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Quinolone-induced cartilage lesions are not reversible in rats

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Abstract The reversibility of quinolone-induced cartilage lesions has not been studied in detail. We treated five groups of five to seven juvenile Wistar rats (male and female; age: 5 weeks) with 2×600 mg ofloxacin/kg by gastric intubation on 1 day only (9:00 a.m. and 5:00 p.m.) and studied the knee joints histologically 3 days, 1, 3, 8 and 17 weeks later. In addition, joint cartilage specimens from vehicle-treated control rats ($n = 21$) at corresponding age were examined. Cartilage lesions such as matrix swelling, loss of proteoglycans and horizontal clefts were found in nearly all knee joints (26 of 27 joints; incidence: 96%) of the ofloxacin-treated rats. Within the observation period of 4 months the size of these lesions in knee joint cartilage did not decrease significantly. The diameter of the lesions at the time points of evaluation was 1146 $\pm$535, 1713 $\pm$309, 1250 $\pm$585, 1406 $\pm$356, and 1542 $\pm$467 μm, respectively (mean values $\pm$sd). Chondrocyte clusters producing glycosaminoglycans were observed 3 weeks after dosing and at later time points. They are considered to reflect the onset of repair but chondrocyte organization did not normalize during the study period, thus indicating the irreversibility of the effect under the experimental conditions. In principle, long-term joint cartilage damage has to be taken into account when the use of quinolones in children is considered. More detailed pharmacokinetic data are necessary for a reasonable risk assessment approach.

Key words Quinolone · Cartilage lesions · Rat

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Introduction

Fluoroquinolones such as ofloxacin are potent antimicrobial agents widely used in the treatment of infections with gram-negative bacteria (e.g. complicated urinary tract infections, salmonella infections and oral treatment of infections with *P. aeruginosa*). All quinolones of the first generation (e.g. nalidixic acid) as well as the more potent fluorinated derivatives (fluoroquinolones) used today exhibit a chondrotoxic potential and induce arthropathy in juvenile animals (for review see Gough et al. 1992; Stahlmann et al., 1993). Although they are contraindicated in children, their use in pediatrics for the treatment of life threatening diseases has been demanded (Schaad and Wedgwood 1992). Limited clinical data, for example in juvenile cystic fibrosis patients with *P. aeruginosa* infections, have shown a higher incidence of arthralgia after treatment with pefloxacin than after ofloxacin treatment (Pertuiset et al. 1989).

Although a considerable number of animal studies have been published describing quinolone-induced arthropathy in multiple species such as rats (Kato and Onodera 1988a, b), guinea pigs (Bendele et al. 1990), rabbits (Machida et al. 1990), dogs (Schlüter 1986; Burkhardt et al. 1990, 1992) or marmosets (Stahlmann et al. 1990), several aspects remain obscure.

For example, the question of whether or not the effects are reversible is a subject of controversy. Published data are insufficient to elucidate this important aspect. Symptoms such as gait abnormalities in dogs occurred transiently and disappeared even with continued medication. However, despite these clinical observations, cartilage lesions were still present at autopsy (Ingham et al. 1977; Tatsumi et al. 1978).

In other species, such as rats, clinical symptoms are not obvious after treatment with quinolones. Kato and Onodera (1988a) briefly described repair processes in joint cartilage after treatment of immature rats with

ofloxacin. However, this study does not clarify if a single day treatment would be sufficient to induce persistent changes.

Taken together, these data indicate that remission of clinical symptoms has to be distinguished from the reversibility of cartilage damage and it must be considered that species other than the dog do not exhibit symptoms at all. In man, symptomatic arthralgia in association with quinolone treatment has been shown to be reversible in case reports (e.g. Alfaham et al. 1987; Biswal et al. 1993; Samuelson et al. 1993) or retrospective evaluations (e.g. Pertuiset et al. 1989; Chysky et al. 1991). However, results from post mortem evaluation of joint cartilage are very limited (Schaad and Wedgwood 1992) and cartilage lesions in patients with clinical recovery cannot be excluded.

The aim of this study was to obtain and to document systematic data on the reversibility of quinolone-induced arthropathy in rats following oral treatment with ofloxacin on 1 day only.

