Ciprofloxacin Inhibition of Experimental Fracture-Healing*

BY P. M. HUDDLESTON, M.D.†, J. M. STECKELBERG, M.D.†, A. D. HANSSEN, M.D.†, M. S. ROUSE, B.S.†, M. E. BOLANDER, M.D.†, AND R. PATEL, M.D.†, ROCHESTER, MINNESOTA

Investigation performed at Mayo Clinic and Mayo Foundation, Rochester

Abstract

Background: **Fluoroquinolones, such as ciprofloxacin, have an adverse effect on growing cartilage and endochondral ossification in children. This study was carried out to determine whether ciprofloxacin also has an adverse effect on the healing of experimental fractures.**

Methods: **Sixty male 300-gram Wistar rats were divided equally into three groups, which received ciprofloxacin, cefazolin, or no treatment for three weeks, beginning seven days after production of a closed, nondisplaced, bilateral femoral fracture. The serum concentrations of the ciprofloxacin and the cefazolin were 2.4 and 146 micrograms per milliliter, respectively. Radiographic, histological, and biomechanical studies were used to evaluate fracture-healing.**

Results: **Radiographs revealed significantly more advanced healing of the control fractures compared with the fractures in the ciprofloxacin-treated group (average stage, 2.1 compared with 1.5, p = 0.01). The cefazolin-treated group was not different from the controls with respect to radiographic healing (average stage, 1.8 compared with 2.1, p = 0.18). Torsional strength-testing of fracture callus exposed to ciprofloxacin revealed a 16 percent decrease in strength compared with the controls (284 compared with 338 newton-millimeters, p = 0.04) and a 49 percent decrease in stiffness (twenty compared with thirty-nine newton-millimeters per degree, p = 0.001). The biomechanical strength in the cefazolin-treated group was not different from that of the controls. Fracture calluses in the animals treated with ciprofloxacin showed abnormalities in cartilage morphology and endochondral bone formation and a significant decrease in the number of chondrocytes compared with the controls (0.77×10^4 compared with 1.3×10^4 cells per square millimeter, p = 0.004).**

Conclusions: **These data suggest that experimental fractures exposed to therapeutic concentrations of ciprofloxacin in serum demonstrate diminished healing during the early stages of fracture repair. The administration of ciprofloxacin during early fracture repair may compromise the clinical course of fracture-healing.**

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†Department of Orthopedics (P. M. H., A. D. H., and M. E. B.) and Division of Infectious Diseases, Department of Internal Medicine, and the Infectious Diseases Research Laboratory (J. M. S., A. D. H., M. S. R., and R. P.), Mayo Clinic and Mayo Foundation, 200 First Street S.W., Rochester, Minnesota 55905.

The toxic effect of fluoroquinolones on articular cartilage has been described in a number of recent reports[3,21,29]. Cellular changes in articular cartilage, such as chondrocyte death and matrix degeneration, have also been reported following toxic doses of fluorinated quinolone antibiotics[13]. Several laboratory investigations have demonstrated articular cartilage erosions, fissure formation, and cartilage fibrillation associated with fluoroquinolone toxicity[7,20]. Use of fluoroquinolones in children is contraindicated because of these adverse effects on growing cartilage and bones[11]. However, the mechanism of experimentally and clinically induced fluoroquinolone arthropathy has not been defined[19,27,31].

In fracture callus, a rapidly growing cartilaginous complex undergoes intramembranous and endochondral ossification during the fracture repair process. The cartilaginous elements that are an integral part of the fracture callus potentially share the chondrocyte vulnerability to fluoroquinolone toxicity that is present in proliferating (growing) juvenile articular cartilage. Indeed, in our clinical practice, we have observed an increase in the prevalence of delayed union and nonunion when ciprofloxacin has been used in the early period of fracture-healing. As a consequence of these observations, this study was initiated to investigate the potentially adverse effect of ciprofloxacin on experimental fracture-healing.

Materials and Methods

Study Design

Sixty male 300-gram Wistar rats (Harlan Sprague Dawley, Indianapolis, Indiana) were arbitrarily assigned to three groups of twenty animals each. One experimental group was treated with ciprofloxacin, one was treated with cefazolin, and the third group was the control group. The procedures and the handling of the rats were reviewed and approved by the Institutional Animal Care and Use Committee. All rats received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health[12]. The rats were fed a standard chow (Lab Diet; PMI Feeds, St. Louis, Missouri), housed in a twelve-hour night-day-cycle environment, and allowed water without restriction. Nondisplaced bilateral fractures of the femur were established with use of a three-point bending device according to the model of Bonnarens and Einhorn[5].

