

Proteoglycan and Collagen Biochemical Variations during Fluoroquinolone-Induced Chondrotoxicity in Mice

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Although fluoroquinolone antibacterials have a broad therapeutic use, with a relatively low incidence of severe side effects, they have been reported to induce lesions in the cartilage of growing animals by a mechanism that remains unclear. This study was undertaken to determine the potentially deleterious effect of a high dose of pefloxacin (400 mg/kg of body weight) on two main constituents of cartilage in mice, i.e., proteoglycans and collagen. Variations in levels of proteoglycan anabolism measured by in vivo [³⁵S]sulfate incorporation into cartilage and oxidative modifications of collagen assessed by detection of carbonyl derivatives were monitored after administration of pefloxacin. Treatment of mice with 1 day of pefloxacin treatment significantly decreased the rate of biosynthesis of proteoglycan for the first 24 h. However, no difference was observed after 48 h. The decrease in proteoglycan synthesis was accompanied by a marked drop in serum sulfate concentration and a concomitant increase in urinary sulfate excretion. The decrease in proteoglycan synthesis, also observed ex vivo, may suggest a direct effect of pefloxacin on this process, rather than it being a consequence of a low concentration of sulfate. On the other hand, treatment with pefloxacin for 10 days induced oxidative damage to collagen. In conclusion, this study demonstrates, for the first time, that pefloxacin administration to mice leads to modifications in the metabolism and integrity of extracellular proteins, such as collagen and proteoglycans, which may account for the side effects observed. These results offer new insights to explain quinolone-induced disorders in growing articular cartilage.

Fluoroquinolones are potent antimicrobial agents that are widely used to treat infections of the respiratory or urinary tract, skin, and soft tissues. Some infrequent adverse reactions occur during their use, and one of the most important concerns is their potential chondrotoxicity in young patients. Indeed, because of their chondrotoxicity in growing animals (4, 8, 16, 43), quinolones are contraindicated for children and adolescents. Chondrotoxicity has been observed experimentally in growing animals after treatment with first-generation quinolones (21) and with fluoroquinolones (3, 23, 31, 44). Histologic changes were similar in all animals, with lesions occurring, as a rule, during growth (15), except with some fluoroquinolones (8, 5), which also produced characteristic lesions in skeletally mature dogs.

The specific mechanism responsible for the quinolone-induced arthropathy remains unclear, although several explanations have been postulated since its first description approximately 20 years ago (21). Some authors have suggested that chondrocytes are the primary site of quinolone toxicity (24, 45). Others postulated that quinolones interfere directly with the extracellular matrix of joint cartilage (1). More recently, chelation of magnesium ions by quinolones, resulting in changes in the functions of integrin receptors on the chondrocyte surface, was suggested (14, 43). In vitro studies of cartilage from various species have shown an inhibition of the synthesis of either collagen or glycosaminoglycans (3, 23, 24). Moreover, experimental data suggested compromised mitochondrial integrity (20, 24) and an early stimulation by fluoroquinolones of the oxidative metabolism within immature articular chondro-

cytes (19, 47), suggesting the generation of reactive oxygen species. The hypothesis that an oxidative stress occurs and participates in the pathophysiological effect seems attractive, as cartilage undergoes a chronic hypoxia resulting from the absence of vascularization.

In the present work, the effect of a single dose of pefloxacin (400 mg/kg of body weight) on the biosynthesis of proteoglycans, as revealed by in vivo ³⁵S incorporation, in articular cartilage in mice was investigated. Indeed, ³⁵S incorporation allows the study of the sulfatation of glycosaminoglycan chains covalently bound to the core protein. This approach allowed us to evaluate the time course of the pefloxacin-induced changes on proteoglycan synthesis. Moreover, we determined the oxidative modifications of collagen by monitoring administration of pefloxacin for 1 and 10 days and by measuring carbonyl derivatives. The long half-life of collagen (7) may allow the detection of damage induced by reactive oxygen species over several days.

MATERIALS AND METHODS

Animals. Three- to 4-week-old male Swiss mice weighing 15 to 20 g (Charles River, Saint-Aubin-lès-Elbeuf, France) were housed in solid-bottomed plastic cages designed to allow easy access to standard laboratory food and water ad libitum. The animals were kept on a cycle of 12 h of light and 12 h of dark in a temperature-controlled chamber and were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee and those of the National Institutes of Health for laboratory animal welfare.

