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Magnesium Deficiency Induces Joint Cartilage Lesions in Juvenile Rats Which Are Identical to Quinolone-Induced Arthropathy

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Quinolones accumulate in cartilage, and because they form chelate complexes with divalent cations, they possess the potential to induce a deficiency of functionally available magnesium. To test the hypothesis that quinolone-induced arthropathy is caused (or aggravated) by magnesium deficiency in cartilage, we induced magnesium deficiency by feeding juvenile rats a magnesium-deficient diet for 9 days and treated the rats with single oral doses of ofloxacin (0, 100, 300, 600, or 1,200 mg/kg of body weight) during this period. Additional groups of juvenile rats on a normal diet were treated with ofloxacin correspondingly. Typical cartilage lesions (e.g., swollen matrix, cleft formation) were found in knee joints of all magnesium-deficient rats, including those without ofloxacin treatment. Lesions in these groups were not distinguishable from lesions induced by a single dose of 600 mg of ofloxacin per kg of body weight or higher in rats on a normal diet. Ofloxacin levels in plasma after 600 mg/kg of body weight were approximately 10-fold higher than those in humans during therapy with this quinolone. Lesions in rats treated with ofloxacin plus magnesium deficiency were more pronounced than those in rats with normal magnesium concentrations. After intake of a magnesium-deficient diet for 9 days, the magnesium concentration in serum (mean ± standard deviation) was 0.18 ± 0.05 mmol/liter (control on normal diet, 0.82 ± 0.10 mmol/liter). Magnesium concentrations in bone (femur) and cartilage (processus xiphoideus) samples were 64.7 ± 10.5 and 14.3 ± 3.9 mmol/kg of dry weight, respectively, which corresponded to approximately 50% of the concentrations measured in controls on a normal diet. It was concluded that quinolone-induced arthropathy is probably caused by a deficit of available magnesium in joint cartilage due to the formation of quinolone-magnesium chelate complexes. If juvenile patients must be treated with quinolones for serious infections, it seems prudent to ensure that these patients do not have a disturbed magnesium balance.

Quinolone-induced arthropathy is an unusual toxic effect observed with all known quinolones. Cartilage lesions are inducible in juvenile animals of multiple species, such as dogs (3, 4, 31), rats (14, 29), nonhuman primates (29), and others (9). The chondrotoxic potential of quinolones in humans under therapeutic conditions is low. Most juvenile patients have shown no signs of arthropathy after treatment with these drugs (1, 26). On the other hand, symptoms of arthropathy in juvenile and even adult patients have been described in a considerable number of case reports (e.g., references 5 and 24). Since most of these patients had cystic fibrosis—a disease which itself can be associated with arthropathy—the causal relationship in these observations and the indications for quinolone therapy in pediatrics remain matters for discussion (6, 25, 28).

Recently, we hypothesized that quinolones might affect magnesium-dependent integrins of the β_1 subfamily in articular cartilage as the primary event of quinolone-induced chondrotoxicity (27, 28). Quinolones show the following important prerequisites for such an effect: (i) they are potent chelating agents for magnesium, and (ii) they accumulate in articular cartilage. As a result, a localized deficiency of (functionally available) magnesium might occur in joint cartilage and cause arthropathy.

To test our hypothesis, we induced magnesium deficiency by feeding juvenile rats a magnesium-deficient diet and simultaneously treated the animals with ofloxacin. The results were surprisingly unequivocal and gave new information on a possible mechanism of quinolone-induced arthropathy. Secondly, and may be more importantly, our results open perspectives on how to optimize the treatment of juvenile patients with quinolones and to minimize the risk of arthropathy.

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MATERIALS AND METHODS

Treatment of rats. Five groups of 35-day-old male and female Wistar rats (4 to 7 rats in each group; total, 28 rats) were treated with a single dose of ofloxacin (100, 300, 600, or 1,200 mg/kg of body weight) by gastric intubation. The drug solution was prepared by suspending commercially available tablets (Tarivid) in a 2% starch solution. Control animals received the vehicle only. Rats were kept in Macrolon cages at 23 ± 1°C with 50 ± 5% relative humidity and a constant light-dark schedule (light from 7 a.m. to 7 p.m.). They received Altromin 1324 pellet feed (Mg^{2+} content, >1,000 mg/kg) and tap water ad libitum. Five additional groups (each group, 6 to 7 rats; total, 32 juvenile rats) were fed a magnesium-deficient diet (Altromin, C1035) starting on postnatal day 28 and treated with a single dose of ofloxacin (100, 300, 600, or 1,200 mg/kg of body weight) by gastric intubation. Atomic absorption spectrophotometric examination of the diet revealed an Mg^{2+} content of 84 mg/kg. Experimental conditions were otherwise identical for these rats.

