A comparison of effects of floroquinolones on fracture healing (An experimental study in rats)

Kırık iyileşmesi üzerine florokinolonların etkilerinin karşılaştırılması (Sıçanlar üzerinde deneysel bir çalışma)

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AMAÇ
Çalışmanın amacı, florokinolonların oluşturulan femur kırıkları üzerine etkilerini histopatolojik olarak test etmek ve karşılaştırmaktır.

GEREÇ VE YÖNTEM
Her bir grupta beşer denek olmak üzere toplam yirmi beş Wistar sıçanı beş gruba ayrıldı. Tüm gruplarda manuel olarak bilateral femur kırığı oluşturuldu. Kontrol (C) grubu olarak, ilk gruba herhangi bir ilaç verilmedi. İkinci grup norfloksasınle (N), üçüncü grup ofloksasinle (O), dördüncü grup pefloksasinle (P) ve son grup da siprofloksasinle (C) tedaviye alındı. İndüktlenen femur kırıkları sonrası yedinci günde antibiyotik tedavisine başlandı. Tüm gruplarda tedaviye yirminci gün son verildi. Femur kırıklarının indüksiyonundan dört hafta sonra tüm sıçanlar aşırı doz eter ile feda edildi ve femurlar kallus dokusuyla birlikte blok halinde çıkarılarak histopatolojik olarak değerlendirmeye alındı.

BULGULAR
Kontrol grubunda kırıkların ortalama iyileşme derecesinin tüm diğer antibiyotik gruplarından yüksek olduğu bulundu. Grupların ortalama iyileşme dereceleri sırasıyla, kontrolde 5 (n:8), ofloksasinde 4.1 (n:7), siprofloksasinde 3.9 (n:8), norfloksasinde 3.4 (n:9) ve pefloksasinde 2.6 (n:10) olarak saptandı. Kontrol ve norfloksasin grubu dışında tüm antibiyotik grupları arasında istatistiksel olarak anlamlı olmakla birlikte değişken farklılıklar olduğu saptandı.

SONUÇ
Kinolon grubu antibiyotiklerin kırık iyileşmesini etkilerini saptamak amacıyla Wistar sıçanlarında yaptığımız çalışmaya dahil edilen tüm florokinolon grubu antibiyotiklerin kontrol grubuna göre histopatolojik olarak kırık iyileşmesini anlamlı derecede geciktirdiği sonucuna varılmıştır.

Anahtar Sözcükler: Kırık iyileşmesi, florokinolonlar, karşılaştırmalı çalışma

BACKGROUND
The objective of the present study was to test and compare the effect of floroquinolones on fracture healing as assessed histopathologically.

METHODS
A total of twenty five Wistar rats were arbitrarily assigned to five groups with five animals each. Bilateral closed femoral fracture was constructed manually in all groups. The first group did not receive any drug as control (C). The 2nd, 3rd, 4th, and the last group were treated with norfloxacin (N), ofloxacin (O), pefloxacin (P) and ciprofloxacin (Ci) respectively. Antibiotic administration was started on the 7th day after the fracture incident. All the treatments were discontinued twenty days after the incident all the rats were sacrificed , and the fracture calluses together with affected femurs were resected en bloc at the fourth week after fracture.

RESULTS
Average healing grades of control group was higher than all the other antibiotic groups. Mean healing grades of control (5 ; n:8), ofloxacin (4.1; n:7), ciprofloxacin (3.9; n:8), norfloxacin (3.4 ; n:9) and pefloxacin groups (2.6 ; n:10) were recorded.. Statistically significant differences between antibiotherapy groups (excluding. norfloxacin) and the control group were detected.

CONCLUSIONS
The current histopathological study has shown that all the studied floroquinolones retarded fracture healing in rats.

