Synergistic Effect of Ofloxacin and Magnesium Deficiency on Joint Cartilage in Immature Rats

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Single high oral doses of fluoroquinolones (e.g., 1,200 mg of ofloxacin/kg of body weight) are chondrotoxic in juvenile rats. Characteristic cartilage lesions are detectable as early as 12 h after treatment. Since this dosing regimen does not reflect the therapeutic situation, we studied the effects of a 5- or 7-day treatment with ofloxacin at lower oral doses (10, 30, and 100 mg/kg twice a day [b.i.d.]) on joint cartilage in 4-week-old rats. We additionally investigated whether the effects of ofloxacin under these conditions are enhanced in animals kept on a magnesium-deficient diet during treatment. Knee joints were examined histologically. The concentrations of ofloxacin and magnesium were determined in plasma and cartilage. The lowest ofloxacin dose at which cartilage lesions occurred in animals on a standard diet was 100 mg/kg b.i.d. for 5 days. Peak plasma ofloxacin levels were approximately 10 mg/liter in these rats and thus were in the same range as the levels in the plasma of humans during therapy with high doses of ofloxacin. Treatment with 30 mg of ofloxacin/kg b.i.d. for 7 days caused no cartilage lesions in rats on a standard diet, but lesions did occur in 10 of 12 rats that were simultaneously fed a magnesium-deficient diet. Magnesium concentrations in bone, plasma, and cartilage from animals on an Mg2⁺-deficient diet were significantly lower than those in the controls. The concentration in plasma from animals on an Mg2⁺-deficient diet was 0.27 ± 0.03 mmol/liter, whereas it was 0.88 ± 0.08 mmol/liter in plasma from rats on a standard diet (means ± standard deviations). Ofloxacin treatment did not change the total magnesium concentrations in tissues, as determined with ashed samples. The incidence of ofloxacin-induced lesions was higher in the magnesium-deficient animals, suggesting a synergistic effect. These results must be taken into account for a benefit-risk evaluation if ofloxacin is considered for use in the pediatric population.

Quinolones have the potential to cause joint cartilage damage in juvenile animals. Due to their arthropathogenic effects, the use of these antibacterials is contraindicated in children and adolescents. Clinical experience with some fluoroquinolones (e.g., ciprofloxacin), mainly in children with cystic fibrosis, has shown that no obvious acute toxicity occurs under these conditions; however, these findings cannot be generalized for other fluoroquinolones because major differences exist in pharmacokinetics and possibly also in toxicodynamics (16, 18; R. Stahlmann and H. Lode, Letter, Lancet 3352:1313, 1998).

Investigations with immature animals and single high doses allow us to study the mechanism or other aspects of quinolone-induced chondrotoxicity, such as the latency period after dosing for such lesions to occur. Such an experimental setup, however, does not reflect the clinical situation (13). We now present data on a new experimental approach that corresponds to a standard clinical situation. Our studies were designed to answer the following questions: (i) is it possible to induce cartilage lesions in immature rats by treating them with multiple low, oral doses of ofloxacin (given twice a day [b.i.d.]) that lead to concentrations in plasma similar to those obtained in the plasma of humans during therapy with ofloxacin, and (ii) is there a synergistic effect on joint cartilage when the animals receive multiple, low (nonchondrotoxic) doses of ofloxacin and are simultaneously fed for a short period of time a magnesium-deficient diet which is not sufficient by itself to induce pronounced cartilage lesions? Background for the second part of the study were the findings that lesions resembling those observed after quinolone treatment can also be induced by feeding immature rats a magnesium-deficient diet for a period of 9 to 15 days (14, 19) and that supplementation with magnesium and/or tocopherol diminishes quinolone-induced arthropathy in immature rats (17). Furthermore, the effects of ofloxacin on Achilles tendons were more pronounced in rats on a magnesium-deficient diet than in rats on a standard diet (12).

To provide a basis for an extrapolation of the results to humans, the concentrations of the quinolone were measured in the plasma and joint cartilage of juvenile rats on days 1 and 3 of treatment, as well as on day 6, i.e., 1 day after the last day of treatment of those rats that were studied histologically. To determine the effect of the diet on mineral concentrations in target organs, magnesium concentrations were measured in plasma, cartilage, and bone at the end of the treatment periods.