Microscopy. All rats were sacrificed 72 h after treatment with the quinolone, and the knee joints were investigated by light and electron microscopy. For sample preparation, standard methods were applied. Briefly, knee joints were fixed in formalin, demineralized in EDTA, embedded in paraffin, and examined by light microscopy after toluidine blue staining. For electron microscopy, tissue samples were fixed in 1% glutaraldehyde and 1% tannic acid and subsequently in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy (Zeiss EM 10).

Biochemical analysis. Calcium and magnesium concentrations were measured in three different samples obtained from each animal: (i) blood plasma obtained immediately before preparation by puncture of a tail vein, (ii) processus xiphoi-

TABLE 1. Magnesium and calcium concentrations in plasma, bone, and cartilage from immature rats on a normal or magnesium-deficient diet plus treatment with ofloxacin or the vehicle[a]

Element measured	Mean concn (± SD) in:					
	Plasma (mmol/liter)		Femur (mmol/kg of dry wt)		Processus xiphoideus (mmol/kg of dry wt)	
	Normal diet	Mg^{2+}-deficient diet	Normal diet	Mg^{2+}-deficient diet	Normal diet	Mg^{2+}-deficient diet
Mg	0.82 ± 0.1	0.18 ± 0.05[b]	135.6 ± 13.9	64.7 ± 10.5[b]	23.4 ± 4.4	14.3 ± 3.9[b]
Ca	2.58 ± 0.20	2.89 ± 0.19[b]	4.7 ± 0.5[c]	4.5 ± 0.4[c]	399 ± 89	352 ± 98

[a] The normal diet was given to 28 rats, and 32 received the Mg-deficient diet. Data from both sexes and all ofloxacin treatment groups were combined. Ofloxacin treatment had no dose-related influence on the magnesium concentrations. Similarly, no significant differences in magnesium concentration were observed between males and females. Magnesium concentrations in plasma were significantly reduced to approximately 25% of the control value in all rats receiving the magnesium-deficient diet. Reduction of magnesium concentrations was not as pronounced in bone and cartilage samples (reduction to approximately 50% of the control values).
[b] Significantly different from the corresponding group on a normal diet ($P < 0.01$; t test).
[c] These data are measured in moles per kilogram of dry weight.

deus cartilage, and (iii) femur joint cartilage plus bone. Plasma was deproteinized and diluted with 10% trichloroacetic acid–0.175% $LaCl_3$. Processus xiphoideus and femur samples were lyophilized and ashed in a Plasma Processor 200-E (Technics, Munich, Germany), and the ashes were dissolved in 0.1 N HCl–0.175% $LaCl_3$. Mg^{2+} and Ca^{2+} were measured by atomic absorption spectrophotometry (Philips SP9).

RESULTS

Calcium and magnesium concentrations. The magnesium concentration in plasma was reduced from 0.82 ± 0.10 mmol/liter in rats on a normal diet to 0.18 ± 0.05 mmol/liter in rats on the magnesium-deficient diet (mean ± standard deviation). Reduction of the magnesium concentrations in cartilage (processus xiphoideus) and femur samples was not as pronounced: at the end of the 9-day period of the magnesium-deficient diet, the concentrations were 64.7 ± 10.5 mmol/kg of dry weight in bone (femur) and 14.3 ± 3.9 mmol/kg of dry weight in cartilage (processus xiphoideus). Corresponding concentrations in the controls on a normal diet were 135.6 ± 13.9 and 23.4 ± 4.4 mmol/kg of dry weight, respectively.

In all of the samples analyzed, there was no clear-cut influence of the single doses of ofloxacin given 3 days before sample collection compared with the vehicle treatment. Also, there were no significant differences between female and male rats.

Calcium concentrations in the plasma of magnesium-deficient rats showed a statistically significant ($P < 0.01$; t test) increase (normal diet, 2.58 ± 0.20 mmol/liter; magnesium-deficient diet, 2.89 ± 0.19 mmol/liter). Calcium concentrations in bone and cartilage samples were not significantly different between the two groups of rats fed the normal diet or the magnesium-deficient diet (Table 1).