For the first phase of the experiment, two groups of five mice received by gavage either a single dose of pefloxacin dihydrate mesylate (400 mg/kg, 10 μl/g in saline solution; kindly donated by Rhône-Poulenc Rorer Laboratories, Neuilly/Seine, France) or saline only and a concomitant intraperitoneal injection of Na₂³⁵SO₄ (2 μCi/g of body weight; Isotopchim; Amersham, Les Ulis, France). They were decapitated 2, 8, 16, 24, or 48 h later. In the second phase of the experiment (24 to 48 h), mice were given a single oral dose of pefloxacin (400 mg/kg) and 24 h later they received an intraperitoneal injection of radioactive sulfate (2 μCi of Na₂³⁵SO₄/g). Mice were sacrificed 48 h after pefloxacin administration (Fig. 1). Blood samples were collected, and the femoral head caps

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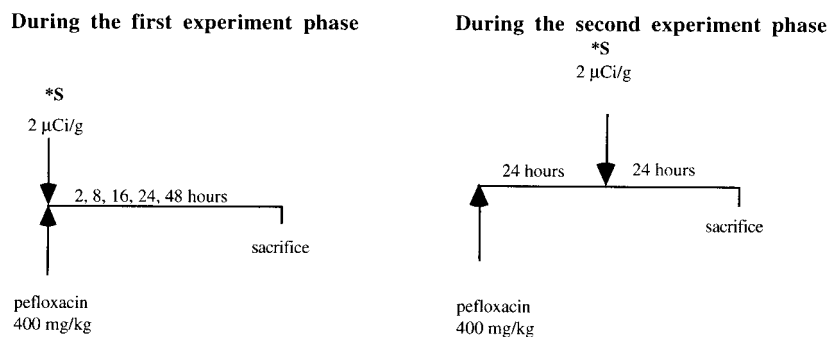


FIG. 1. Effects of pefloxacin administration on the kinetics of ^{35}S incorporation in the blood and cartilage of mice *in vivo*.

(FHCs) and patellae were dissected out. Cartilage samples were fixed overnight in 1 ml of cetylpyridinium chloride (Sigma) in phosphate-buffered formalin. Patellae were decalcified in 5% formic acid for 6 h, and then the central area of each patella was sampled with a 1-mm-diameter biopsy punch. The punched-out portions of the patellae (articular cartilage), the remaining peripheral parts of the patellae (fibrocartilage), and FHCs were dissolved overnight in Soluene-350 (Packard, Rungis, France). Blood samples (10 μl) were decolorized by adding 100 μl of propan-2-ol and 100 μl of H_2O_2 (30%). The amount of [^{35}S]sulfate incorporated in each sample was counted by liquid scintillation spectrometry (Hionic Fluor and Packard). In both treated and untreated mice, the ^{35}S contents in blood paralleled those in the corresponding sera. Therefore, blood samples were used as equivalents of serum samples in measurements of inorganic radio-sulfate content. Results are expressed as the differences between the mean amounts of ^{35}S incorporated in FHCs and patellae from animals treated with pefloxacin and values for control animals treated with saline solution.

Measurement of inorganic sulfate. The content of inorganic sulfate was measured by turbidimetry, as described by Krijgsheld et al. (26, 27). To 500 μl of serum containing 0.1 to 1.3 mM inorganic sulfate, 2 ml of trichloroacetic acid solution (5% wt/vol) was added, and the mixture was allowed to stand at room temperature for 10 min. After centrifugation, 1 ml of the clear supernatant was mixed into 250 μl of BaCl_2 reagent (20 g of $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ and 100 g of dextran in 1 liter of distilled water) in a disposable semimicrocuvette, which was stirred. After 35 min, the absorbance at 360 nm was recorded against a blank consisting of 1 ml of supernatant and 250 μl of reagent containing 100 g of dextran/liter of distilled water. The amount of inorganic ^{35}S in the serum was measured as described by De Vries et al. (12). After determination of serum sulfate concentration by turbidimetry, the contents of the cuvettes were centrifuged and the radioactivities associated with the pellet (inorganic sulfate fraction in the form of precipitable $\text{Ba}^{35}\text{SO}_4$) and the supernatant were counted. In normal and treated mice, 97% of ^{35}S was in the inorganic form $^{35}\text{SO}_4^{2-}$.