MATERIALS AND METHODS

Quinolone treatment and histology. Wistar rats were kept in Macrolon cages at a room temperature of 23 ± 1°C, a relative humidity of 50% ± 5%, and a constant light-dark schedule (light from 0700 to 1900 h). For treatment with ofloxacin, commercially available tablets (Tarivid; Hoechst, Frankfurt, Germany) containing 400 mg of the drug were suspended in a 2% starch suspension. The freshly prepared suspension was administered by gastric intubation at a volume of 10 ml per kg of body weight.

A group of immature rats (n = 30) was treated with a single dose of 1,200 mg of ofloxacin/kg. The animals were killed 3, 6, 9, 12, 24, 36, and 48 h after treatment; and their knee joints were studied histologically to obtain information.
on the minimal latency period between dosing and the appearance of a chondrotoxic effect.

Additional rats (total, n = 40) were treated b.i.d. with ofloxacin at one of three dose levels (10, 30, or 100 mg/kg) or with the vehicle (starch solution). The animals received a standard diet or a magnesium-deficient diet (Altromin, Lage, Germany) for 5 days; in addition, two groups on a standard or a magnesium-deficient diet were treated with 30 mg of ofloxacin/kg b.i.d. for 7 days (total, n = 36). One day later the rats were killed and their knee joints were prepared and examined histologically after the samples were stained with toluidine blue (Merck, Darmstadt, Germany).

The knee joints were fixed in formalin (10%), decalcified in EDTA solution (10%; pH 7.4), dehydrated in an alcohol series, and embedded in paraffin. Series of 40 to 50 slices (<1 mm) were prepared from predilection sites of one knee joint of each animal and stained with an aqueous 1% solution of toluidine blue.

Fluoroquinolone kinetics in juvenile rats. Groups of three to six juvenile rats (age, 4 to 5 weeks) were treated with 100 mg of ofloxacin/kg as described above. The animals were decapitated after 0.75, 1.5, 3, and 6 h; and blood samples were obtained with hematoцит capillaries coated with sodium heparin. Blood was centrifuged and plasma was stored at −20°C until analysis; in addition, joint cartilage samples (femoral head) were collected from these rats. Plasma samples were deproteinized with acetonitrile and analyzed by high-pressure liquid chromatography (HPLC). Bone and cartilage samples were extracted with a 10-fold volume (wt/vol) of 0.1 M phosphoric acid for 20 h at 4°C. The extraction efficiency was 85% for a single extraction compared to those for three repeat extractions.

HPLC analysis. All samples were analyzed by the HPLC method described by Borner et al. (2). Briefly, separation was performed on a cation-exchange column (125 by 4.0 mm [inner diameter]; particle size, 5 μm; Nucleosil 100-5SA). The mobile phase consisted of 750 ml of acetonitrile and 250 ml of 0.01 mol/L phosphoric acid per liter (vol/vol), to which sodium hydroxide had been added (final concentration of sodium, 23 mmol/L [pH 3.8]). Ofloxacin was determined by spectrophotometry (excitation wavelength, 295 nm; emission wavelength, 480 nm). The flow rate was 1.5 mL/min, and the retention time was 4.7 min.

Pharmacokinetic analysis of the ofloxacin concentrations in plasma and cartilage was performed by using the TopFit program (4).

Mineral analysis. Mg²⁺ concentrations were determined in blood plasma, femoral bone (samples were taken from the diaphysis and contained bone marrow), and joint cartilage (pooled samples were taken from both femoral heads of one rat).

For Mg²⁺ quantification, plasma samples were deproteinized and diluted with 10% trichloroacetic acid–0.175% LaCl₃. Cartilage samples were lyophilized, ashed in a plasma processor (Technics, Munich, Germany), dissolved in 0.1 N HCl–0.175% LaCl₃, and measured by atomic absorption spectrophotometry (Phillips SP9). Freeze-dried bone was dissolved by heating with 10 N HNO₃, and the Mg²⁺ concentration was measured by atomic absorption spectrophotometry after appropriate dilution and addition of LaCl₃.

**RESULTS**

A single high dose of 1,200 mg of ofloxacin/kg caused cartilage damage in the knee joints of all rats studied at least 12 h after dosing, but the defects were not detectable in the first 9 h after dosing (Table 1). Figure 1 shows an example of a knee joint of a juvenile rat studied 24 h after treatment. The tibial part of the knee joint is damaged, whereas the femoral part cannot be distinguished from that of a vehicle-treated control rat or an untreated rat. Interestingly, we observed no gradual increase in the frequency of the severity of the lesions when we studied the effect at 3-h intervals, but we observed a rather abrupt change from normal tissue to damaged cartilage between 9 and 12 h.