Materials and methods

Ofloxacin treatment

Commercially available ofloxacin-containing tablets (Tarivid 200, Hoechst AG, Frankfurt/Main, Germany) were suspended in 2% starch solution. A total of 27 juvenile Wistar rats (14 female and 13 male rats; body weight: 58.4 $\pm$ 5.6 and 59.3 $\pm$ 7.6 g, respectively; age: 5 weeks) was treated by gastric intubation with 2×600 mg ofloxacin/kg on 1 day at 9:00 a.m. and 5:00 p.m. Control animals ($n = 21$; nine female and 12 male rats; body weight: 66.8 $\pm$ 6.9 and 66.4 $\pm$ 7.8 g, respectively) received the vehicle only. At 3 days and 1, 3, 8 and 17 weeks after dosing (i.e. at 5.5, 6, 8, 13 and 22 weeks of age) the rats were killed by decapitation and the knee joints were prepared for histological examination.

Histology

Joints were fixed in formalin, decalcified in EDTA (10%), dehydrated in the alcohol series and embedded in paraffin. Sections (7 μm) were cut with a microtome (Reichert-Jung No. 1140, Heidelberg, Germany) and stained with an aqueous solution of 1% toluidine blue (Merck, Darmstadt, Germany). From each joint 40–50 serial sections were prepared from the predilection sites and inspected under a light microscope. The size of the lesions was measured in the specimen within the series which exhibited the most pronounced extension of the lesion (horizontal clefts, areas of matrix alterations or scars). The diameter of the knee joints was determined at the position of the epiphyseal growth plate.

Results

Light microscopy

Incidence and site of lesions

Figure 1 shows normal knee joint cartilage from a 5-week-old rat. Figures 2–5 give typical examples of ofloxacin-induced cartilage lesions as observed during the study period. A total of 36 characteristic cartilage lesions (matrix swelling, horizontal cleft formation, decreased matrix staining with toluidine blue) was found in 26 of the 27 knee joints examined (incidence: 96%; Table 1). In all 26 damaged knee joints lesions were observed predominantly in the caudal part of the femoral condyles. In ten animals both the tibia and the femoral condyles (caudal parts) were affected (Table 1).

Reversibility and repair

The lesions observed 1 week after treatment were indistinguishable from lesions in the group studied 3 days after dosing (Fig. 2). Three weeks after treatment, chondrocyte clusters with cells occasionally arranged in rings were observed in the vicinity of the lesions. Clefts were found 2 months after treatment and lesions persisted in the cartilage matrix throughout the entire study period of 4 months (Fig. 5).

The number of lesions per knee joint (Table 1) and the size of the lesions did not decrease throughout the observation period. In sagittal sections, the maximal diameter of the cartilage lesions was 1146 $\pm$ 535 μm 3 days after treatment and 1542 $\pm$ 467 μm in knee joints examined 4 months later (Table 2). The diameter of the proximal tibial condyle was approximately 4–5 mm in juvenile and 5–6 mm in adult rats.

Table 1 Incidence and localization of joint cartilage lesions in rats after treatment with 2×600 mg ofloxacin/kg on 1 day only. The rats were treated at 5 weeks of age and killed 3 days, 1, 3, 8 and 17 weeks after treatment

Time after treatment	Cartilage lesions after treatment with ofloxacin Localization				Lesions per joint [median (range)]	Incidence
	Tibia		Femur			
	c[1]	v[1]	c[1]	v[1]		
3 days	4	1	6	1	2 (1–2)	100% (7/7)
1 week	1	0	5	0	1 (1–2)	100% (5/5)
3 weeks	1	0	4	1	1 (1–2)	100% (5/5)
8 weeks	1	0	4	0	1 (0–2)	80% (4/5)
17 weeks	2	0	5	0	1 (1–2)	100% (5/5)
Total	9	1	24	2	1 (0–2)	96% (26/27)

