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Effect of pentoxifylline on fracture healing: an experimental study

Pentoksifilinin kırık iyileşmesi üzerine etkisi: Deneysel çalışma

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Objectives: This study aims to investigate the effect of pentoxifylline on fracture healing in an experimental animal model.

Materials and methods: Sixty-one male, Wistar-Albino rats were divided randomly into two groups as the pentoxifylline and control groups. Standard, closed femoral shaft fractures were established in all rats using a three-point bending device under general anesthesia. The rats were administered either pentoxifylline or isotonic NaCl injections everyday, beginning after production of fracture until they were sacrificed. Ten rats (11 rats in the pentoxifylline group on the 14th day) in each group were sacrificed on the 7th, 14th and the 21st days and clinical, radiological, and histological examinations were performed to evaluate bony union.

Results: Radiological evaluation of callus did not reveal any significant difference between the control and the pentoxifylline groups in the first, second and the third weeks. However histological callus formation was significantly superior in pentoxifylline group compared to the control group at the end of the first week and callus formation was better in the control group in the third week.

Conclusion: Pentoxifylline can be used to accelerate fracture union in early phases. Because of its hematological effects pentoxifylline accelerates the hematoma stage of fracture healing. But it inhibits fracture union in the later stages, presumably due to its anti-inflammatory effect. This should be taken into consideration during the clinical use of this drug.

Key words: Fracture healing; models, animal; pharmaceutical preparations.

Amaç: Bu çalışmada, bir deneysel hayvan modelinde pentoksifilinin kırık iyileşmesi üzerine etkisi araştırıldı.

Gereç ve yöntemler: Altmış bir erkek Wistar-Albino sıçan pentoksifilin ve kontrol olarak iki gruba ayrıldı. Genel anestezi altında, üç nokta baskı uygulama cihazı kullanılarak tüm sıçanlarda standart, kapalı femur cisim kırıkları oluşturuldu. Sıçanlara kırık oluşturulduktan sonra başlanarak sıçanlar sakrifiye edilene kadar her gün, pentoksifilin ya da izononik NaCl enjeksiyonları yapıldı. Her grupta 10 sıçan (pentoksifilin grubunda 14. günde 11 sıçan) 7., 14. ve 21. günlerde sakrifiye edildi ve kemik kaynamasının değerlendirilmesi için klinik, radyolojik ve histolojik incelemeler yapıldı.

Bulgular: Kallusun radyolojik incelemesinde kontrol ve pentoksifilin grupları arasında birinci, ikinci ve üçüncü haftalarda anlamlı fark bulunmadı. Buna karşın histolojik olarak ilk haftada kallus oluşumunun pentoksifilin grubunda kontrol grubuna kıyasla anlamlı ölçüde olarak daha iyi olduğu, üçüncü haftada ise kontrol grubunda kallus oluşumunun daha iyi olduğu görüldü.

Sonuç: Pentoksifilin erken aşamalarda kırık kaynamasının hızlandırılması amacıyla kullanılabilir. Hematolojik etkisi nedeniyle, kırık iyileşmesinin hematom fazını hızlandırmaktadır. Daha ileri safhalarda ise muhtemelen anti-enflamatuvar özelliklerinden dolayı kaynamayı yavaşlatmaktadır. İlacın klinik kullanımında bu durumun göz önünde bulundurulması gerekmektedir.

Anahtar sözcükler: Kırık iyileşmesi; modeller, hayvan; farmasötik müstahzarlar.

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Knowledge of the factors that affect fracture healing not only helps to prevent those that have a negative impact on fracture healing but also enables the use of those that can induce faster and more qualified union to obtain positive results. Many of these factors with positive or negative impact on fracture healing are known. In orthopedics, pentoxifylline, a peripheral vasodilator, is widely used to maintain the vitality of grafts and other vascularized tissues used for reconstruction. We suppose that the hematoma stage of fracture healing might be promoted by pentoxifylline, whose rheogenic effects may decrease the viscosity of the blood, which might increase the quantity of the hematoma formed within the fracture region. In our study, the efficacy of pentoxifylline on bone union is investigated in a rat model to see whether its use has an adverse effect or not.