Ex vivo measurement of proteoglycan synthesis. Proteoglycan synthesis was assayed as described by Van den Berg et al. (50). Briefly, pefloxacin (400 mg/kg daily, 10 $\mu\text{l/g}$) was administered orally to mice, which were killed by cervical dislocation 24 or 48 h after drug administration. The FHCs and patella samples were carefully dissected and were incubated in RPMI 1640 culture medium (200 μl /patella or FHC) containing gentamicin (50 $\mu\text{g/ml}$), L-glutamine (2 mM), and $\text{Na}_2^{35}\text{SO}_4$ (10 $\mu\text{Ci/ml}$). After incubation for 2 h at 37°C in a 5% CO_2 atmosphere, FHCs and patellae were washed with isotonic saline solution and fixed overnight in 0.5% cetylpyridinium chloride in phosphate-buffered formalin. Patellae were decalcified and sampled with a 1-mm-diameter biopsy punch as described above. Samples were then dissolved overnight in Soluene-350. Results are expressed as the differences between the mean amounts of ^{35}S incorporated in FHCs and patellae from animals treated with pefloxacin and those for the control animals treated with the vehicle alone.

Collagen extraction. Articular cartilage was harvested from the FHCs of mice treated with pefloxacin (400 mg/kg/day) for 1 or 10 days. Collagen was extracted from FHCs, which were washed with water and neutral salt solution (0.05 M Tris-HCl [pH 7.4], 0.9% [wt/vol] NaCl) to remove the salt-soluble material. The tissue was crushed and continuously stirred in a solution of 4 M guanidine-HCl in 0.05 M sodium acetate (pH 5.8) at 4°C for 24 h to remove proteoglycans (48). After centrifugation for 30 min at $30,000 \times g$, the residue was collected and washed three times with 0.05 M acetic acid. The collagen residue was added to a solution of 1 mg of pepsin per ml of 0.5 M acetic acid in a weight ratio of 1/10 (sample to pepsin) and stirred for 2 days at 4°C (32). Undigested solid material was removed by centrifugation at $30,000 \times g$, for 30 min. This method of pepsin digestion was chosen experimentally to extract more than 90% of the collagen, as determined by measurement of the hydroxyproline content in the supernatant after pepsin digestion and in FHCs after acid hydrolysis (52). Protein concentration was determined by the Lowry assay, with bovine serum albumin as the standard (30).

Protein derivatization with DNPH and by SDS-PAGE. Collagen (50 μg) was treated with an equal volume of 0.5 mM dinitrophenylhydrazine (DNPH) (in 0.1

M sodium phosphate buffer, pH 6.3) for 1 h at room temperature. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (28) with 1-mm-thick 6% slab gels (16- by 18-cm format). The same amount of collagen (15 μg) was loaded into all lanes. After SDS-PAGE, the gels were transferred onto Immobilon-P membranes with a Trans-Blot electrophoretic transfer apparatus according to the method of Towbin et al. (49). Immunochemical detection of protein carbonyls was performed as described by Keller et al. (25) and Shacter et al. (40). Briefly, the blots were incubated with 0.1% Ponceau S (wt/vol) in 5% acetic acid (vol/vol) and destained in methanol until the bands appeared. Then blots were incubated with bovine serum albumin (3%) for at least 90 min, followed by incubation at room temperature with Sigma rabbit anti-dinitrophenyl antibodies (diluted 1:2,000 in 9 mM Tris-HCl [pH 9.0], 154 mM NaCl, 0.05% Tween 20 [vol/vol] [TBST]). The primary antibody was removed, and the blots were washed three times (10 min each time) with TBST. The blots were incubated with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma) (diluted 1:5,000 in TBST) for 90 min at room temperature. After washing of the blots with TBST three times (10 min each time), oxidized proteins were revealed by the addition of 5-bromo-4-chloro-3-indolylphosphate (BCIP)-nitroblue tetrazolium. The intensities of the bands were quantified with densitometry analysis software (NIH Image), and results are expressed in arbitrary units.

Statistical analysis. Biochemical data are presented as means \pm standard errors of the means (SEM). Groups were compared by two-way analysis of variance, with a P of <0.05 taken as the significance level.

RESULTS

In vivo effects of a single dose of pefloxacin on the contents of ^{35}S in blood and cartilage in mice. (i) First-phase experiment (early effects). The effect on proteoglycan synthesis of a single oral dose (400 mg/kg) of pefloxacin depended on the tissue studied. In both treated and control mice, the amount of radioactivity in blood decreased with time until 16 h whereas that in FHCs and patellae increased with a maximum reached at 16 to 24 h (Fig. 2 and 3). This increase was followed by a decrease until 48 h after the injection. Eight hours after injection, pefloxacin-treated animals displayed less radioactivity than control animals in blood (37% less), in cartilaginous structures and FHCs (36% less), in patellar central cartilage (23% less), and in patellar peripheral fibrocartilage (26% less). Similar differences were observed at 16 and 24 h and persisted after 48 h in the central cartilage and peripheral fibrocartilage of patellae but not in blood or FHCs. The amount of radioactivity in blood was 34% lower in the treated mice than in the control mice. Throughout these tests, urinary excretion of ^{35}S was twofold higher in the treated mice than in the controls (data not shown).