Corresponding lesions of joint cartilage were also detectable in two of five juvenile rats after 5 days of treatment with 100 mg of ofloxacin/kg b.i.d. Histological analysis of knees from these rats did not reveal unequivocal differences between the lesions seen after the administration of 100 mg/kg b.i.d. and the lesions seen after the administration of single high doses, as described above. The control animals and those rats that had been treated with 30 mg/kg for 5 days showed an intact cartilaginous layer without any indication of arthropathy (Table 2).

Feeding of a magnesium-deficient diet for 5 days is not sufficient to induce lesions in knee joint cartilage in immature rats; after 7 days, one of six animals exhibited a small lesion.

**TABLE 1. Incidence of joint cartilage lesions in juvenile rats after treatment with a single high dose of ofloxacin**

<table>
<thead>
<tr>
<th>Time (h) after administration</th>
<th>No. of rats with lesions&lt;sup&gt;a&lt;/sup&gt;/No. of rats treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0/4</td>
</tr>
<tr>
<td>6</td>
<td>0/5</td>
</tr>
<tr>
<td>9</td>
<td>0/5</td>
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<tr>
<td>12</td>
<td>4/4</td>
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<td>24</td>
<td>4/4</td>
</tr>
<tr>
<td>36</td>
<td>4/4</td>
</tr>
<tr>
<td>48</td>
<td>4/4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ofloxacin dose, 1,200 mg/kg, administered once.

<sup>b</sup> All lesions were located in the tibial part of the knee joints.

**FIG. 1. Knee joint cartilage from a 5-week-old rat studied 24 h after treatment with a single dose of 1,200 mg of ofloxacin/kg. The section was stained with toluidine blue. A cartilage lesion (white arrow) has developed in the tibial part of the knee joint, whereas the femoral part is normal and corresponds to that for a control. Arrowheads mark the joint surface; asterisks mark bone; the white star marks a meniscus.**

**TABLE 2. Incidence of joint cartilage lesions in juvenile rats after treatment with multiple low doses of ofloxacin**

<table>
<thead>
<tr>
<th>Treatment period and ofloxacin dose (mg/kg)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of rats with lesions&lt;sup&gt;b&lt;/sup&gt;/No. of rats treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5 days</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/5</td>
</tr>
<tr>
<td>10</td>
<td>0/5</td>
</tr>
<tr>
<td>30</td>
<td>0/5</td>
</tr>
<tr>
<td>100</td>
<td>2/5</td>
</tr>
<tr>
<td><strong>7 days</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/6</td>
</tr>
<tr>
<td>30</td>
<td>0/12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Doses were given orally twice daily (0900 and 1700 h).

<sup>b</sup> All except one of the lesions were located in the femoral part of the knee joints; one lesion was in the tibial part of the knee joint and was detected in a rat on magnesium-deficient diet after treatment with the 100-mg/kg dose for 5 days.

<sup>c</sup> Statistically significant difference in comparison to the results for the group of rats kept on a standard diet (P < 0.01 by Fisher’s exact test).
(loss of stainability and desmasking of collagen fibers, but no clefts) without having been treated with ofloxacin. When ofloxacin was given in addition to the magnesium-deficient diet, a synergistic effect was obvious (Fig. 2). After treatment with ofloxacin at 30 mg/kg, we detected cartilage lesions in 1 of 5 magnesium-deficient rats treated for 5 days and in 10 of 12 magnesium-deficient rats treated for 7 days but in none of 5 rats and in none of 12 rats kept on a standard diet and treated with 30 mg of ofloxacin/kg for 5 and 7 days, respectively (Table 2).

No accumulation of ofloxacin during treatment was observed either in plasma or in joint cartilage. Peak concentrations were observed 45 min after dosing in plasma and 90 min after dosing in cartilage; on day 1, however, a peak concentration in cartilage was reached 45 min after dosing. Concentrations in plasma and cartilage were not significantly different on the three experimental days (days 1, 3, and 6) of treatment, when peak concentrations of $10.9 \pm 5.7$, $13.9 \pm 6.5$, and $8.0 \pm 2.5$ mg/liter, respectively, were measured in plasma and peak concentrations of $32.5 \pm 22.1$, $35.4 \pm 7.3$, and $27.7 \pm 11.7$ mg/kg, respectively, were measured in cartilage. The corresponding area under the concentration-time curve (AUC) values ranged from 37.2 to 48.1 mg·h/liter for plasma and 153 to 186 mg·h/kg for cartilage. The data are compiled in Fig. 3.