MATERIALS AND METHODS

Seventy-two male Wistar-Albino rats were used with approvals from the Ethics Committee of Metin Sabancı Baltalimanı Bone Diseases Training and Research Hospital and from the Experimental Animals Ethics Committee of the Istanbul University Cerrahpaşa Medical School. The study was conducted at the Experimental Animals Research Laboratory of the Istanbul University Cerrahpaşa Medical School according to The Guide for the Care and Use of Laboratory Animals.

The mean age of the rats included in the study was 2.9 months (2.5-3.2 months) and their mean body weight was 190 grams (172-213 grams). The animals were randomly divided into six groups and 12 animals were placed in each cage. None of the animals received antibiotic prophylaxis during or after the surgery. After surgery one animal died in one cage and two animals died in each of the other cages. No other animal was lost during the rest of the study.

Six groups were designated as A, B, C, D, E and F. After the loss following the operations, the study went on with 11 rats in cage E and 10 rats in each of the other cages. Starting from the operation day, pentoxifylline (Trental®, Aventis, İstanbul, Turkey) was administered intraperitoneally with a dosage of 50 mg/kg/day in group D, E and F. Pentoxifylline was administered for seven days in group D, 14 days in group E and 21 days in group F. In A, B and C group rats, 0.5 cc isotonic sodium chloride (NaCl) was injected intraperitoneally beginning immediately after the fracture formation. A and D groups of rats were killed on the seventh day, B and E groups of rats on the 14th day, and C and F groups of rats on the 21st day. As an euthanasia method, high dose ether vapor was used. After the rats were killed, their left femurs were disarticulated from their hip and knee joints. Soft tissues on the femoral bone were peeled off gently from the bone without any harm to the callus tissue. All of the left femurs were studied regarding clinical, radiological and histological aspects.

Surgical technique

Rats were operated under ketamine anesthesia which is a method defined by Bonnarens.[1] A femoral channel was prepared by means of a 1 mm Kirschner wire (Hipokrat®, İzmir, Türkiye) that was inserted through the femoral condyles and a 0.8 mm Kirschner wire (Hipokrat®, İzmir, Türkiye) was inserted into this channel. In order to constitute standard closed-fracture after surgery, the guillotine method defined by Bonnares and Einhorn[1] was used. Fracture formation was confirmed clinically and radiologically (Figure 1). The rats were then injected with NaCl or pentoxifylline depending on the study groups.

Radiological, clinical and histological evaluations

Following clinical assessments, direct X-rays of the sacrificed femurs were rated according to the Goldberg classification system.[2] Scoring was performed by two different independent orthopedists (Figure 2). Clinical evaluation of union was performed as indicated by Dimar et al.[3] Histological classification of healing was done according to the histological healing scale published by Huo et al.[4]

Statistical evaluation

Statistical analysis in this study was performed with GraphPad Prisma Version 3 package software (GraphPad Software, Inc. San Diego, CA, USA). Data

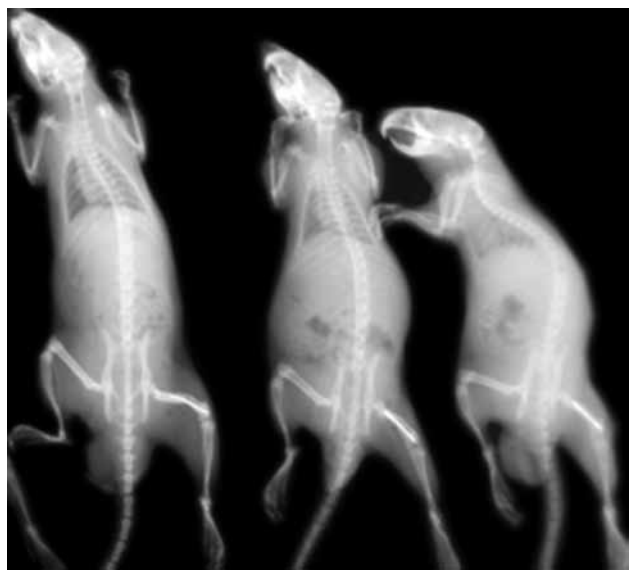

Figure 1. Confirmation of fractures by standard X-rays of the femurs after union is achieved.

