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Effects of diclofenac and tenoxicam on distraction osteogenesis

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Abstract
Introduction In the management of bone defects, pseudoarthrosis, deformities, chronic osteomyelitis and in extremity lengthening procedures, the technique of distraction osteogenesis (DO) has been frequently used. In this experimental animal study, the effects of two non-steroidal anti-inflammatory drugs, i.e., diclofenac and tenoxicam, on the outcomes of distraction osteogenesis are investigated.
Materials and methods In this study, 30 mature New Zealand-type male rabbits (2.5–4.5 kg) were used. The rabbits were randomized into three groups, each consisting of ten animals. Under optimal operating conditions, a pre-reconstructed circular external fixator was applied on the right tibias of rabbits, and osteotomy was performed with a Gigli saw just below the tibial tuberosity. After seven postoperative days, distraction was initiated at a rhythm of 2×0.5 mm/day. During the 10 days of distraction, adjunctive therapy was not instituted for group I (control group). For 10 days, group II received i.m. diclofenac sodium (0.5 mg/kg per day) and group III was treated with tenoxicam (8 mg/kg per day i.m.). At the end of 3 weeks postoperatively, five rabbits from each group were killed for histologic examinations. The remaining rabbits were killed at the end of eight postoperative weeks for biomechanical and histological analysis. Besides, radiological examinations were performed at the end of 3, 6 and 8 weeks postoperatively for the radiologic evaluation of calluses. For statistical evaluations between groups, Kruskal–Wallis variance analysis, and for intergroup assessments, Mann–Whitney test were performed.
Results For radiological evaluations, the scoring system developed by Lane and Sandhu, and for histopathological assessments, the grading system of Huddlestone et al. were used. Biomechanical tests were realized using torsional loading. During the first 3 weeks, the groups did not differ much in the radiological parameters. However, in the diclofenac group and in especially the tenoxicam group, the histological scores were lower than in the control group. Radiological images obtained at the end of 6 weeks demonstrated inadequate consolidation in the diclofenac and tenoxicam groups when compared with the control group. At the end of 8 weeks postoperatively, in consideration of biomechanical, radiological and histological tests, significantly worse regenerates were obtained in the diclofenac and tenoxicam groups.
Conclusions During the distraction osteogenesis period, diclofenac and tenoxicam affected the quality of regenerate unfavorably. This effect is sustained during all periods of bone healing. However, this finding should be supported by experimental and human studies.

Keywords Distraction osteogenesis · Diclofenac · Tenoxicam · Ilizarov

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Introduction

The technique of "distraction osteogenesis (DO)," which is unique in the regeneration of bone, is used frequently in the treatment of bone loss, pseudoarthrosis, chronic osteomyelitis and leg length discrepancy and deformity. According to Ilizarov [15], who introduced this technique and transferred his experiences into the world of orthopedics, certain principles must be obeyed. Important principles to be considered are preservation of blood circulation of the bone segment to be lengthened, stable external fixation, optimal latency period before distraction, 0.25 mm lengthening with four times per day and allowing time for the consolidation of the regenerate segment.

After introduction of this method into the Western world, many authors have investigated the factors effecting the success of distraction osteogenesis [9, 16, 26, 27]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are recommended and widely used for the control of postoperative pain [2, 6, 17]. In addition, NSAIDs are used to manage pain for patients with acute or chronic joint arthritis and degenerative disease. Although the effect of prostaglandin on bone healing has not been clearly defined, some studies report its function as bone resorption. Subsequently, resorption of fracture edges possibly enhances pluripotential cells differential, which contributes to bone healing. On the other hand, prostaglandin is one of the chemotactic agents, which induce inflammatory phase of bone healing [4, 11]. The main effects of NSAIDs are based on the interruption of prostoglandin (PG) synthesis by inhibition of the enzyme cyclo-oxygenase [5, 7, 13, 18, 21, 23]. Therefore, they lose their effect as mediators in the inflammatory process. Studies related with fracture healing have claimed that NSAIDs have adverse effects on inflammatory phase of fracture healing by inhibition of PG synthesis, but do not have any effect on the mineralization process of fracture healing [4, 7, 8, 10, 11, 23]. The administration of these NSAIDs was associated with impaired healing related to the inhibition of prostaglandin synthesis. However, little is known of the effects of NSAIDs on distraction osteogenesis. This is, to our knowledge, the first study to investigate the effects of diclofenac and tenoxicam on distraction osteogenesis. The present study was designed to provide radiologic, histologic and biomechanic information about the influence of these drugs on bone healing in distraction osteogenesis.