[1] *c* caudal; *v* ventral

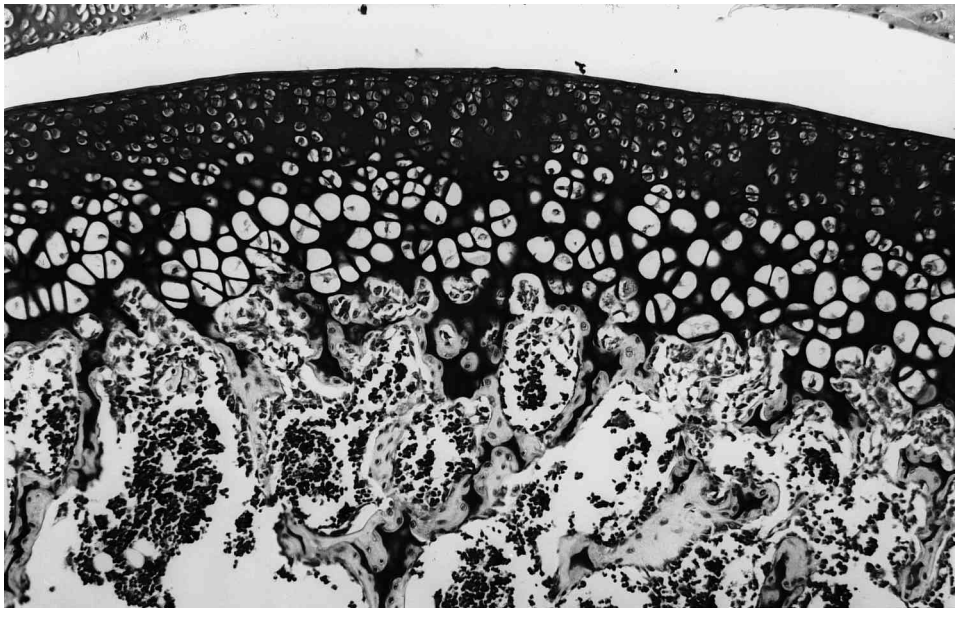

Fig. 1 Control: knee joint cartilage from an untreated juvenile Wistar rat (age: 5 weeks). × 110. Characteristic chondrocyte distribution and regular staining of the cartilage matrix with toluidine blue. Hypertrophic chondrocytes as observed in the deep zone of juvenile cartilage are not observable in adult cartilage

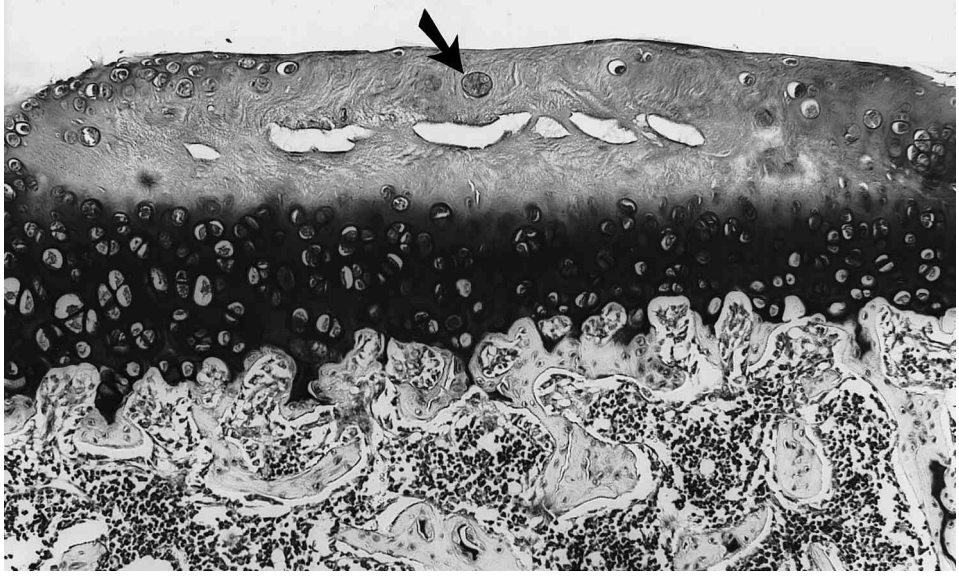

Fig. 2 Characteristic joint cartilage lesion 1 week after treatment with ofloxacin (2 × 600 mg/kg; age: 6 weeks). × 110. Horizontal cleft formation in the cartilage matrix, extensive loss of glycosaminoglycans around the lesion, acellular cartilage matrix; occasionally, chondrocyte clusters were seen 1 week after treatment (*arrow*)

