, the combination of bisphosphonate (Alendronate) 10 mg/day + vitamin D (Calcitriol) 0.5 µg/day to Group C, and vitamin D (Calcitriol) only 0.5 µg/day to Group D. Alendronate was administered after 2 h of fasting to prevent interference with the absorption. Calcitriol was given 2 h after Alendronate dosing.

No animal was lost postoperatively or throughout the follow-up period. There was no wound infection in any case throughout the follow-up. The extremities of the experiment groups were evaluated in terms of fixation during the drug application. The study was terminated on day 28 after the femur fracture. The rats were sacrificed for radiological and histological evaluation. The closed fractured in the left limb was completely removed without damaging the callus tissue in order not to affect the assessment.

Fig. 1 – (a) The device used for fracturing of the rat femur is shown. (b) The middle 1/3 of femur rats were closed fracture through this device.

Fig. 2 – (a) Femoral condyles were revealed by surgical anteromedial longitudinal approach. (b) 0.8 mm Kirschner wire was placed through intercondylar notch.

1.1. Radiological evaluation

Direct radiographs were taken in the antero-posterior position and were scored by 3 different independent orthopedic surgeons (T.K., D.G. and O.T), according to the Goldberg classification; 1 = no fracture healing, 2 = moderate healing, 3 = full healing (Table 1).¹⁴

1.2. Histological evaluations

The histological evaluations were made in the clinic of pathology. After radiological analysis, all the femurs were fixed in 10% formalin solution. They were decalcified at an average of 72 h after the period of fixation with 10% acetic acid, 0.85% NaCl and 10% formalin solution. After removing the K-wire, sections were taken including both distal and proximal parts of the fracture line. The samples were embedded in

paraffin blocks and longitudinal sections of 4–5 μm were cut and stained with hematoxylin–eosin. Histological classification was made according to the Huo et al.'s scale¹⁵ by a pathologist blinded to the groups. The cells and tissues of each cross-section were evaluated at different magnifications ($\times 40$ – $\times 100$).

1.3. Statistical analysis

Statistical analysis of the study data was performed with SPSS 16 for Windows software. Data were stated with descriptive statistical methods (mean, standard deviation, median, minimum and maximum values), and comparisons between the groups were made using the Kruskal–Wallis test. In paired group comparisons, the Mann–Whitney U test was used. A value of $p < 0.05$ was accepted as statistically significant.

Fig. 3 – (a) Kirschner wires for correcting the position by radiography was shown. (b) Fractures were corrected by radiography.

Table 1 – The histological and radiological findings evaluated by three separated orthopedic surgeon and comparison of scores of groups.

Group	Histological scores	Orthopedic surgeon	Orthopedic surgeon	Orthopedic surgeon
A	2.16	1.33	1.16	1.5
B	5.5	2.83	2.67	2.83
C	5.33	2.83	2.83	2.67
D	3.5	1.83	1.5	1.67
p value	0.038	0.512	0.895	0.227

2. Results

2.1. Radiological findings

The mean points determined by 3 different orthopedic surgeons according to the Goldberg classification were 1.33–1.16–1.5 in Group A, 2.83–2.67–2.83 in Group B, 2.83–2.83–2.67 in Group C and 1.83–1.5–1.67 in Group D (Table 1).

No statistically significant difference was determined between the groups in respect of the radiological scores compared with the Kruskal–Wallis test ($p = 0.512$, $p = 0.895$, $p = 0.227$) (Table 1). The treatment with bisphosphonate, bisphosphonate and vitamin D, and vitamin D alone did not have a statistically significant effect on fracture healing.

2.2. Histological findings

The mean histological scores were determined as 2.16 in Group A, 5.5 in Group B, 5.33 in Group C and 3.5 in Group D. Using the Kruskal–Wallis test, the difference between the groups was statistically significant ($p = 0.038$) (Table 1).

In the comparisons of the histological scores between the groups using the Mann–Whitney *U* test, the difference was statistically significant between Groups A and B ($p = 0.017$), Groups A and C ($p = 0.009$), Groups B and D ($p = 0.043$), and Groups C and D ($p = 0.037$). No statistically significant difference was determined between Group B and Group C in respect of histological scores ($p = 0.326$) (Table 2).

In light of these histological findings, fracture healing was found to be significantly more advanced in both Group B and Group C compared with Group A. However, there was no statistically significant difference between Group D and Group A.

