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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

KIRIK UÇ TAZELEME VEYA EKSTRAKORPOREAL ŞOK DALGA TEDAVİSİNİN TİBİA KAYNAMA GECİKMESİNDE ETKİNLİĞİ; DENEYSEL ÇALIŞMA

EFFECTIVENESS OF FRACTURE-END REGENERATION OR EXTRACORPOREAL SHOCK WAVE THERAPY IN DELAY OF TIBIAL UNION: EXPERIMENTAL STUDY

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ÖZET

AMAÇ: Bu çalışmanın amacı rat tibia kaynama gecikmesi modelinde, kırık dinamizasyon işlemine eklenen kırık uç tazeleme veya Ekstrakorporeal Şok Dalga Tedavisinin (ESWT) sonuçlarını karşılaştırmaktır.

GEREÇ VE YÖNTEM: 30 dişi Sprague-Dawley cinsi rat üç gruba ayrıldı. Tüm ratlarda sağ tibia transvers diyafizyal kırık oluşturuldu. Gecikmiş kırık kaynaması modeli amaçlı intramedüller tespit sonrası kırık hat propylene spacer ile distrakte edildi. 6. Haftada tüm gruplardaki propylene spacerlar çıkartılarak distraksiyon alanı dinamize edildi. Kontrol grubunda (Grup 1) sadece dinamizasyon uygulandı. Kırık uç tazeleme grubunda (Grup 2) dinamizasyon sırasında kırık uçları tazelendirildi. ESWT grubunda (Grup 3) dinamizasyonun 24. Saatinde 15 kV 500 şok dalgası uygulandı. 12. haftanın sonunda sakrifikasyonun ardından tüm sağ tibialar radyolojik ve histopatolojik incelemeye alındı.

BULGULAR: Radyolojik ve histopatolojik kaynama skorları Grup 2 ve Grup 3'te kontrol grubuna göre anlamlı derecede yüksekti (P=0.001). Grup 2 ve Grup 3 arasında radyolojik kaynama skoru açısından anlamlı farklılık yoktu (P=0.254). Grup 3'te histopatolojik skorlama Grup 2'ye göre anlamlı derecede yüksekti (P=0.001).

SONUÇ: Rat tibia gecikmiş kaynama modelinde dinamizasyona, kırık uç tazeleme veya ESWT eklenmesi sadece dinamizasyon uygulanan gruba göre daha iyi radyolojik ve histopatolojik sonuçlar elde etmemizi sağlamıştır. Klinik karşılaştırmalı bir çalışma literatür açısından katkı sağlayacaktır.

ANAHTAR KELİMELER: Kırık iyileşmesi, Tibia kırıkları, İntramedüller, Kırık sabitlenmesi

ABSTRACT

OBJECTIVE: The purpose of this present study was to compare the results of fracture regeneration or Extracorporeal Shock Wave Therapy (ESWT) added to the fracture dynamization procedure in the rat tibia delayed union model.

MATERIAL AND METHODS: A total of 30 female Sprague-Dawley Rats were divided into three groups. Right tibia transverse diaphyseal fractures were made in all rats. After the intramedullary fixation for delayed fracture union model, the fracture line was distracted with a propylene spacer, which was removed in all groups at the end of the 6th week, and the fracture line was dynamized. Only dynamization was applied to the Control Group (Group 1). The fracture ends were regenerated during dynamization in the fracture-end regeneration group (Group 2). In the ESWT group (Group 3), 15 kV 500 shock waves were applied at the 24th hour of dynamization. After the sacrification at the end of the 12th week, all right tibiae were taken for radiological and histopathological examinations.

RESULTS: Radiological and histopathological union scores were found to be significantly higher in Group 2 and Group 3 than in the Control Group (P=0.001). No significant differences were detected between Group 2 and Group 3 in terms of radiological union scores (P=0.254). Histopathological scoring was significantly higher in Group 3 than in Group 2 (P=0.001).

CONCLUSIONS: The addition of fracture-end regeneration or ESWT to dynamization in the rat tibia delayed union model allowed us to obtain better radiological and histopathological results when compared to the dynamization group alone. A clinical comparative study will contribute to the literature.

KEYWORDS: Fracture healing, Tibial fractures, Intramedullary, Fracture fixation

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INTRODUCTION

Tibia shaft fractures are common fractures affecting people from all age groups, and accounts for approximately one third of long bone fractures as the most common fracture in this group (1). Since it is often associated with high-energy traumas, it may present with clinical manifestation such as delayed union and nonunion (2). Also, it is the bone that has the most common absence of union among long bone fractures with a combined prevalence of 2.5% (3).

