

Pefloxacin-Induced Achilles Tendon Toxicity in Rodents: Biochemical Changes in Proteoglycan Synthesis and Oxidative Damage to Collagen

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Despite a relatively low incidence of serious side effects, fluoroquinolones and the fluoroquinolone pefloxacin have been reported to occasionally promote tendinopathy that might result in the complication of spontaneous rupture of tendons. In the present study, we investigated in rodents the intrinsic deleterious effect of pefloxacin (400 mg/kg of body weight) on Achilles tendon proteoglycans and collagen. Proteoglycan synthesis was determined by measurement of in vivo and ex vivo radiolabeled incorporation in mice. Collagen oxidative modifications were measured by carbonyl derivative detection by Western blotting. An experimental model of tendinous ischemia (2 h) and reperfusion (3 days) was achieved in rats. Biphasic changes in proteoglycan synthesis were observed after a single administration of pefloxacin, consisting of an early inhibition followed by a repair-like phase. The depletion phase was accompanied by a marked decrease in the endogenous serum sulfate level and a concomitant increase in the level of sulfate excretion in urine. Studies of ex vivo proteoglycan synthesis confirmed the in vivo results that were obtained. The decrease in proteoglycan anabolism seemed to be a direct effect of pefloxacin on tissue metabolism rather than a consequence of the low concentration of sulfate. Pefloxacin treatment for several days induced oxidative damage of type I collagen, with the alterations being identical to those observed in the experimental tendinous ischemia and reperfusion model. Oxidative damage was prevented by coadministration of *N*-acetylcysteine (150 mg/kg) to the mice. These results provide the first experimental evidence of a pefloxacin-induced oxidative stress in the Achilles tendon that altered proteoglycan anabolism and oxidized collagen.

Fluoroquinolones are widely used in clinical practice because of their excellent antibacterial activity, wide spectrum of activity, and high degree of bioavailability. These antibiotics are generally considered well tolerated, although quinolone-induced chondropathy has been observed in young animals of several species (3, 4, 9, 31).

Since 1992, tendinopathy has been described as another side effect in patients treated with fluoroquinolones, with the tendinopathy sometimes resulting in the rupture of the tendon. The cause of this rare ($\leq 1\%$) (7) but severe complication remains unexplained (13, 14, 28). Probably both because of the large number of prescriptions for pefloxacin and because of the high level of diffusion of pefloxacin into tissue, pefloxacin has been the subject of several reports on such secondary effects, and the Achilles tendon seems to be especially vulnerable to fluoroquinolone-promoted tendinopathy (14). The low incidence of this tendon-damaging effect suggests that it may result from some intrinsic effects of fluoroquinolones that could be realized as a result of certain factors, such as age, sex (the male-to-female ratio of those affected is 3:1), concomitant corticosteroid therapy, especially in renal graft patients (27), duration of treatment (26), pathological state, and possibly, other unknown aggravating factors.

In order to characterize and amplify the intrinsic harmful effect of pefloxacin on proteoglycans and collagen, which are

the main biochemical components of the tendon, several investigations used relatively large doses of pefloxacin administered to rodents. As an oxidative event was observed in cartilage (11, 33), we considered the attractive hypothesis that the pathophysiological effects due to pefloxacin administration result from the same effects on both articular cartilage and tendon. On the one hand, the Achilles tendon and articular cartilage are characterized by a low level of or no blood perfusion, respectively, resulting in a low O_2 pressure (18). These conditions render them more susceptible to oxidative stress resulting from an incomplete reduction of oxygen in the mitochondria and the formation of superoxide. In apparent agreement, tendon ruptures occurred at a critical zone which is described as hypovascularized.

In this study, we evaluated the consequences of pefloxacin administration on cellular activity in the Achilles tendon by measuring the anabolism of proteoglycans, which have a fast metabolic turnover rate, after the administration of a single dose of pefloxacin to mice. On the other hand, a possible oxidative stress was assessed by measuring matrix modifications on collagen, which has a low turnover rate and which can retain oxidative alterations for 30 days. Pefloxacin-induced modifications of collagen were compared to those due to ischemia-reperfusion (I-R) of the Achilles tendon in rats. I-R was considered a model of oxidative injury in this tissue, as reactive oxygen species (ROSs) are important mediators of postischemic injury in various tissues (1, 8, 10). Finally, we evaluated the effect of *N*-acetylcysteine, a known antioxidant, on the modifications to collagen induced by pefloxacin. The results obtained provide new insights into the mechanism of fluoroquinolone-induced tendinopathies.