In the immediate proximity of the lesions, the concentration of glycosaminoglycans decreased in the cartilage matrix as observed by reduced toluidine blue staining. This change persisted throughout the entire study period but was more pronounced in cartilage from rats examined 1 or 3 weeks after treatment than in knee joints inspected 8 or 17 weeks later. In cartilage samples from the latter groups, a characteristic finding was chondrocyte clusters with increased pericellular staining of toluidine blue compared to the surrounding damaged cartilage matrix (Figs 4 and 5). There were clefts (Fig. 4) or fissures and acellular spaces in the cartilage matrix, and chondrocytes were not distributed at random as observed in untreated control rats (Fig. 5).

Discussion

Recently, we showed that in 5 weeks old rats a single oral dose of 600 mg ofloxacin/kg induced cartilage lesions in approximately 70% of the rats. After treatment with 300 mg ofloxacin/kg cartilage lesions were not found (Stahlmann et al. 1995). A regimen of 2 × 600 mg ofloxacin/kg administered on 1 day produced cartilage lesions in all knee joints examined (Förster

Table 2 Size of cartilage lesions (μm) in knee joints from rats [mean ± SD; $n = 30$, (proximal tibial condyle: 20, distal femoral condyle: 10)]. Juvenile Wistar rats (age: 5 weeks) were treated orally with 2×600 mg ofloxacin/kg on 1 day only. After 3 days, 1, 3, 8 and 17 weeks groups of five to seven rats were killed and the knee joints inspected under a light microscope. From a total of 36 lesions found, the size could be reliably determined in 30 lesions

Time after treatment with ofloxacin (age in weeks)				
3 days (5,5)	1 week (6)	3 weeks (8)	8 weeks (13)	17 weeks (22)
1146 ±535 ($n = 9$)	1713 ±309 ($n = 5$)	1250 ±585 ($n = 6$)	1406 ±356 ($n = 4$)	1542 ±467 ($n = 6$)

et al. 1995). We also showed before that in juvenile rats single doses of 300 and 600 mg ofloxacin/kg result in mean plasma concentrations of approximately 20 and 30 mg/l, respectively (Stahlmann et al. 1990). Although a dose of 600 mg ofloxacin/kg exceeds the therapeutic dose by a factor of 100, plasma concentrations were only 10 times higher than in humans under therapeutic conditions (Lode et al. 1987).

Under the present experimental conditions, ofloxacin-induced cartilage lesions in rats were not reversible after a 1-day treatment within a period of 4 months. Although glycosaminoglycan producing chondrocyte clusters indicated some repair processes, gross-structural cartilage lesions persisted in adult animals and did not decrease in size.

Studies on quinolone-induced arthropathy in dogs have shown that recovery from clinical symptoms has to be distinguished from morphologic reversibility of cartilage lesions. Quinolone-induced arthropathy was initially observed in dogs (Ingham et al. 1977), since

