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*Eklem Hastalık Cerrahisi*
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# The effects of leukotriene receptor antagonist montelukast on histological, radiological and densitometric parameters of fracture healing

## Lökotrien reseptör antagonisti montelukastın kırık iyileşmesi üzerindeki histolojik, radyolojik ve dansitometrik parametrelerin etkisi

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**Objectives:** In this study we evaluated the effects of montelukast, a leukotriene-receptor antagonist, on fracture healing, and investigated the hypothesis that enhanced fracture healing would be observed with montelukast in a rat tibia fracture model.

**Materials and methods:** Sixty adult (6 months old) female Wistar albino rats (mean weight 220 g, range 210-270 g) were randomly divided into two groups: a montelukast group (n=30) and a control group (n=30). Closed tibia fractures were created and fixed by intramedullary Kirschner wire. The rats were sacrificed three and six weeks after the fractures. Radiological and histological evaluations were performed, and bone mineral density was measured.

**Results:** Three rats died in the montelukast group, whereas only one died in the control group during the study. Initial weight and weight gain at the 3rd and 6th weeks were not significantly different between the groups (p>0.05). Bone mineral densities in the control and study groups were 0.13±0.009 gr/cm$^2$, and 0.13±0.01 gr/cm$^2$ at week three and 0.16±0.02 gr/cm$^2$, and 0.13±0.01 gr/cm$^2$ at week six, respectively. Histopathological scores in the control and study groups were 3.42±0.6, and 3.0±0.0 at week three and 3.5±0.5, and 3.4±0.8 at week six, respectively. Radiological scores in the control and study groups were 1.19±0.6, and 1.0±0.6 at week three and 3.0±0.8, and 2.9±0.9 at week six, respectively. There were no significant differences between the two groups in any parameters evaluated at either time interval (p>0.05).

**Conclusion:** Our study failed to show a possible positive effect of leukotriene receptor inhibition on fracture healing at the 3rd and 6th postoperative weeks.

*Key words:* Experimental study; fracture healing; leukotriene-receptor antagonist; montelukast.

**Amaç:** Bu çalışmada lökotrien reseptör antagonisti montelukastın kırık iyileşmesi üzerine olan etkileri incelendi ve sıçan tibia kırık modelinde montelukastla artmış kırık iyileşmesi görülebileceği hipotezi araştırıldı.

**Gereç ve yöntemler:** Altmış erişkin (6 aylık) dişi Wistar albino cinsi sıçan (ortalama ağırlıkları 220 g, dağılım 210-270 g) rastgele şekilde iki gruba ayrıldı: montelukast grubu (n=30) ve kontrol grubu (n=30). Kapalı tibia kırığı oluşturuldu ve intramedüller Kirschner teliyle fikse edildi. Kırıktan üç ve altı hafta sonra sıçanlar sakrifiye edildi. Radyolojik ve histolojik değerlendirmeler yapıldı ve kemik mineral yoğunlukları ölçüldü.

**Bulgular:** Çalışma sırasında montelukast grubunda üç, kontrol grubunda ise yalnızca bir sıçan öldü. Gruplar arasında hayvanların başlangıç, 3. hafta ve 6. haftadaki ağırlıkları açısından istatistiksel bir fark bulunmadı (p>0.05). Kemik mineral yoğunluğu kontrol ve çalışma grubunda sırasıyla 3. haftada 0.13±0.009 g/cm$^2$ ve 0.13±0.01 g/cm$^2$, 6. haftada ise 0.16±0.02 g/cm$^2$ ve 0.13±0.01 g/cm$^2$ olarak bulundu. Histopatolojik skor kontrol ve çalışma gruplarında sırasıyla 3. haftada 3.42±0.6 ve 3.0±0.0, 6. haftada ise 3.5±0.5 ve 3.4±0.8 olarak bulundu. Radyolojik skor kontrol ve çalışma gruplarında sırasıyla 3. haftada 1.19±0.6 ve 1.0±0.6, 6. haftada ise 3.0±0.8 ve 2.9±0.9 olarak bulundu. Değerlendirilen hiçbir parametre açısından her iki zaman aralığında iki grup arasında anlamlı fark saptanmadı (p>0.05).

**Sonuç:** Bizim çalışmamızda lökotrien reseptör antagonistinin 3. ve 6. haftalarda kırık iyileşmesine olumlu etkisi olmadığı görüldü.

*Anahtar sözcükler:* Deneysel çalışma; kırık iyileşmesi; lökotrien reseptör antagonisti; montelukast.