Antibiotic administration was begun in the experimental groups seven days after the fracture and was continued for three weeks. Fifty milligrams of cipro-

floxacin per kilogram of body weight was administered subcutaneously twice daily at twelve-hour intervals, and fifty milligrams of cefazolin per kilogram of body weight was administered intramuscularly twice daily at twelve-hour intervals. This time-frame was chosen to approximate the clinical conditions of administering ciprofloxacin postoperatively, as in the treatment of a urinary tract or pin-site infection. The antibiotic dosage was selected to lead to peak concentrations of the drug in rat serum approximating those in humans. The control animals received no antibiotic treatment.

Four weeks after the fracture, all animals were killed with an overdose of sodium pentobarbital (Sleep-Away) and the intact fracture calluses were recovered under sterile conditions. The fracture calluses from each treatment group were assigned randomly, with use of statistical software (StatMost; DataMost, Salt Lake City, Utah), to one of four examinations: (1) quantitative cultures to rule out occult infection as a factor in fracture-healing, (2) strength-testing to failure in torsion, (3) histological evaluation, or (4) ultrastructural analysis. After the animals were killed, the soft tissues were removed from the femora, which were then stored in normal saline-solution-soaked gauze at –20 degrees Celsius. The specimens were thawed at room temperature for two hours prior to testing.

Mechanical Testing

Both bone ends were embedded in Cerrobend alloy (Cerrometal Products, Bellefonte, Pennsylvania) heated to just above its melting temperature (47 degrees Celsius). A fifteen-millimeter section of femur centered on the fracture was exposed. A custom jig ensured consistent alignment of the axis of the bone with the axis of the testing machine. Specimens were tested to failure in torsion at room temperature on an electromagnetic testing machine (model 1125; Instron, Canton, Massachusetts) at a rate of 5 degrees per second with no axial load. The maximum torque and torsional rigidity prior to fracture were calculated from a continuous x-y plot.

The biomechanical stage of fracture union was determined according to the method of White et al.[36], which defines stage 1 as failure through the original fracture site, with low stiffness; stage 2 as failure through the original fracture site, with high stiffness; stage 3 as failure partially through the original fracture site and partially through intact bone, with high stiffness; and stage 4 as failure entirely through intact bone, with high stiffness.

Radiographic Study

Immediately after the fracture was produced, radiographs of each rat femur were made to verify the position and characteristics of the fracture. After the animal was killed, the femora were disarticulated and radiographs were made for assessment of fracture-healing. Each fracture specimen was reviewed and classified for callus maturity according to the classification of Goldberg et al.[15]. In that classification, stage 1 indicates nonunion; stage 2, possible union; and stage 3, radiographic union.

Histological Examination

Immediately after the animals were killed, the fracture calluses were removed and were fixed for two to three days in 10 percent neutral buffered formalin followed by two days in Bouin's solution and then decalcified in a 10 percent acetic acid, 0.85 percent NaCl, and 10 percent formalin solution. Specimens were embedded in paraffin, sectioned longitudinally, and stained with hematoxylin and eosin or Masson's trichrome.

The progression of fracture-healing in each specimen was quantified with use of a scale that assigns a grade based on the relative percentages of fibrous tissue, cartilage, woven bone, and mature bone in the callus[1,17]. Grade 1 indicates fibrous tissue; grade 2, predominantly fibrous tissue with some cartilage; grade 3, equal amounts of fibrous tissue and cartilage; grade 4, all cartilage; grade 5, predominantly cartilage with some woven bone; grade 6, equal amounts of cartilage and woven bone; grade 7, predominantly woven bone with some cartilage; grade 8, entirely woven bone; grade 9, woven bone and some mature bone; and grade 10, lamellar (mature) bone. Four slides were examined for each fracture.

Histomorphometric evaluation of trabecular structure and chondrocyte characteristics was carried out with an Osteo Metrics image-analysis system (Atlanta, Georgia), consisting of a Compaq computer (Houston, Texas) interfaced with a microscope and image-analysis software[20,30]. Measurements of trabecular structure and of the size and number (cell density) of hypertrophic chondrocytes were made in the interface region (the region beneath the subperiosteal new bone formed by intramembranous ossification and the ends of the fractured femur). Measurements of the hypertrophic chondrocytes were made in the cartilage within 258.5 micrometers of this interface. Parameters that were evaluated included cell size (in square millimeters) and cell density (in cells per square millimeter). Evaluation of endochondral bone included (1) the area occupied by trabeculae composed of spicules of calcified cartilage covered by osteoid and newly formed bone, (2) the thickness of these trabeculae, and (3) the percentages of bone and calcified cartilage in these trabeculae.

