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Ofloxacin in juvenile non-human primates and rats. Arthropathia and drug plasma concentrations

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Abstract. Arthropathia in juvenile animals is the most important toxic effect induced by quinolones. We conducted pharmacokinetic and morphological studies with ofloxacin on non-human primates (*Callithrix jacchus*, Marmosets) and rats. In the marmoset, electron microscopy and the application of immuno-morphological methods proved to be suitable for the detection of specific alterations in cartilage (e.g. loss of proteoglycanes and altered chondrocytes). Subsequently performed electron microscopic examinations in rats showed similar specific alterations of the femur cartilage surface after multiple oral applications of 600 mg ofloxacin/kg body wt. These results were correlated with pharmacokinetic data obtained for the same species. After single oral application of 100, 300 or 600 mg ofloxacin/kg body wt to 5 week-old rats peak plasma levels were achieved 15–45 min after administration indicating a rapid absorption of the drug. The following peak concentrations were measured for the three doses applied (mean ± SD): 8.9 ± 2.1, 22.6 ± 7.5 mg/l and 33.5 ± 9.8 mg/l, respectively. After 360 min the concentrations were 1.1 ± 0.4, 5.9 ± 2.5 and 15.9 ± 5.1 mg/l, respectively. After subcutaneous injection of 100 mg ofloxacin/kg body wt the mean peak concentration was 27.7 ± 2.6 mg/l after 45 min (0.5 ± 0.2 mg/l after 360 min). In the marmoset higher plasma concentrations were measured with comparable doses. One, 3, and 6 h after the last of nine administrations of 200 mg ofloxacin/kg body wt, the mean (± SD) plasma concentrations were: 42.7 ± 16.7, 40.6 ± 9.5, and 26.5 ± 3.6 mg ofloxacin/l plasma. Typical alterations of the joint cartilage of juvenile rats (e.g. opened chondrocyte cavities, swelling of rough endoplasmic reticulum and mitochondrial swelling in the chondrocytes) were induced by oral administration of ofloxacin at doses that were approximately 100 times higher than therapeutic ones, but led to peak plasma concentrations which were only approximately 10 times above the therapeutic level. Since we found corresponding cartilage alterations in marmosets and rats, this species provides a convenient model for additional studies on chondrotoxic effects of quinolones.

Key words: Ofloxacin – Quinolones – Marmosets – Rats – Arthropathia

Introduction

Fluorinated quinolones have recently gained special interest as a new group of antimicrobial agents (e.g. Editorial 1986; Stahlmann 1988). The new derivatives possess a higher antibacterial activity than older gyrase inhibitors such as nalidixic acid (Wolfson and Hooper 1985). In addition, they exhibit favourable pharmacokinetics and are therefore used not only for treatment of urinary tract infections but also for respiratory tract or soft tissue infections (Hooper and Wolfson 1985).

Arthropathy in juvenile animals is an important toxicological effect regarding all quinolones so far investigated.

Ingham and coworkers (1977) first described gait abnormalities in 3-month-old beagle dogs after oral administration of nalidixic acid or pipemidic acid. The cartilage toxicity must be considered a major drawback of these valuable antibacterial agents. The experimental findings have led to important restrictions in the therapeutic use of quinolones: they are presently contraindicated in children and adolescents in the growing phase and during pregnancy and lactation (Christ and Schmidt 1984). The toxicity and adverse effects of the quinolones have been recently reviewed (Stahlmann and Lode 1988).

Since no data are available on the susceptibility of primates towards this toxic effect, we conducted experiments on quinolone-induced cartilage toxicity in non-human primates (marmosets) as compared to rats. As a model compound we used ofloxacin, a new fluoroquinolone which can be used against systemic infections and which is characterized by metabolic stability and offers

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the best bioavailability (Monk and Campoli-Richards 1987). Therefore, this compound combines the best prerequisites for studies using oral application. Parts of these results have been presented before (Stahlmann et al. 1988a, b).

Materials and methods

Studies on morphological alterations and on plasma levels in marmosets

Six male, juvenile (9- to 18-week-old) marmosets (*Callithrix jacchus*) were treated orally for 5 days (twice daily) with 200 mg ofloxacin/kg body wt. The solution was prepared by suspending commercially available tablets (Tarivid) in a 2% starch solution supplemented with a Vitacombex vitamin solution (10%; v/v), the taste of which was already well-known to the animals. This was applied at a volume of 10 ml/kg body wt. The treated animals were kept with their parents in cages in our primate colony. Maintenance conditions were: room temperature 26 ± 1° C at a relative humidity of 60 ± 5%, constant light/dark schedule (light: from 7 a.m. to 7 p.m.). The animals were fed Altromin pellet feed and tap water ad lib, apples and bananas twice a week. The animals were sacrificed by an overdose of hexobarbital.

For electron microscopic examination one knee joint was prepared and the femur condylae were evaluated macroscopically; subsequently it was immediately fixed in Karnowsky's solution, tannic acid or Ruthenium Red (RR) fixation. Karnowsky's solution consisted of 3% paraformaldehyde plus 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), tannic acid solution of 1% glutaraldehyde in phosphate buffer (0.2 M, pH 7.2) with the addition of 0.5% tannic acid, and RR fixation of 1% glutaraldehyde (see above) with the addition of 1% RR. Dehydration was performed in the alcohol series, embedding in Epon. Sectioning: Reichert ultracut; contrasting: uranyl acetate/lead citrate; electron microscopy: Siemens Elmiskop 101 and Zeiss EM 10.

Immuno-histochemical methods were also applied in these studies. The other knee joint was prepared and frozen in liquid nitrogen and stored at –80° C before cryosections were prepared. The sections were stained using specific antibodies prepared in our institute against different compounds of cartilage matrix (collagen type II, cartilage proteoglycanes, and fibronectin), with the help of a second, fluorescence-labelled antibody (for details cf. Barrach et al. 1980, 1981).

One hour after the ninth application of the drug, blood was taken from a femoralis vein of all animals. Two hours later blood was again taken from three of the animals in the same manner. From the remaining three animals blood was taken by cardiac puncture in hexobarbital narcosis at sacrifice. The remaining three animals were sacrificed 6 h after the last application and blood was again obtained by cardiac puncture.

Plasma concentrations

The determination of ofloxacin concentrations in plasma was performed using a HPLC method as described by Borner (1986).

Pharmacokinetic studies in rats

For these studies juvenile, 5-week-old Wistar rats (Bor: Wisw/spf, TNO) bred at our institute were used. Ofloxacin was given as a single dose either by gavage (10 ml/kg body wt), or subcutaneously. The solution for oral application was prepared by suspending commercially available tablets (Tarivid^R) in a 2% starch solution. The solution for s.c. injection (HOE 280 Lösung zur i.v.-Infusion CH-B:6) was kindly provided by the manufacturer (Hoechst AG, Frankfurt, FRG). We checked the plasma concentrations at five different times after administration of three (100, 300, 600 mg/kg body wt orally) or two (30 and 100 mg/kg body wt s.c.) different doses. At 15, 45, 90, 180 and 360 min after oral administration 12 animals (6 male, 6 female) were sacrificed and blood was obtained by decapitation. In the s.c. experiments the number of animals was two male and two females. Plasma was obtained by immediate centrifugation and stored at –20° C until it could be analysed.