Key Words: Fracture healing, floroquinolones, comparative study

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INTRODUCTION

Floroquinolones are highly potent antibacterial agents with a broad spectrum of activity including high potency against gram-negative bacteria. Application of these drugs in orthopedics has demonstrated a high level of efficacy with a relative lack of significant side effects in the prophylaxis and treatment of bone and joint infections.[1]

Floroquinolones inhibit bacterial cell replication by inhibiting the bacterial enzyme deoxyribonucleic acid gyrase, which is a type II topoisomerase.[2] Even though there are infrequent adverse reactions associated with their usage, one of the most important concerns is their potential for chondrotoxicity. This concern has derived mainly from extrapolation from animal experiments.[3,4] Arthropathy has also been reported in rare case reports in pediatric and adult patients.[5,6] Use of floroquinolones in children is contraindicated because of the adverse effects on cartilage and bones.[7]

In fracture healing progress, cartilaginous complex plays an important role in both intramembranous and endochondral ossification. Thus cartilaginous complex may potentially share the vulnerability of the chondrocytes in growing juvenile articular cartilage to fluoroquinolone toxicity. Experimental delaying of fracture healing with ciprofloxacin has been demonstrated.[8] However, to our knowledge, no study with other quinolones has been reported yet.

The objective of the present study was to test and compare the effects of floroquinolones on fracture healing from histopathological point of view.

METHODS

A total of twenty-five Wistar rats were arbitrarily assigned to five groups with five animals, and ten femurs each. After approval of the ethics committee of the faculty, bilateral closed femoral fractures were established manually in all groups under intramuscular ketamine hydrochloride (50 mg/kg bodyweight) anesthesia. All the fractures were confirmed with direct radiography. The first group did not receive any drug as a control group (C). The second, third, fourth and the last group were treated with norfloxacin (N) ofloxacin (O), pefloxacin (P) and ciprofloxacin (Ci) respectively. The rats were fed a standard chow housed in a twelve hours night day cycle environment and allowed water without restriction.

Table 1: The histological grading

Grade	Histological Evidence
I	All fibrosis tissue
II	More fibrosis than cartilaginous tissue
III	Fibrosis and cartilaginous tissue are almost equal
IV	Excessive cartilage
V	More cartilage than fibrosis
VI	Excessive cartilage with minimal woven bone
VII	Cartilaginous tissue and woven bone are almost equal
VIII	Excessive woven bone with minimal cartilage
IX	Excessive woven bone
X	Excessive woven bone with mature bone
XI	Woven bone and mature bone are almost equal
XII	Excessive mature bone with minimal woven bone
XIII	Excessive lamellar mature bone

Antibiotic administration was started on the 7th day after fracture. Ciprofloxacin was applied as 50 mg/kg twice a day. The dosages were chosen so as to achieve peak drug concentrations in rat serum approximating to those in humans. Groups Ci, P, O and N received ciprofloxacin (50 mg/kg sc bid), pefloxacin (40 mg/kg sc bid) ofloxacin (20 mg/kg sc bid) and norfloxacin (40 mg/kg sc bid) respectively group C (control) received no treatment. All the treatments were discontinued at twenty days post-fracture and all the rats were sacrificied with an overdose of ether inhalation. The fracture calluses together with affected femurs were resected en bloc at the fourth week after fracture.

All the resected femurs were evaluated with direct radiography and the segments were placed in buffered neutral 10% formalin for three days, followed by decalcification with 10% acetic acid. Specimens placed in paraphin blocs were stained with both hematoxylene-eosin (HE) and Masson's trichrom (MT). A new scale based on the amount of fibrotic tissue in callus formation, cartilaginous tissue, woven bone and mature bone were used to evaluate the degree of bone healing process (Table 1).

RESULTS

Two specimens from the control, one specimen from norfloxacin, three from ofloxacin, two from

Table 2: Average histological grades of the specimens

No	Grade	No	Grade	No	Grade	No	Grade	No	Grade
C1	5	O1	NA	Ci1	3	N1	3	P1	2
C2	3	O2	5	Ci 2	NA	N2	3	P2	3
C3	5	O3	4	Ci 3	3	N3	5	P3	3
C4	5	O4	5	Ci 4	5	N4	3	P4	2
C5	6	O5	4	Ci 5	5	N5	3	P5	3
C6	NA	O6	4	Ci 6	5	N6	3	P6	2
C7	6	O7	3	Ci 7	3	N7	4	P7	3
C8	5	O8	NA	Ci 8	4	N8	3	P8	3
C9	NA	O9	4	Ci 9	3	N9	NA	P9	2
C10	5	O10	NA	Ci10	NA	N10	4	P10	3

NA: not available / Ci: ciprofloxacine / O: ofloxacine / P: perfloxacine / C: control

ciprofloxacin were excluded from the study. Rats with fractures of metaphysis and those from which specimens for histopathological examination could not be obtained were excluded from the study. The details of the histopathological findings were shown in table 2.