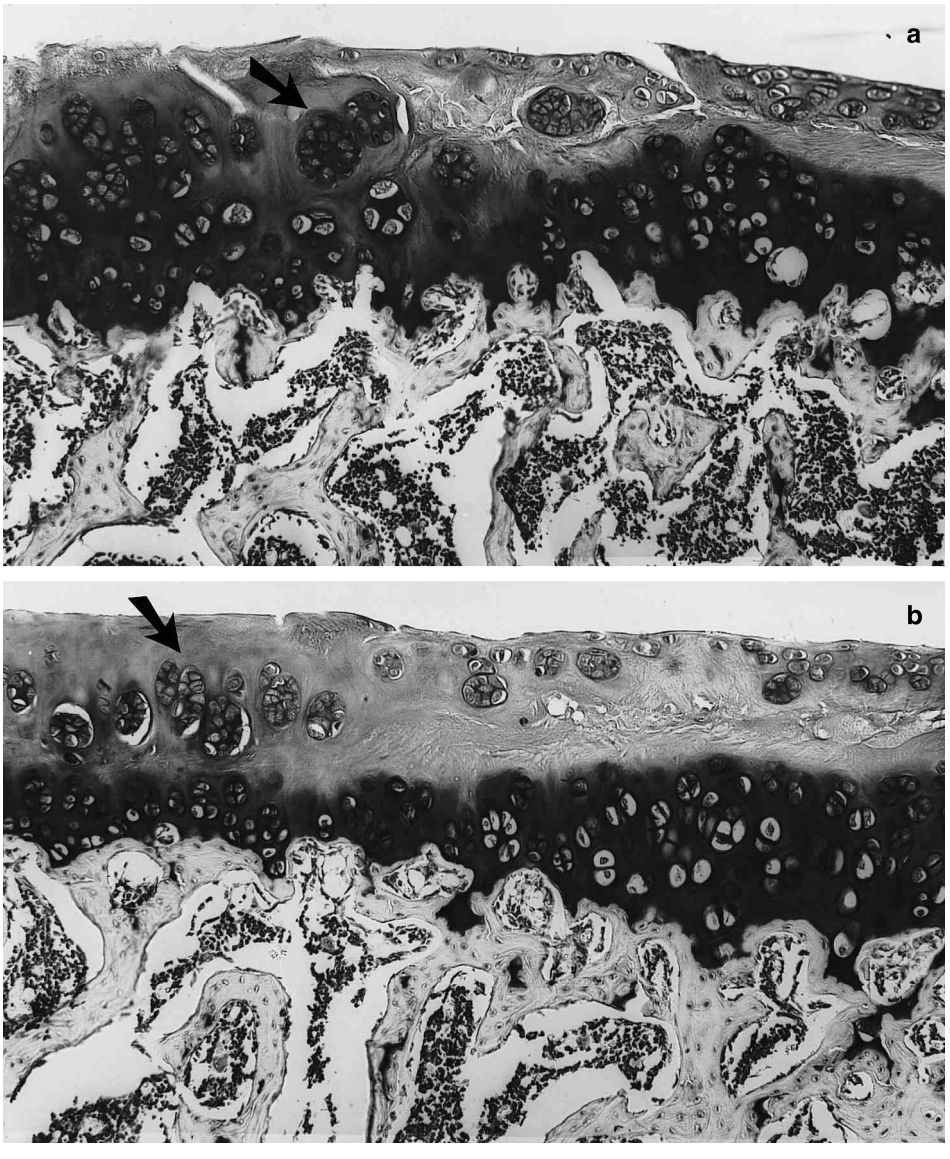

Fig. 3a, b Characteristic examples of joint cartilage lesions 3 weeks after treatment with ofloxacin (2×600 mg/kg; age: 8 weeks). × 110. Horizontal clefts in the cartilage matrix and extensive loss of glycosaminoglycans. Chondrocyte clusters with pronounced pericellular staining for toluidine blue (*arrows*) are increasingly found within acellular spaces of the lesions

