Kafkas Univ Vet Fak Derg
18 (4): 671-676, 2012
DOI:10.9775/kvfd.2012.6270

RESEARCH ARTICLE

The Effects of Ketoprofen and Meloxicam on Bone Healing in Rat Model: A Comparative Dual Energy X-Ray Absorptiometry Study [1]

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[1] *This research was supported by the Scientific Research Project Coordination Unit of Kırıkkale University*

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Makale Kodu (Article Code): KVFD-2012-6270

Summary

Pain control is a common clinical approach in trauma and postoperative care especially complicated orthopedic surgeries to ease the deleterious effects of pain. Various kinds of pain killers have been used, and nowadays nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs for pain control purposes. Prostaglandin-endoperoxide synthetase, also commonly called cyclooxygenase (COX), is one of the key enzymes in prostaglandin bio-synthesis. The COX enzymes have subgroups of enzymes, each of which suppresses different inflammatory mediators. These enzymes are involved in different functions, some of which are essential for continuity of physiological processes. Thus, NSAIDs are expected not to cause any change of functions of some enzymes while suppressing others. Among the COX enzymes, COX-1 is associated with gastrointestinal system functions and gastrointestinal mucosa while COX-2 is associated with inflammation and pain. Like most drugs, NSAIDs have known and possible side effects. In various studies related to NSAIDs, inhibitory effects of conventional NSAIDs with non-selective effects and specific COX-2 inhibitors on bone healing have been reported. In this study, the effects of ketoprofen and meloxicam on bone fracture healing induced in 24 adult male Wistar rats was studied by Dual Energy X-Ray Absorptiometry (DEXA). The results indicates that meloxicam inhibits the fracture healing to some degree.

Keywords: *Cyclooxygenase inhibitors (COX), Ketoprofen, Meloxicam, Dual energy X-Ray Absorptiometry (DEXA), Bone healing, Rat*

Ketoprofen ve Meloksikam'ın Kemik İyileşmesi Üzerine Etkilerinin Rat Modelinde DEXA Ölçümleri ile Değerlendirilmesi

Özet

Günümüzde ağrının kontrol edilmesinin gerekliliği net olarak ortaya konulmuştur. Bu amaçla farklı ilaç grupları kullanılmaktadır, şu an için en yaygın kullanılan ilaç gruplarından birisi de steroid olmayan yangı önleyici ilaçlardır (NSAIDs). Prostaglandin-endoperoxide sentetaz ya da daha sık kullanılan adı ile siklooksijenaz enzimleri (COX) prostoglandin sentezinde anahtar rolü olan enzimlerdir. COX enzimleri üç alt grubu olan bir enzim grubudur ve her alt grup farklı yangı mediatörlerini baskılarlar. Bu enzimlerin farklı fonksiyonları vardır ve bazıları fizyolojik fonksiyonların devamı için gereklidir. Dolayısı ile kullanılacak NSAID ilacın kimi enzim gruplarını baskılarken kimi enzim gruplarının fonksiyonlarında değişim oluşturmaması istenir. Bu enzim gruplarından COX-1 olarak isimlendirilenler gastrointestinal sistem fonksiyonları ve mukozası ile, COX-2 grubu ise yangı ve ağrı ile ilişkilidir. İlaçların çoğu gibi NSAID ilaçların da bilinen yan etkilerine ilaveten olası yan etkileri de vardır. NSAID ilaçlarla ilgili yapılan çeşitli araştırmalarda, hem seçici etkisi olmayan geleneksel NSAID ilaçların hem de spesifik COX-2 inhibitörü ilaçların kemik iyileşmesi üzerinde inhibe edici etkileri rapor edilmiştir. Bu çalışmada 24 adet, Wistar ırkı, erişkin, erkek rat kullanılarak ketoprofen ve meloksikamın kırık iyileşmesi üzerine olan etkileri klinik gözlemler ve Dual Energy X-Ray Absorptiometry (DEXA) ölçümleri ile ortaya konulmaya çalışılmıştır. Elde edilen veriler meloksikamın kırık iyileşmesini bir ölçüde inhibe edebildiğini göstermektedir.