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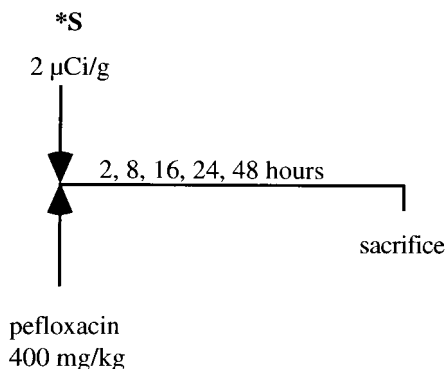


FIG. 1. Kinetics of incorporation of ³⁵S in blood and Achilles tendons in vivo in mice: effect of pefloxacin administration during the first phase (early effects of pefloxacin). *S, Na₂³⁵SO₄.

MATERIALS AND METHODS

Animals. Four- to 6-week-old male Sprague-Dawley rats (weight, 125 to 150 g) and 3- to 4-week-old male Swiss mice (weight, 15 to 20 g) (Charles River, Saint-Aubin-lès-Elbeuf, France) were housed in solid-bottom plastic cages designed to allow easy access to standard laboratory food and water ad libitum. The animals were kept in a 12-h light and 12-h dark cycle in a temperature-controlled chamber.

Kinetics of incorporation of ³⁵S in blood and Achilles tendons in vivo in mice: effect of pefloxacin administration. (i) **Effects during the first phase (early effects).** Mice received by gavage either a single dose (400 mg/kg of body weight daily; 10 μl/g with saline as the vehicle) of pefloxacin dihydrate mesylate (provided by Bellon Laboratories, Neuilly/Seine, France) or saline solution (as a control) (n = 5 per group) and a simultaneous intraperitoneal injection of Na₂³⁵SO₄ (2 μCi/g body weight). Animals were decapitated 2, 8, 16, 24, or 48 h later (Fig. 1). Blood samples were collected, and the Achilles tendons were dissected out and were placed overnight in 1 ml of cetylpyridinium chloride (Sigma) in phosphate-buffered formalin. They were dissolved overnight in Soluene-350 (Packard, Rungis, France). The amount of [³⁵S]sulfate incorporated into each sample was counted by liquid scintillation spectrometry in 4.5 ml of Hionic Fluor (Packard) as the scintillation fluid. Blood samples (10 μl) were decolorized by adding 100 μl of propan-2-ol and 100 μl of H₂O₂. Results are expressed as the difference between the mean percentage of ³⁵S incorporated into the Achilles tendons from pefloxacin-treated animals and the mean percentage of ³⁵S incorporated into the Achilles tendons from the control animals treated with the vehicle alone.

(ii) **Effects during the second phase (24 to 48 h).** Weight-matched mice received by gavage a single dose of pefloxacin (400 mg/kg) and 24 h later received an intraperitoneal injection of radioactive sulfate (2 μCi of Na₂³⁵SO₄/g) (n = 5 per group with two experiments). At 48 h after pefloxacin administration, blood was sampled and Achilles tendons were removed as described above (Fig. 2). The

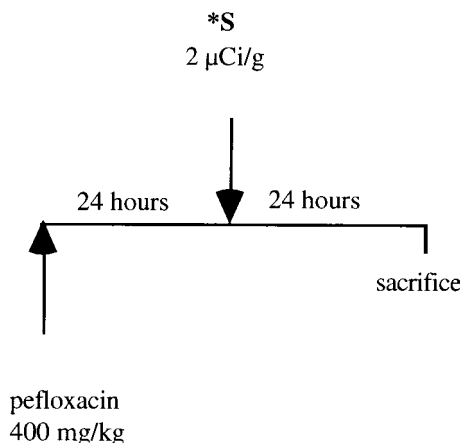


FIG. 2. Kinetics of incorporation of ³⁵S in blood and Achilles tendon in vivo in mice: effect of pefloxacin administration during the second phase (24 to 48 h). *S, Na₂³⁵SO₄.

³⁵S contents in the blood of both treated and untreated mice were identical to those in serum derived from the blood. Thus, blood samples were used and were considered equivalent to serum samples for measurement of circulating inorganic radi sulfate contents.

