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Cefazolin-sodium has no adverse effect on fracture healing in an experimental rabbit model

Deneysel tavşan modelinde sefazolin-sodyumun kırık iyileşmesi üzerine olumsuz etkisi yoktur

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Objectives: This study aims to investigate whether cefazolin-sodium has any adverse effect on fracture healing in an experimental rabbit model.

Materials and methods: The study was performed on 50 male New-Zealand white rabbits. Under general anesthesia, closed double fracture of middle one-third of the tibia-fibula of the left lower extremity of the subjects was produced by manual compression followed by closed reduction of fracture and long leg circular cast was applied. Subjects were divided randomly into five groups including 10 rabbits in each group. The first and second group were administered ciprofloxacin 50 mg/kg SC bid and cefazolin-sodium 50 mg/kg IM on the seventh day of fracture. The third group was applied a single high-dose of vitamin D (50.000 IU/kg) IM following fracture. The fourth group was applied daily vitamin E (alpha tocopherol) 20 mg/kg IM for five days from one hour before the production of fracture. Control group did not receive any treatment before and after fracture. Initial and control X-ray examinations were performed immediately and four weeks after production of fracture, respectively. At the end of the fourth week, animals were sacrificed and a histological examination of the fracture site was performed.

Results: Histological evaluation showed that the histological grade of the fracture healing was significantly lower in the ciprofloxacin group, while it was significantly higher in the cefazolin-sodium, vitamin D and vitamin E groups, compared to control group ($p<0.005$).

Conclusion: Significantly improved histological grade of the fracture healing in subjects treated with cefazolin-sodium than controls suggest that it may be reasonable to choose cefazolin-sodium as an antibiotic therapy for the treatment of infection in patients with bone fractures.

Key words: Cefazolin; ciprofloxacin; fracture healing; vitamin D; vitamin E.

Amaç: Bu çalışmada deneysel tavşan modelinde sefazolin-sodyumun kırık iyileşmesi üzerine olumsuz etkisinin olup olmadığı araştırıldı.

Gereç ve yöntemler: Çalışma 50 adet Yeni Zelanda tipi beyaz erkek tavşan üzerinde gerçekleştirildi. Genel anestezi altında deneklerin sağ alt ekstremitesinde tibia-fibula yaklaşık üçte birlik orta kısmında manuel kompresyon yöntemi ile kapalı çift kırık oluşturulduktan sonra, kapalı yöntemle kırık redüksiyonu sağlandı ve uzun bacak sirküler alçı uygulandı. Denekler her grupta 10'ar tavşan olacak şekilde rasgele beş gruba ayrıldı. Birinci ve ikinci gruplara kırığın yedinci gününde günde iki kere siprofloksasin 50 mg/kg subkutan ve sefazolin-sodyum 50 mg/kg intramusküler olarak uygulandı. Üçüncü gruba kırık sonrasında yüksek dozda (50.000 IU/kg) D vitamini bir kez İM olarak verildi. Dördüncü gruba ise kırık oluşturulmadan bir saat önceden başlayarak, günde bir kere, toplam beş gün süreyle E vitamini (alfa-tokoferol) 20 mg/kg İM olarak uygulandı. Kontrol grubuna kırık öncesinde ve sonrasında herhangi bir ilaç verilmedi. Tüm deneklere kırık oluşturulduktan hemen sonra ve dört hafta sonra başlangıç ve kontrol X-ışını incelemesi yapıldı. Dördüncü haftanın sonunda denekler sakrifiye edildi ve kırık bölgesine histolojik inceleme yapıldı.

Bulgular: Histolojik incelemede siprofloksasin grubunda kırık iyileşmesi histolojik evresinin anlamlı olarak düşük olduğu, sefazolin-sodyum, D vitamini, E vitamini gruplarındaki kırık iyileşmesinin kontrol grubuna göre anlamlı olarak daha fazla olduğu saptandı ($p<0.005$).

Sonuç: Çalışmamızın sonuçlarına göre sefazolin-sodyum grubunda histolojik kırık iyileşmesi evresinin kontrol grubuna göre anlamlı olarak daha iyi olduğunun saptanması, kemik kırığı olan hastalarda enfeksiyon tedavisi için antibiyotik tedavisi olarak sefazolin-sodyumun tercih edilebileceğini düşündürmektedir.

Anahtar sözcükler: Sefazolin; siprofloksasin; kırık iyileşmesi; D vitamini; E vitamini.

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Fracture healing is a sophisticated process, which begins from the time of injury and proceeds until the fractured bone ends unite with mature and organized bone tissue.[1] Although factors that influence fracture healing have been extensively studied, there are some uncertainties about this complex process.[2]

There are several factors identified as having negative or positive consequences on fracture healing.[3-7] Several studies suggested that vitamin D and E influence fracture healing positively[8-10] and others demonstrated that ciprofloxacin, a fluoroquinolone antibiotic commonly prescribed for infections of soft tissue, urinary tract or other regions, has negative consequences on the healing of joint cartilage and bone.[3,11,12] A study of the effects of cefazolin sodium on fracture healing reported that the effect of this antibiotic on fracture healing was not significantly different from the control group.[3]

There are limited number of studies investigating the effectiveness of antibiotics commonly used in orthopedics and traumatology departments for prophylaxis and treatment of the infections.[3,11-14] Therefore, we compared the effects of vitamin D and E, which have well-known positive effects on fracture healing, with ciprofloxacin and cefazolin sodium, which were less studied with regard to their effects on fracture healing but commonly used in patients with a bone fracture. The research question was "Does cefazolin sodium have any adverse effect on fracture healing in an experimental rabbit model?"