Light microscopy. Figure 1A to C shows representative examples of light microscopic evaluation of joints from immature rats on normal and magnesium-deficient diets with or without additional ofloxacin treatment. The lesions induced by magnesium deficiency could not be distinguished microscopically from ofloxacin-induced alterations and were found in all animals of this group. However, in contrast to the ofloxacin-induced lesions, clefts induced by magnesium deficiency were consistently located in the ventral part of the distal femoral condyle while the proximal tibial condyles were not affected. Treatment of magnesium-deficient rats with the drug aggravated the damage. Typically, in these animals lesions were not found in only one part of the joint but often affected the femoral and tibial cartilage.

We distinguished three different stages of severity of cartilage damage: (i) swollen matrix with unmasked collagen fibers and decreased cell density, (ii) one cartilage fissure in only one part of the joint, and (iii) multiple fissures in one or both parts of the joint. The results of the histological examination are summarized in Table 2.

Electron microscopy. Figure 2a is an electron microscopic picture of rat cartilage from animals on a normal diet without ofloxacin treatment (control): a typical chondrocyte is characterized by a normally formed nucleus, mitochondria, endoplasmic reticulum, and other intracellular structures. Preparations from rats that received a normal diet and a high dose of ofloxacin (1,200 mg/kg of body weight) show cells which are

TABLE 2. Incidence of cartilage lesions[a] in rats with magnesium deficiency, ofloxacin treatment, or both

Ofloxacin dose (mg/kg of body wt)[b]	Knee joint lesions[c]									
	Normal diet					Magnesium-deficient diet				
	No. of rats	Tibial condyles	Femoral condyles	Total incidence	No. of lesions per joint[d]	No. of rats	Tibial condyles	Femoral condyles	Total incidence	No. of lesions per joint[d]
0	6	0/6	0/6	0/6	0	7	0/7	7/7	7/7	1 (1–2)
300	7	0/7	0/7	0/7	0	6	6/6	4/6	6/6	3 (1–4)
600	7	5/7	0/7	5/7	1 (0–2)	6	5/6	5/6	6/6	2 (1–3)
1,200	4	4/4	3/4	4/4	2 (1–4)	6	6/6	5/6	6/6	3 (1–4)

[a] One knee joint from each animal was studied. The lesions were swelling of the cartilage matrix, unmasked collagen fibrils, and cleft formation.
[b] Rats in the control group received the vehicle only. Rats that received 100 mg of ofloxacin per kg of body weight were not evaluated histologically.
[c] The values shown are number of knee joints affected/number of knee joints examined.
[d] The median of number of cartilage lesions per joint (tibial and femoral condyles) is shown. The values in parentheses indicate the minimum and maximum numbers of lesions detected in one joint.