Measurement ex vivo of proteoglycan synthesis. An assay for measurement of proteoglycan synthesis was performed as described by Van den Berg et al. (36). Briefly, pefloxacin dihydrate mesylate was administered orally to mice (400 mg/kg daily; 10 μl/g), which were killed by cervical dislocation 24 or 48 h after drug administration. The Achilles tendons were carefully dissected and were incubated as described above by using RPMI culture medium (200 μl/patella or femoral head cap) containing gentamicin (50 μg/ml), L-glutamine (2 mM), and Na₂³⁵SO₄ (10 μCi/ml). After incubation for 2 h at 37°C in a 5% CO₂ atmosphere, the tendons were washed with isotonic saline solution and were fixed overnight in 0.5% cetylpyridinium chloride in phosphate-buffered formalin. The samples were then dissolved overnight in Soluene-350.

Experimental model of I-R of Achilles tendons of rats. In the experiments with the I-R model, the animals were separated into four groups (with five rats per group). Pefloxacin was administered to rats in group 1 (400 mg/kg/day) for 7 days, and I-R was achieved on the 4th day of pefloxacin treatment. Rats in group 2 were submitted to tendon I-R without pefloxacin treatment. Rats in group 3 were given pefloxacin for 7 days and underwent sham surgery. Control rats from group 4 that underwent sham surgery received saline solution. Anesthesia was achieved by intraperitoneal administration of ketamine (Imalgem; 0.1 ml/100 g). Midline incisions of 3 cm were made over the right and the left Achilles tendons, and the tendons were isolated from the surrounding fascia. To promote ischemia, the myotendinous portion and the calcaneal insertion were ligated by a suture technique (with Ethicon sutures). The skin was then sutured. Two hours later, the ligations were removed while the rats were under anesthesia to allow reperfusion and the skin was again sutured.

Collagen extraction. Collagen extraction was performed with rat and mouse Achilles tendons, which were washed for 1 h at 4°C with water and neutral salt solution (0.05 M Tris-HCl, 0.9% NaCl [pH 7.4]) to remove soluble material. The residue was added to a solution of 1 mg of pepsin per ml in 0.5 M acetic acid at a ratio of 1/10 (sample/pepsin), and the mixture was stirred for 2 days at 4°C (24). Undigested solid material was removed by centrifugation at 30,000 × g for 30 min. The operational conditions for pepsin digestion were chosen to be sufficient to extract ≥90% of the collagen, as determined by measuring the hydroxyproline content of the supernatant after pepsin digestion and of the tendons after acid hydrolysis (37). The protein concentrations of the samples were determined by the assay described by Lowry et al. (22) by using bovine serum albumin as the standard.

Protein derivatization with DNPH and by SDS-PAGE. The fraction extracted from the Achilles tendon (50 μg) was treated with an equal volume of 0.5 mM dinitrophenylhydrazine (DNPH; in 0.1 M sodium phosphate buffer [pH 6.3]; Sigma) for 1 h at room temperature. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of collagen was performed as described by Laemmli (19) in 1-mm-thick 6% slab gels (16- by 18-cm format). The same amount of collagen (15 or 7 μg) was loaded into all lanes. After SDS-PAGE, the gels were transferred onto Immobilon-P membranes (Sigma) with a Trans-Blot electrophoretic transfer apparatus as described by Towbin et al. (34). Immunochemical detection of protein carbonyls was performed as described elsewhere (16, 29). Briefly, the blots were incubated with 0.1% (wt/vol) Ponceau solution (Sigma) in 5% (vol/vol) acetic acid and were destained in methanol until bands appeared. Then the blots were incubated with bovine serum albumin (3%) for at least 90 min, followed by an incubation at room temperature with rabbit anti-dinitrophenyl antibodies (diluted 1:2,000 in 9 mM Tris-HCl [pH 9.0], 154 mM NaCl, 0.05% [vol/vol] Tween 20 [TBST]; Sigma). The primary antibody was removed, and the blots were washed three times for 10 min each time with TBST. The blots were incubated with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G (diluted 1:5,000 in TBST; Sigma) for 90 min at room temperature. After washing the blots with TBST three times for 10 min each time, oxidized proteins were revealed by the addition of 5-bromo-4-chloro-3-indolylphosphate-nitroblue tetrazolium. Optical densities were acquired with an image analysis system (NIH Image 1.54).