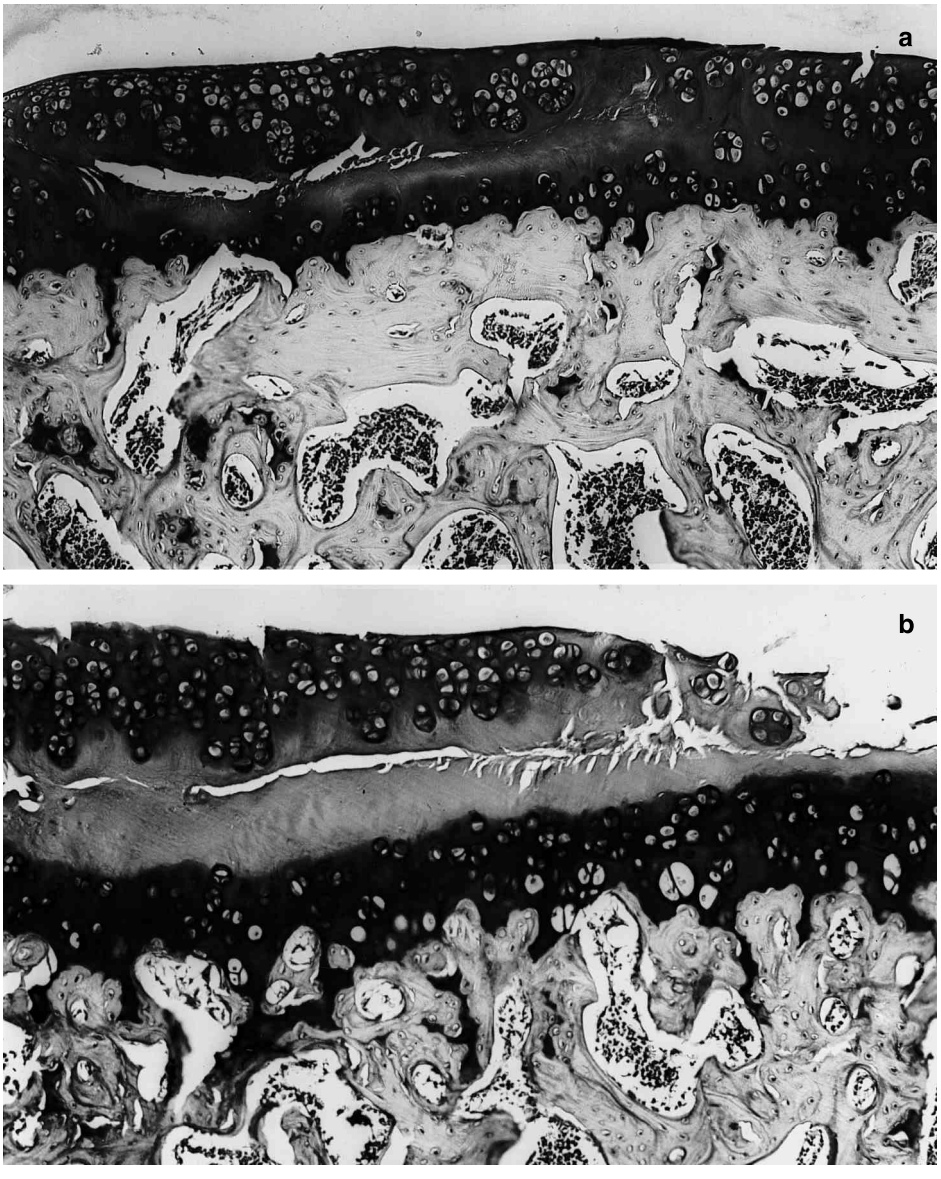

Fig. 4a, b Characteristic examples of joint cartilage lesions 8 weeks after treatment with ofloxacin (2×600 mg/kg; age: 13 weeks). $\times 90$ (**a**), $\times 145$ (**b**). Persisting horizontal clefts, chondrocyte clusters. In some samples (**a**), the loss of glycosaminoglycans is not as pronounced as in samples investigated 3 weeks after treatment (see Fig. 3)

dogs exhibit symptoms such as pain, joint stiffness, staggered gait and reluctance to rise from a sitting position during treatment. Symptoms appeared 2 days after the onset of dosing and were most pronounced from day 3 to day 14. Clinical recovery was then observed within 1 month even with continued dosing. These first descriptions were confirmed by Tatsumi et al. in 1978. Doses as low as 15 mg pipemidic acid administered twice daily for 90 days were sufficient to induce blisters and erosions in all the joints of all dogs studied, but clinical symptoms were not associated with these gross-structural lesions. Histological examination of the knee joints revealed cartilage injury such as cleft formation and erosion of the joint surfaces also in dogs which had recovered from clinical symptoms in both studies (Ingham et al. 1977; Tatsumi et al. 1978). Tatsumi et al. (1978) reported that blisters persisted until 30 days after a 1-week-treatment period with pipemidic acid. The authors also mentioned that the areas at which blister or erosion seemed to be formed were still found as scars 4 years after treatment with pipemidic acid.