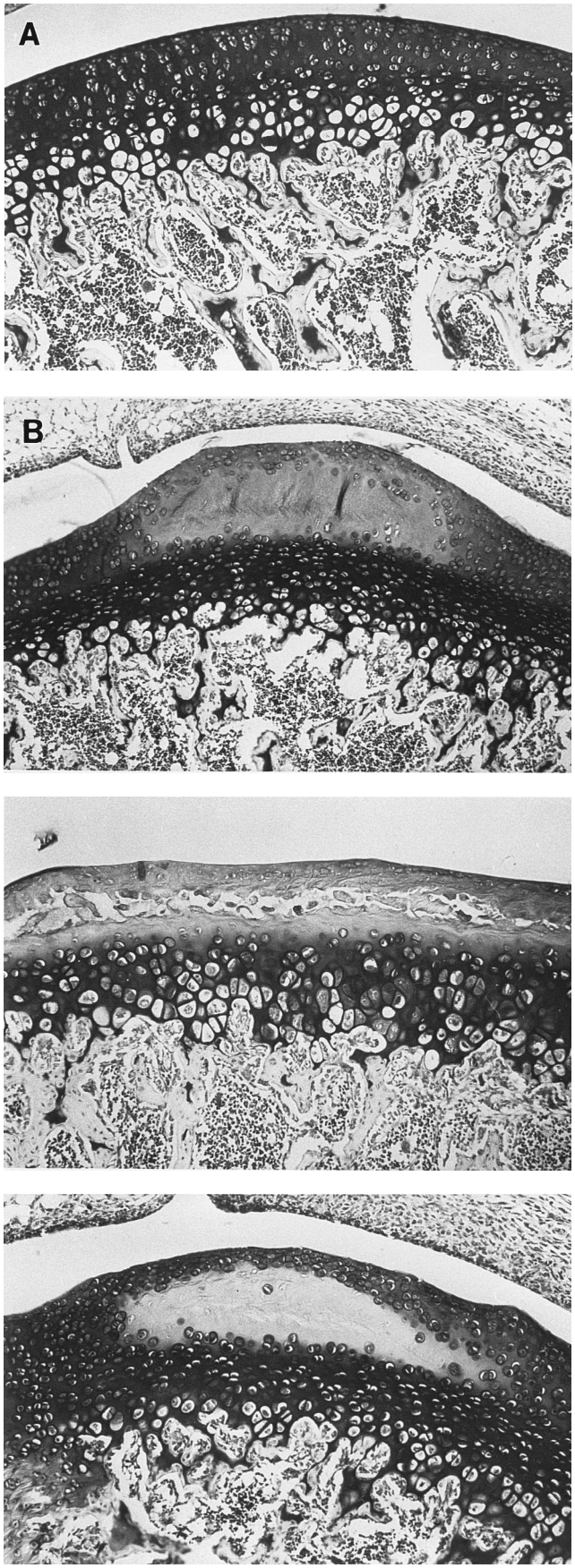

FIG. 1. Light microscopy of toluidine blue-stained articular cartilage from knee joints of immature rats. (A) Normal diet, no ofloxacin (control; magnification, ×75). (B) Normal diet plus treatment with one dose of 1,200 mg of ofloxacin per kg of body weight (magnification, ×78). Shown are three examples of cartilage alterations. Matrix swelling, demasked collagen fibers, cleft formation, and loss of proteoglycans in the vicinity of a lesion (as indicated by decreased staining with toluidine blue) were observed in all of the rats treated with 1,200 mg of ofloxacin per kg of body weight and most of the animals treated with 600 mg of ofloxacin per kg of body weight. (C) Magnesium-deficient diet, no ofloxacin (magnification, ×77). Shown are three typical examples of cartilage alterations. Magnesium deficiency induces lesions in cartilage similar to those caused by treatment with ofloxacin.