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

RESULTS

In vivo effects of a single dose of pefloxacin on the content of ³⁵S in Achilles tendons of mice. (i) **Effects during the first phase (early effects).** The effect of a single oral dose (400 mg/kg) of pefloxacin on proteoglycan synthesis in mice, as measured by the incorporation in vivo of ³⁵S given intraperitoneally at the same time as pefloxacin, varied with the tissue studied. In both treated and control mice, the amount of radioactivity in blood decreased with time, whereas in the Achilles tendon it increased to a maximum at 16 to 24 h (Fig. 3) and

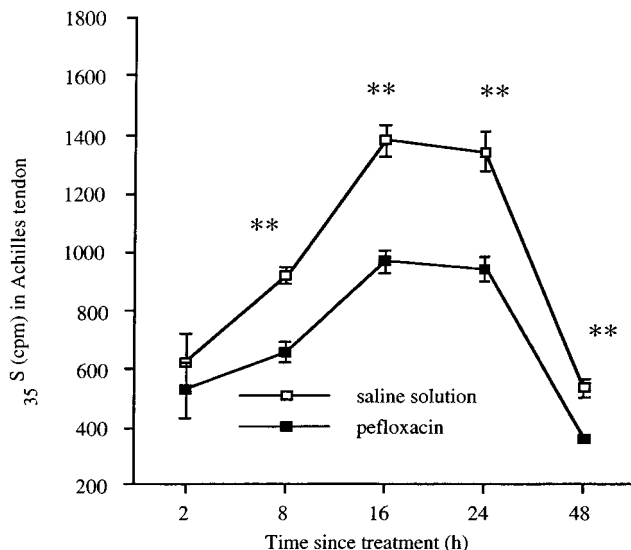


FIG. 3. Effect in mice of a single oral dose (400 mg/kg) of pefloxacin on the *in vivo* incorporation of simultaneously administered ^{35}S into the Achilles tendon. Control animals were given saline solution instead of pefloxacin. Values for ^{35}S are means \pm SEMs ($n = 4$ experiments). *, $P < 0.05$, and **, $P < 0.01$, versus controls (Student's *t* test).

then decreased until 48 h after the injection. The radioactivity in the Achilles tendon was 29% less in treated animals than in control animals 8 h after pefloxacin administration. Similar differences between treated animals and control animals were seen at 16 and 24 h and persisted until 48 h. Throughout these assays, the urinary excretion of ^{35}S was twofold higher in the treated mice than in the control mice (data not shown).

(ii) **Effects during the second phase (24 to 48 h).** Forty-eight hours after the administration of a single dose of pefloxacin (400 mg/kg) and 24 h after injection of ^{35}S , proteoglycan synthesis was higher in the Achilles tendons of the experimental group than in the Achilles tendons of the control group (27%), as measured by incorporation of ^{35}S (Fig. 4). Identical results

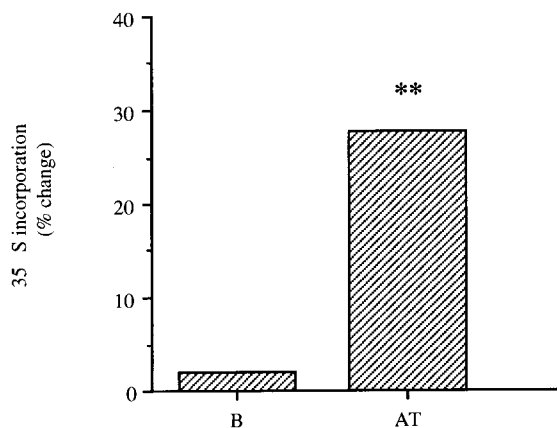


FIG. 4. *In vivo* effects in mice of a single oral dose (400 mg/kg) of pefloxacin on proteoglycan synthesis as indicated by changes in ^{35}S incorporation 48 h after pefloxacin administration and 24 h after intraperitoneal administration of ^{35}S (2 $\mu\text{Ci/g}$). Control animals received saline only. Values are percent differences in ^{35}S incorporation in treated mice and controls ($n = 2$ experiments each with five mice). B, blood; AT, Achilles tendon. *, $P < 0.05$, and **, $P < 0.01$, versus controls (Student's *t* test).

(increase by 32%) were obtained with the fibrocartilage of the peripheral part of the patellae (data not shown). The amounts of radioactivity in blood were identical in both experimental mice and control mice.

Ex vivo effects in mice of a single dose of pefloxacin on the content of ^{35}S in Achilles tendon 24 and 48 h after administration of a single oral dose (400 mg/kg). Pefloxacin decreased by 29% the level of *ex vivo* incorporation of ^{35}S in the Achilles tendons 24 h after administration (data not shown). In addition, the level of proteoglycan synthesis in the Achilles tendons returned to the control level 48 h after pefloxacin administration.