Figure 2. X-rays of the sacrified femurs.

evaluation used the Kruskal Wallis test in intergroup comparisons, Dunn's multiple comparison test in sub-group comparisons, the Mann-Whitney U-test in pair group comparisons, and chi-square and Fisher's exact tests in the comparison of qualitative data. Significance in the results were evaluated at the level of $p<0.05$.

RESULTS

Rats included in the group administered pentoxifylline and in the control group not administered pentoxifylline were killed on the seventh, 14th and 21st days. Among the control groups using isotonic NaCl, mean scores were calculated as 1.50 and 1.95 in group A, 1.95 and 2.20 in group B, 2.35 and 2.50 in group C according to the Goldberg classification system. On the other hand, among the trial goups in which pentoxifylline was used, mean scores were determined as 1.65 and 2.25 in group D, 2.22-2.27 in group E, and 2.30 and 2.05 in group F.

After radiological assessment, the femurs were put through macroscopic motion examination. Among the control groups administered isotonic NaCl, mean scores were found to be 0.7 in group A, 1.2 in group B, 1.7 in group C. On the other hand, among trial groups administered pentoxifylline, means scores were calculated as 0.8 in group D, 1.36 in group E and 1.7 in group F. Macroscopically, infection in two rats was observed only in group F.

Following radiological and clinical examinations, femurs prepared adequately were put through histological evaluation. Among control groups administered isotonic NaCl, mean scores were found to be 2.35 in group A, 6.97 in group B and 8.85 in group C, while the mean scores were 5.02 in group D, 6.79 in group E and 7.17 in group F among trial groups administered pentoxifylline.

In statistical analysis, control group and pentoxifylline group were studied both in themselves and in comparison between. Radiological and histological results of control and pentoxifylline groups were statistically significant with respect to the results of the first, second and third weeks according to the Kruskal Wallis test ($p<0.05$). In radiological evaluations, statistically significant increases were determined particularly in weeks one and three in control groups, and in weeks one and two in pentoxifylline groups with Dunn's multiple comparison test. Radiologically, no statistically significant difference between the results of the pentoxifylline and control groups was observed according to the Mann-Whitney U-test.

When histological results were evaluated, a statistically significant increase among the results of the first, second and third weeks appeared in comparisons between control and pentoxifylline groups using Mann-Whitney U-test. Comparisons of the first weeks' results determined that the increase in the pentoxifylline group was significantly higher. No statistically significant difference was seen for the second week, while the amount of union was significantly higher in the control group compared to the pentoxifylline group in the third week.

Infection rates determined during histological evaluation were compared using the Mann-Whitney-U test. Although no significant difference was determined, rates were observed to be within limits. In the pentoxifylline group, infection was determined in the first week in six rats (60%), in the second week in two rats (18%), in the third week in four rats (40%).

In the light of these findings, it was determined that the use of pentoxifylline had no statistically significant radiological impact on fracture healing. According to the histological findings, it was observed that the quantity of union was significantly higher in the first week for the pentoxifylline group while it was significantly higher in the third week for the control group. Infection determined histologically was numerically higher in the pentoxifylline group although there was no statistically significant difference between this group and controls.

DISCUSSION

Pentoxifylline, a phosphodiesterase inhibitor derived from xanthine is a vasodilator. Unlike most of the peripheral vasodilators, pentoxifylline exhibits rheologic effects in blood and decreases blood viscosity.[5] The efficacy of pentoxifylline in treatment is essentially due to its potential to increase blood flow and tissue oxygenation with its haemorheologic effects.[6]

In a rat study, Kurtoğlu et al.[7] demonstrated that problems in newborns such as low birth weight and decreased bone density caused by the use of maternal nicotine could be resolved with pentoxifylline. The experimental study of Xu et al.[8] in 2005 demonstrated that the use of pentoxifylline had a preventive efficacy against intrauterine fetal growth and skeletal development retardation. Another animal study demonstrating that pentoxifylline increases bone formation in rats was published by Kinoshita et al.[9]

It is not clearly known if pentoxifylline increases osteoblasts or osteoclasts. An article published by Takami et al.[10] in 2005 demonstrated that phosphodiesterase inhibitors both increased osteoclasts and the transformation of osteoclasts into osteoblasts. In spite of that, Horiuchi et al.[11,12] studied the effects of pentoxifylline on new bone formation in an animal model and demonstrated that pentoxifylline increased new bone formation through increasing bone morphogenic protein (BMP)-2.[11,12] Tsutsumimoto et al.[13] found out that pentoxifylline could be used in order to increase bone formation.