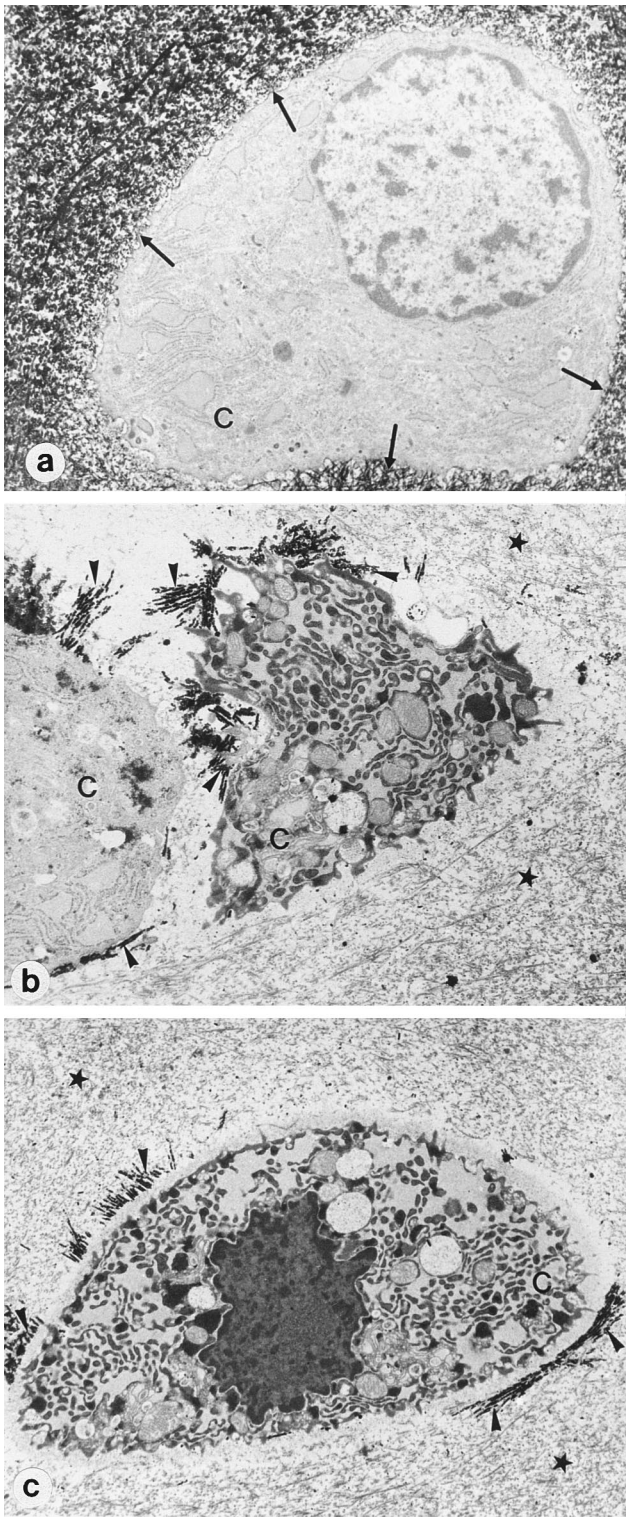

FIG. 2. Electron microscopy of knee joints from immature Wistar rats. (a) Normal diet, no ofloxacin (control). This electron micrograph of chondrocytes in the midzonal articular cartilage shows a typical chondrocyte (C) with a smooth surface and numerous cavities in the rough endoplasmic reticulum. The well-developed matrix (★) is closely attached to the cell membrane (arrows). The tissue was fixed in 1% glutaraldehyde and 1% tannic acid and then in 1% osmium tetroxide and stained with uranyl acetate and lead citrate. (b) Normal diet plus treatment with one 1,200-mg dose of ofloxacin per kg of body weight. In this ultrathin section through the midzonal articular cartilage, the cells are necrotic. A chondrocyte (C) is detached from the surface of the cartilage cell cavity. Note the typical formation of electron-dense bundles of aggregates (arrowheads) at the membrane of the chondrocyte. (c) Magnesium-deficient diet. This ultrathin section through the midzonal articular cartilage shows that magnesium deficiency induced alterations similar to those caused by treatment with ofloxacin. A typical finding is the electron-dense, bundle-like aggregates (arrowheads) at the cell membrane of a chondrocyte (C). Magnification, ×5,740.

necrotic and exhibit characteristic large, electron-dense, bundle-like aggregates on the cell surface and inside the chondrocytes (Fig. 2b). In magnesium-deficient rats, the same typical alterations—such as the electron-dense aggregates mentioned above—were also detectable in these preparations (Fig. 2c).

DISCUSSION

Quinolone-induced arthropathy can be induced in immature animals of multiple species (3, 4, 9, 14, 29). Nevertheless, it has been argued that this effect does not occur in humans, although several reports have suggested a relevance also for humans. This discrepancy is due to the fact that from casuistic reports or limited retrospective studies no causal relationship between drug exposure and symptoms can be proven, especially since most of the juvenile patients treated with quinolones had cystic fibrosis—a disease which is itself associated with arthropathy.

Since quinolones possess the unique combination of a high level of activity against gram-negative pathogens—including *Pseudomonas aeruginosa*—plus good bioavailability after oral administration (20), more and more pediatricians have demanded limited use of these drugs in pediatrics (1, 25). Certainly, the chondrotoxic potential of quinolones under therapeutic conditions is low. Most juvenile patients have shown no clinical signs of arthropathy after treatment with a quinolone. However, many questions regarding the use of quinolones in pediatric medicine remain unanswered: e.g., (i) which drug of this class is most suitable for pediatric use, (ii) what influence do dosage and length of treatment period have, (iii) why are juveniles more susceptible than adults, and (iv) how can the safety of therapy with these drugs in children be increased?

The chondrotoxic potentials of the different drugs of this class probably differ. The highest incidence of arthropathy in humans reported to date is 14% in patients with cystic fibrosis who were treated with pefloxacin, whereas in a similar group treated with ofloxacin no corresponding adverse effects were observed (23). Schaad and Wedgwood (26) found no evidence of arthropathy in 18 patients treated with ciprofloxacin; however, in a retrospective study with more than 600 children, ciprofloxacin-associated arthropathy was reported in 1.3% of the children (6).