Influence of pefloxacin treatment (400 mg/kg/day) on carbonyl derivative formation in type I collagen in mice. We examined the collagen extracted from the Achilles tendons of both control animals and mice treated with pefloxacin for 7 days. SDS-PAGE and immunoblotting of tendon collagen showed the two characteristic bands from the $\alpha 1(\text{I})$ and $\alpha 2(\text{I})$ chains of collagen type I. Figures 5A and B show that the collagen extracted from the tendons of mice treated with pefloxacin for 7 days had a higher carbonyl content than the collagen extracted from the tendons of control mice. This was observed for both $\alpha 1(\text{I})$ and $\alpha 2(\text{I})$ chains.

Influence of pefloxacin treatment (400 mg/kg/day) and of an experimental tendinous I-R on carbonyl derivative formation in type I collagen in rats. Tendons were submitted to a 2-h ischemia followed by a reperfusion for 3 or 7 days. The tendons were analyzed for their collagen contents under the conditions described above. Before the experimentation with this model, pefloxacin was administered orally to the rats 7 days (twice at 200 mg/kg/day), whereas the experimental I-R was achieved on the 4th day. An increase in the level of carbonyl derivatives from collagen type I was observed in the tendons of rats which received pefloxacin for 7 days, and a similar effect was observed in rat tendons submitted to I-R (Fig. 6A and B). This response was observed for the $\alpha 1(\text{I})$ chains only, as there was no significant increase in the carbonyl contents of the $\alpha 2(\text{I})$ chains. The same increase was observed when rats were first treated with pefloxacin and then submitted to an ischemia (2 h)-reperfusion (3 days) of the tendon.

Influence of coadministration of pefloxacin (400 mg/kg/day) and N-acetylcysteine (150 mg/kg/day) on carbonyl derivative formation in type I collagen in mice. We examined the collagen extracted from the Achilles tendons of both control animals and mice treated with pefloxacin (400 mg/kg/day) and N-acetylcysteine (150 mg/kg/day) for 10 days. The increase in the carbonyl content of collagen observed in mice treated with pefloxacin was prevented by the simultaneous administration of N-acetylcysteine. No changes were observed between control animals and rats which received N-acetylcysteine only (Fig. 7A and B).

DISCUSSION

Fluoroquinolone derivatives are characterized by good tissue penetration, a broad antibacterial spectrum, and a relatively low incidence of serious side effects. However, quinolones may have adverse effects on the musculoskeletal system, but with a very low incidence (1% or less [32]). Recently, adult or old-age tendinopathy, sometimes resulting in spontaneous rupture, has been considered a side effect of treatment with these drugs. The Achilles tendon seems to be especially affected, but other targets may also be damaged (20). Pefloxacin has been the subject of several studies of such damage, probably both because of the large number of prescriptions for pefloxacin and because of the high level of diffusion of pefloxa-