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Arachidonic acid is the precursor of prostaglandins and leukotrienes, which are lipid-signaling molecules. The cyclooxygenase enzyme is involved in the conversion of arachidonic acid to prostaglandins. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase activity and lead to the impairment of fracture healing in animal models. Furthermore, they can reduce heterotopic bone formation, and may impair fracture healing in humans.[1,2]

Analogous to cyclooxygenase, 5-lipooxygenase (5-LO) converts arachidonic acid into two major groups of leukotrienes: LTB4 and cysteinyl leukotrienes 'LTC4, LTD4, LTE4, and LTF4'. Cysteinyl leukotrienes have been reported to be negative regulators of mesenchymal cell differentiation.[3] Based on this report, Wixted et al.[4] tested the hypothesis that specific CysLT1 receptor inhibition promotes mesenchymal cell differentiation during fracture repair. Wixted et al.[4] also reported an increase in callus size, chondrocyte proliferation, and differentiation during early bone fracture healing.

Montelukast is a selective reversible Cys-LTl-receptor antagonist used in the treatment of asthma.[5] We hypothesized that montelukast would enhance fracture healing in the rat tibia fracture model at three and six weeks after the fracture.

## MATERIALS AND METHODS

Sixty female adult Wistar albino rats (6 months old, mean weight 220 gr, range 210-270 gr) were randomly divided into two groups: a montelukast group (30 rats) and a control group (30 rats). Rat weights (initial weight and weight at week 3 and week 6) were recorded. The Abant İzzet Baysal University Ethics Committee approved the experimental design and all procedures. Rats were housed in a temperature-controlled environment (22-24 ºC) with free access to water and lights were turned on from 08.00 hours to 20.00 hours. All animals were fed a commercial diet during the experiment.

The montelukast solution was prepared by adding 1 ml of saline to a calculated number of montelukast granules based on the weight of the animals. The montelukast group received 1 ml of solution containing 1 mg/kg montelukast intraperitoneally (IP) 30 minutes before surgery and then daily for six weeks. In the control group, 1 ml of saline was administered IP 30 minutes before surgery and then daily for six weeks.

All rats were anesthetized by the intraperitoneal administration of 60 mg/kg of ketamine and 10 mg/kg of xylazine. The left hind limb was shaved and washed with a povidone iodine solution. The middle of the diaphysis of the tibia was fractured using a custom-made three-point bending device as described previously.[6] Under aseptic conditions, a medial parapatellar incision was created. The patella was dislocated laterally, and the medullary canal was entered through the intercondylar area and reamed with a 21-gauge needle. The fracture was reduced and fixed with a 0.7 mm stainless steel pin inserted through the medullary canal. The proximal portion of the pin was cut flush with the knee joint so as not to interfere with knee function. The patellar dislocation was reduced and the soft tissue and skin were closed with 4-0 Vicryl resorbable sutures in two layers. The animals were caged in pairs and allowed to walk freely after surgery. Radiographs of the fractures were obtained at week three and week six. Two independent observers examined blinded radiographs. Radiographic examination was dependent on a 4-grade scale for each bone. The grading scheme was based on the bridging of the fracture by callus and cortical bone. One point was assigned for each aspect of the bridging (right callus and cortex, left callus and cortex) across the fracture site.[7]

Three rats died in the montelukast group (two on the 1st day, one in the 5$^{th}$ week), whereas only one died in the control group (in the 4$^{th}$ week) during the study. Fourteen of the animals in each group were sacrified with high doses of thiopental in the 3$^{rd}$ week whereas all remaining animals were sacrified in both groups in the 6$^{th}$ week (13 animals in the montelukast group, 15 in the control group). Tibial bones from each group were removed for histopathological examination and dual-energy X-ray absorptiometry measurement and embedded into a fixative solution of 10% formalin.

One investigator who was blinded to the study groups performed histological analysis. Bones were embedded in paraffin, and five longitudinal serial sections, each 5 $\mu$m thick, were taken from the injured site 'core area' of bones. Hematoxylin and eosin (H-E) staining was performed. The histological grading of fracture healing was performed according to the 5-grade system (Table I).[8] Bone mineral density (BMD) was measured using Lunar-DPX-IQ (Madison, WI, USA) with small animal software. The instrument was set to 76.0 kV and 150 $\mu$A, collimation was fine (with a

**TABLE I**

Histological grading of fracture healing scores

| Histological evaluation | Grade |
|---|---|
| Pseudoarthrosis formation | 0 |
| Incomplete cartilaginous union | 1 |
| Complete cartilaginous union | 2 |
| Incomplete bony union | 3 |
| Complete bony union | 4 |

**TABLE II**

Results for bone mineral density, histopathological scores, radiological scores and weight changes

| | Bone mineral density (gr/cm²) | | Histopathological scores | | Radiological scores | | Weight changes | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3rd week | 6th week | 3rd week | 6th week | 3rd week | 6th week | Pre-operative | 3rd Week | 6th week |
| Control group | 0.13±0.0 | 0.16±0.0 | 3.42±0.6 | 3.5±0.5 | 1.19±0.6 | 3.0±0.8 | 220 | 235 | 245 |
| Montelukast group | 0.13±0.0 | 0.13±0.0 | 3.0±0.0 | 3.4±0.8 | 1.0±0.6 | 2.9±0.9 | 220 | 240 | 247 |
| *p* | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

standard region of interest mode of 0.3x0.3 cm area), and the sample interval was 1/64.