Materials and method

In this study, 30 mature New Zealand-type male rabbits (mean weights 3.5 kg: 2.5–4.5 kg) were used. Before initiation of the study, approval from the Ethics Committee of Gaziosmanpasa University, School of Medicine was obtained. The study was realized in the "Center of Experimental Animals" of the same institution. Rabbits were randomized into three groups, each consisting of ten animals. As a prophylaxis, cefazoline sodium (20 mg/kg per day i.m.) was injected preoperatively and at 8 h postoperatively. Interventions were performed under ketamine (10 mg/kg i.m.) and xylazine (8 mg/kg i.m.) anesthesia. Firstly, skin, subdermis and periosteum were exposed with an anteromedial longitudinal incision on the right tibias. A Gigli saw was passed subperiostally. Then a pre-reconstructed circular fixator was applied (Fig. 1). After application of the fixator, the Gigli saw was used for osteotomy (Fig. 2). Subsequently, the periosteum and skin were closed properly. After a 7-day latency period, distraction was applied for each tibia at a rhythm of 2×0.5 mm/day. However, for group I (control group), treatment was not instituted, while group II received diclofenac sodium (0.5 mg/kg i.m.) for 10 days and group III was treated with tenoxicam (8 mg/kg per day i.m.). At the end of 10 days, distraction was terminated. At the end of 3 weeks postoperatively, five rabbits in each group were killed with high doses of intravenous sodium pentothal and evaluated radiologically and histologically. In the sixth postoperative week, radio-