(ii) Second-phase experiment (late effects). In these second-phase experimental group, 48 h after a single dose of pefloxacin (400 mg/kg) and 24 h after injection of ^{35}S , proteoglycan synthesis was not significantly different from that in controls in patellar cartilage or FHCs (Fig. 4). The amounts of radioactivity in blood were similar in the treated mice and in the controls.

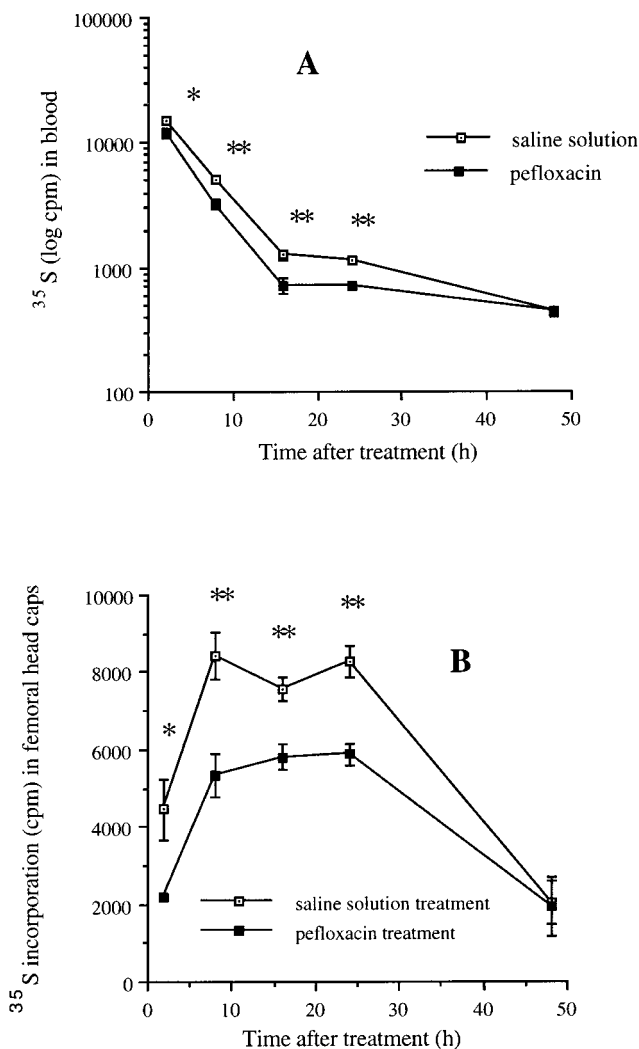


FIG. 2. Effects in mice of treatment with a single oral dose (400 mg/kg) of pefloxacin on the incorporation of simultaneously administered ³⁵S in vivo in blood (log plot) (A) and FHCs (B). Controls received the same volume of saline solution instead of pefloxacin. Values for ³⁵S incorporation are means ± SEM of results from four experiments. *, *P* < 0.05; **, *P* < 0.01 versus controls, by Student's *t* test.

Effect of pefloxacin on the endogenous inorganic-sulfate concentration in mice sera 24 h after drug administration. An acute pefloxacin administration (400 mg/kg) caused a marked decrease (30%) in the endogenous sulfate concentration in serum (Fig. 5). The concentration of inorganic sulfate decreased from the physiologic level in serum (0.95 mM) to approximately 0.66 mM and returned to the control level after 48 h.

Ex vivo effects of a single dose (400 mg/kg) of pefloxacin on the ³⁵S contents in the cartilage of mice. (i) Twenty-four hours after a single oral dose. The ex vivo levels of incorporation of ³⁵S into FHCs and patellar central cartilage decreased by 22 and 25%, respectively, 24 h after pefloxacin administration (Fig. 6A).

(ii) Forty-eight hours after a single oral dose. Proteoglycan synthesis in FHCs and patellar central cartilage returned to control values 48 h after pefloxacin administration (Fig. 6B).