Average healing grade of the control group was higher than all the other antibiotic groups. Mean healing grades of the groups were as follows: control 5 (n:8), ofloxacin 4.1 (n:7), ciprofloxacin 3.9 (n:8), norfloxacin 3.4 (n:9) and pefloxacin 2.6 (n:10). (Figure.1)

Histopathological examination of the control group under lower magnification revealed external callus formation with subperiostal bone and cartilage. formation. Also endochondral ossification with vascular invasion of cartilage was seen in calluses between bone and cartilage. (Figure. 2) Examination of ofloxacin group also revealed growing patterns of fibrous tissue in callus. Examination of ciprofloxacin group also revealed growing patterns of equal fibrous and cartilaginous tissue with excessive cartilaginous callus formation with a mean healing degree lower than that of the ofloxacin group. The results of norfloxacin group were similar to those of ciprofloxacin group. The degree of healing in pefloxacin group was quite lower than that of the other groups. Excessive fibrous tissue with minimal

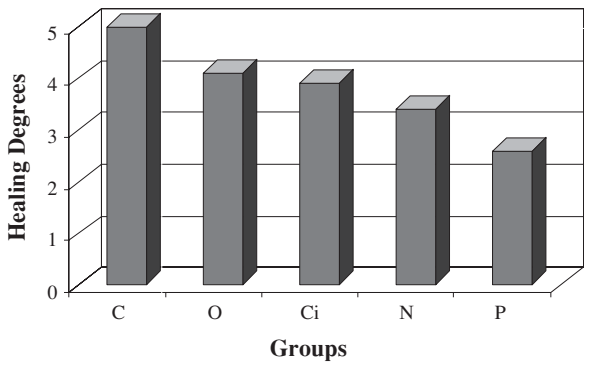

Figure 1: Mean histological grades of the groups (C: Control, O: Ofloxacin, Ci: Ciprofloxacin, N: Norfloxacin, P: Pefloxacin)

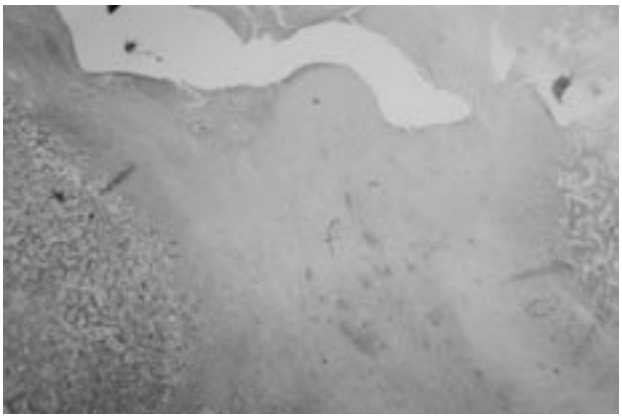

Figure 2: Callus maturation of control group in fourth week (HE X 50) (Abbr: c=cartilaginous area, f=fibrous area)

Figure 3: Differences of thickness and cartilage content maturation specialties in the control (a), ofloxacin (b), ciprofloxacin (c), norfloxacin (d) and pefloxacin (e) groups. (MT X 125)