Anahtar sözcükler: *Siklooksijenaz inhibitörleri (COX), Ketoprofen, Meloksikam, Dual enerji X ray absorbtiometry (DEXA), Kemik iyileşmesi, Rat*

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INTRODUCTION

During the last two decades, studies and reports clearly indicated that pain causes physiological abnormalities that have harmful effects on organisms. Once it is understood pain control one of medical priorities in patients, the pain medications as well as their modes of use and related procedures have been under investigation. In past, non-steroidal anti-inflammatory drugs (NSAIDs), having a moderate analgesic effects, were only used in osteo-arthritis. However, the new generation NSAIDs have a wide range of use as the side effects were minimized. As in anti-inflammatory agents and pain killers, NSAIDs are commonly used in post operative care of small animals to control inflammation and pain as well as to alleviate pain caused by diseases such as osteoarthritis [1-3]. The higher success rate of NSAIDs compared to other narcotic pain killers has been proved through comparative studies conducted in human and animals [4-8].

Prostaglandin-endoperoxide synthetase, commonly called cyclooxygenase (COX), is the key in bio synthesis of prostaglandins. Cyclooxygenase represents a group of enzymes. The COX-1 enzymes are associated with gastro-intestinal system mucosa and implemented in gastro-intestinal functions. On the other hand, the COX-2 enzymes, defined by Daniel Simmons in 1988, are responsible inflammation and pain [9-11]. After characterization of COX-2 enzymes, researchers identified another enzyme group in the dog brain and called it COX-3 [12]. Although the COX-3 enzymes seemed different than COX-1 and COX-2, later they were defined as COX-1 derivatives upon revealing that they are controlled by the same gene [12]. The COX-3 enzyme can be inhibited by the drugs with a COX-1 and COX-2 inhibition effects such as paracetamol [12].

Several researchers, who conducted research regarding NSAIDs effects on bone fracture healing using direct or radiological methods, reported that NSAIDs did not cause a quantitative difference in callus formation [13-15]. However, some studies reported some qualitative difference through histological studies. These studies indicated that NSAIDs retards callus maturation [16-18], however, the mechanism of COX-2 inhibitor effects on bone healing and bone metabolism has not been clearly defined yet. In the mean time, it is known that COX-2 enzymes have some effects on intramembranous and endochondrial ossification [19] and are required in bone healing [20].

In this study, it was aimed to investigate possible inhibitory effects of the NSAID ketoprofen and meloxicam at post-operative doses in bone healing in a rat femur fracture model through bone mineral density (BMD) measurement techniques.

MATERIAL and METHODS

The animal experiments proceeded upon obtaining an approval from the Kırıkkale University Ethical Council for Animal Experiments (2009/13). A total of 24 male Wistar rats (GATA, Turkey) with a mean weight of 296.8 g were randomly allocated into three groups; ketoprofen group (n=8), meloxicam group (n=8), and control/placebo (n=8). The animals were kept in pairs in wire-topped plastic cages in a 12-h light and 12-h dark cycle and fed ad libitum allowing free access to tap water and standard laboratory rodent diet (Ankara Yem/Kırıkkale).

For surgery the animals were anesthetized with intra-peritoneal administration of a combination of xylazine HCl (Alfazyne 20 mg/ml Egevet, Izmir, Turkey) and ketamine HCl