Data presented in this report indicate that quinolone-induced arthropathy is most probably caused by interference of the drugs with extracellular magnesium in joint cartilage, since a dietarily induced magnesium deficiency caused cartilage lesions which were identical to quinolone-induced lesions. The organ specificity of the effect can be explained by the facts that quinolones accumulate in cartilage (21) and that ion disturbances are not as readily balanced in poorly nutrient-supplied joint cartilage as in other tissues. The ofloxacin doses used in this study are higher than therapeutically applied doses. However, we showed before that with gastric intubation of 600 mg of ofloxacin per kg of body weight—representing approximately 100 times the usual human dose—concentrations in the plasma of immature rats were only approximately 10-fold higher than concentrations measured in humans (17, 29). This relationship between dose and kinetics must be taken into

account when toxicological and therapeutic data are compared.

The ultrastructural changes observed in this study have not been reported before in association with quinolone treatment or magnesium deficiency. The similarity of the findings in both groups of animals (ofloxacin treatment and magnesium deficiency) gives further proof that a lack of functionally available magnesium is the primary event in the development of quinolone-induced arthropathy.

It is well known that magnesium is important for many physiological actions, e.g., activities of hundreds of enzymes. However, these lesions of joint cartilage induced by magnesium deficiency have not been described before. Intake of a magnesium-deficient diet for several days does almost exclusively reduce extracellular magnesium (10). The activity of integrin receptors of the β_1 subfamily is known to depend strongly on extracellular concentrations of calcium and/or magnesium (8, 16, 18). Since we have shown that the expression of these receptors, which are important for cell-matrix interactions, in joint cartilage is altered by ofloxacin treatment (27), it is reasonable to assume that by magnesium deficiency—induced either by dietary means or by the chelating activity of quinolones—the activity of these receptors is impaired, causing cell damage and/or degeneration of the matrix which eventually leads to severe damage of articular cartilage.

Any reasonable explanation for the mechanism of quinolone-induced arthropathy must take into account the fact that this toxic effect is closely correlated to the antibacterial action of the drugs, since all tested quinolones with antimicrobial activity induce arthropathy in juvenile animals. Detailed studies on the molecular mechanism of the action of these antimicrobial agents on bacterial DNA have shown that they bind to the gyrase-associated nucleic acid via a magnesium ion (22). This finding suggests that, in general, quinolones must have a pronounced affinity for magnesium ions to exhibit antibacterial activity.

Data from other areas have indeed indicated that quinolones possess a pronounced chelating capacity for magnesium ions. For example, bioavailability of ciprofloxacin is reduced by more than 90% if the drug is taken simultaneously with antacids containing magnesium and aluminium (12). Similar pharmacokinetic interactions with other drugs of this class have been shown (7, 19). Microbiological studies have shown that magnesium reduces the antibacterial activity of the drugs in vitro (19).

Several other possible mechanisms for quinolone-induced arthropathy have been postulated since the first description of this unusual toxic effect approximately 15 years ago (13, 31). Some investigators have favored the idea that chondrocytes are the primary site of quinolone toxicity, possibly by interference of the drugs with mammalian DNA (15, 30). On the other hand, Bendele and coworkers have published data which indicate that quinolones interfere directly with the intercellular matrix of joint cartilage (2). For none of these possible mechanisms have convincing experimental data been published to prove these assumptions.

Our data indicate that a pronounced magnesium deficiency in the extracellular matrix of cartilage is most probably the reason for quinolone-induced arthropathy. Ofloxacin-induced arthropathy was aggravated by magnesium deficiency, and the electrolyte imbalance alone was sufficient to induce severe arthropathy in juvenile rats. It remains unclear, however, if alterations of the function of integrin receptors or other events subsequently lead to tissue damage. Besides such specific reactions, a general cytotoxic effect must be discussed, since it has been shown that magnesium deficiency generally enhances cytotoxicity by increasing membrane permeability, particularly for calcium (11).

These new aspects of the mechanism of quinolone-induced arthropathy also offer the opportunity to select the most appropriate quinolone for pediatric use: both concentrations achieved in cartilage and affinity for magnesium should be low for such a compound. In a clinical situation, it will be of interest to check the magnesium concentration in plasma closely if patients have arthropathy in association with quinolone treatment. Our findings indicate that if juvenile patients must be treated with quinolones for serious infections, it is prudent to ensure that these patients do not have a disturbed magnesium balance. It is tempting to speculate that exogenously administered magnesium has a protective function. Corresponding experiments with the rat model are under way in our laboratory.

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