Bone healing remains one of the issues in orthopedics whose details have not been fully comprehended. Factors affecting fracture healing and acceleration of healing are often subjects of research. Various studies have been conducted particularly on the acceleration of fracture healing. The effects of frequently used drugs on fracture healing have comprised an important part of the literature and the effects of some drugs on fracture healing have been almost definite.[14-16] Accelerating the union process is one the major targets of studies on bone healing. The effects of a great variety of drugs have been evaluated with experimental animal models.

Various pharmaceutical agents and factors are being studied with the purpose of accelerating fracture healing. At present, among the most popular agents are BMP[17] which have started to be used clinically in cases of nonunion and delayed union.[18] Growth hormones (GH) are also among the agents studied in this domain. Schinder et al. in 2002 studied the effects of systemic growth hormone and local administration of IGF–1 and TGF-β 1 on fracture healing. All administered agents enhanced healing compared to the control group.[19] Türk et al.[20] in 2004 studied the effect of vitamin E on fracture healing and reported that vitamin E positively affects fracture healing. A search of the literature showed that the effects of pentoxifylline on fracture healing has not been studied. This drug is frequently used in the treatment of circulatory problems and in cases that require increasing blood flow due to its peripheral vasodilator effects. This study assumed that mediators and cytokines involved in bone repair are transferred to the fracture area through the increase in blood flow resulting in an increase in their concentrations.

The fracture healing model used in this study has been used in many studies in the literature.[21-23] Injections to rats were performed every day at the same time by the same investigator. Injections in the control group standardized the stress factor for all animals. Radiological evaluations were performed by two orthopedists independent from the study. It was observed that the evaluation findings of both were in accord with each other. Although no statistical difference between controls and pentoxifylline groups was shown in this study, a numerically higher rate of union was determined in the pentoxifylline group compared to the control group in the first and the second week, but was totally opposite in the third week. Histologically, the quantity of the union in the control group was seen to be higher than the pentoxifylline group with statistically significant differences between the first and the third week. While the quantity of union was higher in the pentoxifylline group in the first week, the quantity of union in the control group was found to be higher in the third week. In the literature, it has been reported that pentoxifylline enhanced new bone formation thorough increasing BMP-2.[11,12] Early term results are concordant with this information. The late term tendency toward negative effects of pentoxifylline has not been previously reported.

We propose that the hematoma stage of fracture healing which is the first phase might be promoted because of pentoxifylline and its rheogenic effects, decreasing the viscosity of blood and in turn increasing the quantity of hematoma formed within the fracture region. For the late term, we believe that the anti-inflammatory effects of pentoxifylline might delay fracture healing similar to other nonsteroidal antiinflammatory drugs. In addition, although a statistically significant difference was not obtained, higher infection rates were determined in the pentoxifylline group. It is known that among the most important reasons for nonunion are infections in the fracture region. Particularly with a review of the results of the third week, 40% infection rate was found in the pentoxifylline group versus 0% in the control group. This condition can be considered as another factor that could lead to union delay.

The lack of biomechanical studies in our evaluations can be considered as the inadequacy of our study. Although biomechanical investigations have been conducted in some of the studies on fracture healing, the effects of pentoxifylline on fracture healing have not been investigated biomechanically due to the lack of availability in the laboratory where we conducted our study.

Results we have obtained from our study show us that the use of pentoxifylline accelerates healing histologically in the early phases of fracture healing. However, for the succeeding periods, this acceleration decreases and even reverses. Radiologically, it has been seen that it had no effect on fracture healing. Though not statistically significant, infection rates were also higher in the pentoxifylline group. In conclusion, pentoxifylline might be used safely in early periods for patients in whom vascularized grafts or other tissues are used, but care must be taken in long-term administration due to the high infection rates and subsequent delay in union. In patients who must receive long-term pentoxifylline because of peripheral circulatory disorders, delays in fracture healing or the possibility of infections may increase. Hence, frequent follow-ups may be required for long-term clinical administration.

Declaration of conflicting interests

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