Magnesium concentrations in plasma, cartilage, and bone were consistently and significantly ($P < 0.05, t$ test) lower in rats receiving a magnesium-deficient diet than in rats receiving a standard diet (Table 3). As an example, in the control group that had received a standard diet, the concentration of Mg$^{2+}$ in plasma was $0.88 \pm 0.08$ mmol/liter, whereas it was $0.27 \pm 0.03$ mmol/liter in the group fed an Mg$^{2+}$-deficient diet without ofloxacin treatment. Ofloxacin treatment had no effect on total magnesium concentrations in tissues, as determined with ashed samples.

**DISCUSSION**

Most of the results of studies on the chondrotoxicity of ofloxacin in rats published so far were obtained after the administration of single doses of up to 3,000 mg/kg (5, 13). Under such conditions, we showed that cartilage lesions in knee joints are present as soon as 12 h after dosing, whereas 3 h earlier no changes had been detectable histologically. By treatment with multiple doses, we found cartilage lesions after the adminis-
istration of considerably lower doses of 100 mg/kg, which are not sufficient to induce chondrotoxicity when given as single doses, as has been shown before in our laboratory (14).

Therefore, it can be concluded that besides the developmental phase of the animal and the dose of the quinolone, the duration of treatment plays an important role in the development of chondrotoxicity. Interestingly, we found no differences in the histological features of cartilage defects after the administration of a single high dose or multiple low doses.

It appears to be noteworthy that the chondrotoxic effect seen in juvenile rats after the administration of multiple doses cannot be explained by an accumulation in plasma or cartilage. The peak concentrations in plasma on days 1, 3, and 6 after the administration of multiple doses of 100 mg/kg (mean values, between 8.0 and 13.9 mg/liter [Fig. 3]) are in agreement with results published earlier after treatment of juvenile rats with single doses of 100 to 600 mg/kg (13).

Pharmacokinetic investigations with ofloxacin in adult patients yielded average peak concentrations in plasma of 3.5 mg/liter after the administration of oral doses of 400 mg. The corresponding AUC was 28 mg · h/liter (8). Similar levels were obtained in 5- to 14-year-old children after oral treatment with 7.5 mg of ofloxacin/kg: the maximum concentration in serum was 5.7 mg/liter and the AUC was 26.5 mg · h/liter, but after intravenous infusion of 7.5 mg of ofloxacin/kg the peak concentrations varied from 7.3 to 12.0 mg/liter (95% confidence interval) (1). Thus, after the administration of high therapeutic doses to humans the concentrations of ofloxacin are in the same range as those in rats after the administration of oral doses of 100 mg/kg of body weight.

Concentrations in joint cartilage were consistently two- to threefold higher than the corresponding concentrations in plasma. Our data also show that during the treatment period the accumulation of ofloxacin does not occur either in plasma or in joint cartilage. The peak concentrations and AUC values in plasma and cartilage did not exhibit significant differences on day 1, 3, or 6 of treatment (Fig. 3). These data confirm previous findings that quinolone concentrations measured in cartilage are higher than those measured in plasma (15).

Data on quinolone concentrations in human cartilage are scarce. Meissner and coworkers (10) examined patients who had received a single dose of 200 mg of ofloxacin during hip surgery and 12 h after intravenous injection measured a concentration of 2.18 ± 0.45 mg/liter in joint cartilage, which was considerably higher than the corresponding level in plasma.

The chondrotoxic effect of Mg2+ deficiency induced in juvenile rats has been observed after feeding of an Mg2+-deficient diet for at least 9 days (14, 19). This led to cartilage lesions that were morphologically identical to the lesions observed after quinolone administration. In this study we investigated the knee joints after 5 and 7 days of magnesium deficiency with and without ofloxacin treatment. After 5 days of Mg2+ deficiency, the corresponding changes in the joint cartilage could not be recognized, but the feeding of such a diet for 7 days induced one slight lesion in the knee joint of one rat. Due to the rapid growth of the juvenile rats at this developmental stage, additional days of feeding of a magnesium-deficient diet have a major impact on the magnesium status of the animals. The combination of a magnesium-deficient diet for 7 days plus the administration of ofloxacin at nonchondrotoxic