Electron microscopy was performed on fracture callus specimens fixed in Trump's fixative (1 percent glutaraldehyde and 4 percent formaldehyde in 0.1-molar phosphate buffer, pH 7.2)[25]. Tissues were rinsed for thirty minutes in three changes of 0.1-molar phosphate buffer at pH 7.2 and then postfixed for one hour in phosphate-buffered 1 percent OsO_4. After three rinses for thirty minutes in distilled water, tissues were stained *en bloc* with 2 percent uranyl acetate for thirty minutes

TABLE I
CHARACTERIZATION OF HEALING OF EXPERIMENTAL FRACTURES*

Group	Radiographic Stage† (N = 20)	Biomechanical Stage‡ (N = 20)	Histological Grade§ (N = 4)
Control	2.1 ± 0.16	2.7 ± 0.26	5 ± 0.9
Cefazolin#	1.8 ± 0.17 (p = 0.18)	2.1 ± 0.21 (p = 0.07)	4.5 ± 1.1 (p = 0.17)
Ciprofloxacin#	1.5 ± 0.15 (p = 0.01)	1.75 ± 0.26 (p < 0.01)	3.5 ± 1.1 (p = 0.06)

*The values are given as the mean and the standard error.

†Stage 1 = fracture callus without bridging bone, stage 2 = fracture callus with bridging bone, and stage 3 = fracture callus with the fracture line no longer seen.

‡Stage 1 = failure through the original fracture site, with low stiffness; stage 2 = failure through the original fracture site, with high stiffness; stage 3 = failure partially through the original fracture site and partially through intact bone, with high stiffness; and stage 4 = failure entirely through intact bone, with high stiffness.

§Grade 1 = fibrous tissue in callus, grade 2 = predominantly fibrous tissue with some cartilage, grade 3 = equal amounts of fibrous tissue and cartilage, grade 4 = all cartilage, grade 5 = predominantly cartilage with some woven bone, grade 6 = equal amounts of cartilage and woven bone, grade 7 = predominantly woven bone with some cartilage, grade 8 = entirely woven bone, grade 9 = woven bone and some mature bone, and grade 10 = lamellar (mature) bone.

#The p values represent the significance of the difference compared with the control group.

at 60 degrees Celsius. After *en bloc* staining, the tissues were rinsed three times in distilled water, dehydrated in progressive concentrations of ethanol and 100 percent propylene oxide, and embedded in Spurr's resin[33]. Thin (ninety-micrometer) sections were cut with a Reichert Ultracut E ultramicrotome (Leica Microsystems, Wetzlar, Germany), placed on 200-mesh copper grids, and stained with lead citrate. Micrographs were made with a JEOL 1200 EXII electron microscope (Tokyo, Japan) operating at sixty kilovolts.

Microbiological and Pharmacokinetic Studies

The doses of cefazolin and ciprofloxacin were chosen to reproduce therapeutic concentrations achieved in clinical practice and determined through serum drug assays. Preliminary studies in rats demonstrated that subcutaneous administration of fifty milligrams of ciprofloxacin per kilogram of body weight and intramuscular administration of fifty milligrams of cefazolin per kilogram of body weight achieved concentrations in serum at one hour of 2.4 and 146 micrograms per milliliter, respectively. These doses are equivalent to peak concentrations observed in humans after oral administration of ciprofloxacin and intravenous administration of cefazolin[26].

The presence of any viable bacteria at the fracture sites was determined with the techniques of Washington[35]. After the animals were killed, the fracture specimens were weighed, immediately frozen at –70 degrees Celsius, and then pulverized. The pulverized bone was suspended in nutrient broth and vortexed, and serial dilution aliquots were subcultured on sheep blood agar culture plates. The blood agar plates were incubated for forty-eight hours at 37 degrees Celsius in a 5 percent carbon dioxide atmosphere. The plates were inspected after incubation for bacterial growth. There were no positive bacterial cultures of fracture callus suggestive of a postoperative infection in any group.

Statistical Analysis

Statistical analyses were performed under the null hypothesis of no histological, radiographic, or biomechanical distinction among fractures exposed to ciprofloxacin, fractures exposed to cefazolin, and controls. Torsion strength was analyzed with use of the Student t test (two-tailed, unpaired for unknown variance) and analysis of variance between experimental groups. Histomorphometric parameters were analyzed with analysis of variance. The Wilcoxon rank-sum test was used for nonparametric data to analyze the results of radiographic and biomechanical stages of fracture-healing.