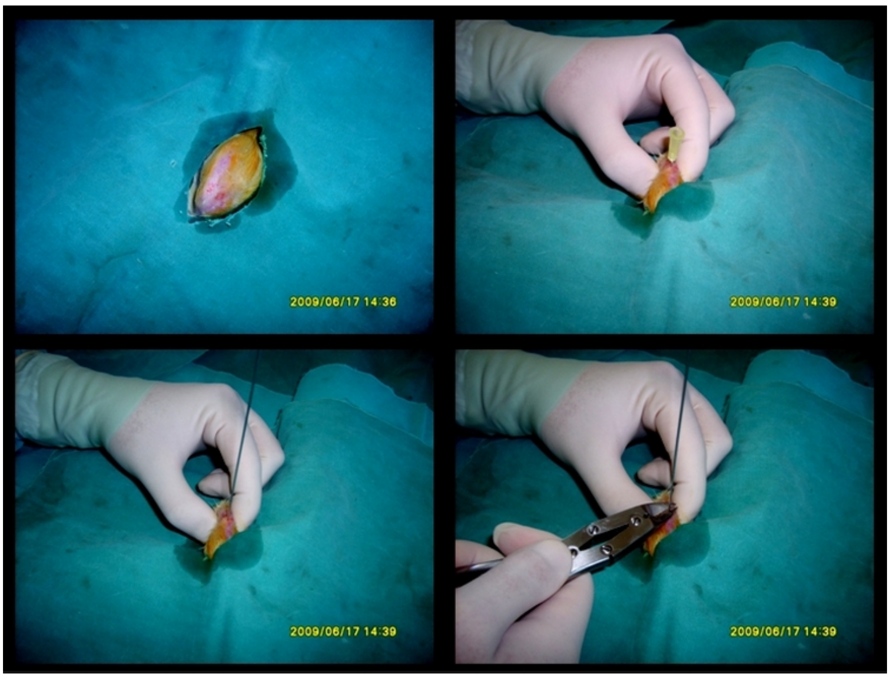

Fig 1. Some steps of the operation procedure

Şekil 1. Operasyondan bazı aşamalar

(Alfamine 100 mg/ml Egevet, Izmir, Turkey). After shaving the skin and aseptic wash, a 20G1½ (Microlance™ 0.9X40 Nr.1 TW PM) cannula was inserted into the medullary canal through the intercondylar area just medial to the patellar tendon in front of the cruciate ligaments. Then the cannula was removed, a Kirschner wire (0.8 mm) was placed into medullary canal, and then the wire was cut just near the femoral condyle.

The femur was subjected to a standardized closed midshaft fracture using a specially designed fracture forceps. Upon collecting the postoperative radiogram of each fracture, the precision of the fracture models was examined.

All animals in the ketoprofen (Tobrofin, Provet Veterinary Products Ltd, İstanbul Turkey) group were given 0.5 mg/100 g body weight intraperitoneally twice daily for 21 days, and the first injection was made just prior to surgery. The animals in the meloxicam (Maxicam, Sanovel, İstanbul, Turkey) group were given 0.1 mg/100 g body weight and the animals in the placebo group were given a corresponding volume of saline intraperitoneally. The doses for meloxicam and ketoprofen were calculated using the recommended doses for dogs use. Such doses were proved to be adequate in earlier study [21]. To prevent inflammation and ulceration in the gastrointestinal system, 0.1 mg/100 g omeprazol (Losec ampul Astra/ Sweeden) was administered intraperitoneally [22,23]. All animals were euthanized by a thiopental sodyum (Pental 1 g, İ.E Ulagay, İstanbul) overdose [24].

Three weeks after surgery, the bone density at the fracture site was measured in separated femurs (after removing the implant in the femur) using a dual energy x-ray absorptiometry (DEXA) machine, (Siemens PIXIMUS, Germany). Small animal (research mod) software was used to determine the bone mineral content (BMC) and bone

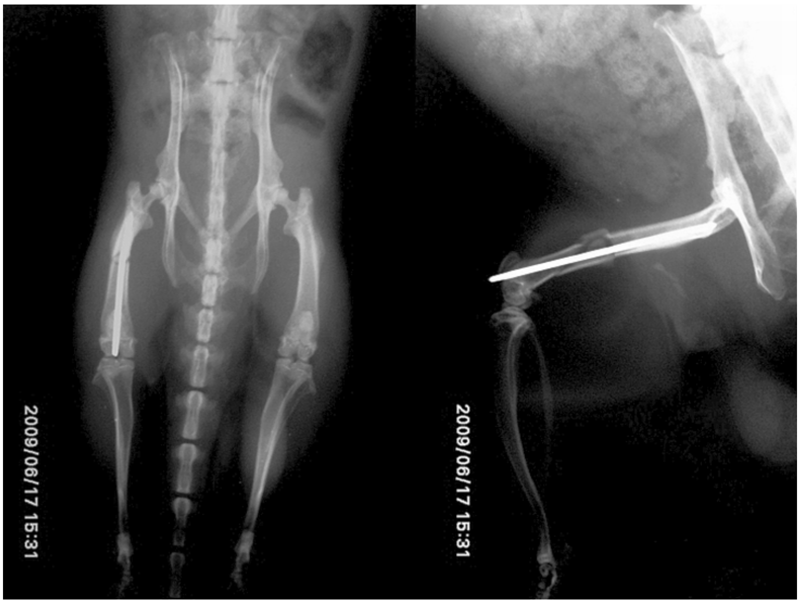

Fig 2. Postoperative anterioposterior and mediolateral radiological view of the fracture