Morphological studies in rats

Four-week-old male rats were treated orally with 600 mg ofloxacin/kg body wt twice daily for 5 days. The animals were kept in Macrolon^R cages at a room temperature of 23 ± 1° C and 50 ± 5% relative humidity, constant light/dark schedule (light: from 7 a.m. to 7 p.m.), Altromin 1324 pellet feed and tap water ad lib. At the end of the treatment period the animals were sacrificed, the knee joints were prepared, fixed in Karnowsky's solution and examined electron microscopically.

Results

Immuno-morphological studies in marmosets

Macroscopically no clear-cut lesions could be detected under our experimental conditions. However, after preparing serial sections of a marmoset knee joint, small blisters were detected which were then further examined using immuno-morphological techniques.

With this methodological set-up we observed a blister-like detachment of the upper cartilage layer (Fig. 1), and a distinct increase in the fibronectin content in the blister-capsule and the surrounding area (Fig. 2). Fibronectin could also be demonstrated immunomorphologically in some areas without the occurrence of blisters.

A loss of cartilage-specific proteoglycanes can be demonstrated in a variety of areas (Fig. 3).

Electron microscopic studies in marmosets

In a follow-up study to these investigations the cartilage preparations were examined electron microscopically to ascertain whether the light microscopic results could be verified. Using Ruthenium Red- and tannic acid-fixed preparations we were able to confirm the results obtained by antibody staining. In contrast to the light microscopic investigations, which only revealed localized alterations, electron microscopic inspection showed specific alterations throughout the whole preparation, which were more pronounced in certain areas.

A loosening and loss of matrix on the surface of the joint cartilage (Fig. 4), and an opening of cartilage cell cavities accompanied by loss of cells was also observed. The chondrocytes in deeper layers exhibited a ballooning of the endoplasmic reticulum, shrinkage and necroses (Fig. 5*a*). In the matrix beneath the cartilage surface a loss of Ruthenium Red-positive granules which represent proteoglycanes was detectable. Figure 5*b* and 5*c* show examples of a necrotic chondrocyte and a cell-free cavity in the matrix just beneath the surface.

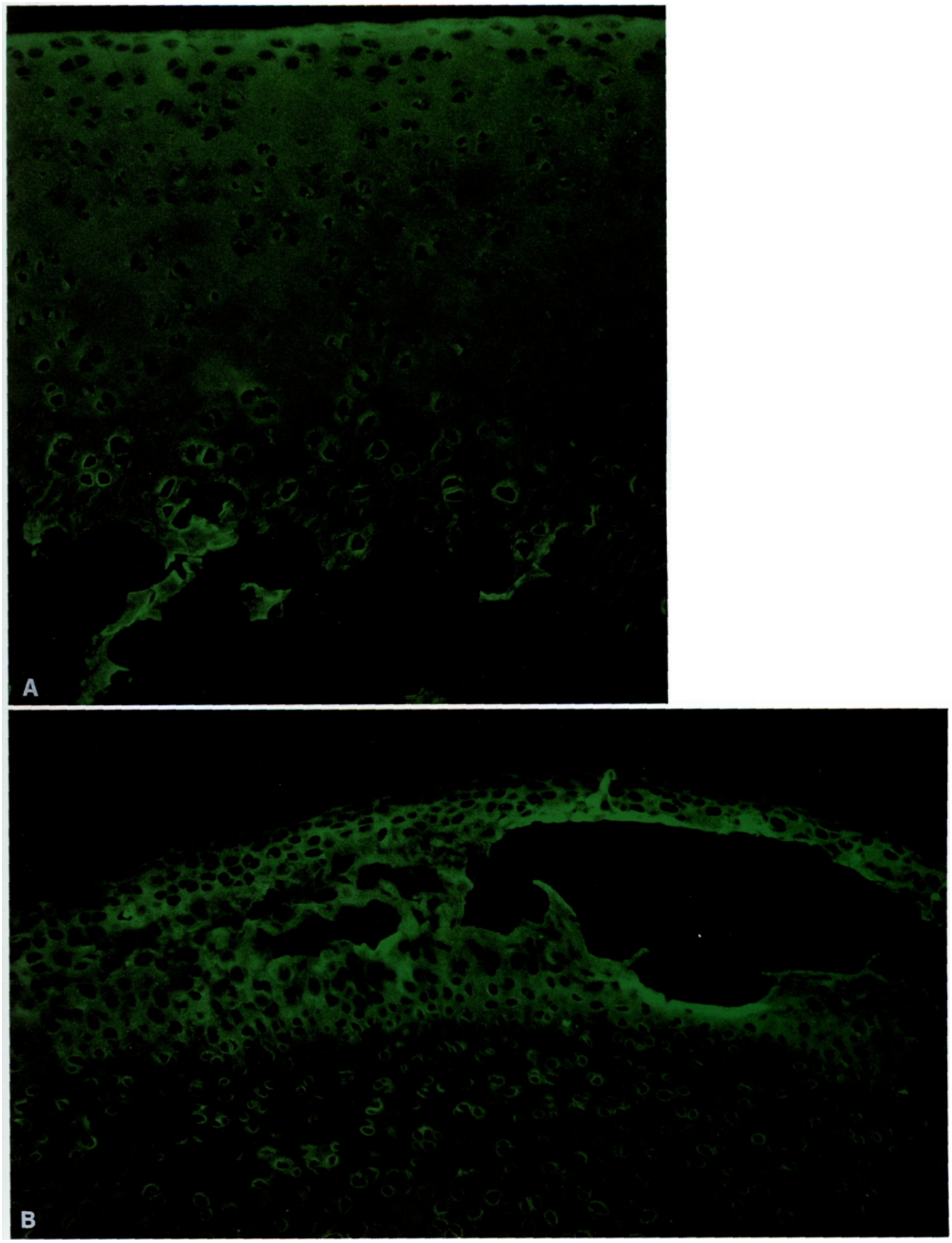

Fig. 1. Immunofluorescence microscopic picture of cartilage from the knee joint of a juvenile marmoset. **A.** Untreated control. Demonstration of the binding of fluorescence-labelled antibodies against collagen type II diffusely distributed in the cartilage matrix. X 200. **B.** Like **A**, but treated with ofloxacin (200 mg/kg body wt twice daily for 5 days). Distinct blister formation with detachment of the superficial layer of the joint cartilage. X 120