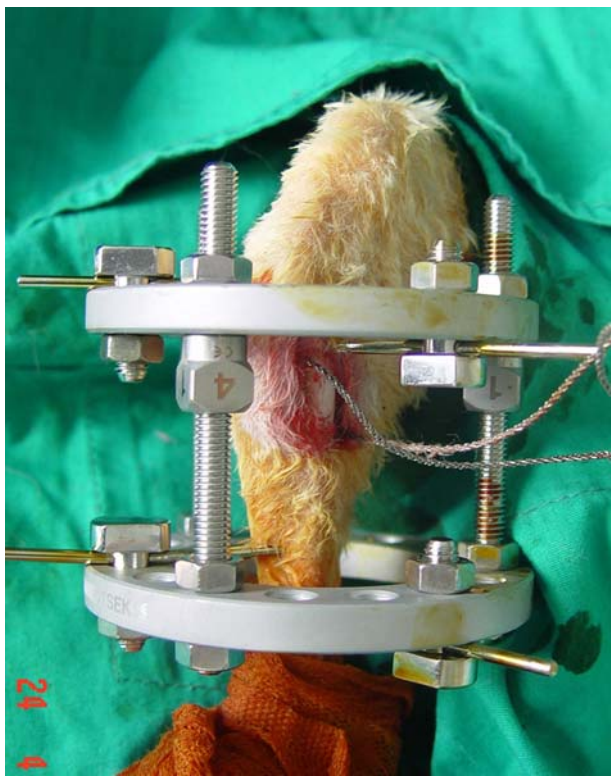


Fig. 1 Application of circular fixator

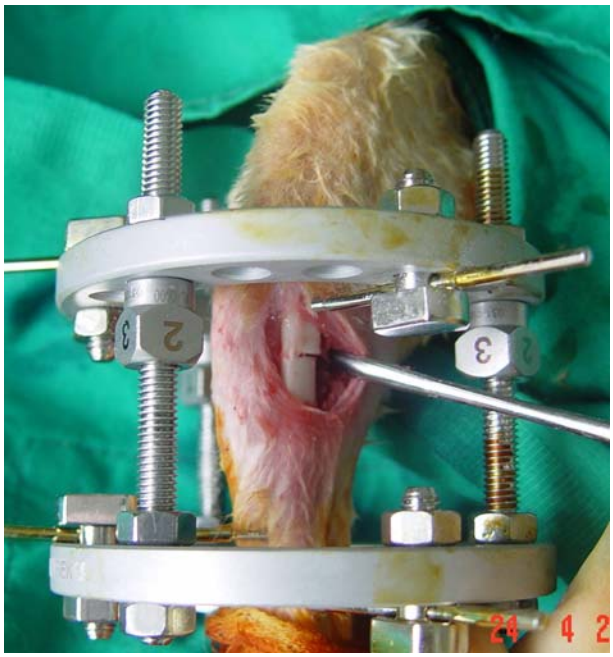


Fig. 2 Gigli saw osteotomy

logical assesments were done on the remaining five rabbits. The remaining five rabbits in each group were killed similarly in the eighth postoperative week, and radiological, histological and biomechanical investigations were carried out.

After killing the rabbits, all tibias were harvested, stripped from their soft tissue and sent to a pathologist unaware of the allocation of rabbits into groups. For histological evaluation, the extremities dissected from their soft tissues were fixed in 10% buffered formaldehyde solution for 48 h and then treated with a rapid decalcification solution (formol potassium dichromate 3 g/l, nitric acid 40 ml/l, ethyl alcohol 270 ml/l) (Assistain, GBL, Turkey) for 12 h. After the decalcification procedure, the tibias were sampled longitudinally from distracted segments. For each tibial segment, four cuts were taken from the distraction area and routine histological examinations were done. After routine histological preparations, the samples were embedded in paraffin. From paraffin blocks, sections of 4 μm width were taken and stained with hematoxylene–eosin. Sections stained with hematoxylene–eosin were examined under light microscope. For each subject, four cuts from the distraction area were taken for analysis. For radiological evaluation, the scoring system developed by Lane and Sandhu [20] (Table 1), and for histopathological assessment, the grading system of Huddlestone et al. [14] were used (Table 2).

Biomechanical tests were performed with Materials Testing Machine (Lloyd Instruments Ltd, Sagensworth, Fareham) using torsional loading forces at a rhythm of 2 mm/min.

Table 1 Radiological evaluation system [20]

Bone formation	Points
Lack of bone formation	0
Bone formation filling 25 % of the defect	1
Bone formation filling 50 % of the defect	2
Bone formation filling 75 % of the defect	3
Bone formation filling 100 % of the defect	4
Union	
No union	0
Onset of union	1
Complete radiological union	2
Remodeling	
None	0
Formation of intramedullary channel	1
Formation of cortex	2
Maximal total score	10
Bone formation	4
Proximal union	2
Distal union	2
Remodeling	2

Table 2 Histological evaluation system [14]

Points	Histological findings
1	Fibrous tissue
2	Predominantly fibrous tissue with some cartilage
3	Equal amounts of fibrous and cartilage tissue
4	All cartilage
5	Predominantly cartilage tissue with some woven bone
6	Equal amounts of cartilage and woven bone
7	Predominantly woven bone with some cartilage
8	Entirely woven bone
9	Woven bone and some mature bone
10	Lamellar (mature) bone

For statistical evaluations of all groups, Kruskal–Wallis variance analysis, and for intergroup assessment Mann–Whitney *U* test (Bonferroni correction was used) were performed. Statistical significance ($P < 0.05$) was determined in consideration of 95% confidence limits.

Radiological evaluations at the end of 3, 6 and 8 weeks were performed separately by the investigators in charge (CS, ME, TG) without regarding assignment of study groups or allocation of numbers.