Effect of a pefloxacin treatment (400 mg/kg/day) on carbonyl derivative formation in type II collagen. Oxidized proteins as

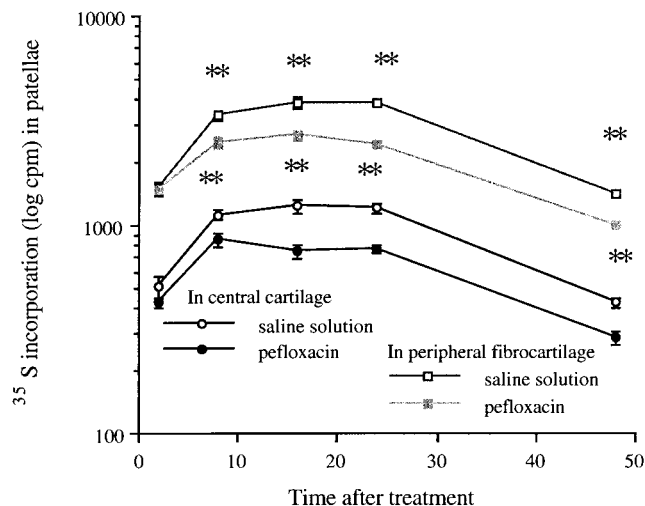


FIG. 3. Effect in mice of the same treatment described for Fig. 1 on the incorporation of ³⁵S in patellar central cartilage and peripheral fibrocartilage (log plot). Controls received the same volume of saline solution instead of pefloxacin. Values for ³⁵S incorporation are means ± SEM of results from four experiments with five mice each for levels of radioactivity incorporated. *, *P* < 0.05; **, *P* < 0.01 versus controls, by Student's *t* test.

revealed by Western blot analysis were used to determine the susceptibility of collagen to oxidative modification due to pefloxacin treatment. Tissues from control animals and those treated with pefloxacin for 1 or 10 days were assayed. Collagen was extracted from FHCs and analyzed by SDS-PAGE, after incubation with DNPH. We observed a characteristic band corresponding to the α1(II) chains (Fig. 7). No difference was observed 24 h after a single dose of pefloxacin. A higher carbonyl derivative content, measured by densitometry, was observed in collagen obtained from the FHCs of mice treated daily with pefloxacin for 10 days (400 mg/kg/day) (Fig. 7 and 8).

DISCUSSION

Although fluoroquinolones have a broad therapeutic potential and a relatively low incidence of serious side effects, they

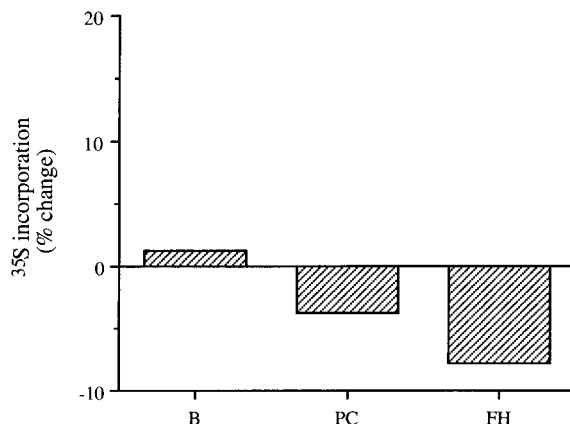


FIG. 4. Proteoglycan synthesis measured by the in vivo changes in levels of incorporation of ³⁵S 48 h after a single oral dose (400 mg/kg) of pefloxacin and 24 h after intraperitoneal administration of ³⁵S (2 μCi/g). Values are percentages reflecting the difference in ³⁵S incorporation in treated mice from that in controls receiving the same volume of saline only (means of results from two experiments with five mice each). B, blood; PC, central patellar cartilage; FH, FHCs.

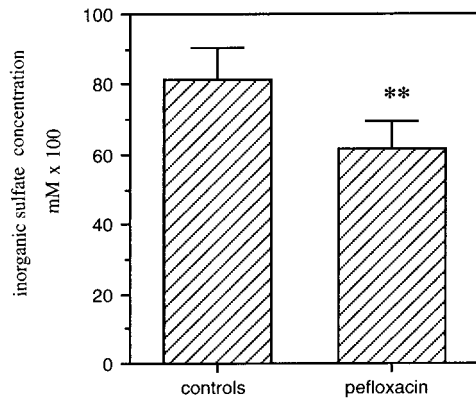


FIG. 5. Effect of pefloxacin (400 mg/kg) administered orally to mice on the concentration of inorganic sulfate 24 h after drug administration. Values for inorganic sulfate are means \pm SEM of sulfate concentrations from three experiments. **, $P < 0.01$ versus controls, by Student's t test.

are known to induce lesions of cartilage in growing animals. Pefloxacin has also been reported to induce side effects on articular cartilage in adult dogs, resulting in arthropathy (5, 8, 16). Numerous in vitro studies of cartilage from various species have shown an inhibition of the synthesis of either collagen or glycosaminoglycans under these conditions (3, 23, 24). The