Delayed union of the fracture or nonunion causes many functional and psychosocial problems affecting the quality of life of patients negatively such as chronic pain, permanent joint stiffness, and inability to lose weight because of inactivity (4). Also, multiple surgeries and long hospital stays cause high additional costs (5). Alternative methods such as electrical stimulation, low-intensity ultrasound, and Extracorporeal Shock Wave Therapy (ESWT) are used in patients who have delayed union and nonunion, along with the use of many surgical techniques such as dynamization, fracture-end regeneration and fixation, grafting, and fixation (6 – 9).

Extracorporeal Shock Waves were first used in the treatment of urinary tract stones with lithotripsy in 1984 (10). It then started to be used for the treatment of a wide range of orthopedic diseases such as calcific tendinitis of the shoulder, plantar fasciitis, avascular necrosis of the femoral head, delayed union and nonunion treatment in the following years (11, 12). ESWT increases microcirculation and inflammation in the target tissue, and activated inflammation stimulates cell growth and proliferation with a hypervascular effect (13). Mesenchymal stem cell migration and differentiation also help increase bone mass and strength by promoting angiogenesis. In delayed union or nonunion cases, all these mechanisms have curative effects in favor of union to the fracture line (14).

In this study, the purpose was to compare the surgical fracture-end regeneration or ESWT methods added to dynamization in rats in which tibia diaphyseal delayed union models were created.

MATERIALS AND METHODS

Study Design: This study was carried out with the permission of Trakya University Faculty of Medicine Ethics Committee Experimental Animal and Research Laboratory (Approval date and number: 2013.05.01). A total of 30 female Sprague-Dawley rats (3-4 months old with an average weight of 250 grams) were obtained from the Experimental Animal and Research Laboratory of our institution. Animals were fed ad *libitum* with normal rat chow in the experimental laboratory. A two-step surgical protocol was applied to all animals.

1st Surgical Procedure: All surgical procedures were performed by one single surgeon (M.K.). All the rats were anesthetized with intraperitoneal injection of 50 mg/kg Ketamine HCL (Ketalar, Pfizer, USA) in the first surgery. After the necessary batticon staining and covering, the tibia was approached through a midline anterior incision. A standard transverse fracture was created in the tibial midshafts with the help of a mini cutter motor (Small battery drive 2, DePuy Synthes). Fibula was broken with manual bending, and was fixed with the intramedullary Kirschner wire (K-wire-0,8mm-TST Medical, İstanbul, Türkiye). The periosteum of the broken line was stripped, and a propylene spacer was inserted to distract it by 2 mm (15). The layers were closed in line with the anatomical plan after irrigation. No movement restriction was applied to the rats. The steps of the first surgery are shown in **Figure 1**. After the first surgery, the rats were followed for 6 weeks. **Figure 2** shows the radiographs after the first surgery and at 6 weeks.

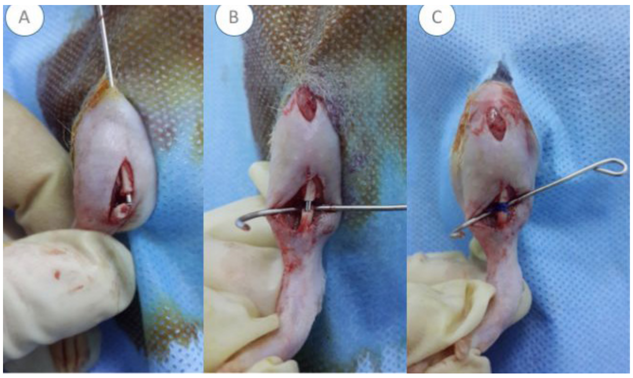

Figure 1: A) Standard, transverse tibia shaft fracture; **B)** Distracted fracture ends over the K-wire; **C)** Propylene spacer placed between fracture ends

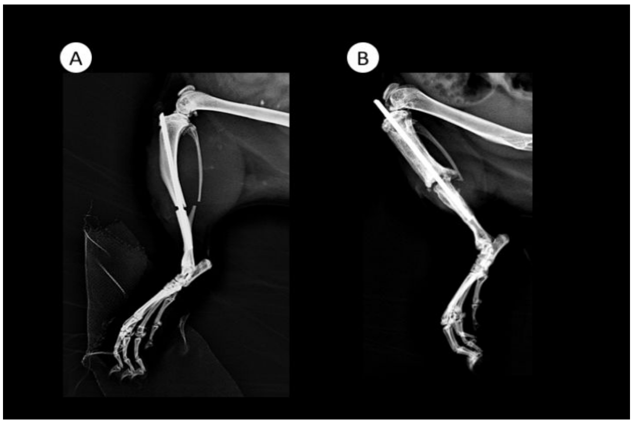

Figure 2: A) Lateral view of tibia in a rat from any of the groups after 1st surgery, distracted fracture ends are seen; **B)** Lateral view of tibia in a rat from any of the groups 6 weeks after 1st surgery, pseudarthrosis formation is visible