Şekil 2. Oluşturulan kırığın AP ve ML radyolojik görünümü

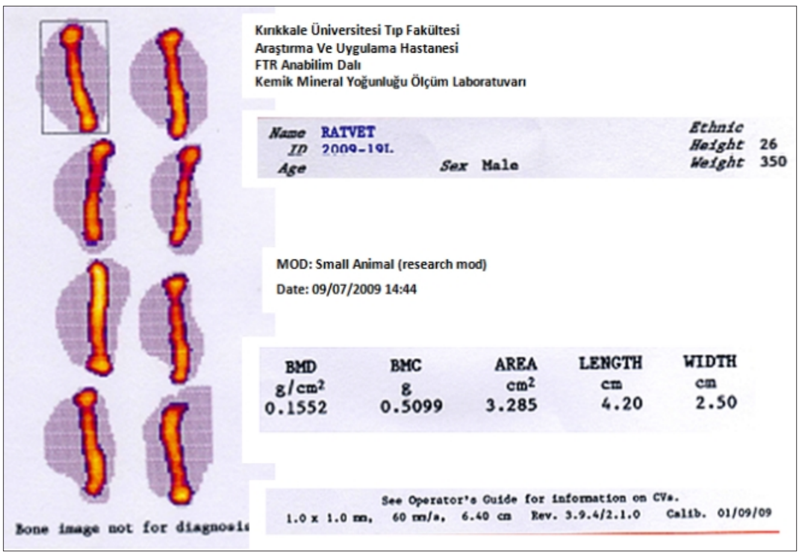

Fig 3. DEXA scan results of the one of the case

Şekil 3. Olgulardan birinin DEXA tarama sonuçları mineral density (BMD). The stability of the machine was controlled by means of the calibration phantom, which was regularly scanned during the study period. All DEXA scans were performed by the same operator. Each femur was scanned in a craniocaudal direction. The region of interest (ROI) was considered as the entire femur.

There were invasive methods in the past to evaluate bone density; however, the current technology provides non-invasive methods based on ultrasonography and gama or X-ray technologies. It is currently feasible to determine BMD and BMC values using non-invasive methods including radiogrametry, single photon absorptiometry, dual photon absorptiometry, dual energy absorptiometry and quantitative computed tomography [40-48]. As study by Paniagua et al.[49] claimed that DEXA scanning is adequate to determine BMD in rats and the data generated were reliable and reproducible while radiogrametry, SPA, DPA and QCT were more precise techniques.

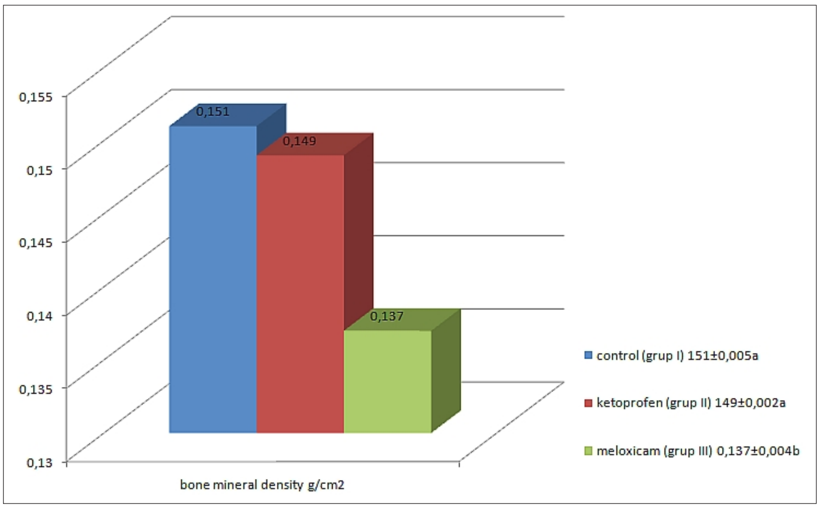

Fig 4. The graphic of the bone mineral density (BMD) of the groups)