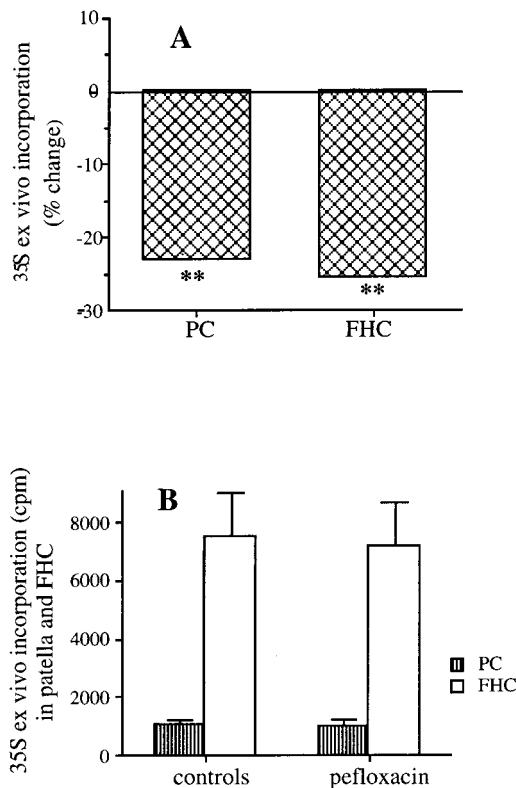


FIG. 6. Effects of pefloxacin on proteoglycan synthesis measured by the changes in the levels of incorporation of ^{35}S ex vivo 24 h after a single oral dose (400 mg/kg), relative to the levels of incorporation in controls receiving the same volume of saline solution (A), and 48 h after a single oral dose (400 mg/kg), relative to the levels of incorporation in controls receiving the same volume of saline only (B). Duplicate experiments were performed with eight mice each. PC, central patellar cartilage.

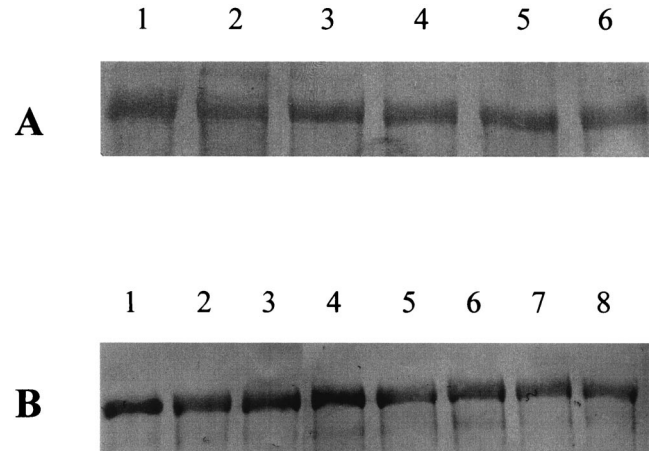


FIG. 7. Effect of pefloxacin administration on carbonyl derivative formation in the collagen of articular cartilage revealed by immunochemical detection. Fifteen micrograms of DNPH-derivatized protein was loaded into each lane. (A) Lanes 1 to 3, articular cartilage collagen of mice treated with a single dose of pefloxacin (400 mg/kg); lanes 4 to 6, articular cartilage collagen of mice given saline solution. (B) Lanes 1 to 4, articular cartilage collagen of mice which received pefloxacin (400 mg/kg/day) for 10 days (duplicate loads); lanes 5 to 8, articular cartilage collagen of mice receiving saline solution.

damage to articular cartilage promoted by pefloxacin may result from an oxidative stress (17, 47). As free radicals may alter the extracellular matrix, we determined levels of collagen oxidation through the formation of carbonyl derivatives and modifications of cellular activity by proteoglycan anabolism by measuring ^{35}S incorporation in an in vivo mouse model. The doses of pefloxacin administered to mice in this study were much higher than those usually delivered to humans but are common in such toxicological studies. Nevertheless, half-lives of this drug ranged from 1.9 h in mice to 8.6 h in humans (36). Thus, pefloxacin is metabolized more rapidly in mice, and this dose may allow a sufficient tissular diffusion.