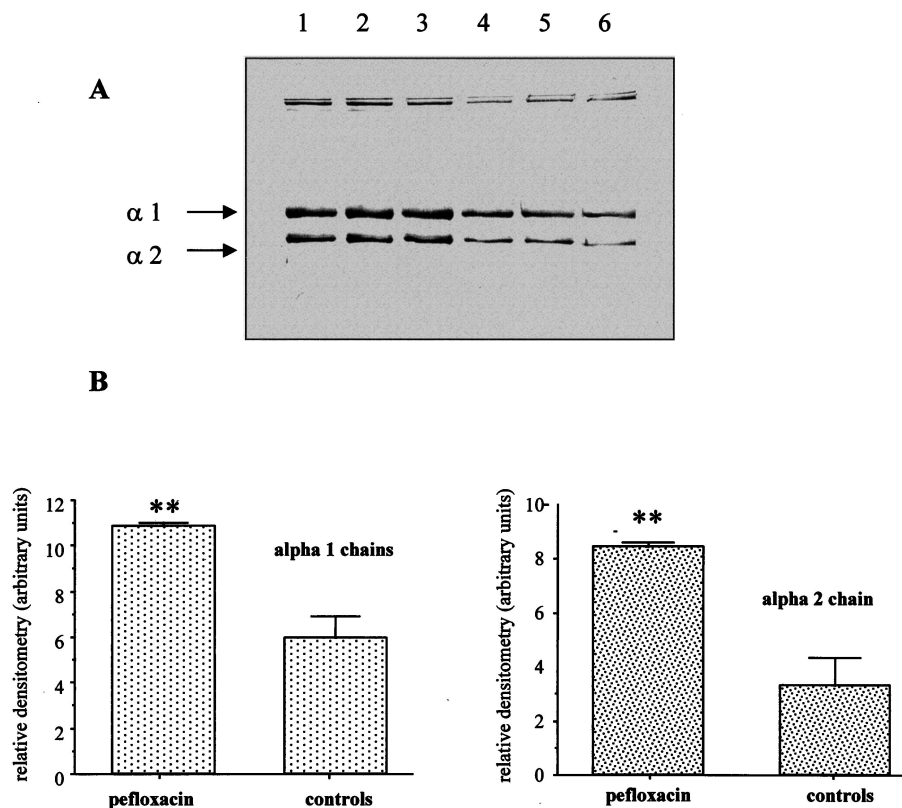


FIG. 5. Immunochemical detection of carbonyl groups in Achilles tendon collagen of mice after pefloxacin administration. A total of 10 μ g of DNPH-derivatized protein was loaded onto each lane. (A) Lanes 1 to 3, Achilles tendon collagen of mice treated with pefloxacin (400 mg/kg/day) for 7 days; lanes 4 to 6, Achilles tendon collagen of control mice. (B) Densitometric analysis of the immunoblots in panel A. Values are means \pm standard deviations. *, $P < 0.05$, and **, $P < 0.01$, versus controls (Student's t test).

cin into tissues (5, 15). In this study, we demonstrated for the first time that pefloxacin induces an oxidative stress on proteoglycans and collagen, which are the main constituents of tendon, by using relatively large doses of pefloxacin in order to amplify the drug effects. Measurement of proteoglycan anabolism in mice revealed modifications of the cellular activity after administration of a single dose of pefloxacin, and tissue alterations were confirmed by detection of an increase in the oxidation markers of collagen after several days of treatment of rats.

The pefloxacin-induced alterations in the Achilles tendon were revealed in mice by measurement of the level of ^{35}S incorporation in vivo, which is a highly sensitive and reproducible assay for proteoglycan synthesis. This approach allows quantification of both the early and the late changes in proteoglycan synthesis induced by pefloxacin by using various time delays between pefloxacin administration and ^{35}S injection. In control mice, very low levels of radioactivity remained in the blood 24 h after ^{35}S injection (30), whereas in the tendon, the radioactivity peaked at between 16 and 24 h. When mice received either pefloxacin or saline solution and a simultaneous intraperitoneal ^{35}S injection, the amount of radioactivity in the tissue studied was significantly lower in pefloxacin-treated mice than in control mice at every time during the first phase (after 8, 16, 24, and 48 h). As demonstrated in a previous work (30), pefloxacin induced a marked fall in the endogenous serum sulfate level. This effect was similar to the effects of salicylate on cartilage, as determined by using different times between drug and radiolabeled sulfate administrations (6). In order to

determine whether the decrease in ^{35}S incorporation during the first phase results from a direct effect of pefloxacin or from the lower concentration of sulfate, we evaluated the effects of pefloxacin in ex vivo experiments (data not shown). In these ex vivo studies, by using an identical sulfate concentration for both the controls and the assays, an inhibition of ^{35}S incorporation was observed 24 h after pefloxacin administration (-29%), as was also demonstrated in vivo. Therefore, this depletion phase in proteoglycan synthesis seems to be a direct effect of pefloxacin on tissue metabolism rather than a result of a depletion of the sulfate in serum.

Moreover, 48 h after a single pefloxacin administration (400 mg/kg) and 24 h after ^{35}S injection, pefloxacin induced an increase in the level of ^{35}S in the Achilles tendon (27%) and also in the peripheral fibrocartilage of the patellae (32%; data not shown). Under these conditions, proteoglycan synthesis suggests the onset of a tissue-specific repair process in response to a previous deleterious effect. All these changes suggest a fibroproliferative response, presumably in an attempt to repair the tendon defect resulting from the depletion phase.