Şekil 4. Çalışma gruplarına ait kemik mineral yoğunluk (BMD) ölçümlerine ait grafik

RESULTS

All animals tolerated the surgery well. Grossly, there was no evidence of infection, and new bone formation was evident in all 24 animals at the time of euthanasia.

Data are presented as mean values ± standard deviation (SD). The data were first analyzed for normality and homogeneity of variance. The normality test revealed that the data was normally distributed and the Levene test showed that the variances are homogeneous. The groups were compared using one way analysis of variance (ANOVA) and LSD post hoc test ($P<0.05$). All analyzes were done by SPSS (SPSS Inc., Chicago, IL) for Windows, version 15.

The DEXA measurements performed post-operatively at the 3rd week indicated that the rats in the meloxicam group, BMD is significantly lower compared to those of the other groups ($P=0.03$). However, there were no difference between ketoprofen and control groups ($P>0.05$).

DISCUSSION

The effects of various generations of NSAIDs on bone metabolism and bone healing have been investigated by researchers for the past half century. As the results of several studies revealed that the conventional non-selective COX inhibitors and selective COX-2 inhibitors retards bone fracture healing by impeding remodeling and mineralization at healing sites [25-36]. Thus, their use especially in patients with elderly and metabolic disorders related to bone metabolism [37-39].

In the present study, we investigated the effects of two different generations of NSAIDs on bone mineral density, and thus, bone healing rate in a rat model of femur fracture. The results indicated that meloxicam, a more COX-2 selective inhibitor, have an inhibitory effects on BMD; however, such data should be further confirmed by biomechanical tests. Although a relationship between BMD and callus strength has been suggested, the value of biomechanical test for callus strength is indispensable [50].

The reason for use of ketoprofen and meloxicam in our study is that these drugs are commonly used in veterinary practice as well as the fact that ketoprofen is an inhibitor of COX-1 and COX-2 while meloxicam is a more COX-2 selective inhibitor. In a study conducted in dogs revealed that there was no difference between for ketoprofen and meloxicam for alleviation of pain and blood coagulation rate [51]. Thus, these two drugs can be considered as alternatives to each other.

Upon testing various COX-2 inhibitors, Simon et al.[52] and Leonelli et al.[53] reported that COX-2 inhibitors retard bone fracture healing. The most frequently studied NSAIDs with claimed effects include the selective COX-2 inhibitor celecoxib and rafecoxib as well as the non-selective NSAID ibuprofen, ketorolac, and indomethasin.

In a study by Dimmen et al.[18], rats receiving parecoxib at doses equivalent to perioperative doses in human for a week had lower BMD at the fracture line [18]. In the same study, the difference in BMD between placebo and parecoxib receiving rats were reduced at the end of the 6th week; however, the bone resistance measured biomechanically was still weaker in parecoxib receiving rats compared to placebo receiving rats.

Beck et al.[54] evaluated the bone fracture healing in rats based on BMD parameter, diklofenac and tramadol, which are conventional NSAIDs, and concluded that the degree of bone healing inhibition was higher in diclofenac receiving rats compared to control and tramadol receiving rats. In the same study, the BMD value was calculated from CT measurements following removal of the intramedullar pin in euthanized animals, not based on DEXA scanning.

In conclusion, ketoprofen does not inhibit bone fracture healing and bone mineralization based on the DEXA scanning data indicating no difference between the ketoprofen receiving and control rats. On the other hand, meloxicam considerably inhibit bone fracture healing and bone mineralization based on the data indicating a significant difference between the meloxicam receiving rats and the others.

These results support the notion suggesting there is direct relationship between COX-2 enzymes and bone fracture healing and mineralization. However, as indicated earlier biomechanical tests should also be conducted to reveal clinical inhibition.

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