MATERIALS AND METHODS

Fifty male New Zealand White rabbits weighing 2000-2600 g (mean 2200 g) were used in the present experimental study. The animals were fed standard laboratory diet throughout the study. Animals were housed in standard conditions of Animal Laboratory in normal room temperature (24 °C) of our university under supervision of veterinary doctor.

Fracture and reduction of fracture were done under general anesthesia in all animals. Under general anesthesia with the mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg),[15] closed double fracture of the middle one-third of the tibia and fibula was produced in the animals. All fractures were produced in the right leg of the animals to standardize the method. Each fracture was produced by manual compression using both hands according to the method described by Allen et al.[16] Animals in which an open fracture resulted were excluded from the study. Following the fracture, closed reduction was performed and a long leg circular cast was applied.[1,3]

Animals were divided randomly into five groups of 10 rabbits in each group. Animals in the first and second group respectively received twice daily 50 mg/kg ciprofloxacin subcutaneously (s.c.) and 50 mg/kg cefazolin sodium intramuscularly (i.m), for three weeks after the seventh day of fracture.[3] The third group of animals received a single high-dose of (50000 IU/kg) vitamin D i.m after the production of fracture.[9] Animals in the fourth group received one daily 20 mg/kg vitamin E (alpha-tocopherol) i.m for total five days, initiating treatment one hour before production of the fracture.[8] The control group did not receive any treatment before or after the fracture.

An initial X-ray evaluation was performed in all animals after the production of the fracture. Bone continuity more than 25% of the tibial diameter was considered as radiological healing criterion.[17]

Animals were sacrificed at four weeks after the fracture via intraperitoneal injection of high-dose phenobarbital, and then control X-ray examination was performed. The material from the fractured extremity was prepared for histological evaluation using light microscopy. Bone tissue was prepared with a decalcification technique, being initially fixed in 10% formalin for three days, placed in Bouin's solution for two days followed by placing in 10% acetic acid, 85% NaCl, 10% formalin solution for decalcification, for an average of 15 days.[1] Fragments were rinsed in tap water, and after appropriate fixation and cutting procedures, stained with hematoxylin-eosin and evaluated histologically. Histologic gradings were made by the same pathologist who was blind to animal groups.

A grading technique for the progression of fracture healing was used in histological evaluation, in which grade I indicated only fibrous tissue; grade II, predominantly fibrous tissue with a small amount of cartilage; grade III, equal amounts of fibrous tissue and cartilage; grade IV, only cartilage; grade V, predominantly cartilage with a small amount of new bone (woven bone); grade VI, equal amounts of cartilage and new bone development; grade VII, predominantly new bone with a small amount of cartilage; grade VIII, entirely new bone development; grade IX, new bone and a small amount of mature bone; and grade X, lamellar (mature) bone tissue.[18,19]

Statistical procedures were performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA) for Windows version 11.0 software. Kruskall-Wallis test was used to find the difference between groups and Mann Whitney-U test to identify the group that resulted in significant difference.

TABLE I

Number of rabbits in groups according to histological grades of fracture healing

Histological grade of fracture healing	Ciprofloxacin	Cefazolin-sodium	Vitamin D	Vitamin E	Control	*p*
3	7	–	–	–	–	p<0.001
4	1	–	–	–	–	
5	–	–	–	–	8	
6	–	–	–	8	–	
7	–	8	–	–	–	
8	–	–	8	–	–	

RESULTS

Ten animals were excluded from the study because of open fracture (n=5), death before the completion of study (n=4) or infection at the fracture site (n=1). The study was completed with eight animals in each group. Grades of fracture healing on histological evaluation were as follows: grade III in seven of eight animals and grade IV in one of eight animals in the ciprofloxacin group; grade VII in eight of eight animals in the cefazolin sodium group; grade VIII in eight of eight animals in the vitamin-D group; grade VI in eight of eight animals in the vitamin-E group; and grade V in eight of eight animals in the control group (Table 1).

Radiographic evidence of callus formation was present in the fractures of all of the animals. When attempting to cut the fracture line in order to confirm this radiological healing before the decalcification procedure, callus could not be cut with lancet in any of the animals. Moreover, callus did not allow any movement between the broken ends of the fracture, and manual compression with three fingers of a hand did not produce a fracture.

There was a significant difference between the groups in histological fracture healing ($p<0.001$). In paired comparisons of the groups, the grade of histological fracture healing was significantly lower in the ciprofloxacin group compared to the four other groups ($p<0.005$). It was significantly different between the cefazolin sodium group compared to the vitamin-D, E and control groups; being significantly greater than the vitamin-E and control groups ($p<0.005$), significantly greater in the vitamin-D group compared to the cefazolin sodium, vitamin-E and control groups ($p<0.005$); and significantly greater in the vitamin-E group compared to the control group ($p<0.005$).