Results

According to these results, at the end of the third postoperative week, a statistically significant difference was

not detected during intergroup evaluations for radiologic evaluation ($P > 0.05$). However, histopathologically, callus formation was significantly worse in groups II and III ($P = 0.002$) (Table 3). In radiograms taken at the end of 6 weeks, consolidations in groups II and III were statistically worse than those found in group I ($P = 0.008$) (Table 4; Fig. 3a–c). Radiological and histological results obtained at the end of 8 weeks demonstrated lower quality regenerates in groups II and III ($P = 0.009$) compared to the control group, while groups II and III did not differ significantly ($P > 0.05$). During histopathological examinations, entirely woven bone and some mature bone and patchy areas of bone marrow formation were observed in the distraction area of the control group. Trabecular bone tissue was the predominant component in callus tissue (Fig. 4a). In contrast, in the tenoxicam and diclofenac groups, predominately cartilage tissue with some woven bone was seen. In addition, fibrous and fibrocartilage tissue with chondrocysts were observed (Fig. 4b, c).

Table 3 Mean histological scores of the groups in the third and eighth weeks

Groups	Third week ($P = 0.002$)	Eighth week ($P = 0.009$)
Group I ($n = 5$)	3.4 ± 0.5	8.6 ± 0.5
Group II ($n = 5$)	1.6 ± 0.5	5.8 ± 0.4
Group III ($n = 5$)	1.4 ± 0.5	5.6 ± 0.5

Table 4 Mean radiological scores of the groups in the third, sixth and eighth weeks

Groups	Third week ($P > 0.05$)	Sixth week ($P = 0.008$)	Eighth week ($P = 0.009$)
Group I ($n = 5$)	2.2 ± 0.8	6.8 ± 0.4	8.8 ± 0.4
Group II ($n = 5$)	2.0 ± 0.7	4.0 ± 0.7	6.2 ± 0.4
Group III ($n = 5$)	2.0 ± 1.0	3.6 ± 0.5	6.0 ± 0.7

When the results of the biomechanical tests were evaluated, a significant difference was found between group I and the treatment groups (group II and III) with respect to failure of torsional loading strain ($P = 0.001$). However, a statistically significant difference could not be detected between group II and III for this parameter ($P = 0.05$) (Table 5).

Discussion

Although many different methods of treatment are available, a mutual consensus has not been established for the management of deformities of long bones, chronic osteomyelitis, leg length discrepancy and bone loss. In the reconstruction of long bones, intramedullary nailing, muscular flaps with free pedicles, vascular/avascular fibula graft and Papineau grafting techniques are being frequently used. However, prolonged hospital stay, and longer durations of therapy, higher costs and frequency of complications are the striking disadvantages of these interventions [25].

Distraction osteogenesis technique, which was introduced by Ilizarov [15], has been used extensively in the Western world from the late 1980s. This technique has gained popularity in that it can correct various disorders of the bone and also leads to lesser number of complications. In the treatment of bone defect, distraction osteogenesis not only establishes union but also compensates for the length discrepancy by employing distraction at the same time, thus achieving reorientation and realignment of the extremity [25].

Histological findings of distraction osteogenesis were reported by Aronson [3]. This distraction process was recognized as "intramembranous ossification." Then, type I collagen fibers are formed, which are aligned parallel to the lengthening vector and the