Results

Radiographic Analysis

The ciprofloxacin-treated fractures had significantly less bridging bone and thus less advanced radiographic healing in the fracture callus compared with the control fractures (average stage[15], 1.5 compared with 2.1, p = 0.01) (Table I). The fractures treated with cefazolin were not significantly different from the controls with regard to radiographic healing (average stage, 1.8 compared with 2.1, p = 0.18). The fractures treated with cefazolin had more advanced healing than those treated with ciprofloxacin (average stage, 1.8 compared with 1.5, p = 0.18), but the difference was not significant.

Mechanical Testing

Biomechanical analysis of fracture-healing showed that the control fractures had a significantly higher mean stage[36], indicative of more advanced fracture-healing, than the ciprofloxacin-treated fractures (2.7 compared with 1.75, p < 0.01) (Table I). Comparison of the controls with the cefazolin-treated fractures revealed no significant difference in this parameter (2.7 compared with 2.1, p = 0.07). The cefazolin-treated fractures had a higher mean stage of healing than the ciprofloxacin-treated fractures (2.1 compared with 1.75,

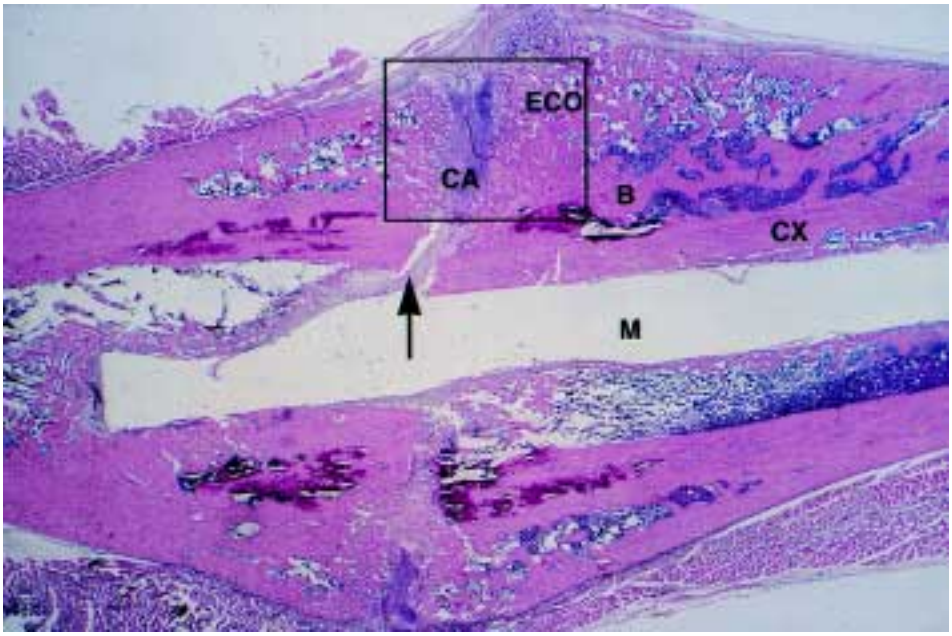

FIG. 1-A

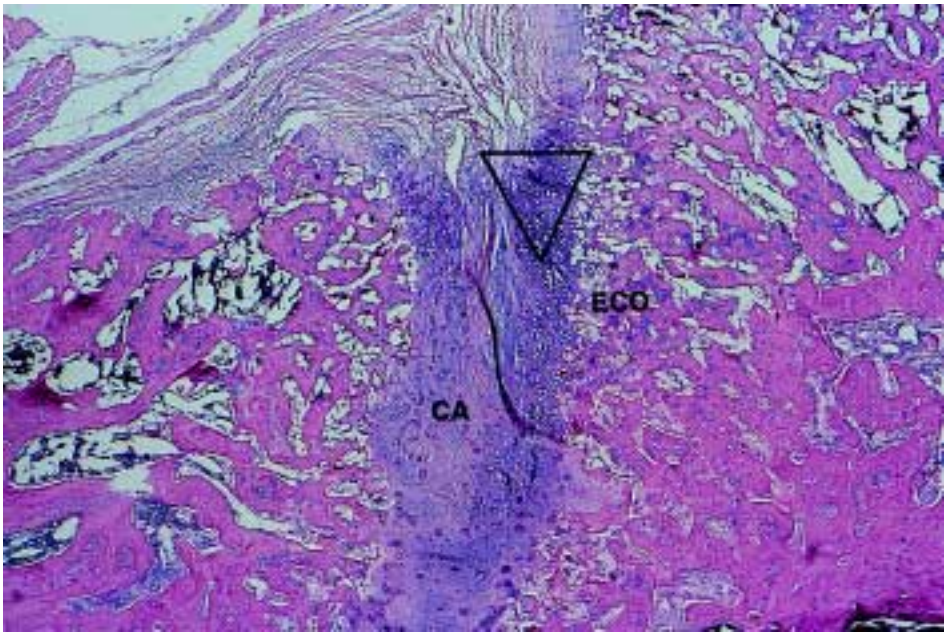

FIG. 1-B

Histological appearance of the fracture callus from a control rat after four weeks of healing. A low-magnification photomicrograph (Fig. 1-A) shows the formation of an external callus consisting of subperiosteal bone and cartilage. The marrow