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Quinolone Arthropathy Induced by Ofloxacin in Juvenile Rats: A Light Microscopic Study

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Abstract: This study was conducted to examine the development, progression and reversibility of cartilage lesions in juvenile rats that had been given ofloxacin, a fluoroquinolone. Four-week–old rats were treated with an oral dose of 900 mg/kg/day of ofloxacin for 7 days. The animals were sacrificed at different (1st day, 1st week, 2nd week, 4th week, 8th week, 12th week, 16th week) after the completion of the administration and the knee joints were prepared for light microscopic analysis. The lesions were demonstrated in the articular cartilage of all the animals. Acellular, oedematous matrix areas, and cavity formations were observed. These lesions progressed to extensive erosions at the end of the fourth month. Thus we concluded that arthropathy induced by ofloxacin is not reversible and the damage is persistent with increasing severity over time.

Key Words: Fluoroquinolone, ofloxacin, articular cartilage, chondrotoxicity, rat

Introduction

Quinolones have recently gained considerable therapeutic interest, since new fluorinated derivatives possess a higher antibacterial activity and favourable pharmacokinetic properties. However, in addition to their valuable antibacterial properties, all quinolones tested so far show arthropathic potential in the articular cartilage of juvenile animals. This unusual type of toxicity has restricted the use of fluoroquinolones in children, adolescents, and during pregnancy and lactation (1–3).

Bailey et al. (4) reported the first case of nalidixic acid arthralgia in 1972. Since then conflicting reports have appeared in the literature, either presenting quinolone arthropathy during clinical trials (5-10) or, by retrospective studies, documenting quinolone–treated patients revealing neither laboratory and radiological signs nor clinical symptoms of arthropathy attributable to quinolone exposure (9–22).

Results of experiments have shown that dogs, rats, rabbits, guinea pigs, marmosets, mice and chicks developed quinolone arthropathy (23–29). Animal studies have indicated that quinolone arthropathy is a prepubescent disease. Although quinolones cause arthropathy in young beagle dogs of about 3 months old, they fail to cause such an abnormality in mature beagle dogs of 8–12 months or older (27,30). A similar age–related difference in susceptibility to cartilage toxicity has also been demonstrated in rats (25,31).

Susceptibility to quinolone arthropathy is dose-dependent (27,30,32–34), and the chondrotoxic doses differ among the species and quinolone derivatives (3,27,35–40). There are comparative animal studies on the minimum arthropathogenic doses of different quinolones, but very little is known about the quinolone concentrations which induce lesions in articular cartilage of humans (41). The potential toxic differences between the drugs also remain unclear.

Although there have been a large number of studies exploring the mechanisms of quinolone arthropathy development (1–3,10,23–31,42–47), studies on the reversibility of the lesions are limited (24,25,27,48). The present study was designed to examine whether quinolone arthropathy is reversible or not. For this purpose, the articular cartilage of juvenile rats were examined under a light microscope at different time intervals, after the administration of ofloxacin had been completed, to demonstrate the development, progression and reversibility of the arthropathy over time.

Materials and Methods

Eighty-four 4–week–old Sprague–Dawley rats (42 male, 42 female) were obtained from the Experimental Animal Breeding and Research Centre, Uludag University Faculty of Medicine, Bursa, Turkey with the approval of the ethics committee. They were housed four or five to a cage. Food and water were provided ad libitum.

A preliminary study was conducted to determine the arthropathogenic dose of ofloxacin (OFLX). Twenty juvenile rats (4 weeks old) were divided into four groups (n=5) and were given OFLX for 7 days. We observed 0/5, 3/5, 4/5 and 5/5 rates of arthropathy in the groups that were given 30, 300, 600 and 900 mg/kg of OFLX, respectively (data not shown). In order to examine the development and reversibility of the lesions in the articular cartilage of all the animals, we decided upon a dose of 900 mg/kg /day. Ofloxacin powder (OFLX V 57), obtained from Turk Hoechst Company (Istanbul, Turkey), was suspended in distilled water (final concentration of 900 mg/mL). The rats were weighed at the beginning and on every other day for the calculation of dosing.