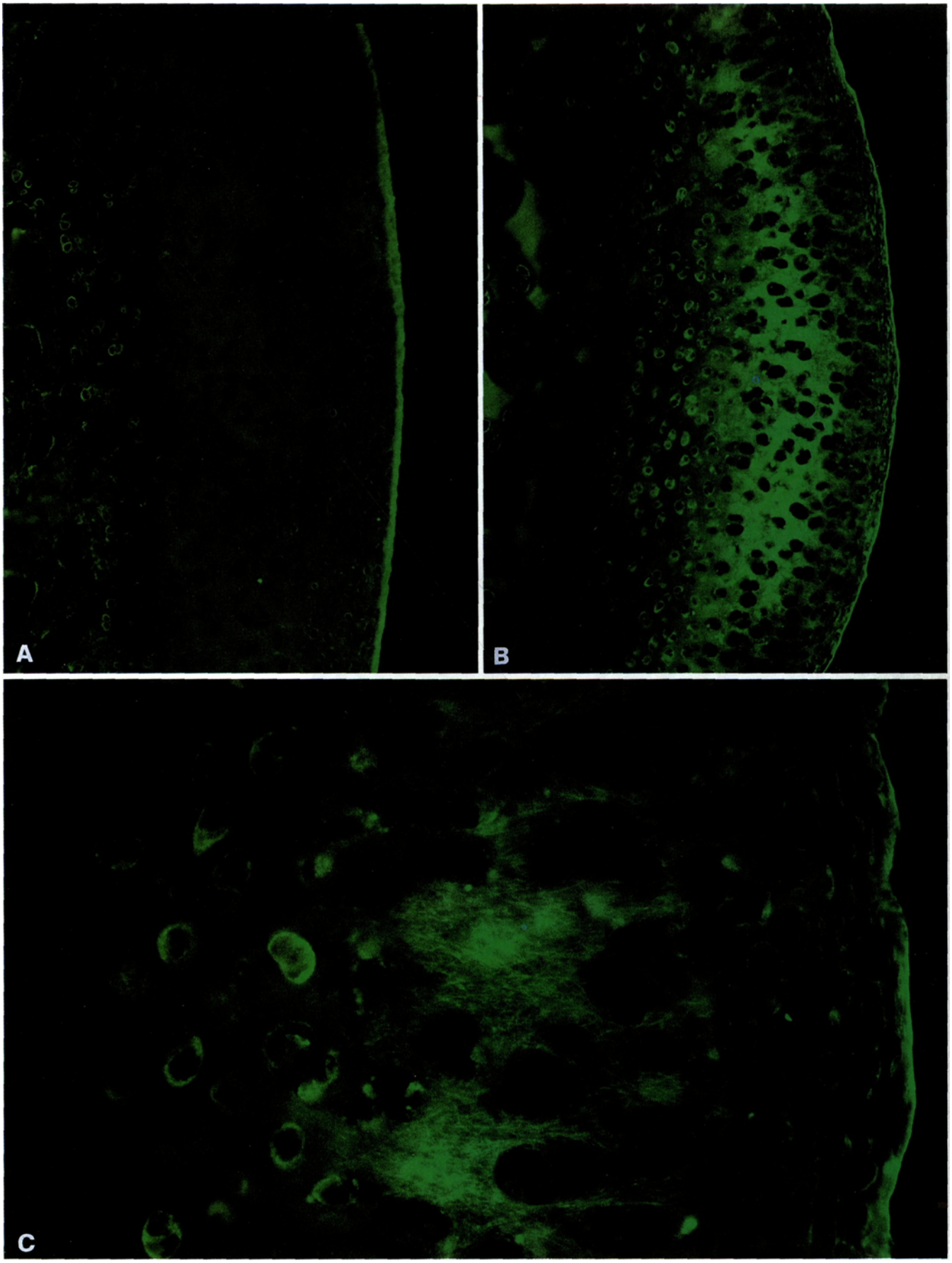

Fig. 2. Immunofluorescence microscopic pictures of the knee joint of a juvenile marmoset. Demonstration of the binding of fibronectin antibodies. **A.** Untreated control. Binding can only be demonstrated in the vicinity of osteogenesis areas and joint surface. X 126. **B.** Ofloxacin-treated (see Fig. 1 B). Sectional plane in the vicinity of a blister. Clearly stronger binding of antibodies in the cartilage matrix. X 126. **C.** Like **B**, but higher magnification. Binding of fibronectin antibodies to fibrous structures in the cartilage matrix. X 500