FIG. 3. Concentrations (means ± standard deviations) of ofloxacin in cartilage and plasma of rats treated with multiple doses of ofloxacin (100 mg/kg). Concentrations were determined by HPLC in samples taken 45, 90, 180, and 360 min after the administration of an oral dose. Trend lines were established with the Microsoft Excel program. Concentration profiles are shown for days 1 (A), 3 (B), and 6 (C) of treatment. Ofloxacin did not accumulate either in plasma or in cartilage. The highest concentrations in plasma were observed at the first time point studied (45 min after dosing). The concentrations in cartilage were considerably higher than those in plasma and peaked at 45 min (A) or 90 min (B and C).
doses proved to be chondrotoxic in most rats. In contrast, after a 5-day treatment period and by use of the same experimental design described above, the incidence of cartilage lesions was lower; only one of five animals was affected. This result corroborates the theory that the administration of multiple doses as well as additional Mg\(^{2+}\) deficiency increases the chondrotoxic effect. Magnesium is essential for a large number of biochemical processes in cartilage, and it is not possible to identify a single reaction that would be responsible for the cartilage defects; but as we have hypothesized earlier, a disturbance of the integrin function must be considered a primary target. Integrin expression on chondrocytes was affected after treatment with ofloxacin or during magnesium deficiency (3).

The chelate-forming properties of quinolones and their affinities for magnesium have been described by several groups of investigators (6, 7, 9, 11). In order to be able to better ascertain the Mg\(^{2+}\) conditions during treatment with low ofloxacin doses and simultaneous Mg\(^{2+}\) deficiency, the magnesium concentrations in plasma, cartilage, and bone were determined. We could confirm that by feeding an Mg\(^{2+}\)-deficient diet for 5 days, a substantial decline in the concentration of this mineral could be induced in tissues, especially in cartilage. The concentrations in plasma, cartilage, and bone were significantly reduced in the Mg\(^{2+}\)-deficient group (with and without the addition of ofloxacin) compared with those in the control group fed a standard diet.

In summary, the data presented here reveal that, besides the dose and developmental phase, the duration of treatment is also a determinant of quinolone-induced chondrotoxicity. The experimental design used in this study corresponds to a possible clinical situation: treatment of a patient with two doses of a fluoroquinolone per day with simultaneous magnesium deficiency, although in patients magnesium deficiency will usually occur more chronically and will not be as pronounced as it was in our animal model. The pharmacokinetic data show that the level of exposure to ofloxacin under our experimental conditions corresponds to that achieved with high-dose ofloxacin therapy in humans. Typical lesions can be induced by the administration of low, nonchondrotoxic doses of ofloxacin if the animals are simultaneously fed a magnesium-deficient diet. These data are therefore of considerable clinical relevance, and it appears to be justified to ascertain the magnesium status of those patients who develop joint or tendon complaints while receiving quinolone therapy.

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REFERENCES


### TABLE 3. Magnesium concentrations in plasma, cartilage, and bone of rats fed a standard diet and a magnesium-deficient diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ofloxacin dose (mg/kg)</th>
<th>No. of rats</th>
<th>Magnesium concn (no. of samples) in:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma (mmol/liter)</td>
<td>Cartilage (mmol/kg [dry wt])</td>
</tr>
<tr>
<td>+Mg(^{2+})</td>
<td>0</td>
<td>5</td>
<td>0.88 ± 0.08 (5)</td>
<td>36.08 ± 6.51 (4)</td>
</tr>
<tr>
<td>-Mg(^{2+})</td>
<td>0</td>
<td>5</td>
<td>0.27 ± 0.03(4)</td>
<td>22.93 ± 4.63(4)</td>
</tr>
<tr>
<td>+Mg(^{2+})</td>
<td>100</td>
<td>5</td>
<td>1.04 ± 0.22 (5)</td>
<td>47.26 ± 14.59(3)</td>
</tr>
<tr>
<td>-Mg(^{2+})</td>
<td>100</td>
<td>5</td>
<td>0.55 ± 0.22(5)</td>
<td>24.69 ± 2.60(4)</td>
</tr>
</tbody>
</table>

* +Mg\(^{2+}\), magnesium-sufficient diet; -Mg\(^{2+}\), magnesium-deficient diet.

** Table 3. Magnesium concentrations in plasma, cartilage, and bone of rats fed a standard diet and a magnesium-deficient diet. **