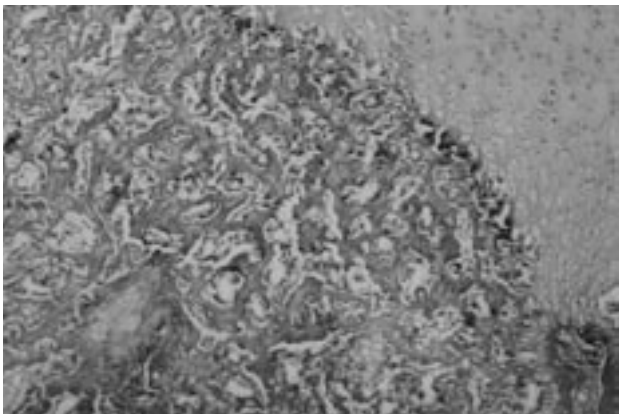

Figure 3a

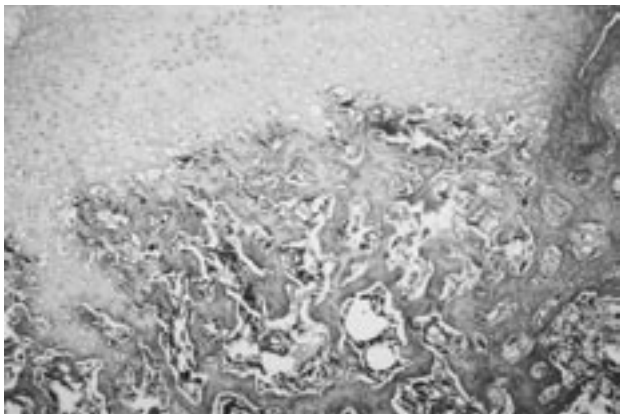

Figure 3c

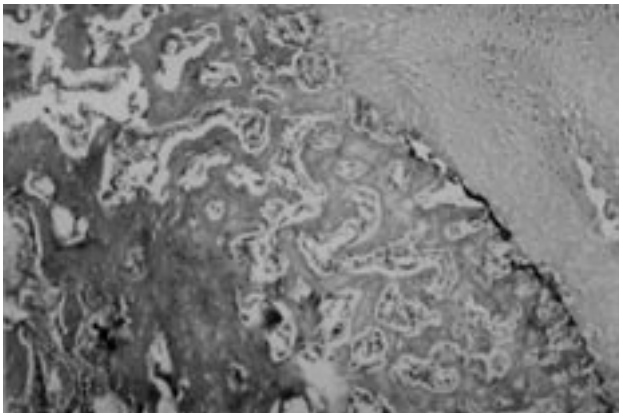

Figure 3b

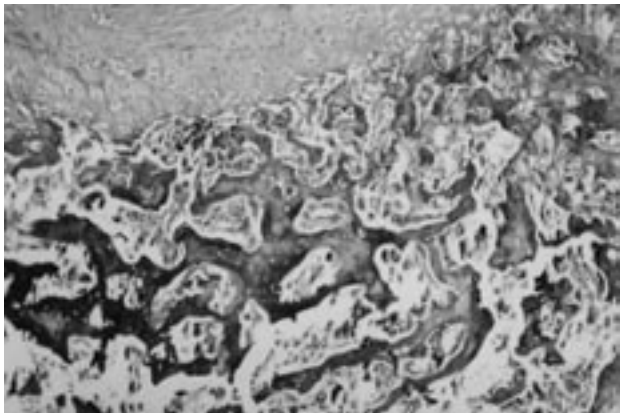

Figure 3d

cartilage was observed. Also vascular formation in fibrous tissue was apparent. (Figure. 3a,b,c,d,e)

During microscopic examination under higher magnification, endochondral ossification front revealed more chondrocytic abnormalities in the antibiotic groups than those of the control group. These abnormalities included decreased number of chondrocytes, relatively more immature chondrocytes with various shapes and dimensions. In the control group, chondrocytes were mature and more uniform in shape. In the antibiotic group, especially with pefloxacin, fewer number of chondrocytes with pleomorphism and immaturity were observed.

Serial sections of the tissue samples stained with MT revealed that new trabecules had contained more cartilage in antibiotic groups. Also relative trabecular atrophy and dominancy of cartilage were apparent in norfloxacin and pefloxacin groups.

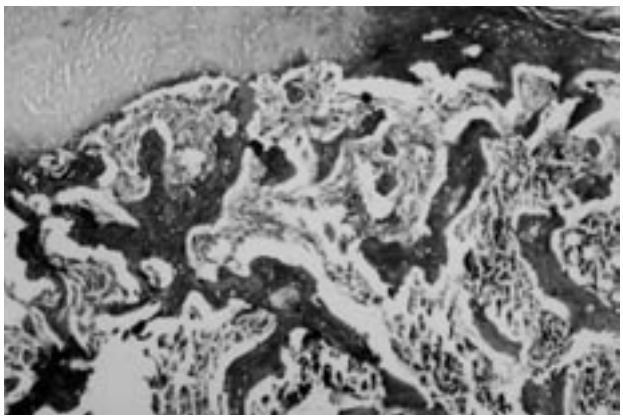

Figure 3e

Statistical analyses of the groups was performed with Kruskal Wallis analysis of variance and the difference between experimental groups was assessed with Mann-Whitney U test. The difference between the antibiotic groups, and the control group exclud-