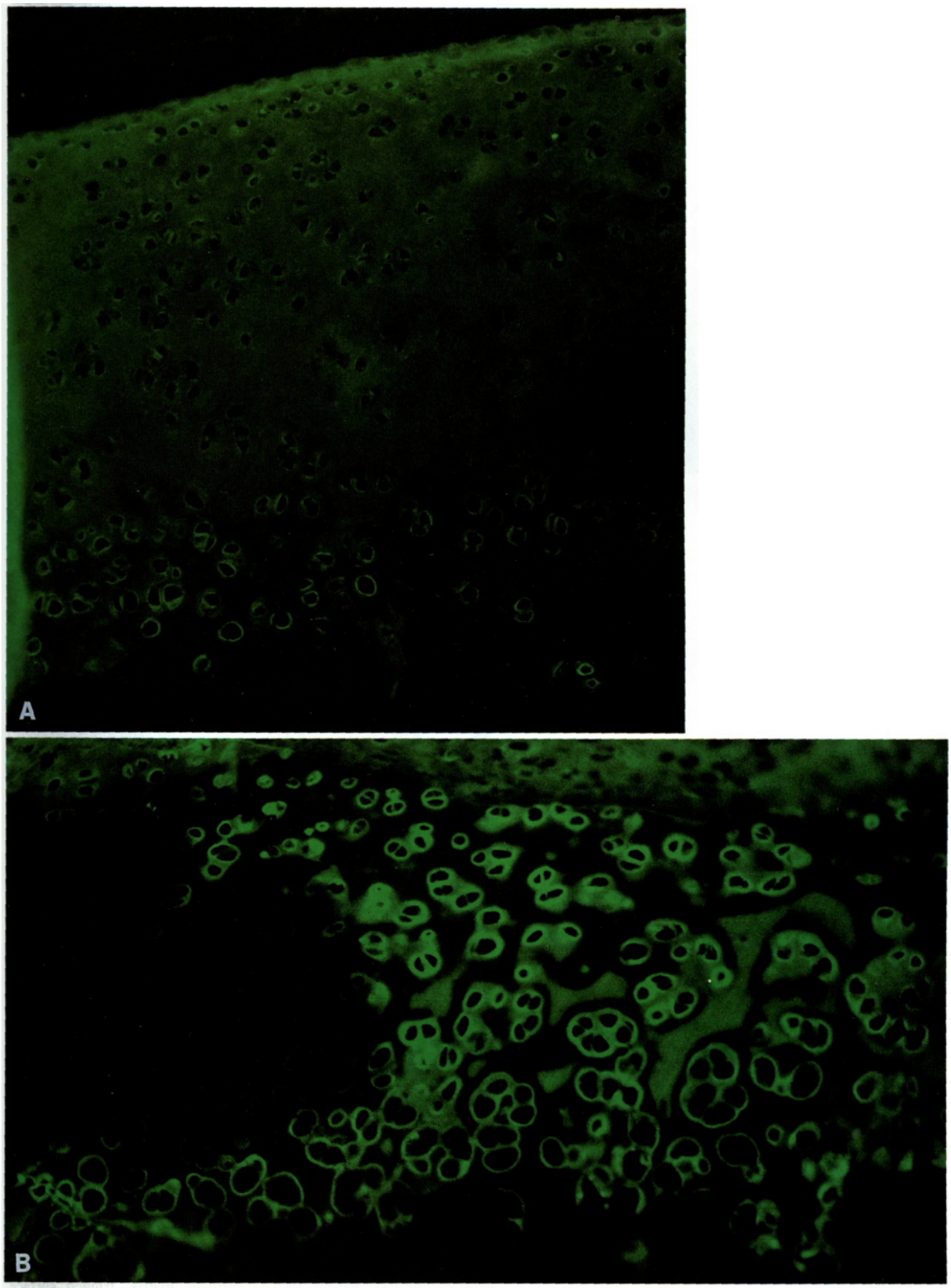

Fig. 3. Immunofluorescence microscopic pictures of the knee joint of a juvenile marmoset. Demonstration of the binding of cartilage proteoglycane antibodies. **A.** Untreated control. Even distribution of proteoglycanes in the cartilage matrix. X 200. **B.** Ofloxacin-treated (see Fig. 1*B*). Loss of proteoglycanes in defined areas. X 200

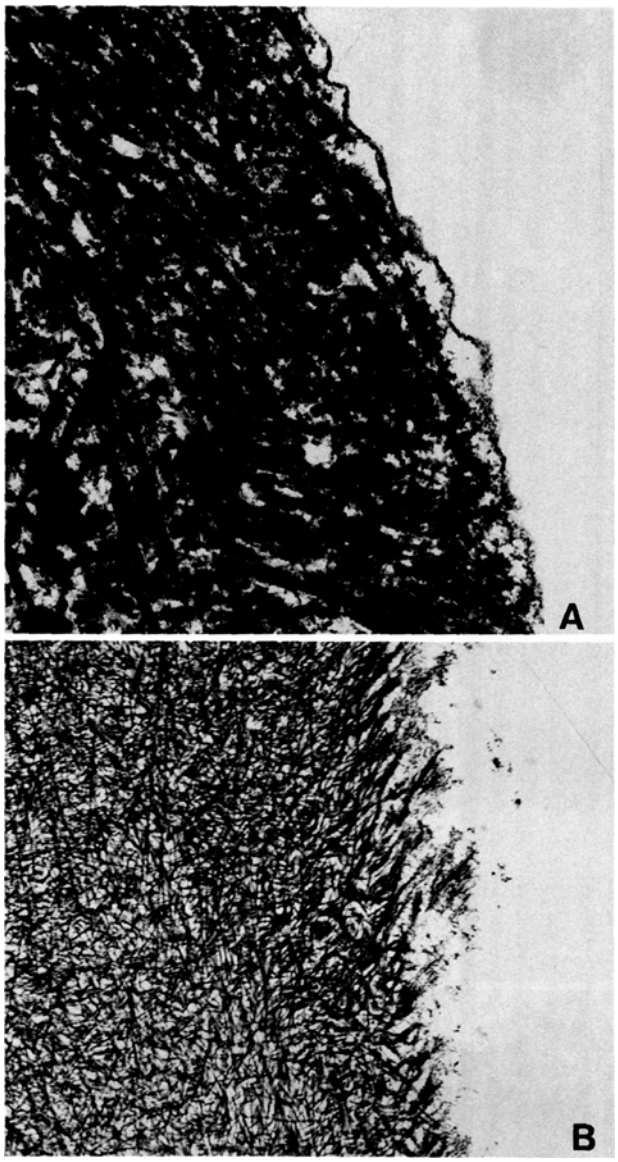

Fig. 4. Electron microscopic pictures. Knee joint of a juvenile marmoset. Cartilage surface of the distal femur. Tannic acid fixation. **A.** Untreated control. Smooth, membrane-like, delineated cartilage surface. Dense packing of collagenous fibrils. X 60000. **B.** Ofloxacin-treated (see Fig. 1 *B*). Low magnification. Loosely packed cartilage surface. X 15000

Electron microscopic studies in rats

After we had observed the changes described in the marmoset, we systematically and more closely studied rat tissue. Very similar or even identical changes could be demonstrated in electron microscopic studies on femur joint cartilage of juvenile rats.

In the SEM picture opened cavities of the cartilage cells at the surface of the joint, partly filled with cell debris, are visible (Fig. 6).