The rats were divided into 2 groups at the beginning of the experiment. The experimental group (28 male, 28 female) received 900 mg/kg/day of ofloxacin through an oragastric tube for 7 days. Control rats (14 male, 14 female) received the same amount of distilled water for 7 days. Following the completion of drug administration, the experimental and control groups were divided into 7 subgroups (for experimental subgroups n=8, for control subgroups n=4). They were sacrificed by exsanguination under ether anaesthesia on Day 1 (Group 1), on Week 1 (Group 2), at 2 weeks (Group 3), at 4 weeks (Group 4), at 8 weeks (Group 5), at 12 weeks (Group 6) and at 16 weeks (Group 7) after the last administration of OFLX. Samples of the articular cartilage of the knee joints were prepared for histological examination as follows. The specimens were fixed in 10 % neutral buffered formaldehyde at room temperature for 1 week. Decalcification was performed in neutral EDTA (Merck, Darmstadt, Germany) solution, with several changes of the solution, for 7–10 days and calcium oxalate testing was used to confirm that decalcification had been completed as described previously (49). Then the tissue blocks were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin. Sections of 5–7 µm were cut with a sliding microtome and stained with Harris' haematoxylin and eosin (49), safranin-O (50), toluidine blue (49) and Masson's trichrome (49). The morphological differences observed in the slides were evaluated according to the lesion scoring system used by Burkhardt et al. (45) (Table 1). In each group the mean scores were calculated. Then the statistical differences between the groups were estimated by using Kruskal–Wallis and Dunn's Multiple Comparison Tests. The slides were photographed with a Zeiss Axioscope MC 80 photomicroscope.

Table 1. The scoring system used for the evaluation of the lesions (adapted from Burkhardt et al.(45)).

MICROSCOPIC EVALUATION	POINTS
Structural changes	
No lesion identified	0
Vesicle	1
Surface over vesicle raised	1
Abnormal clumping of collagen	
Fibres perpendicular to surface	1
Fibres parallel to surface, not compressed	2
Fibres parallel to surface, compressed	3
Surface detached	4
Cellular changes (chondrocytes)	
Normal	0
Shrunken cytoplasm and/or nuclei	1
Hypocellularity (generalised)	2
Hypercellularity (generalised)	3
Clusters of chondrocytes	3
Spindle-shaped cells	4
Uptake of toluidine blue and/or safranin-O stains	
Normal	0
Slight reduction	1
Moderate reduction	2
Severe reduction	3
No dye noted	4

Results

In the present study, articular cartilage lesions were observed in all of the 56 rats that were given 900 mg/kg/day of OFLX for 7 days. The incidence of lesions did not differ between male and female rats. The lesions were unifocal, and 74.1% of them were seen in the femoral condyles and 25.9% were in the tibial condyles. The articular cartilages of the knee joints of the control rats were histologically normal in appearance (Figure 1).

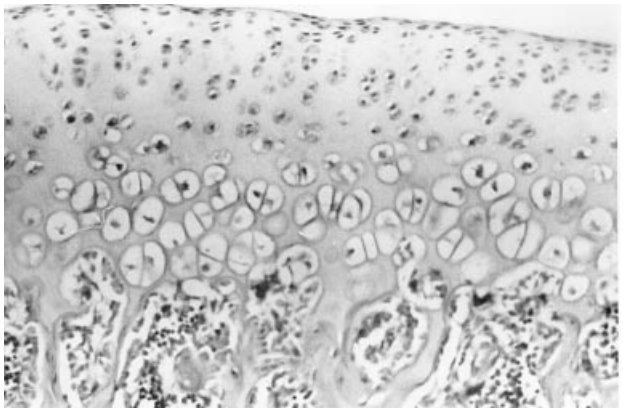

Figure 1. The normal histological appearance of articular cartilage of a 5-week-old rat from the second control group. (H&E, X 200).