cavity shows the space left after removal of the rod that was used to stabilize the fracture. A high-magnification view (Fig. 1-B) of the boxed region in Fig. 1-A shows basophilic-stained tissue with large cells, indicating that this tissue is hypertrophic cartilage. Vascular invasion of the hypertrophic cartilage and the formation of spicules of bone and cartilage indicate that new bone is forming by endochondral ossification. The hypertrophic cartilage completely fills the space between regions of endochondral bone formation. CA = cartilage, ECO = endochondral bone, B = intramedullary bone, CX = cortex, M = intramedullary cavity, arrow = fracture site, and open arrowhead = hypertrophic chondrocytes (original magnification × 25 [Fig. 1-A] and × 125 [Fig. 1-B]).

$p = 0.18$), but the difference also was not significant.

Torsional testing revealed a 16 percent decrease in strength (284 compared with 338 newton-millimeters, $p = 0.04$) and a 49 percent reduction in stiffness (twenty compared with thirty-nine newton-millimeters per degree, $p = 0.001$) in the ciprofloxacin-treated group compared with the controls (Table II). The ciprofloxacin-treated specimens were also weaker than the specimens exposed to cefazolin with regard to torque strength (284 compared with 327 newton-millimeters, $p = 0.09$) and stiffness (twenty compared with thirty-six newton-millimeters per degree, $p = 0.01$); however, the difference was significant only for the stiffness parameter. The cefazolin-treated fractures were not significantly