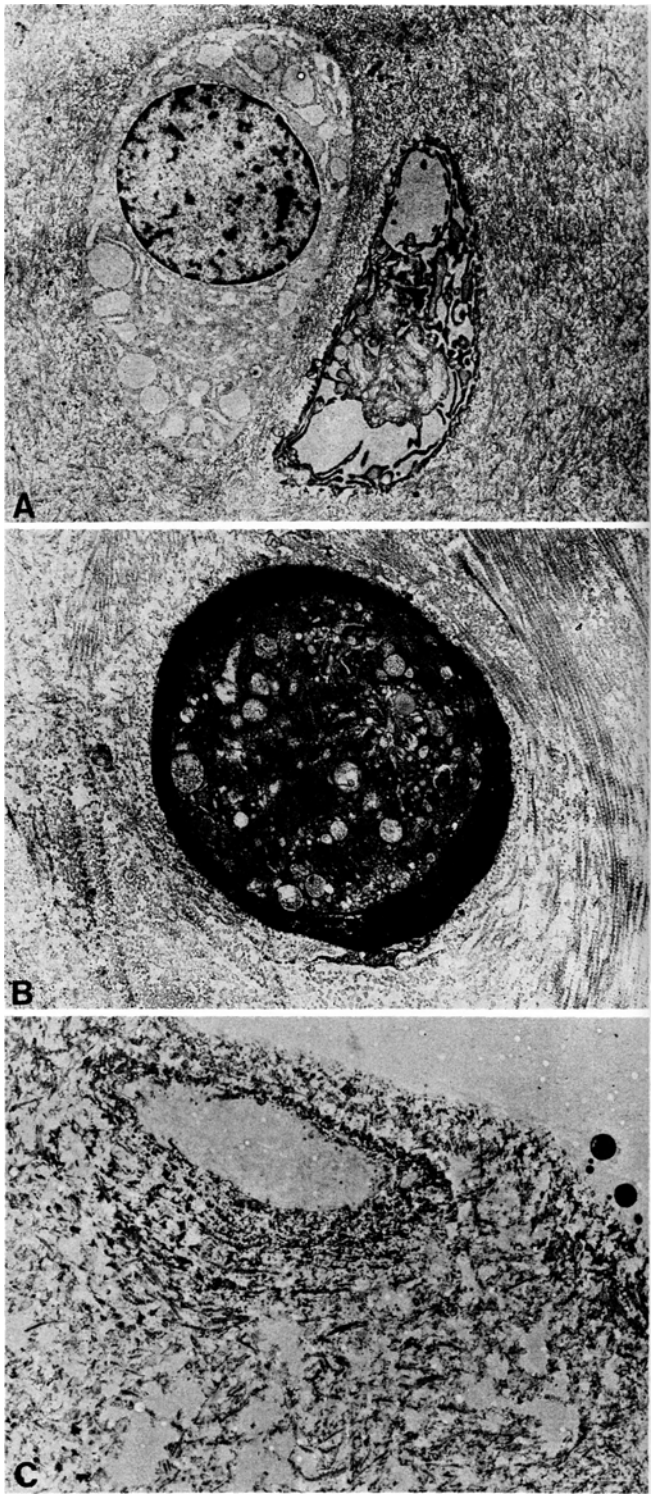

Fig. 5. Electron microscopic pictures. Knee joint of a juvenile marmoset. Ruthenium red fixation. Ofloxacin-treated (see Fig. 1 *B*). **A.** Two cells showing a different degree of lesion. The left cell exhibiting still translucent hyaloplasm, the right cell showing a densification of the hyaloplasm and pronounced dilatation of endoplasmic cavities. X 25000. **B.** Necrotic chondrocyte with pronounced densification of the hyaloplasm. Parallel arrangement (fibre formation) of fibrils. X 35000. **C.** Empty chondrocyte cavity, less densely packed cartilage matrix, fringed surface. X 35000

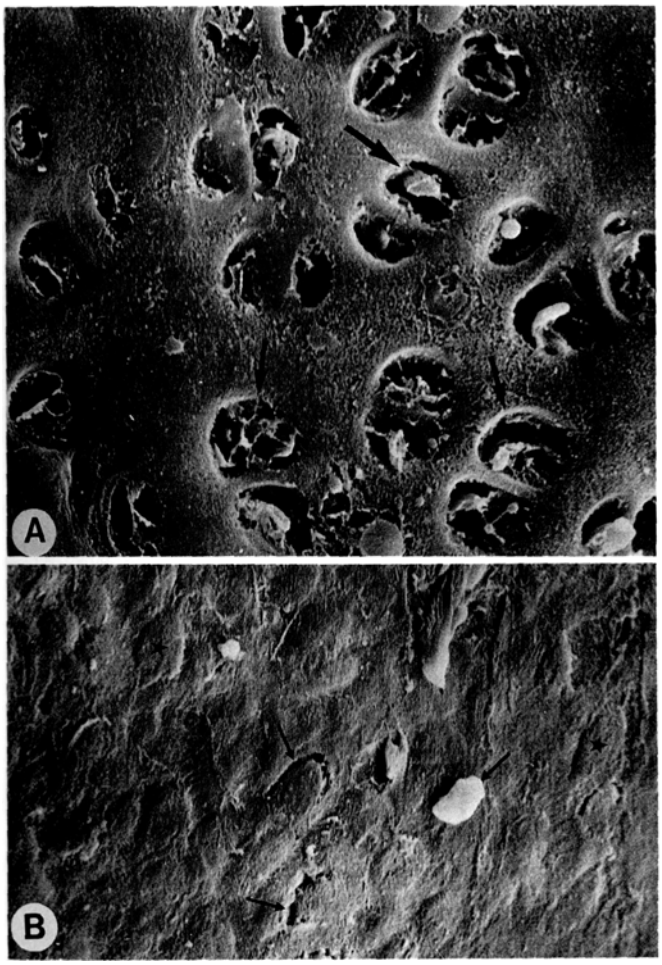

Fig. 6. Scanning electron microscopic pictures. Knee joint of a juvenile rat. **A.** Treated orally with ofloxacin (600 mg/kg body wt twice daily for 5 days). *Arrows*: opened chondrocyte cavities with or without cell remnants. X 2300. **B.** Untreated control. *Arrows*: pollution and shrinking artefacts. X 1800

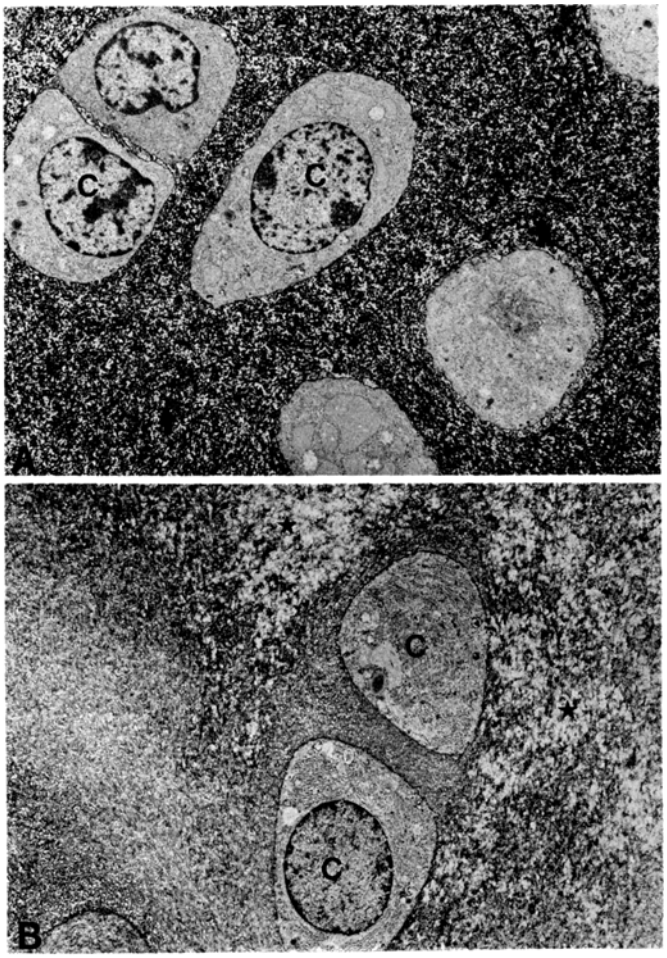

Fig. 7. Transmission electron microscopic pictures. Knee joint of a juvenile rat. **A.** Untreated control. *C* = chondrocyte tightly embedded in the matrix. X 8000. **B.** Treated orally with ofloxacin (600 mg/kg body wt twice daily for 5 days). *Black star* = areas with loss of matrix which resemble "moth-eaten" structures. *C* = chondrocyte. X 8000

In the TEM pictures of the femur cartilage the matrix close to the chondrocytes appears to be normal, and numerous Ruthenium Red-positive granules are discernible. In other areas, often at a certain distance from the cells, a loss of matrix is observed. These areas resemble "moth-eaten" structures (Fig. 7).

Furthermore, besides a fringed surface of the matrix of the articular cartilage, in the cytoplasm of the chondrocytes swellings of rough endoplasmic reticulum and mitochondria are typical findings (Fig. 8).

Figure 9 gives a comparison of chondrocytes beneath the cartilage surface from a rat and a marmoset preparation. Pericellularly matrix-free spaces can be seen.