Kato and Onodera (1988a) studied the reversibility of ofloxacin-induced cartilage lesions in male Sprague-Dawley rats. Histological changes after a 7-day treatment period were not documented. The authors described lesions such as slightly irregular joint surfaces, acellular matrix areas, large chondrocyte clusters and narrowed cavities persisting up to 10 weeks after treatment. Since the authors observed repair processes in joint cartilage during their study, the alterations were considered to be reversible.

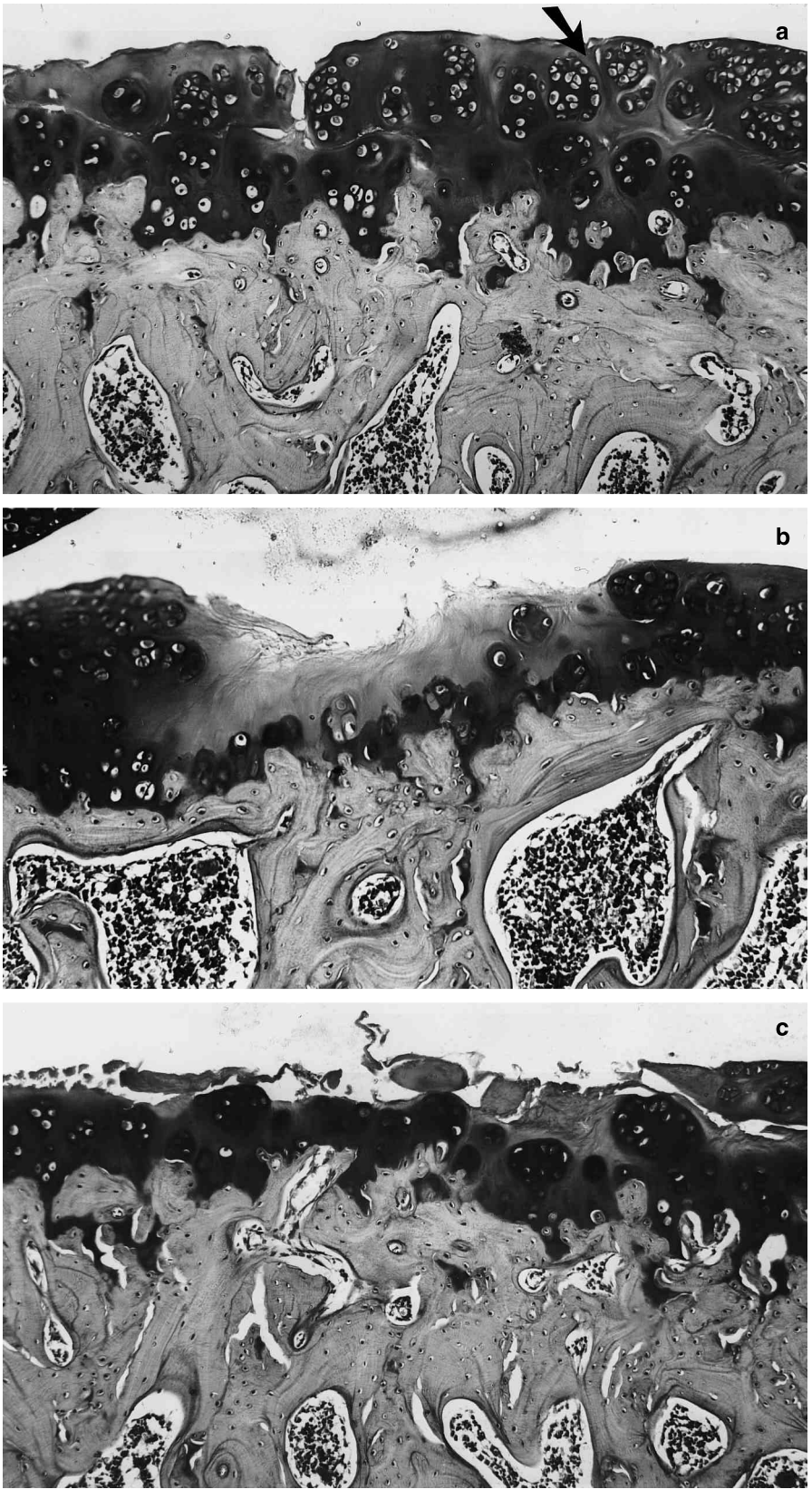

Fig. 5a, b, c Characteristic examples of joint cartilage lesions 17 weeks after treatment with ofloxacin (2×600 mg/kg; age: 22 weeks). $\times 110$. (**a**), $\times 145$ (**b**), $\times 110$ (**c**). Persisting fissures (**a**), scars (**b**) and clefts (**c**) in the cartilage matrix. Chondrocyte clusters with increased pericellular staining for toluidine blue (**a**; *arrow*). There are still acellular areas within the matrix and chondrocytes are not distributed at random as observed in untreated controls (cf. Fig. 1)

Dogs are apparently more sensitive to quinolones than rats. These discrepancies between the species might be due to pharmacokinetic and/or pharmacodynamic differences. Compared to dogs, quinolone-induced arthropathy in rats occurs at higher doses. Gait abnormalities were not observed, but cartilage lesions are characterized by very similar histological findings in both species. Therefore the juvenile rat is a suitable model to study quinolone-induced arthropathy. However, the consideration of differences in pharmakokinetics is an essential prerequisite if interspecies comparisons are made.

Only one comparative toxicokinetic study with two quinolones in rats, rabbits and dogs is available, which showed that cartilage lesions in dogs occur at lower concentrations than in other species: the lowest arthropathogenic dose produced maximal plasma concentrations of 5.1 mg and 16.1 mg norfloxacin/l in dogs and in rats, resulting in respective cartilage concentrations of 6.9 and 20.6 μg norfloxacin/g tissue after 7 days of treatment (Machida et al. 1990). In juvenile rabbits, levofloxacin cartilage concentrations at the lowest arthropathogenic dose were 12.2 ± 5.8 μg/g tissue (Kato et al. 1995).

Meissner et al. (1990) reported a mean concentration of 2.2 ± 0.5 mg ofloxacin/l in articular cartilage (assuming a cartilage density of 1 kg/l) from four patients undergoing hip replacement. These concentrations were measured 12 h after infusion of a single dose of 200 mg ofloxacin.