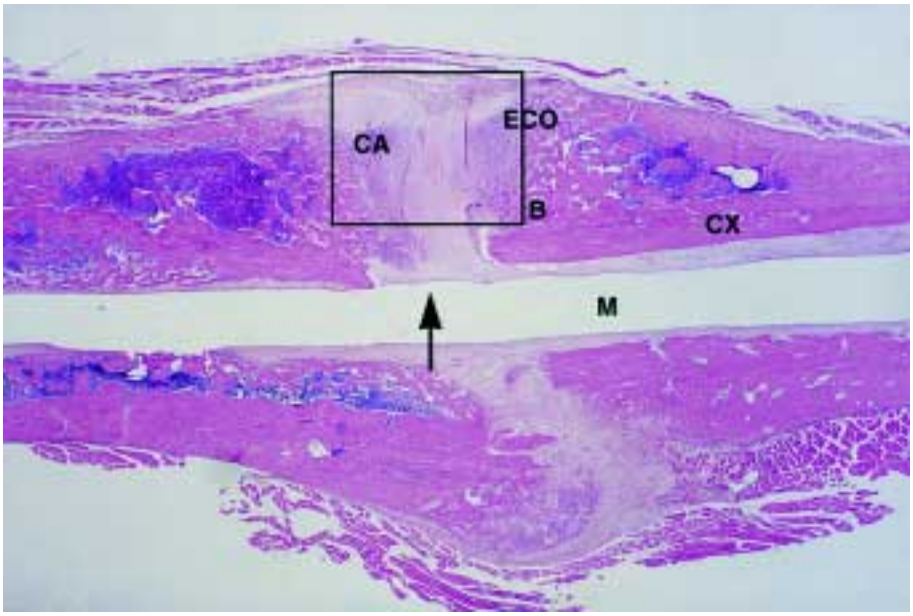

FIG. 2-A

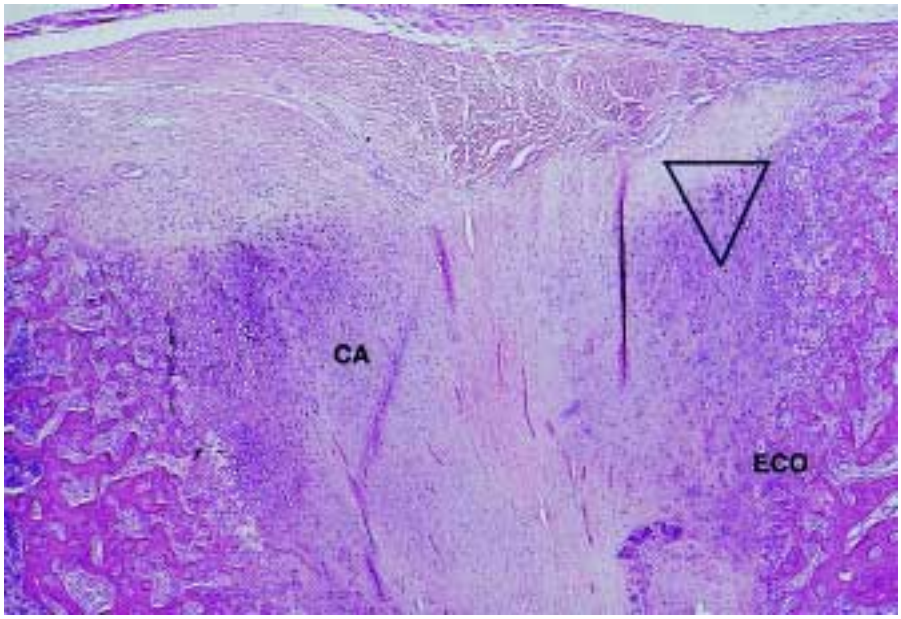

FIG. 2-B

Histological appearance of the fracture callus from a ciprofloxacin-treated rat after four weeks of healing. A low-magnification photomicrograph (Fig. 2-A) shows subperiosteal bone and cartilage in an external callus. The lack of basophilic staining in the center of the callus suggests that this region is not completely filled with cartilage. The marrow cavity shows the space left after removal of the rod used to stabilize the fracture. A high-magnification view (Fig. 2-B) of the boxed region in Fig. 2-A also shows vascular invasion, hypertrophic cartilage, and bone formation by endochondral ossification. The loss of basophilic staining, the flattened cell morphology, and the decreased cellularity confirm the presence of poorly differentiated chondrocytes and fibrous tissue in the center of the callus. CA = cartilage, ECO = endochondral bone, B = intramedullary bone, CX = cortex, M = intramedullary cavity, arrow = fracture site, and open arrowhead = hypertrophic chondrocytes (original magnification × 25 [Fig. 2-A] and × 125 [Fig. 2-B]).

different from the control fractures with regard to either torsional strength ($p = 0.64$) or stiffness ($p = 0.55$).

Histological Analysis

The control fractures had a higher mean grade[17] of histological healing than the cefazolin-treated fractures (5 compared with 4.5, $p = 0.17$) and than the ciprofloxacin-treated fractures (5 compared with 3.5, $p = 0.06$), indicating a trend but not a significant difference (Table I).

Low-magnification histological analysis of the control fractures revealed formation of an external callus consisting of subperiosteal bone and cartilage (Fig. 1-A). Hypertrophic cartilage filled the entire space between the subperiosteal bone regions of the callus. Vascular invasion of the hypertrophic cartilage associated with endochondral ossification occurred in the callus at the