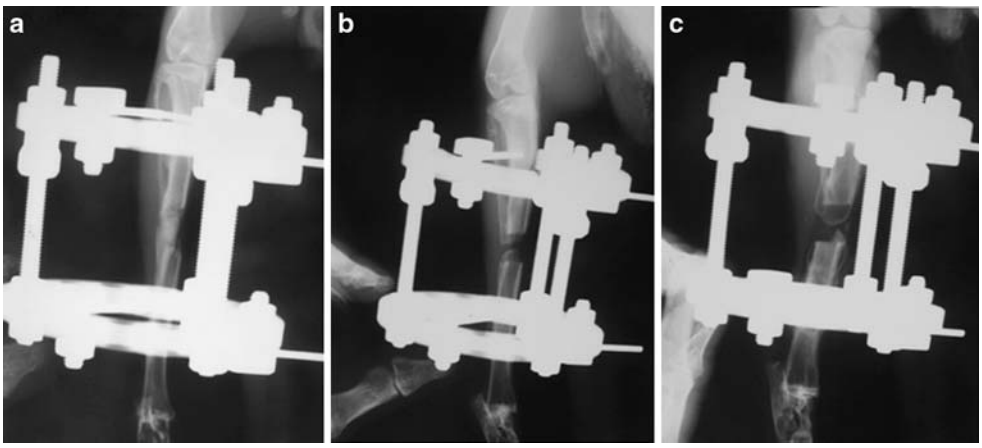


Fig. 3 Radiological appearance of the three groups at the end of 6 weeks. **a** Control group, **b** diclophenac group, **c** tenoxicam group

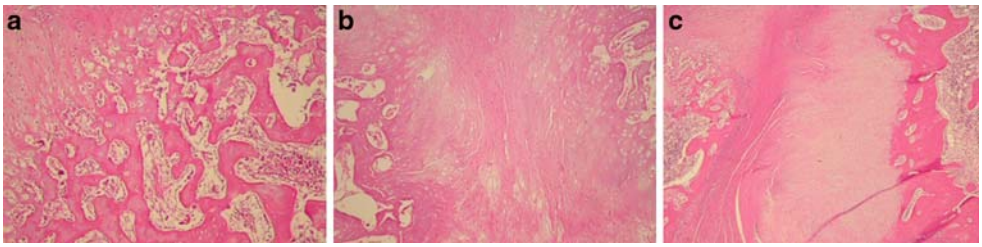


Fig. 4 **a** Histological appearance of control group at the end of 8 weeks. The callus formation consisted of mostly trabeculary bone in the group of control killed in the eighth week. Chondroid tissue areas were limitedly observed in the callus (HE, ×20). **b** Chondroid tissue constituted nearly the whole of the callus in the group of diclophenac killed in the eighth week (HE, ×20). **c** Chondroid tissue formed the majority of the callus formation in the group of tenoxicam, killed in the eighth week (HE, ×8)

Table 5 Mean torsional loads (*N*) of the groups in the eighth week

Groups	Eighth week ($P = 0.001$)
Group I ($n = 5$)	170.8 ± 4.9
Group II ($n = 5$)	62.4 ± 7.1
Group III ($n = 5$)	52.8 ± 7.7

P values for the Tables 3, 4 and 5 demonstrate the statistical differences between control and drug administrated groups (diclofenac sodium and tenoxicam)

longitudinal axis of bone. Subsequently, columns formed by collagen fibers are joined along their transverse axis and appear like a honeycomb. During this period, mesenchymal cells differentiate to chondroblasts, cartilage tissue ossifies, and the periosteum of the bone also participates in this process. When the distraction process is terminated, trabecules are formed in the matrix localized between bone ends; osteon formation and osteoblastic activity increase immensely and mineralization begins to take place. These newly formed trabecules align parallel to the axis of lengthening, join with each other through bone bridges and the bone rapidly remodels.

The principles of accomplishment of high quality regenerate with distraction osteogenesis were put forth fully by Ilizarov [15]: avoiding trauma to periosteum during corticotomy, low-energy corticotomy, a stable osteosynthesis, 7–10 days of latency period, distraction applied at rhythms of 1 mm/day and 4 × 0.25 mm and waiting period for consolidation.

In our study, distraction procedures in accordance with these principles, except two, were applied for all groups. We lengthened the tibias at a rhythm of 2 × 0.5 mm/day instead of 4 × 0.25 mm/day. In a study conducted by Sen et al. [24], they concluded that even at a rhythm of 1 mm/day, regenerates of higher quality can be obtained than those achieved with 0.5 mm/day. On the other hand Kojimoto et al. [19] demonstrated that preservation of periosteum rather than endosteum is more important for callus formation. Gigli saw osteotomy recommended by Paley and Tetsworth [22] reinforced this argument. In our study, we believe that lengthening at a rhythm of 2 × 0.5 mm/day and Gigli saw osteotomy do not affect callus formation adversely because we accomplished in obtaining a good quality regenerate tissue in the control group.