The relevance of data from animal studies for man is still unclear. From clinical experience, it appears as if the arthropathogenic potential of quinolones at therapeutic doses in humans is low, although there might be some important differences between the individual drugs. In a retrospective evaluation of ciprofloxacin-treated juvenile patients, Chysky et al. (1991) reported on eight episodes of arthralgia in 613 treated patients (1.3%) which were associated with joint swelling and pain. However, in all patients arthralgia was reversible after discontinuation of the drug. Radiological examination of nalidixic acid-treated juvenile patients after therapy gave no evidence for drug-related cartilage damage (Schaad and Wedgwood-Krucko 1987; Adam 1989). An arthropathy incidence of 14% has been observed after treatment with pefloxacin in nine of 64 juvenile cystic fibrosis patients but all episodes were reversible (Pertuiset et al. 1989).

Several case reports exist describing symptoms of arthropathy after quinolone treatment which generally were reversible (for review: Stahlmann et al. 1993). In contrast, one case of severe cartilage damage requiring hip and knee joint replacement has been described in a 17-year-old boy after pefloxacin treatment (Chevalier et al. 1992). However, the causal role of quinolone treatment in all of these patients is unclear, since most of the case reports describe juvenile patients suffering from cystic fibrosis, a disease which is associated with arthropathy itself (Bourke et al. 1987).

In summary, the present study provides the first detailed histological data on the irreversibility of quinolone-induced cartilage damage after short-term treatment in animals. Although there was evidence for some repair process, gross-structural cartilage lesions such as horizontal clefts or fissures, scars and matrix alterations persisted and were found even 4 months after treatment. The size of these lesions did not decrease. These data indicate that in principle the risk of quinolone-related long-term joint cartilage damage has to be taken into account when the use of quinolones in children is considered. However, it remains to be established if therapeutic doses of quinolones could induce such lesions in man. More detailed pharmacokinetic data should allow a scientifically better founded risk assessment.

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