Plasma concentrations

The mean peak plasma concentrations were achieved 15–45 min after gavage. After oral application of 100, 300 or 600 mg ofloxacin/kg body wt the following peak concentrations (mean ± SD) of ofloxacin in plasma were achieved (12 rats): 8.9 ± 2.1, 22.6 ± 7.5, 33.5 ± 9.8 mg/l respectively. After 6 h the concentrations were: 1.1 ± 0.4, 5.9 ± 2.5, 15.9 ± 5.1 mg/l for the three doses tested.

No sex-dependent differences in plasma levels were detectable under these experimental conditions.

Figure 10 shows the pharmacokinetics of ofloxacin in juvenile rats after oral and subcutaneous application, giving the mean values ± SD.

Although similar maximum levels were reached, the drug was more rapidly eliminated after s.c. injection than after oral intubation. Plasma levels 360 min after s.c. application of 100 mg/kg body wt were 0.5 ± 0.2 mg/l and after oral administration 1.1 ± 0.4 mg/l. Twelve samples from this part of the study were first analysed in another laboratory (Prof. Klaus Borner, Institut für klinische Chemie und klinische Biochemie, Klinikum Steglitz der Freien Universität Berlin). A parallel analysis of the same samples was then conducted in our laboratory. The correlation of the data ($r = 0.995$) was good (data not shown).

In the marmoset only limited information could be gained so far on the pharmacokinetics of ofloxacin. We

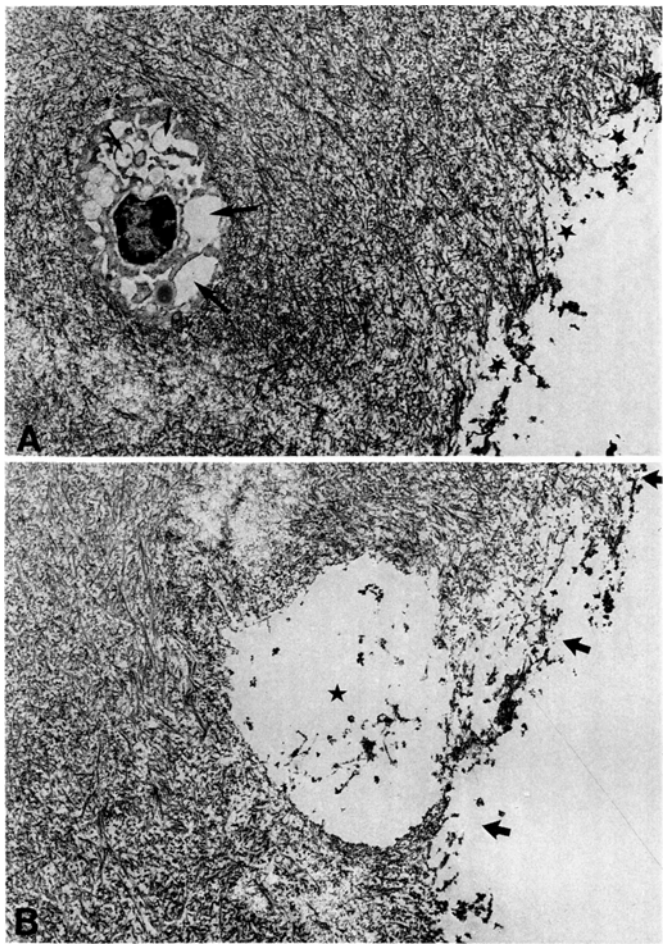

Fig. 8. Transmission electron microscopic pictures. Knee joint of a juvenile rat treated orally with ofloxacin (600 mg/kg body wt twice daily for 5 days). **A.** Fringed surface of articular cartilage, swelling of rough endoplasmic reticulum (*big arrows*) and mitochondrial swelling (*small arrows*). Fringed surface (*stars*). X 12000. **B.** Strongly dispersed surface of articular cartilage, empty chondrocyte cavity (*star*) just beneath the surface (*arrows*). X 12000

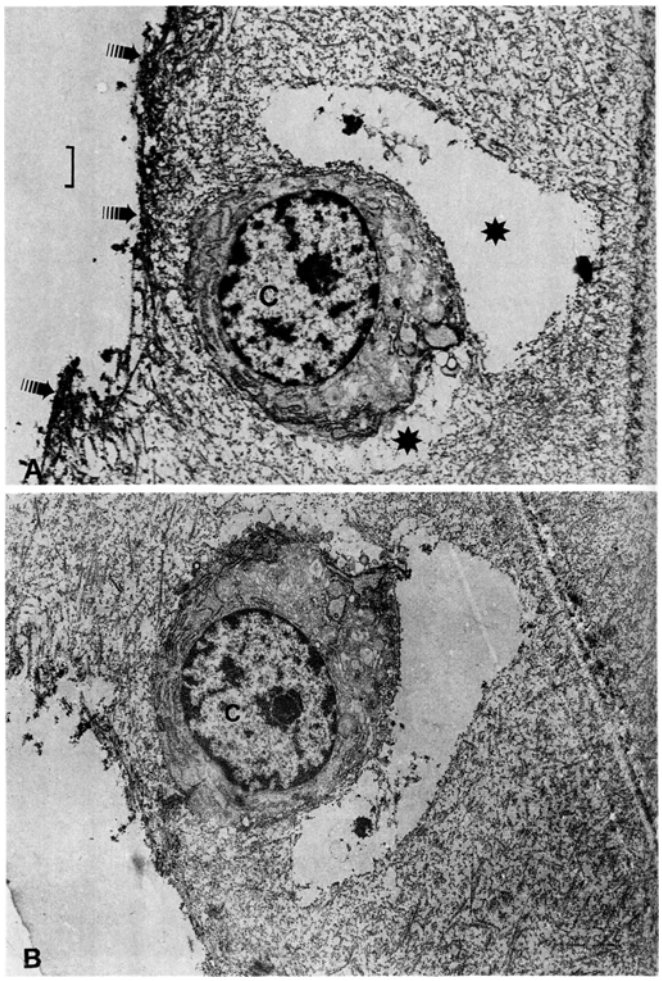

Fig. 9. Transmission electron microscopic pictures. Comparison of ofloxacin-induced effects in two species. **A.** Knee joint of a *juvenile rat* treated orally with ofloxacin (600 mg/kg body wt twice daily for 5 days). Effects observed very closely resemble those found in marmosets (cf. Fig. 9B). X 16000. **B.** Knee joint of a *juvenile marmoset* treated orally with ofloxacin (200 mg/kg body wt twice daily for 5 days). The contact between matrix and cell is lost; pericellularly matrix-free spaces occur. X 16000

measured higher concentrations in the marmoset plasma, although only one third of the dose used in rats was applied (Table 1).

Discussion

The results of the studies described in this paper show that quinolone-induced arthropathia is a relevant toxic effect not only in dogs and rats, but also in non-human primates. The application of immuno-morphological and electron microscopic methods proved to be suitable for the detection of specific alterations in cartilage. Subsequent studies with rats revealed that very similar morphological alterations can also be induced by ofloxacin in this species.