The depletion phase in proteoglycan synthesis observed after pefloxacin administration also appeared in articular cartilage. However, 48 h after pefloxacin administration and 24 h after ^{35}S injection, the level of ^{35}S incorporation in cartilage did not differ from that in controls (30). Therefore, we postulate that repair-like responses to the early inhibition of proteoglycan synthesis promoted by pefloxacin are regulated differently in tendon and fibrocartilage than in cartilage. Our results also suggest that a single dose of pefloxacin induced a

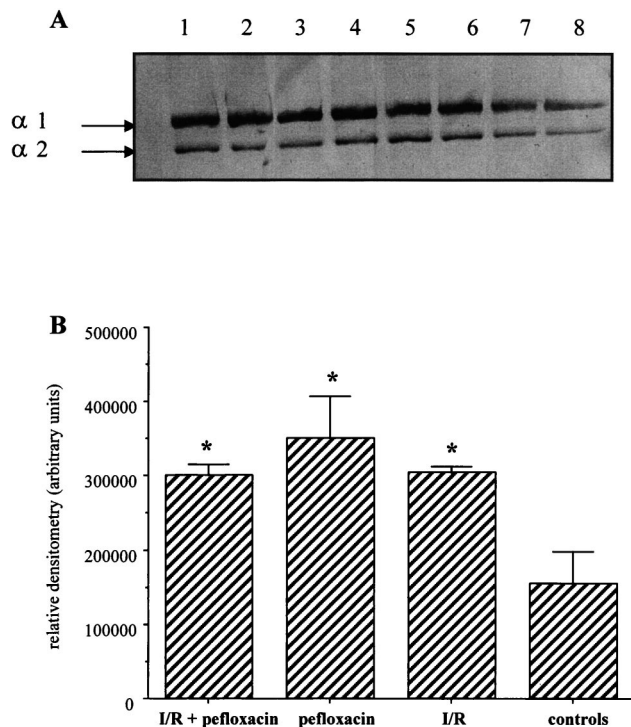


FIG. 6. Immunochemical detection of carbonyl groups in Achilles tendon collagen of rats after pefloxacin administration and ischemia (2 h) and reperfusion (3 days). A total of 15 μ g of DNP-derivatized protein was loaded onto each lane. (A) Lanes 1 and 2, Achilles tendon collagen of rats treated with pefloxacin (400 mg/kg/day) for 7 days; for this group I-R and sham surgery were done on the 4th day of pefloxacin treatment (duplicate loads); lanes 3 and 4, Achilles tendon collagen of rats that underwent sham surgery and that were treated with pefloxacin (400 mg/kg/day) for 7 days (duplicate loads); lanes 5 and 6, Achilles tendon collagen of rats submitted to a tendinous I-R (duplicate loads); lanes 7 and 8, Achilles tendon collagen of rats that underwent sham surgery and that received saline solution for 7 days. (B) Densitometric analysis of the immunoblots in panel A. Values are means \pm standard deviations. *, $P < 0.05$, and **, $P < 0.01$, versus controls (Student's t test).

cellular damage which leads to a biphasic effect on Achilles tendon proteoglycan synthesis: a transitory decrease followed by a repair-like response.

Together, the results of the present study suggest that fluoroquinolone-induced tendinopathies occur by the same pathophysiological pathways as those described for cartilage (12, 24). Previous reports suggested compromised mitochondrial activity, and a precocious stimulation of the oxidative metabolism within immature articular chondrocytes was also described (11, 33), with both of these resulting in the generation of reactive oxygen species. Therefore, we looked for an eventual pefloxacin-induced oxidative stress on the collagen isolated from the Achilles tendon. We observed in mice that after a 7-day pefloxacin treatment, an increase in the level of carbonyl derivatives was observed in type I collagen, indicating oxidative damage. These oxidative changes were observed only after at least 5 days of pefloxacin administration (data not shown). We attach particular importance to the similar effect that appeared during Achilles tendon I-R, which leads to ROS production, which is the main mediator of postischemic injury in various tissues. A synergistic increase in carbonyl formation was not detected after pefloxacin treatment was applied during an I-R, perhaps indicating a saturation of collagen-oxidizable sites. Curiously, pefloxacin induced an oxidative modification in both $\alpha 1(I)$ and $\alpha 2(I)$ chains in mice, whereas only $\alpha 1(I)$ chains