The time course study of ^{35}S incorporation showed that in control mice, very little radioactivity remained in the blood 24 h after the ^{35}S injection and that the highest values of radioactivity in the other tissues studied were obtained between 16 and 24 h. When administered simultaneously with ^{35}S , pefloxacin induced a decrease in ^{35}S incorporation in all tissues studied. By contrast, 48 h after a single (400-mg/kg) pefloxacin administration and 24 h after the ^{35}S injection, proteoglycan synthesis in patellar cartilage or FHCs did not significantly differ from that in controls. This reversible change in proteoglycan synthesis in cartilage may reflect a repair process, suggesting that cells recovered their normal functions. This apparent recovery differs from the results of Ingham et al. (21), who observed long-lasting cartilage lesions still present at autopsy. Förster et al. (13) also described irreversible lesions induced by ofloxacin in rats. On the other hand, Kato and Onodera (23) briefly described repair-like processes in the joint cartilage of immature rats that had been treated with ofloxacin. The changes in sulfate incorporation reversibility that we observed 48 h after a single administration of pefloxacin are in agreement with the results of a study of the uptake of $^{35}\text{SO}_4$ by cultured rabbit chondrocytes incubated with levofloxacin (24).

The present data demonstrate a marked fall in the endogenous serum sulfate level 24 h after an acute administration of pefloxacin and a concomitant increase in sulfate urinary excretion. More precisely, the total decrease in serum SO_4^{2-} con-

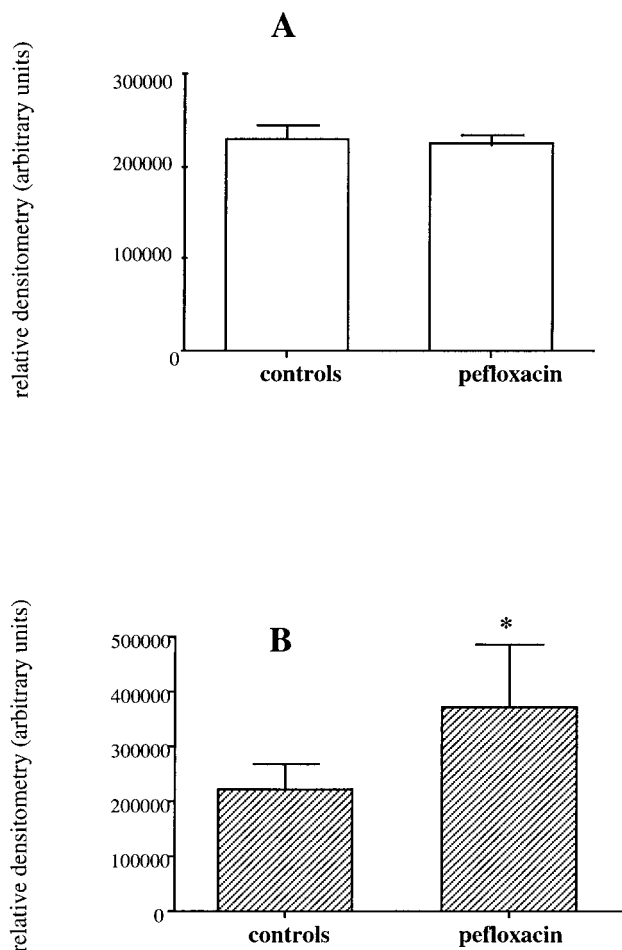


FIG. 8. Effect of pefloxacin administration on carbonyl derivative formation in articular cartilage collagen. Shown is a densitometric analysis of the blots in Fig. 7. Values are means \pm standard deviations of results from two experiments. *, $P \leq 0.05$ versus controls, by Student's t test.

centration at 24 h paralleled the decrease in serum ^{35}S content. Consequently, the specific activities of circulating [^{35}S]sulfate remained identical in both pefloxacin-treated and control mice. A similar situation was also reported in another study of salicylate effects on cartilage, which used a different duration between drug and radiolabel administrations (12). These results suggest that pefloxacin may inhibit the renal absorption of sulfate or may require sulfation for its elimination. Other possible mechanisms for the reduced sulfate incorporation are a direct action of pefloxacin on chondrocyte metabolism and a drug-induced decrease in sulfate availability.