Following a single dose administered to healthy volunteers the following mean peak plasma concentrations were measured (Monk and Campoli-Richards 1987): after 200 mg, 2.6 mg/l; after 400 mg, 4.0–5.6 mg/l; after 600 mg, 6.8–6.9 mg/l. The usually administered therapeutic doses are 200–400 mg.

Our data indicate that in both species investigated (rats and marmosets) the peak plasma concentrations leading to cartilage alterations were approximately 10 times higher than those reached under therapeutic conditions in man.

After oral administration of 100, 300 or 600 mg ofloxacin/kg body wt to rats the elimination half-life seems to be longer after higher doses, but the slower decline of the plasma concentration probably reflects a prolonged absorption. After a dose of 600 mg/kg the elimination half-life was approximately 5 h, which is very similar to the value reported for man (Lode et al. 1987).

This result clearly shows how important pharmacokinetic investigations are as a part of toxicological studies. By comparison of the doses applied in experimental studies with those used therapeutically a risk assessment is not possible. Only a comparison of the dose- and time-dependent serum concentrations in humans with serum and

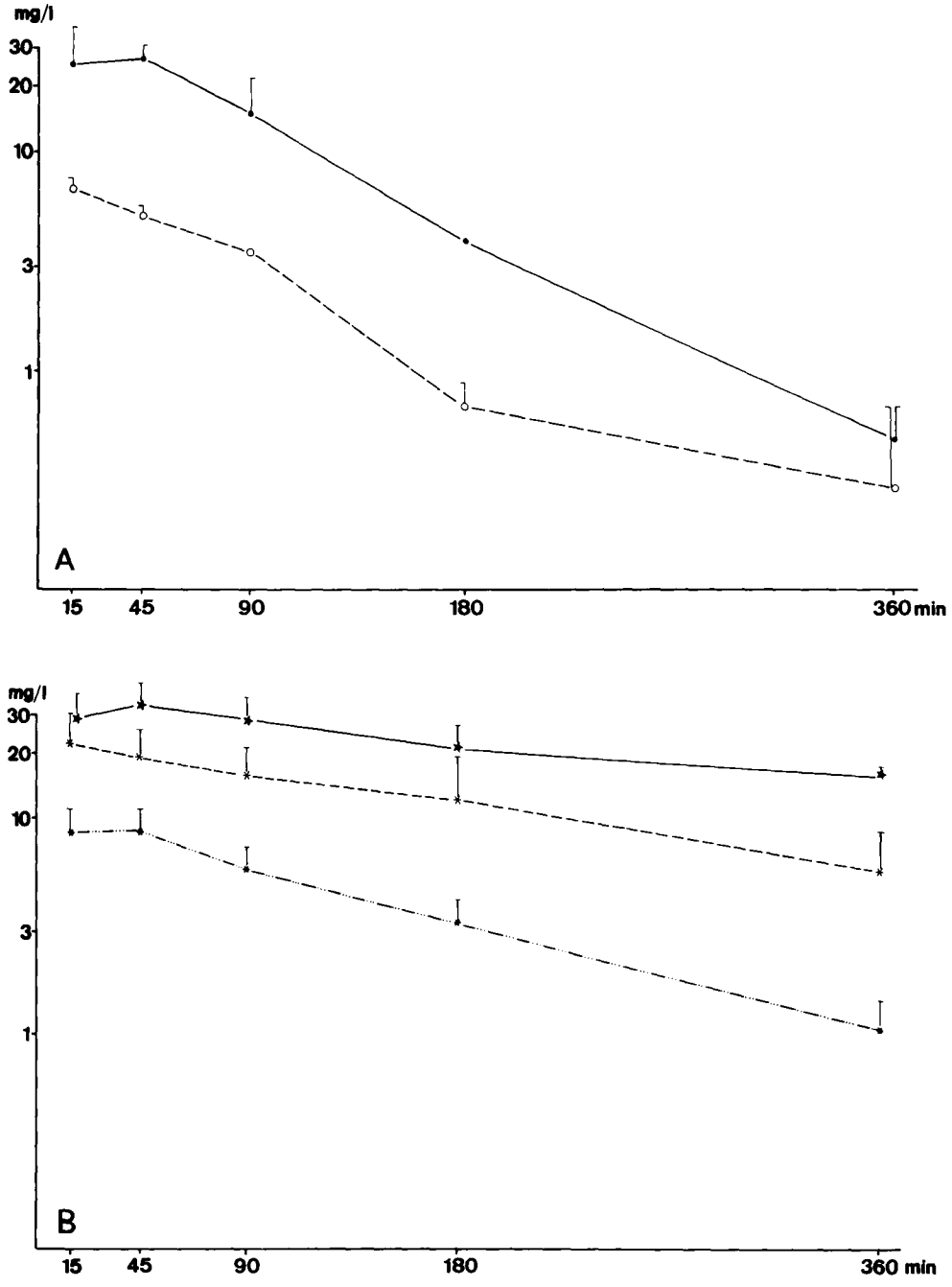

Fig. 10. Plasma concentrations (mg/l) of ofloxacin in juvenile rats. **A.** Plasma concentrations ($n = 40$) after s.c. injection of 30 (○), or 100 (●) mg/kg body wt. Each point represents the mean value ± standard deviation of 4 samples. **B.** Plasma concentrations ($n = 180$) after oral intubation of 100 (●), 300 (✳), and 600 (●) mg/kg body wt. Each point represents the mean value ± standard deviation of 12 sample

tissue concentrations achieved under experimental conditions provides a basis for a risk assessment approach (Neubert et al. 1987; Neubert 1988).

Most articles published so far in scientific journals on this type of drug-induced arthropathia describe effects induced by the "older" quinolones, e.g. pipemidic acid or nalidixic acid. So far morphological studies combined with systematic pharmacokinetic investigations have not been published for any compound.

Ingham and coworkers (1977) first noticed lameness in immature dogs after daily administration of nalidixic, oxolinic and pipemidic acid. They described lesions in the joints of the animals noticed at autopsy after 6 months administration, in spite of the fact that the animals had been clinically normal except for the first month. Microscopically they observed hypertrophy of the chondrocytes, with large cell nests and multinucleate cells.

Gough et al. (1979) confirmed these results in another study with oxolinic and pipemidic acid in juvenile dogs. Both compounds induced gross alterations of the articular cartilage in major synovial joints.

Japanese authors reported similar effects with pipemidic acid at lower doses: daily oral administration of 2×50 mg pipemidic acid/kg body wt caused lameness in juvenile beagle dogs which was most pronounced 3–7 days after start of treatment. After administration of 2×15 mg/kg body wt daily cartilage erosions were still detectable but without clinical symptoms. No effect was

Table 1. Ofloxacin plasma concentrations in juvenile marmosets after multiple oral administration of 200 mg/kg body wt

Animal No.	Body wt (g)	Plasma concentrations (mg/l) after:		
		1 h	3 h	6 h
1.	170	56.4	43.1	22.7*
2.	185	18.9	44.9*	–
3.	140	46.3	47.3	29.8*
4.	120	36.3	31.6*	–
5.	120	33.1	26.3	27.0*
6.	100	65.0	50.6*	–
Mean ± SD		42.7 ± 16.7	40.6 ± 9.5	26.5 ± 3.6

Blood was taken from a femoralis vein
* = blood was taken after anaesthesia by cardiac puncture

seen after 2× 10 mg pipemidic acid/kg body wt. At autopsy performed 30 days after a treatment period of one week (2× 500 mg/kg body wt daily), blisters were still visible in the cartilage and 4 years after treatment the investigators detected scars in the joint cartilage where blisters and erosions used to occur under treatment. Mechanical pressure was shown to enhance pipemidic acid-induced cartilage damage in dogs (Tatsumi et al. 1978).