**Statistical analysis**

The Mann-Whitney U-test was used to test differences between the two groups in terms of radiological and histological scores and rat weights. The t-test was used to test differences in bone mineral density measurements. A p-value of <0.05 was considered statistically significant. Data means and standard deviations were reported.

## RESULTS

Weights of all rats were recorded and summarized in table II. Initial weights and weight gains in the 3rd and 6th weeks were not significantly different between the groups (p>0.05). Bone mineral densities were found in control and study groups 0.13±0.009 gr/cm² and 0.13±0.01 gr/cm² at week 3 (p>0.05), and 0.16±0.02 gr/cm², and 0.13±0.01 gr/cm² at week 6 respectively (p>0.05). Radiological scores were found in control and study groups 1.19±0.6 and 1.0±0.6 at week 3 (p>0.05), and 3.0±0.8, and 2.9±0.9 at week 6 respectively (p>0.05; Table II; Fig. 1a, b). Histopathological scores were found in control and study groups 3.42±0.6 and 3.0±0.0 at week 3 (p>0.05), and 3.5±0.5, and 3.4±0.8 at week 6 respectively (p>0.05; Fig. 2a, b).

## DISCUSSION

Analogous to cyclooxygenase, 5-lipooxygenase (5-LO) converts arachidonic acid into two major groups of leukotrienes. Montelukast is a selective reversible Cys-LTl-receptor antagonist used in the treatment of asthma. It was reported that the CysLT1-receptor antagonist montelukast reversed ischemia reperfusion-induced oxidant responses and improved microscopic damage by possible inhibition of neutrophil recruitment and pro-inflammatory mediators.[5] On the other hand, 5-LO metabolite 'leukotrienes' act as negative regulators of mesenchymal cell differentiation.[3] Fracture repair is an inflammatory process, and metabolites affecting leukotriene metabolism may affect this process.[9] 5-LO enzyme inhibition with AA-861 (a leukotriene-receptor inhibitor) has been reported to accelerate endochondral ossification depending on early increases in callus cartilage, bone formation, and callus cell proliferation. 5-LO inhibition has led to increases in bone formation at the fracture site seven days after the fracture but lower callus areas 21 days after the fracture, indicating callus bone bridging and onset of callus bone remodeling.[9-11] In another study, montelukast was reported to enhance early chondrogenesis and increase callus size. Enhanced chondroid formation was present as early as day seven and sustained to day 14 after the fracture, indicating rapid mesenchymal cell differentiation and sustained chondrocyte proliferation.[9] However by day 21, all treatment groups had bridged the fracture ends and remodeled the callus to the woven bone. This may suggest that early increases in chondrogenesis may occur at the expense of a delay in osteogenesis.[4] We could not find differences in fracture healing parameters either at week three or at week six that supported the hypothesis. Our histologic results in the 3rd week showed that both groups had incomplete bone union in most of the animals, whereas complete bone union was present in the 6th postoperative week in most of

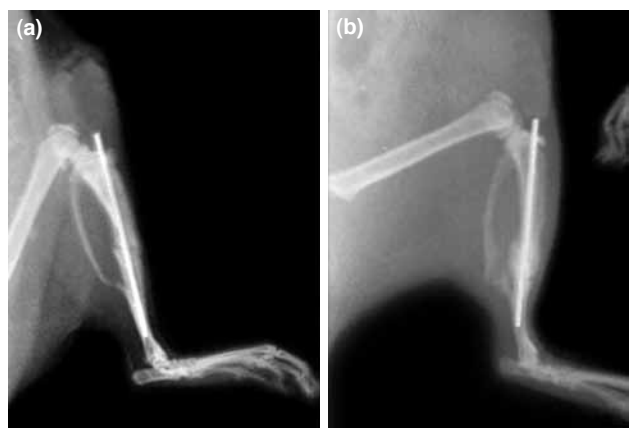


**Figure 1. (a)** Postoperative 3rd week radiography of surgically treated closed tibia fracture in montelukast treated group. No callus formation is seen (left). **(b)** Postoperative 6th week radiography of surgically treated closed tibia fracture in montelukast treated group. Callus formation and complete bone union is seen (right).