In order to determine if the decrease in ^{35}S incorporation results from a direct effect of pefloxacin on cartilage or from a low concentration of sulfate, we evaluated the pefloxacin effects in an ex vivo system. Using the measured sulfate concentration, we confirmed that pefloxacin inhibits proteoglycan synthesis 24 h after its administration and that this response is reversed after 48 h, as demonstrated in vivo. These results confirm previous work dealing with the in vitro effects of fluoroquinolones on articular chondrocytes (3, 24). A decrease in the ex vivo level of ^{35}S incorporation into the articular cartilage 12 and 24 h after a single administration of ofloxacin (900 and 2,700 mg/kg) has also been described (23). Therefore, the decrease in ^{35}S incorporation we observed during in vivo experiments seemed to be the result of a direct effect of pefloxa-

cin on tissue metabolism rather than the result of a low concentration in sulfate. Thus, these results demonstrate that a single administration of pefloxacin induces early cellular damage which leads to a transitory decrease in proteoglycan anabolism. This matrix component can be replaced relatively quickly, as opposed to collagen, which is much less readily released, but when degradation of collagen does occur, the structural integrity of the tissue is lost (7). Therefore, we investigated the possible oxidative influence of pefloxacin on collagen, which can participate in the damage produced during several days of treatment.

Several reports showed an early stimulation of oxidative metabolism due to fluoroquinolone in immature articular chondrocytes (18, 19, 47), possibly resulting in the generation of reactive oxygen species. Accordingly, compromised mitochondrial integrity has also been described (20, 35). In an attempt to demonstrate in vivo oxidative damage due to pefloxacin, we measured the content of protein carbonyl in purified collagen. It is well known that oxidative stress increases the protein carbonyl content by a direct oxidative attack on amino acid side chains (11). Another mechanism is the modification of side chains by lipid peroxidation products. Therefore, the measurement of protein carbonyls is usually considered a marker of oxidative injury (38, 42). In the present study, we report for the first time that pefloxacin induces oxidative damage to cartilage collagen. An increase of collagen carbonyl derivative content was observed in mice treated for 10 days, whereas no changes appeared after an acute administration of pefloxacin. This oxidative damage to collagen was observed after 5 days of daily treatment. Identical results have been obtained with rats in the same experimental condition (data not shown). These results suggest that repeated administrations of pefloxacin induce the production of oxidizing species in the extracellular matrix of articular cartilage and that this production promotes some oxidative damage to collagen. These oxidizing species may be oxygen-derived reactive species that are formed as a result of modifications in chondrocyte mitochondrial and respiratory activities, as suggested by previous work (18, 19). The formation of oxidative species is sufficient to promote alterations to connective tissue macromolecules, as was observed in collagen. Oxygen-reactive species formation may also explain the inhibition observed on the synthesis of proteoglycan in vivo and ex vivo in mice (46). In this way, previous data demonstrated that reactive oxygen species alter cartilage metabolism and structure when they are overproduced (37). It has been established that oxygen-derived reactive species may promote extracellular matrix modifications, by intensive cross-linking and polymerization of the protein (34, 39, 51) or by activation of latent metalloproteinases (2, 29). Moreover, in vitro localization of radical formation within a collagen molecule was observed at particular sites (17). It was also established that oxidants can directly degrade soluble collagen and at low levels can modify collagen, making it susceptible to proteolytic degradation (10, 33).

These results clearly demonstrated that pefloxacin induced two effects on cartilage, one at the cellular and one at the matrix level. Both effects may contribute to cartilage damage. Cartilage homeostasis is regulated, in part, by the interaction of chondrocytes with their extracellular matrix and depends on interplay between integrins and matrix components related to "outside-in" signaling (9). The oxidative stress induced by pefloxacin may modify cartilage organization by altering mitochondrial function (6), the intracellular redox state, and some cellular signaling pathways (22). For instance, the expression of intercellular adhesion molecule 1 has been shown to be modulated by oxidative stress (53). Moreover, it has been observed

that chondrocytes adjacent to fissures in articular cartilage in rats treated with fluoroquinolone have a reduced integrin expression (14), an observation which was confirmed with mice (41). Moreover, cartilage of growing animals may be more susceptible to quinolones than that of adults, as chondrocytes must produce a greater quantity of matrix macromolecules leading to cartilage structural integrity. Also, pefloxacin-induced oxidative stress may modify growing cartilage organization in a faster and more deleterious way than in mature cartilage.

In conclusion, our results indicate that the oral administration of a high dose of pefloxacin to mice induces modifications of the extracellular matrix, as was observed with collagen, suggesting the production of oxygen-derived reactive species. This study also revealed early cellular changes, as evidenced by modifications of proteoglycan anabolism. This double impact, one at the cellular level and one at the matrix level, offers new insights to explain quinolone-induced disorders in articular cartilage. Owing to metabolic needs, a fluoroquinolone-induced oxidative stress may alter immature cartilage organization more seriously than that of mature cartilage. Nevertheless, these results should be considered with care in relation to the adverse effects of pefloxacin reported for humans. On the other hand, further study would be necessary to evaluate the potentially protective effect of antioxidants.

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