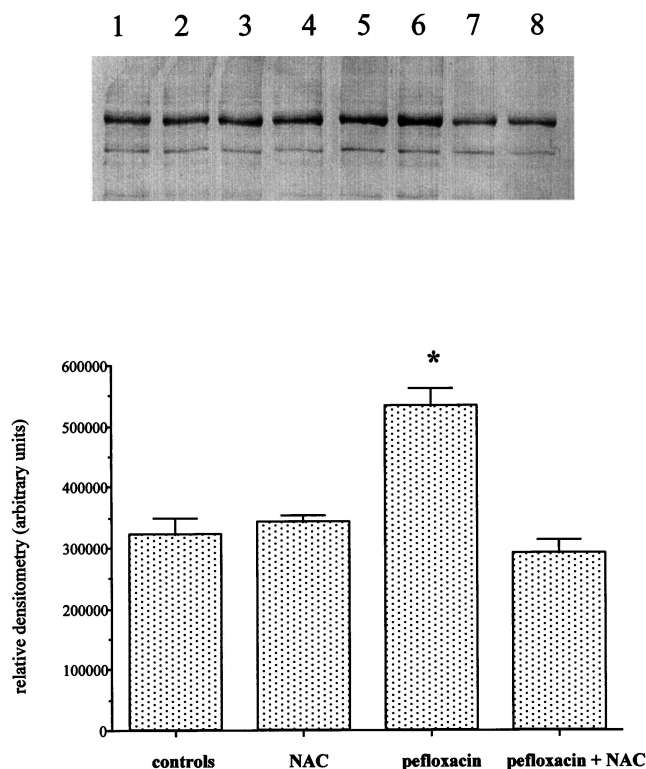


FIG. 7. Immunochemical detection of carbonyl groups in Achilles tendon collagen of mice after coadministration of pefloxacin (400 mg/kg/day) and *N*-acetylcysteine (NAC; 150 mg/kg/day) for 10 days. A total of 10 μ g of DNP-derivatized protein was loaded onto each lane. (A) Lanes 1 and 2, Achilles tendon collagen of controls receiving saline solution for 10 days (duplicate loads); lanes 3 and 4, Achilles tendon collagen of mice receiving *N*-acetylcysteine (150 mg/kg/day) for 10 days (duplicate loads); lanes 5 and 6, Achilles tendon collagen of mice receiving pefloxacin (400 mg/kg/day) for 10 days (duplicate loads); lanes 7 and 8, Achilles tendon collagen of mice receiving pefloxacin (400 mg/kg/day) and, simultaneously, *N*-acetylcysteine (150 mg/kg/day) for 10 days (duplicate loads). (B) Densitometric analysis of the immunoblots in panel A. Values are means \pm standard deviations. *, $P < 0.05$ versus controls (Student's t test).

were affected in rats. Moreover, this study demonstrated that *N*-acetylcysteine, a thiol antioxidant, prevented the pefloxacin-induced oxidative modifications of Achilles tendon collagen. We also observed that pefloxacin treatment induced oxidative damage on type II collagen from articular cartilage (30). These results suggest that the hypoxic conditions of the cartilage and tendon could lead fluoroquinolones to particular redox levels, allowing the generation of free radicals which promote cell and tissue damages. In particular, superoxide has been shown to exert direct deleterious effects on collagen fibers and could oxidize susceptible amino acids in collagen and change the protein conformation (24). In the same way, ROSs could be toxic to matrix components or could act as activators of metalloproteinases (2, 21).

In conclusion, the present study provides new insights into fluoroquinolone-induced tendinopathies. A single large dose of pefloxacin promoted a change in proteoglycan synthesis, with a precocious inhibition followed by a repair-like phase. We also reported that pefloxacin induced oxidative damage to collagen that was similar to that resulting from an experimental I-R of the tendon and that was prevented by the simultaneous administration of *N*-acetylcysteine, therefore suggesting in both cases the involvement of ROSs. Individual factors of

susceptibility such as participation in sports, age, or corticosteroid therapy possibly do not allow the tendon to repair adequately, resulting in irreversible matrix alterations that might explain the occurrence of tendinopathies that sometimes result in the rupture of the tendon (35). Accordingly, in vitro experiments with cultured tendon cells have confirmed the clinical observations that the administration of other drugs in parallel with fluoroquinolones can increase the risk of tendon rupture (17). The doses of pefloxacin administered to rodents in this study are much larger than those usually delivered to humans. Nevertheless, the half-lives of this drug range from 1.9 h in mice to 8.6 h in humans (25). Thus, pefloxacin is metabolized more rapidly in mice and this dose may allow a sufficient diffusion into tissue. Therefore, the results presented here should be considered with care in relation to the adverse effects of pefloxacin reported in humans.

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