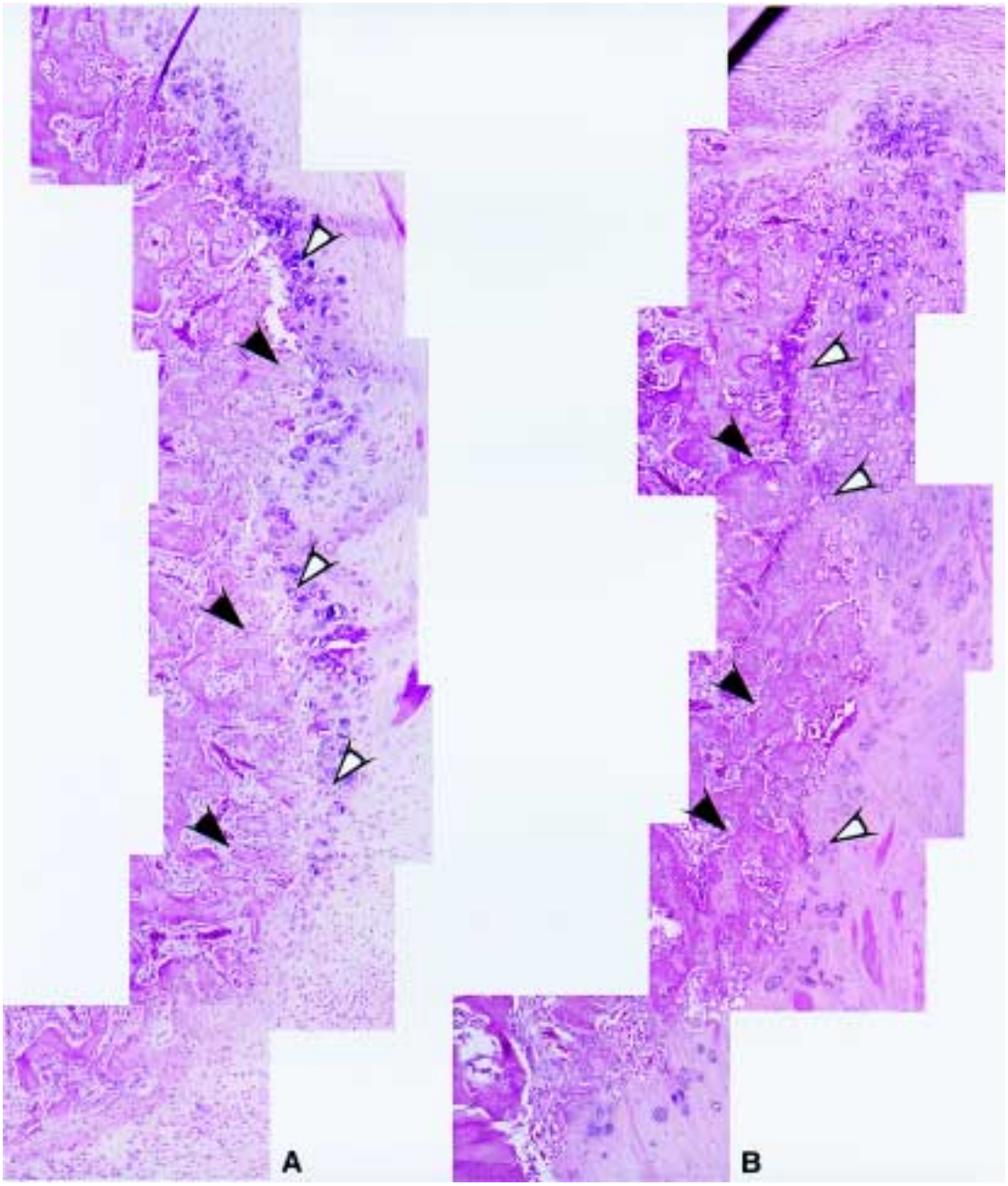

FIG. 3

High-magnification photomicrographs showing the histological appearance of the endochondral ossification front in calluses from control (A) and ciprofloxacin-treated (B) animals. In the specimen from the ciprofloxacin-treated animal, the hypertrophic chondrocytes appear larger and the cartilage matrix is less cellular. The thickness of the new bone trabeculae is decreased, and marrow cellularity is reduced. Open arrowheads = hypertrophic chondrocytes, and closed arrowheads = bone trabeculae with a cartilage core (hematoxylin and eosin, original magnification × 250).

interface of bone and cartilage. At higher magnification, this bone-cartilage interface was seen to have the histological characteristics of endochondral ossification, including vascular invasion into regions of hypertrophic chondrocytes, os1teoid deposition on spicules of calcified cartilage, and formation of spicules of cartilage covered by woven bone (Fig. 1-B). With the continued deposition of bone, the cartilage-bone spicules thickened into the normal trabecular bone in the hard callus. Evaluation of the specimens from the cefazolin-treated animals revealed the same pattern of external callus formation, vascular invasion of hypertrophic cartilage, and endochondral ossification as was seen in the callus from the control animals.

The specimens from the ciprofloxacin-treated animals had the same general pattern of external callus formation, vascular invasion of hypertrophic chondrocytes, and endochondral ossification (Fig. 2-A). However, differences were observed between the calluses from these animals and the calluses from the cefazolin-

TABLE II
MECHANICAL PROPERTIES OF THE EXPERIMENTAL FRACTURE CALLUS*

Group	No. of Fractures	Torque (*N-mm*)	Stiffness (*N-mm per degree*)
Control	20	338 ± 17	39 ± 5.2
Cefazolin†	20	327 ± 16 (p = 0.64)	36 ± 3.3 (p = 0.55)
Ciprofloxacin†	20	284 ± 8 (p = 0.04)	20 ± 1.3 (p = 0.001)

*The values are given as the mean and the standard error.

†The p values represent the significance of the difference compared with the control group.

treated and control animals. Higher-magnification histological evaluation of specimens from the ciprofloxacin-treated animals displayed a central portion of the soft callus incompletely filled with cartilage (Fig. 2-B). This region appeared less cellular, and the cells exhibited a flattened, fibroblast-like morphology.