Table 3a: The comparisons of statistical significance between groups

Groups	Result
C-N	p:0.023*
C-O	p:0.072
C-P	p:0.002*
C-Ci	p:0.015*
N-O	p:0.331
N-P	p:0.009*
N-Ci	p:0.363
O-P	p:0.004*
O-Ci	p:0.109
P-Ci	p:0.065

(*: Statistical significance, p<0.005)

Table 3b: Means ± SDs of the groups

Group	Number of cases	Mean	SD
C	8	4.0000	0.9258
N	9	3.2222	0.4410
O	9	3.4444	0.5270
P	10	2.4000	0.6992
Ci	8	3.0000	0.5345

(SD.: Standard deviation)

ing norfloxacin was statistically significant. However variable results were recorded for the differences among antibiotic groups (Table 3a). Means and standard deviations were shown in table 3b.

DISCUSSION

Floroquinolone group antibiotics are prescribed commonly for the treatment of urinary tract, soft tissue or other common infections. The adverse effects of floroquinolones on immature cartilage have been extensively investigated.[1.9.10.11] The toxic effects of quinolones tend to occur especially in the larger weight bearing joints such as the hip and knee.[11,12,13] Degeneration of the cartilage matrix has been observed after as few as two oral doses of ciprofloxacin.[10]

The repair process of the fracture involves development of the cartilaginous complex, which undergoes intramembranous and endochondral ossification similar to those of maturing cartilage of young animals. Huddleston et al.[8] have studied the toxic effects of floroquinolon on cartilage in fracture callus in an experimental study. In their study, ciprofloxacin was compared with cefazolin in rats with bilateral closed femoral fracture. The results were evaluated according to histological, radiological and physical examinations. In all examinations, fracture healing rate was decreased in the ciprofloxacin group. Histological examination of the fracture calluses obtained from the ciprofloxacin treated animals showed progressive formation of cartilage and subperiostal bone and replacement of cartilage by endochondral ossification. Besides they showed decreased number and size of the chondrocytes in the fracture calluses in the ciprofloxacin group. Also they demonstrated striking electron microscopic evidence of chondrocyte death in calluses exposed to ciprofloxacin. They expressed that negative effect is associated with a direct toxic effect on chondrocytes. This adverse effect on chondrocyte function then leads to an inefficient conversion of cartilage to bone, which is manifested by decreased mechanical properties of the fracture callus.[9] In the present study, greater number of chondrocytic abnormalities in antibiotic groups than the control group comply with these previous results.

Other possible mechanisms include action of quinolones as a DNA gyrase inhibitor.[14,15,16,17] In addition to the inhibition of DNA, other possible mechanisms of quinolone chondrotoxicity include alterations in DNA synthesis or repair.[3,10,18] The most pronounced inhibitory effect is related to the secretion of glycosaminoglycans and collagen components by the chondrocyte matrix Accordingly Mont et al.[2] stated that ciprofloxacin affects cell replication in adult human chondrocytes in vitro. Ciprofloxacin caused a decrease in cell proliferation as measured by [3H]-thymidine uptake and bromodeoxyuridine labeling where decrease of uptake is explained by a toxic effect on cells. Also it has been shown that the quinolones inhibit eukaryotic deoxyribonucleic acid polymerase alfa and beta terminal deoxyribonucleotidyl transferase activity.[19]

Mont et al.[2] showed that, ciprofloxacin does not effect synthesis of proteoglycans notably. Accordingly no obvious effect was noted in immunocytochemical staining for type I procollagen, type II collagen, keratan sulfate or unsulfated

chondroit in culture. Stahlman et al.[6] demonstrated loss of cartilage specific proteoglycans with ofloxacin.treatment.

The present study has shown that all the studied floroquinolones inhibit fracture healing. Further studies will be planned to understand why pefloxacin has possessed the most dramatic effect. Therefore quinolone group antibiotics, which are used widely in clinical practice, should be administered cautiously in orthopedics and traumatology.

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