In addition to frequent usage of this technique (DO), many studies have been performed to increase the success rates of this technique. Especially, mechanical and chemical factors effecting distraction osteogenesis were stressed, and favorable and unfavorable characteristics of this technique have been investigated. Waanders et al. [27] reported that strains and cyclic loading forces acting on the distracted segment are important factors for rapid bone formation. Jarka et al. [16] observed that methotrexate had no effect on distraction osteogenesis. Shimazaki et al. [26] found that low-intensity ultrasound enhanced maturation of the distracted bone segment.

Nonsteroidal anti-inflammatory agents have been used by many orthopedic surgeons for the relief of pain. The effects of NSAIDs, especially on bone healing, have been investigated, but authors have not achieved any consensus. Akman et al. [1] reported no effect of diclofenac sodium on bone healing, while Göksan et al. [12] achieved the same conclusion for tenoxicam. In contrast, Beck et al. [4] reported that diclofenac significantly delayed the healing of fractures, while Giardino et al. [11] mentioned unfavorable effects of tenoxicam on bone healing. Although some studies indicate no effect of NSAIDs on bone healing, majority of studies demonstrated slower healing and delayed union [1, 4, 5, 7, 8, 10–13, 16, 18, 21, 23]. On the other hand, we could not encounter any experimental study investigating the effects of diclofenac sodium and tenoxicam on distraction osteogenesis, so far. Therefore, our study is the first investigation on this subject.

The widely accepted unfavorable effects of NSAIDs on bone metabolism are due to their inhibition of cellular activities on endosteal bone surfaces, differentiation of mesenchymal cells and decreases in local immunologic and inflammatory blood flow by inhibiting prostaglandin synthesis [4, 8, 9, 11, 13, 18, 23]. In distraction osteogenesis, consensus states that this inflammatory process continues throughout the lengthening procedure. In our study, in order to observe the effects of NSAIDs on the outcomes of distraction osteogenesis, we administered optimal doses of diclofenac and tenoxicam during 10 days of lengthening procedure. The doses administered were below the recommended daily doses of tenoxicam and diclofenac [4, 11]. Despite lower doses used, histological results obtained at the end of the third week demonstrated that callus formation relatively deteriorated. At the end of the third week, radiological findings did not differ between groups, which we attributed to our osteotomy method used in these experiments. In a study done by Kojimoto et al. [19], endosteal revascularization could be achieved only after 14 postoperative days due to deterioration of medullary perfusion. In Gigli saw osteotomies, because of deterioration of medullary perfusion, differences between the groups at 3 weeks could not be detected.

It is accepted that anti-inflammatory drugs given after callus formation do not affect the healing process, also known as bone remodeling [13, 23]. However, radiological, histological and biomechanical results at the end of 8 weeks showed more significant deterioration of rabbits in groups II and III than those of the control group. We thought that these findings could result from the harmful effects of NSAIDs on the differentiation of mesenchymal cells. Numerous chondrocytes and immature bone tissue encountered during histological examination in groups II and III may validate our opinion. A study conducted by Giardino [11] is consistent with our results. They reported that a substantial reduction may lead to bone resorption and may be one reason for the impairment of cellular differentiation, because acute inflammation phase of bone healing is reduced after NSAID administration. Therefore, we may observe this effect during all periods of bone healing. We believe that even after a long time, inadequate callus formation is a very important finding. However, this result should be supported with more experimental studies. Furthermore, NSAIDs should be used with meticulous care, particularly in patients whose regenerate tissue is possibly poor due to local deficiency of blood perfusion at the distraction area (smoker, diabetic, arterial occlusive disease, etc.).

Various studies have shown unfavorable effects of NSAIDs on early phases of callus formation. With the present study, firstly we demonstrated that unfavorable effects of NSAIDs continue to the end of 8 weeks. We believe that our results give some messages to the reader to perform new studies.

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