2nd Surgical Procedure: The second surgical procedure was initiated at the end of the 6th week after the same anesthesia steps. The distracted area was reached by entering through the old incision area. The existing propylene spacer was removed. Animals were randomly divided into groups of 10. For Group 1 (Dynamization) to be the Control Group, the distracted line was closed in such a way that the fracture ends were in contact without any additional procedures. Perfusion was provided with the help of a minicurette to regenerate the distracted line fractured ends, and then the fractured ends were closed to contact each other in Group 2 (Dynamization-fracture-end regeneration). In Group 3 (Dynamization-ESWT), the distracted line was closed to contact the broken ends without any additional procedures, and ESWT (Stonelith V5 ESWT device-PCK, Ankara, Turkey) 500 shock waves with a power of 15 kV were applied to the fracture line skin under fluoroscopic control and under anesthesia using 50 mg/kg Ketamine HCL (Ketalar, Pfizer, USA) injection at 24 hours(16). No movement restriction was applied to the rats. One rat died of unknown cause in Group 2 after the second surgery, and was excluded from the study.

Radiological Evaluation: At the end of the 12th week, radiographic examinations were performed after the sacrification procedures. Radiographs were evaluated according to the Lane-Sandhu Radiological Scoring by a radiologist who was unaware of the study groups (17). **Figure 3** shows the radiographs of tibia specimens of all three groups.

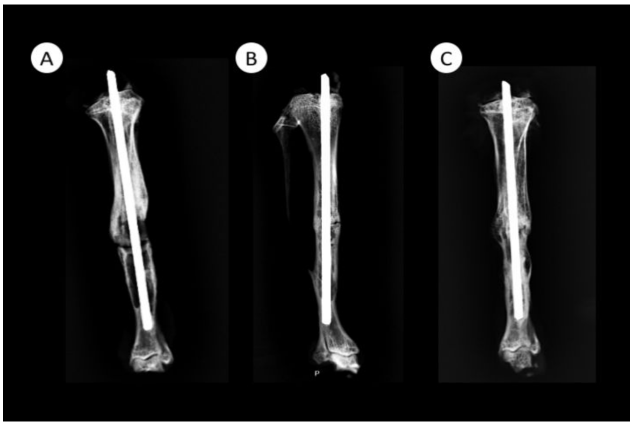

Figure 3: Tibia specimen X-rays obtained from the rats 6 weeks after the 2nd surgeries, **A:** Group I; **B:** Group II; **C:** Group III

Histopathological Evaluation: All tibiae of the rats were surgically dissected in all three groups after the sacrification and radiological examinations, and were separated from the surrounding muscle tissues. Both proximal and distal disarticulation were performed. The tibia specimens, from which the fixative material was removed, were then decalcified in 15% formic acid solution. The specimens with 6 μm thickness sections were prepared by a histology specialist who was unaware of the study groups after the Hematoxylin and Eosin (H&E) Staining and were evaluated under the X10 magnification light microscope according to the scoring system of Allen et al. (18).

Ethical Committee

This study was carried out with the permission of Trakya University Faculty of Medicine Ethics Committee Experimental Animal and Research Laboratory (Approval date and number: 2013.05.01).

Statistical Analysis

The Kruskal-Wallis Test was used to compare histological and radiological scores between the groups. The Mann-Whitney U Test with Bonferroni Correction was used for multiple comparisons. The data evaluation was performed by using "Microsoft Excel" and "SPSS 20.0" (SPSS Inc., Chicago, IL, USA) programs. Significance level was accepted as $p<0.05$.

RESULTS

According to the radiological scoring system, the bone formation scores were found to be significantly higher in Group 2 and Group 3 than in Group 1, which was the Control Group (p=0.002). No significant differences were detected between Group 2 and Group 3. Radiological union scores were found to be significantly higher in Group 2 and Group 3 than in Group 1. No significant differences were detected between Group 2 and Group 3. Radiological bone remodeling scores were found to be significantly higher in Group 2 and Group 3 than in Group 1, and there were no significant differences between Group 2 and Group 3. Although there were statistically significant differences between Group 1 and Group 3 in terms of total radiological scores, no significant differences were found between Group 2 and Group 3. **Table 1** shows the results of the radiological examination in all three groups.