Only limited information is available on the cartilage-damaging effects of newer fluoroquinolones.

Some data on the toxic effects of quinolones have been published by the drug-producing companies. From a comparative study on the arthropathogenic potential of nalidixic acid and newly developed fluoroquinolones after oral administration to juvenile rats, the author draws the conclusion that ciprofloxacin was least effective. However, since no pharmacokinetic data are available it remains unclear whether this is due to differences in absorption and/or metabolism or to a different intrinsic activity of the drugs (Schlüter 1986).

Ofloxacin is the derivative which offers the highest bioavailability of the fluoroquinolones known so far. This probably explains why in comparison to other quinolones rather low doses of ofloxacin can already induce cartilage alterations. A 1-week treatment period with 10 mg/kg (dogs) or 300 mg/kg body wt (rats) was sufficient to produce blisters and erosions in articular cartilage (Monk and Campoli-Richards 1987).

Kato and Onodera (1988a, b) recently published results on morphological cartilage alterations in juvenile rats after oral application of ofloxacin (900 mg/kg body wt daily for 7 days). They found blisters and/or erosions in the articular cartilage of the humeral trochlea, femoral condylae, and femoral head of 4- to 6-week-old rats. No changes were noted in rats aged 8 or 10 weeks. After high doses (1000 and 3000 mg ofloxacin/kg body wt) histological changes (atrophy and deformation of the nuclei of chondrocytes) were detected as early as 5 h after application. Aggregated nuclear chromatin was interpreted as altered DNA synthesis and dilation of RER cisternae was interpreted as a sign of increased protein synthesis. Degenerated cells with vacuolated and partially disintegrated cytoplasma were also seen in the middle zone. These lesions were followed by edema of the matrix accompanied with markedly decreased stainability with safranin-O, and a cavity was later formed by liquefaction of the cartilage. They found the changes to be principally reversible, but – although repair was effective in the femoral condylae – in the humeral trochlea extensive erosion still remained after the 10-week recovery period (treatment: 900 mg ofloxacin/kg body wt for 7 days); proliferation of chondrocytes around the lesion chiefly contributed to the repair.

The results of our EM studies show clear-cut matrix alterations and a loss of proteoglycanes. Since the cartilage consists of a functional unit of different types of collagens, proteoglycanes and glycoproteins, the loss of any of these partners may lead to a disturbance of the whole system. In this way the collagen architecture, which is stabilized by proteoglycanes, largely breaks down at the joint surface. Consequently, dehiscences may occur under static stress, and, in the extreme case, large blisters may develop.

From our results it cannot be decided whether alterations in the matrix or a cellular damage is the first event in the sequence leading to arthropathia in experimental animals. We like to hypothesize that the *mitochondrial DNA* in chondrocytes may be the primary target which is affected by the drugs, subsequently leading to the cellular damage. Several aspects might support such a hypothesis:

1. Although not studied extensively so far, cartilage tissue seems to be a "deep compartment" for quinolones. For example, relatively high concentrations have been measured with ciprofloxacin in skeletal muscle, cartilage and in liver and kidney of rats after treatment with radioactively labelled material (Siefert et al. 1986a). Since it is well-known that ciprofloxacin is not metabolized to a great extent in the rat (Siefert et al. 1986b) it can be assumed that the original compound achieves high concentrations in cartilage. The unusually high "volume of distribution" observed in man (Höffken et al. 1985) may be explained by the high concentrations reached in these tissues.

Similar results have been published with ofloxacin (Okazaki et al. 1984; Sudo et al. 1984, 1986). During a 3-week treatment period on the 3rd, 7th and 14th day of treatment the concentrations of ofloxacin in tracheal cartilage were – except for liver and kidney – higher than in all other organs. Three and 7 days after the last dose the concentrations in tracheal tissue were significantly higher than in all other organs. A high volume of distribution has also been shown for ofloxacin in volunteers (Lode et al. 1987)

2. Gyrase, a bacterial enzyme that controls bacterial DNA, is the primary target of all quinolones known so far (Gellert 1976). In contrast to the mammalian DNA which is organized in the form of chromosomes, striking similarities exist between bacterial DNA and mammalian *mitochondrial DNA*. Both forms of DNA exist as circular super-twisted helices, and gyrase-like enzymes have also been postulated to be responsible for the organisation of mitochondrial DNA (Clayton 1982). Melcion and Cordier (1986) first postulated that mitochondrial DNA may be inhibited by quinolones.

3. Of the cells of the mammalian organism, chondrocytes have an especially low number of mitochondria. They are smaller than in cells of many other tissues.

The high concentrations of the drugs in this tissue on the one hand, and the low number of mitochondria in chondrocytes on the other hand, might give an explanation as to why a damage of mitochondrial DNA manifests itself as "chondrotoxicity". Such a biochemical reason for the toxic effect would give an explanation why all quinolones known so far exhibit chondrotoxicity.

If our assumption were true, the toxic effect would be strictly bound to the antibacterial activity of the drug itself. It might be discussed that the chondrotoxicity is induced by a metabolite of the drugs. However, metabolites are probably not responsible for the chondrotoxicity, since the effect is observed with all quinolones in several species and metabolism differs strongly between the drugs and in different species.

The significance of the experimental findings on quinolone-induced arthropathia for Human remains unclear. No corresponding effects were noticed in children treated with older derivatives such as nalidixic acid at a time when this toxic potential was not yet known (Schaad et al. 1987). There are few publications on children and adolescents treated with recently developed quinolones but *no* arthropathy was noted. On the other hand, several case reports exist on arthralgia after quinolone therapy; however, the causal relationship remains unclear in these casuistics (e.g. McDonald and Short 1964; Bailey et al. 1972, 1983).

For pefloxacine it was recently recommended that the pediatric benefits associated with the marked antibacterial activity of the drug should be weighed against the risks associated with arthralgia whenever the condition of the patient is grave and vital decisions for a favourable prognosis for the sick child are necessary (Chevais et al. 1987).

There are numerous open questions on the problem of quinolone-induced arthropathia, e.g., further pharmacokinetic and morphological investigations are necessary to answer the important question of a threshold concentration in cartilage at which alterations can primarily be observed. Since corresponding cartilage alterations were found in marmosets and rats, further systematic studies on the phenomenon of quinolone-induced chondrotoxicity can conveniently be conducted in rats to provide a rational basis for a risk assessment approach.

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