The lesions, located in the middle zone, were characterised by acellular, oedematous matrix areas, and markedly decreased stainability with safranin–O and toluidine blue in groups 1 and 2 (Figure 2). In some of the sections there were fissures that contained slightly eosinophilic homogenous material, clumps of collagen fibrils and clumps of granular extracellular material. Around these lesions there were normal and necrotic chondrocytes, which were determined by their shrunken cytoplasm and/or nuclei. Cavity formation was clearly seen in groups 3, 4 and 5 (Figures 3 and 4). Chondrocytes adjacent to the cavity were shrunken and had pyknotic nuclei. There were also chondrocyte clusters containing 6–10 cells. Matrix staining with toluidine blue and safranin–O exhibited a decreased intensity. Only the clusters of chondrocytes were focally surrounded by a dark purple matrix whereas shrunken cells were not. The articular surface over the cavity was irregular and slightly elevated towards the articular cavity. The cartilage tissue covering the roof of the cavity showed a severe reduction

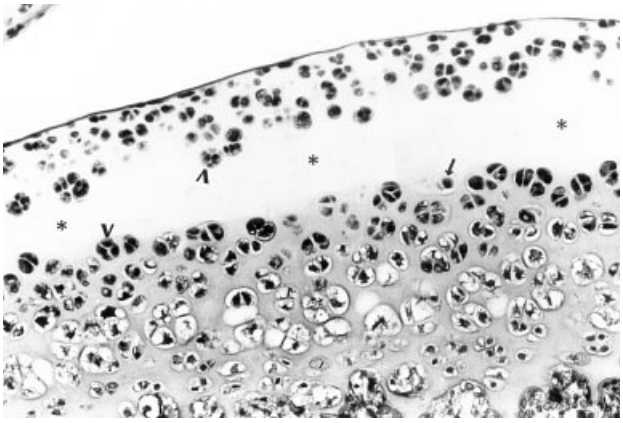

Figure 2. The histological appearance of articular cartilage on day 1 (Group 1). (H&E, X 200). The lesion was located in the middle zone, characterised by acellular, oedematous matrix areas and decreased stainability (asterisk). Necrotic chondrocytes with shrunken cytoplasm and/or nuclei (arrow), and chondrocyte clusters (arrowhead) were present.

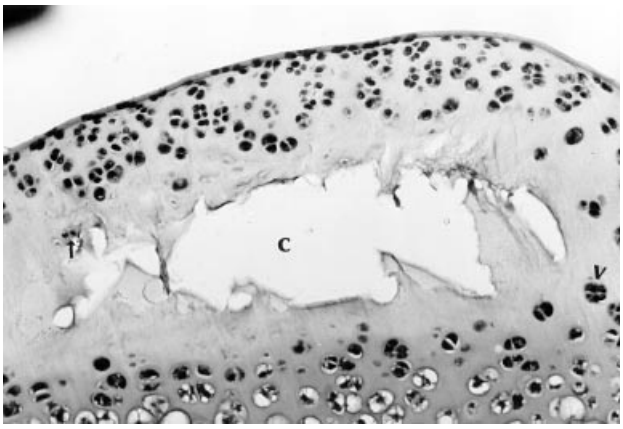

Figure 3. The histological appearance of articular cartilage at 2 weeks (Group 3). (H&E, X 200). Cavity formation (c) could be clearly seen. Around the cavity shrunken chondrocytes with pyknotic nuclei (arrow) and chondrocyte clusters containing 6–10 cells (arrowhead) were present.

in staining with toluidine blue and safranin–O. In some cases the surface layer of cartilage was separated from the remaining articular surface resembling a flap. Adjacent to the cavities, unmasked collagen fibrils were oriented parallel to the joint surface and in some of the severely compressed cases, collagen fibril aggregates were observed. In addition to those parallel-running fibrils there were unmasked collagen fibrils running perpendicularly into the cavity.

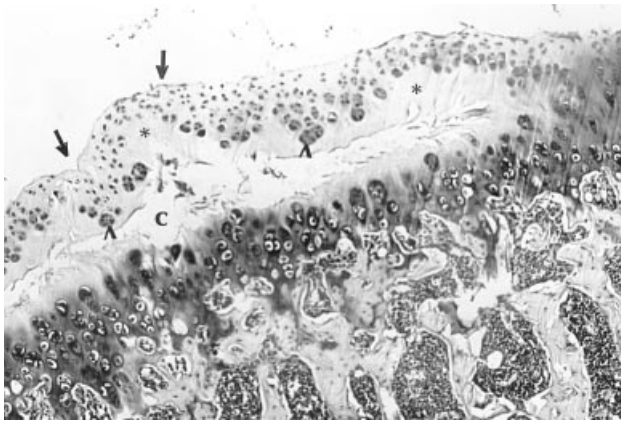

Figure 4. The histological appearance of articular cartilage at 8 weeks (Group 5). (Safranin–O, X 100). The articular surface over the cavity was irregular and slightly elevated towards the articular cavity (arrows). The cartilage tissue covering the roof of the cavity showed severe reduction in staining with safranin–O (asterisk). C; cavity, arrowheads; chondrocyte clusters.