Table 2 – Control group was compared with treated groups according to histological scores.

Group name	Histological scores	p value
SF/Bifosfonat	2/5.5	0.017
SF/Bifosfonat and vitamin D	2/5	0.009
Bifosfonat/vitamin D	5.5/3.5	0.043
Bifosfonat and vitamin D/vitamin D	5/3.5	0.037

3. Discussion

The main aim of studies is to accelerate the process of fracture healing. The effect of various drugs has been examined on experimental animal model studies. Bisphosphonates, which are commonly used in the treatment of postmenopausal osteoporosis, are a potent inhibitor of osteoclast-induced bone resorption. Several studies have recently investigated the effect of this impact on fracture healing. In a study by Fink et al.,¹⁶ in the presence of postmenopausal osteoporosis or non-traumatic fractures, bisphosphonate therapy was shown to reduce the risk of vertebral and non-vertebral fracture. Clement et al.¹⁷ investigated the effect of bisphosphonate on fracture healing in osteoporotic women, who had distal radius fracture and identified an increase in bone mineral density in the fracture field after 8 weeks of bisphosphonate usage in the post-fracture period. The histological findings of the current study showed the fracture healing in the bisphosphonate group to be significantly advanced compared to the control group.

Alendronate was the first approved bisphosphonate for the treatment of osteoporosis in the US (>11 years).¹⁸ It has been reported that in bone biopsies from patients with 10 years alendronate use or 5 years risedronate use, no inhibition was found in progressive bone metabolism.¹⁹ Peter et al.²⁰ emphasized that Alendronate, which is a highly potent amino bisphosphonate and is frequently used in the treatment of osteoporosis, had no negative impact on surgical fracture healing, bone strength or mineralization.²⁰ The findings of the current study support these findings as a statistically significant difference was determined in Group 2 (bisphosphonate) and Group 3 (bisphosphonate and vitamin D) compared with Group 1 (control) according to the histological scores. However, the difference between the histological scores of Group 2 and Group 3 was not statistically significant ($p = 0.326$).

In the current study, there was a statistically significant difference between the histological scores of Group 3 (bisphosphonate + vitamin D) and Group 1 (control) but no statistically significant difference between Group 4 (vitamin D) and Group 1 (control). In a study of patients with an osteoporotic, acute, proximal humeral fracture, Doetsch et al.²¹ administered vitamin D and supplementary calcium to one group and compared these patients with the placebo group. BMD was measured in the fracture field and at the end of 6 weeks, callus formation was higher in the treated group, although there was no difference at the end of 12 weeks. In contrast to the findings of the current study by Ömeroğlu et al.²² stated that in terms of histological scores, the usage of high-dose vitamin D increased the mechanical strength of the callus in fracture healing in healthy rats.

Another study conducted by Von Schacht et al.²³ with a similar design and results to the present study, suggested that the use of a combination of alfacalcidol and Alendronate in osteoporosis treatment reduced the side effects associated with long-term usage of bisphosphonates, thus preventing hypocalcemia and the risk of fracture. In addition, the results of a study by Peris et al.²⁴ were consistent with those of the current study and it was emphasized that 25(OH)D level should be >30 ng/ml to provide a sufficient response to

bisphosphonate treatment. In a recent study, Sugimoto et al.²⁵ stated that Eldecalcitol, which is a derivative of 1α 25(OH) $_2$ D $_3$, or a combination of alfacalcidol and Alendronate, has advantages over Alendronate monotherapy and has a more beneficial anti-osteoporosis effect.

The limitations of the current study were (1) the small sample size was considered to be a restrictive factor in interpretation of the results; however, this number in each group was determined by the Local Ethics Committee. (2) Histopathological evaluation was performed by a single pathologist; (3) the healing was not evaluated by micro computed-tomography; (4) the effect on fracture healing was not shown radiologically. Therefore, there is a need for further experimental and clinical studies using various bisphosphonate and vitamin D combinations to be conducted on a larger number of subjects over longer periods to better reveal the effect on fracture healing.

In conclusion, the use of bisphosphonate and the combination of bisphosphonate + vitamin D was shown to increase fracture healing histologically compared to vitamin D supplementation alone and the control group. Therefore, initiating bisphosphonate treatment after the fracture occurred, has been shown to affect healing positively. Bisphosphonate treatment combined with vitamin D can also be safely used without negative effects on fracture healing.

Conflicts of interest

The authors have none to declare.

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