Table 1: Results of radiologic examination in all groups[8]

Mean±SD (min-max)	**Group 1** (n=10)	**Group 2** (n=9)	**Group 3** (n=10)	***p*** **value**
Histopathological score	0.80±0.63 (0-2)	1.56±0.88 (0-3)	2.70±1.16 (1-4)	0.002

SD: Standard deviation
Level of statistical significance was regarded as p<0.050

The evaluations were made according to the 9 Scoring System of Allen et al. in the histopathological examinations. In the statistical analysis, the histopathological scores were significantly higher in Group 3 than in Group 2 and Group 1, and histopathological scores were higher in Group 2 than in Group 1 at statistically significant levels. **Table 2** shows the results of the histopathological examinations of all three groups.

Table 2: Results of histopathological examination in all groups[9]

Mean∓SD (min-max)	**Group 1** (n=10)	**Group 2** (n=9)	**Group 3** (n=10)	***p*** **value**
Bone formation score	0.60∓1.07 (0-3)	2.11∓1.05 (0-4)	2.70∓0.95 (1-4)	0.002
Union score	0.60∓0.97 (0-2)	2.0∓1.0 (0-4)	2.40∓0.84 (2-4)	0.002
Remodeling score	0.40∓0.83 (0-2)	1.56∓1.33 (0-4)	2.20∓1.13 (0-4)	0.006
Total radiologic score	1.60∓2.75 (0-7)	5.67∓3.16 (0-12)	7.30∓2.75 (3-12)	0.002

SD: Standard deviation
Level of statistical significance was regarded as p<0.050

DISCUSSION

Minimally invasive methods are preferred more frequently than major surgical interventions recently in delayed union and non-union (19). Nail dynamization is a proven method for delayed union and non-union after intramedullary fixation in long bone fractures (20). In the study of Claes et al. in which they investigated the effects of dynamization of the Intramedullary Nail in a rat femur fracture model on the union, they found that there was a significant excess of callus tissue at the end of the 4th week when compared to the static locked group (21). However, we created a delayed fracture model that might require an indication for the adaptation to the patient model we encountered in the clinic because there is no early dynamization indication for every patient in clinical practice, and applied dynamization at the end of the 6th week after fracture and distraction in all groups. We obtained significantly higher radiological and histopathological union scores in Group 2 which underwent fracture end regeneration in the same session and in Group 3, in which ESWT was applied at the 24th hour of dynamization compared to Group 1 in which only dynamization was applied.

Fracture-end regeneration is among the effective methods applied in patients with delayed union or non-union by acting as a biological stimulus (22). As a result of fracture-end regeneration and fixation surgery without grafting performed for lateral condylar nonunion in 16 pediatric patients, Park et al. reported that union was achieved in all patients in an average follow-up period of 45.4 months (23). In the study of Ramoutar et al., when the surgeries performed on nonunion in different anatomical regions were examined, they did not report significant differences in terms of union rates between the groups of plaque fixation after decortication and biological stimulation of the fracture line or plate fixation after grafting (24). Mukhopadhaya et al. performed fracture-end regeneration-autografting and fixation in 23 of 31 symptomatic patients with delayed union or non-union, and applied only fracture-end regeneration and fixation in 8 of them. Union was achieved in all patients at the end of the 3rd month (25). In the present study, significantly higher union rates were detected by adding fracture-end regeneration when compared to the control group, which was only dynamized.

ESWT is another non-union treatment method, and many experimental studies show that ESWT has positive effects on bone healing and cartilage regeneration (26). Chen et al. showed increased expression of TGF-ß1 and VEGF after one single ESWT (0.16 mJ/mm^2, 1 Hz, 500 impulses) in rats with segmental femoral defects (27). The authors also reported that, based on their findings, mesoblast deposition at the bone-cartilage junction can be enhanced by using ESWT, which is a critical step in bone healing. Wang et al. reported increased bone mineral density, callus diameter, angiogenesis and osteogenic growth markers and mechanical strength in bone tibia fractures 12 weeks after the treatment with ESWT (28). Bara et al. reported a successful result by achieving 83% union rate after ESWT in their study conducted with patients who had delayed union and non-union (29). Willems et al. reported the average union rate after ESWT in delayed unions was 86%, in nonunions 73%, and in nonunions after surgery 81%, and reported that ESWT is an effective and safe treatment for delayed unions and nonunions (30). In the present study, we achieved significantly successful radiological and histopathological results by adding ESWT at the 24th hour to dynamization when compared to the control group which only had dynamization.

This study had some limitations. The rat tibia used in the experimental study does not exactly match the human anatomy. The results obtained are insufficient for clinical interpretation. Also, radiological and histopathological evaluations were made in our study; however, micro-tomography and biomechanical evaluation of union tissue quality were not performed. A clinical comparative study will contribute to the literature.

Although dynamization is an effective treatment method alone in delayed union or non-union, better union results were obtained in our experimental study when dynamization was applied along with fracture-end regeneration or ESWT. Also, more successful union was achieved in the group with ESWT added than in the group with fracture-end regeneration.

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