In groups 6 and 7, the lesions obviously altered to extensive erosions (Figure 5). Flap–like separations and openings in the cavity ceiling were observed in most of the cases. The cavity was collapsed in some slides and the cartilage over the cavity became thinner and hypocellular. The decreased staining intensity of the matrix around the lesion still existed. In these groups the cartilage adjacent to the lesion was hypocellular. Although there were still some chondrocyte clusters, they were fewer in number.

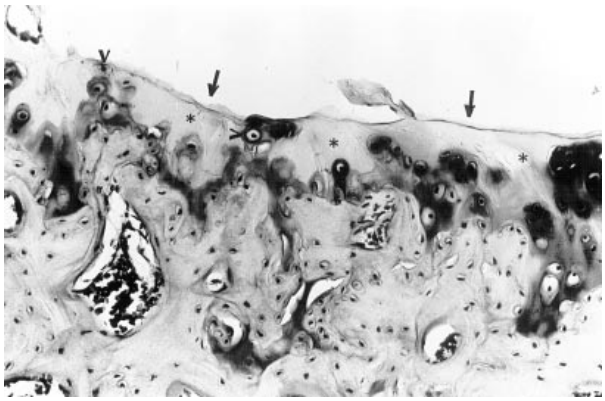

Figure 5. The histological appearance of articular cartilage at 16 weeks (Group 7). (Safranin–O, X 200). In the last group the lesion altered to extensive erosions (arrows). Note that subchondral osseous tissue was covered with only one or two layers of chondrocytes. Reduced staining of cartilage matrix with safranin–O (asterisk), chondrocytes with pyknotic nuclei (arrowhead) were still present.

We also detected some normal chondrocytes and a few shrunken ones. In the last group erosions were dominant. In some slides it was observed that subchondral osseous tissue was covered with only one or two layers of chondrocytes. Collagen fibrils were oriented perpendicularly in a few cases while most of them were parallel and compressed.

There was a statistically significant difference between each experimental group when compared to its time-match control group. Although there was no difference among the control groups at different time points, significant differences were observed when the mean scores of the experimental groups were analysed (Table 2).

Table 2. The mean scores of the control and experimental groups.

	CONTROL	EXPERIMENTAL	
Group 1	0.63±0.38	7.50±0.30	(d)*
Group 2	1.13±0.44	10.75±0.41	(e)*
Group 3	0.25±0.16	11.50±0.40	(b)*
Group 4	0.63±0.38	11.88±0.45	(f, g)*
Group 5	0.25±0.25	12.56±0.33	(c, g)*
Group 6	0.25±0.16	13.44±0.52	(c, g)*
Group 7	0.13±0.13	13.75±0.35	(a, g, h)*

*Significant differences between the experimental groups are:
a vs b= $p<0.05$; b vs d and c vs e= $p<0.01$; g vs d and h vs e= $p<0.001$.

Discussion

In this study, the articular cartilage damage induced by a 7-day treatment of ofloxacin was demonstrated in juvenile rats. We used a dosage of 900 mg/kg/day, which induced lesions in all animals, as the main aim of the study was to detect the reversibility of the induced arthropathy.

The histological findings of the cartilage lesions were in accordance with the studies that have previously been reported (23–27,30,32,45,46). In the experimental groups, degenerated chondrocytes with shrunken cytoplasm and pyknotic nuclei were scattered around the lesion. This finding was considered to be the result of DNA inhibition in the chondrocyte nuclei. Since quinolone arthropathy is a class effect of quinolones, cartilage toxicity seems to be closely related to the antibacterial activity (DNA-gyrase inhibition) of the drugs (51). Kato et

al. (25), have studied the histogenesis of cavity formation in rats. They have shown that condensation of chondrocyte nuclei was the first finding 5 h after a single dose (1000–3000 mg/kg) of OFLX. In another study they showed that the [^{3}H] thymidine binding capacity (an indicator of DNA synthesis) was decreased by 80 % 5 h after a single dose of OFLX, when compared with the control group (47). And with these findings they concluded that OFLX is suppressing the DNA synthesis which results in the degeneration of chondrocytes.