The time to decalcification and subsequent softening of materials derived from the animals in the ciprofloxacin group was three to four days shorter than in the other groups. Although clinical fracture healing was not significantly different between groups, mean decalcification time in the ciprofloxacin group was shorter than mean decalcification time of 10 days in the cefazolin sodium, Vitamin-D and E groups.

DISCUSSION

Results of the present experimental animal model investigating the effects of ciprofloxacin and cefazolin sodium on fracture healing showed that fracture healing was worse in the ciprofloxacin group than in other treatment groups, and was better in the cefazolin sodium, vitamin-D and E groups than in control animals. Besides local and systemic factors, treatment drugs also affect fracture healing.[20,21] Antibiotics are commonly used for the prophylaxis and treatment of pre- and postoperative infections in orthopedics and traumatology clinics. The present study revealed that ciprofloxacin has negative and cefazolin sodium has positive effects on fracture healing.

Ciprofloxacin is an antibiotic used for soft tissue and urinary tract infections, as well as for infections at the fracture site. Ciprofloxacin has a direct toxic effect on chondrocytes, inhibiting maturation of chondrocytes, and preventing subsequent transformation to bone tissue.[1] Similar to the results of Huddleston et al.,[3] we also found that fracture healing was worse in the ciprofloxacin group than in other groups.

The grade of fracture healing in the cefazolin group was two grades greater than in other groups. Although the mechanism of action is not known, the positive effect found in the present study in favor of cefazolin sodium on fracture healing is an important factor for determination of antibiotic in the treatment of infections in patients with a bone fracture.

Vitamin-D3 (1.25 dihydroxycholecalciferol) is essential in synthesis of bone and cartilage, fracture healing and remodeling of the fracture. Ömeroğlu et al.[9] demonstrated that administration of a single dose 50000 IU/kg i.m vitamin-D3 influence fracture healing

positively. Authors suggested that the positive effect of this vitamin on fracture healing results from increased blood flow, increased synthesis and organization of collagen fibrils, proliferation and differentiation of progenitor cells of the bone and mineralization of the matrix.[22] Accordingly, we found a significant histological improvement in the vitamin-D group compared to the control group.

While radiological findings were comparable, histological grade was significantly greater in the vitamin-E group than the control group. It is well known that free oxygen radicals are one of the important factors influencing the fracture healing. Göktürk et al.[23] administered 100 mg/kg Zymosan intraperitoneally to rats in order to generate free oxygen radicals and reported that fracture healing was influenced negatively by this treatment. Evidence of improved fracture healing in vitamin E-treated animals in the present study may be attributed to antioxidant effects of vitamin E that prevent the damage caused by free oxygen radicals at the fracture site.[23]

Plain radiography is commonly used for monitoring of fracture healing. Plain radiography is an inexpensive method, and has the advantage of low exposure to radiation.[24] Osteoblasts are moderately radiosensitive cells. Sensitivity of these cells to radiation increases during fracture healing as a consequence of proliferation of the cells. It was suggested that minimizing the number of control radiographs for monitoring of fracture healing would influence the healing process positively.[25] We attempted to minimize this hazardous effect by taking direct radiographies only at the beginning of the study and at the fourth week.

Fracture healing is commonly studied in experimental animal models, including rabbit, rat, mice and dog models. The experimental rabbit model used in the present study provided the advantage of easier application of circular cast after producing the fracture and performing reduction in comparison to other animal species.

Ciprofloxacin is reported as having adverse effects on fracture healing because of its chondrotoxicity. Huddleston et al.[3] reported that cefazolin sodium had no adverse effects on fracture healing in radiologic and histologic examinations. We found that cefazolin sodium had a positive effect on histologic fracture healing via mechanisms that we could not explain. The positive effect of cefazolin sodium on fracture healing which was detected in our study might be due to a positive effect on chondrocytes or other mechanisms. Differently designed and advanced experimental studies exploring the mechanisms of cefazolin sodium on fracture healing are needed.

Hematoma at the fracture site induces and modulates the cells required for the repair process. It was demonstrated that positioning of the fracture hematoma into the fracture line without any disruption favors fracture healing. Moreover, fracture hematoma is found to be rich in angiogenic factors. Angiogenesis is crucial for normal fracture healing. It was suggested that removal of fracture hematoma affects callus formation negatively and extends fracture healing time.[26] Producing a closed fracture in our experimental study provided the advantage of no loss of fracture hematoma from the fracture site. However, because we produced double fractures of the tibia and fibula, one of the limitations of the present study is the fact that the healing process might be influenced negatively by the probable interposition of interosseous membrane between the broken ends of the fracture. Another limitation of the study is lack of mechanical testing of fracture healing.

In conclusion, the histological grade of fracture healing was greater in animals treated with cefazolin sodium than control animals, suggesting that it may be reasonable to choose cefazolin sodium for treatment of infections in patients with a bone fracture.

Declaration of conflicting interests

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