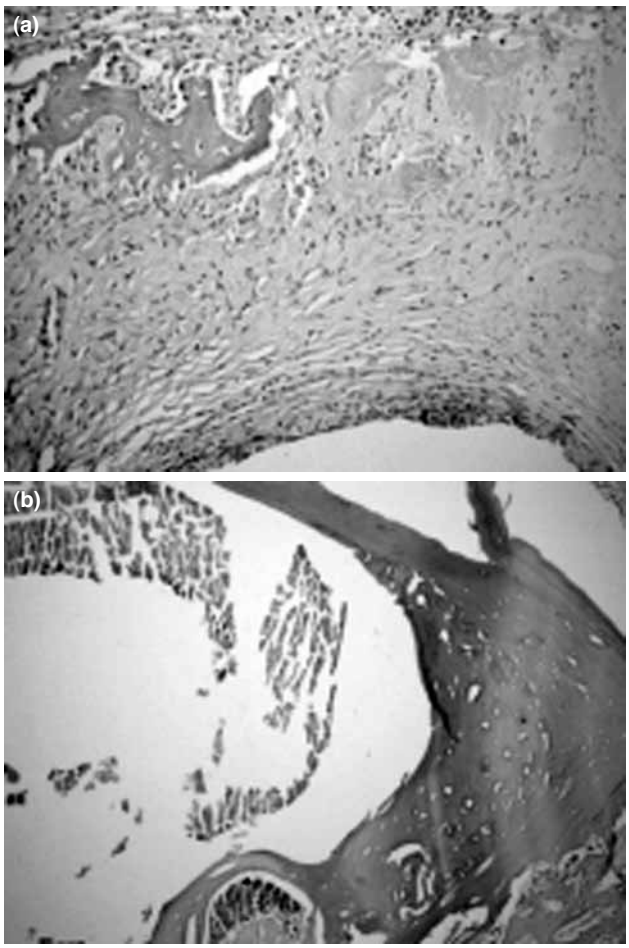


**Figure 2. (a)** Complete cartilaginous and incomplete bony union in montelukast treated group at 3rd postoperative week (H-E x 100; left). **(b)** Complete bony union in montelukast treated group at 6th postoperative week (H-E x 100; right).

the animals. Accordingly, we contend that the positive effect of leukotriene 5-LO inhibition only exists in early phases of bone healing. Furthermore, the difference between the groups equalized between the 14th and 20th days, and no difference could be detected on the 21st day. Moreover, during the remodeling phase (3rd to 6th weeks), we could not detect any histologic difference between the groups, supporting the hypothesis that the effect of montelukast only exists in early phases of fracture healing. Additionally, the results of radiologic and densitometric evaluations support this hypothesis. A limitation of our study was that we did not investigate early phases of bone healing (1st and 2nd weeks). However, our aim was to investigate the late effects of montelukast on bone healing. Another limitation of this study was that we did not perform a biomechanical study to detect the strength of fracture healing. Instead, we measured the bone mineral density of the healing bone. During the process of bone healing, mineralization of the callus leads to higher bone density and bone mass of the callus tissue, leading to increased bone density and correlation of increased bone density with increased strength of fracture healing.[12] In our study, we could not detect any differences between the two groups.

Montelukast sodium is approved for use in adults and children to control symptoms of asthma and for relief of symptoms of indoor and outdoor allergies. An oral dose of 10 mgs is usually used once a day. However, we used montelukast IP 1 mg/kg once a day for two reasons: *(i)* to ensure the exact dosage taken by the animal and *(ii)* because this application method is the preferred method in animal studies with montelukast.[5,13,14] Our hypothesis was that montelukast would enhance fracture healing in rats. The surgically treated closed tibia fracture model was not improved, although AA-861, which is a leukotriene-receptor antagonist, has been shown to enhance fracture healing.[9] First, we think that differences in the effects of these two drugs on fracture healing occurred due to differences in the mechanisms of action and pharmacological effects of AA-861 and montelukast. We suggest that the therapeutic properties of montelukast differ from those of AA-861 and that they should be investigated in further studies. Second, although early chondrogenesis was reported to be enhanced by 5-lipoxygenase inhibition, initiation of osteogenesis or late remodeling of fracture healing may be affected in a different manner. Although 5-lipoxygenase inhibition was reported to enhance early chondrogenesis and increase callus size, it has also been reported that collagen maturation inhibits montelukast, which may also cause retardation in bone union. Inhibition of collagen maturation may also explain why montelukast does not enhance fracture healing in the 3rd or 6th weeks.[15] Further studies are necessary to present the effects of montelukast during the different stages of fracture healing.

In conclusion, our study failed to show that montelukast enhances bone healing at the 3rd and 6th weeks after fracture. However, further studies are needed to clarify its positive effects and mechanisms in the early phases of bone healing.

**Declaration of conflicting interests**

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