The chondrocyte clusters, which were present around the lesions, were thought to indicate that the regeneration process had started in the articular cartilage. The intensive staining with safranin–O and toluidine blue around the chondrocyte clusters has also shown the repair response of the cartilage. Supporting our findings, Kato et al. (47) showed that 24 h after a single dose of OFLX, [^{3}H] thymidine binding capacity increased by 160 % in comparison to the control group, which they explained by the increased proliferation of chondrocytes.

In the articular cartilage, the chondrocytes located in the middle zone have the largest synthesising capacity (52) and mitosis can be seen in this zone in the immature cartilage (3). These cells dealing with active synthesis were sensitive to the DNA gyrase inhibitory effect of the quinolones and were found to be degenerated in our experimental groups.

The decreased staining intensity of the cartilage matrix around the lesion with safranin–O and toluidine blue, which is in agreement with the other studies in the literature (24–26,32,35), was considered to be evidence of a decreased amount of glycosaminoglycans. It is known that these stains specifically stain the glycosaminoglycan compartment of the cartilage tissue. This reduction in the staining intensity can be explained by the decreased synthesis of matrix macromolecules due to the inhibition of DNA synthesis by quinolones. An immunohistochemical study demonstrated that marmosets which were treated with 200 mg/kg OFLX for five days showed a significant decrease in proteoglycan immunoreactivity around the lesion (36). Kato et al. (47) demonstrated a decreased ^{35}S–binding capacity (an indicator of polysaccharide synthesis) 12–24 h after the administration of a single 3000 mg/kg dose of OFLX in rats. Recent immunohistochemical studies have demonstrated increased expression of fibronectin, reduced staining for collagen II and altered expression of integrin after quinolone treatment (43,44).

Very little is known about the reversibility of quinolone arthropathy in the literature. Ingham et al. (24) reported the presence of lesions in the articular cartilage of dogs 3 months after the administration of pipemidic acid. However, their results were based on macroscopic examinations and there were no microscopic details of the lesions. Kato et al. (25) treated juvenile rats with OFLX for 7 days (900 mg/kg/day), and 10 weeks after the last dose they demonstrated that the surface of articular cartilage in the region of repair was irregular and the upper zone of the matrix did not contain any chondrocyte. Furthermore, in some cases large erosions were still remaining in the articular cartilage. The present study histologically demonstrates that the lesions were persisting as great erosions in the articular cartilage even in the fourth month following the termination of the 7-day treatment . Although the follow-up period was longer than in the aforementioned studies, no sign of repair or recovery was observed. The mean group score of the last group was higher than the others. In our opinion the raising of the score from the beginning of the experiments up to the fourth month could be accepted as proof of the deterioration of the lesion.

Comparative approaches on the dosage are trivial, since the bioavailability and pharmacokinetics of quinolones differ considerably among species. For example, juvenile rats treated with 100 times the human therapeutic dose of ofloxacin exhibited only 10 times the plasma concentration of the drug compared with the plasma concentrations in humans under therapeutic conditions (26). In our preliminary study we did not observe any lesions with 30 mg/kg/day of OFLX but the dose of 300 mg/kg/day developed cartilage lesion in the articular cartilage of 3 of the 5 rats. The present study did not include the examination of the reversibility of lesions induced by therapeutic doses. In fact, the cartilage toxicity of quinolones is dose-dependent, and the reversibility of lesions induced by minimal arthropathogenic dose should be studied in the future.

With the findings of this study we suggest that ofloxacin develops a progressive lesion in the articular cartilage of juvenile rats which can be seen even 4 months after the termination of the therapy, and the lesions are irreversible. In conclusion, it should be noted that with any case, in which the quinolones are necessary, paediatricians must be aware of the arthropathogenic

potential and must do the risk/benefit analysis carefully, until the concerns over arthropathy are alleviated.

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