High-power views of the endochondral ossification front showed additional abnormalities in the cartilage and trabecular structures of the calluses from the ciprofloxacin-treated animals (Fig. 3). These abnormalities included a decreased number of chondrocytes, an increase in chondrocyte size, and an increased proportion of cartilage in the newly formed trabecular bone spicules compared with the control specimens. These differences were observed most clearly on the higher-magnification photomicrographs of the endochondral ossification front (Fig. 4).

Serial sections stained with Masson's trichrome clearly showed the structures of the bone and cartilage trabeculae (Fig. 5). The calluses from the ciprofloxacin-treated animals appeared to contain more cartilage and less bone in the newly formed trabeculae than the control calluses.

Due to variability in cell characteristics and trabecular structures between different regions of the same callus, the differences between the calluses from the control and ciprofloxacin-treated animals were difficult to characterize. To quantify these cell characteristics and the trabecular structure at the endochondral ossification front, the size and number of the chondrocytes in the hypertrophic cartilage were determined with histomorphometry (Table III). Because the calluses from the control and cefazolin-treated animals were not significantly different on mechanical testing, the calluses from cefazolin-treated animals were not evaluated with histomorphometry.

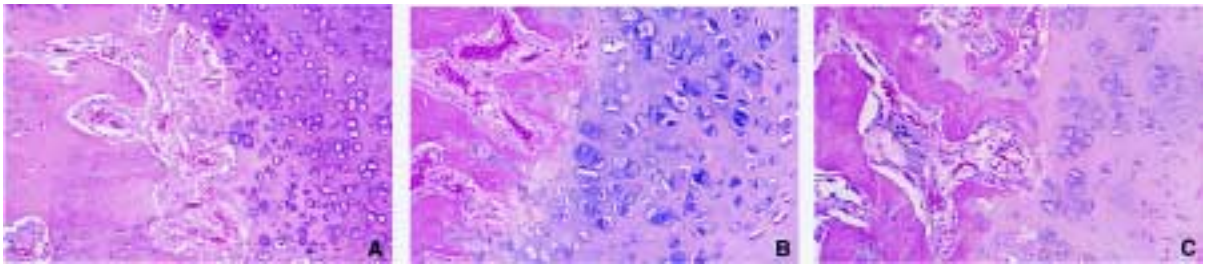

FIG. 4

High-magnification photomicrographs showing the morphology of the chondrocytes at the endochondral ossification front in calluses from control (A) and ciprofloxacin-treated (B and C) animals. Note the differences in the size and number of chondrocytes. In general, the chondrocytes in the ciprofloxacin-treated animals are larger and less numerous. These histological sections also suggest differences in trabecular architecture, but because of poor contrast between bone and cartilage with hematoxylin and eosin staining these differences are not clear (hematoxylin and eosin, original magnification × 500).

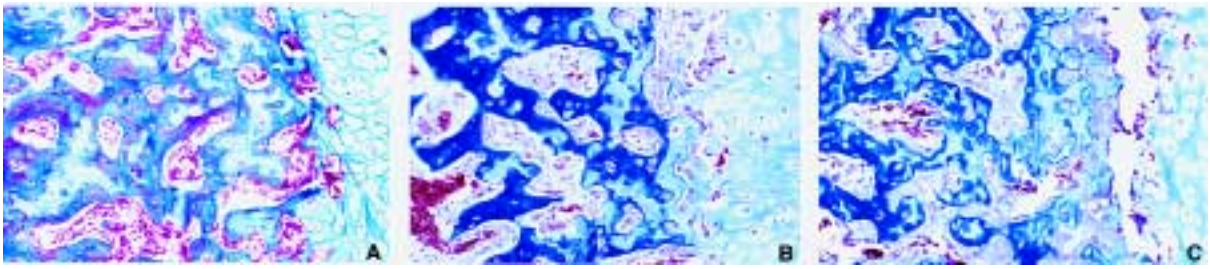

FIG. 5

High-magnification photomicrographs showing the trabecular structure at the endochondral ossification front in calluses from control (A) and ciprofloxacin-treated (B and C) animals. The primary bone spicules are easily identified in the fracture calluses from both groups. The cartilage core in these spicules confirms that there is bone formation by endochondral ossification. Although the calluses are similar in composition, there appear to be differences between them with regard to the fine structure of these primary bone spicules. The trabecular structures in the calluses from the ciprofloxacin-treated animals appear to have thinner cross sections and a higher percentage of cartilage. This impression was confirmed by histomorphometric analysis of the trabecular structures. There also appears to be a subtle difference in the cellularity, with increased numbers of cuboidal cells and less development of the marrow structures in the ciprofloxacin-treated animals (Masson's trichrome, original magnification × 500).

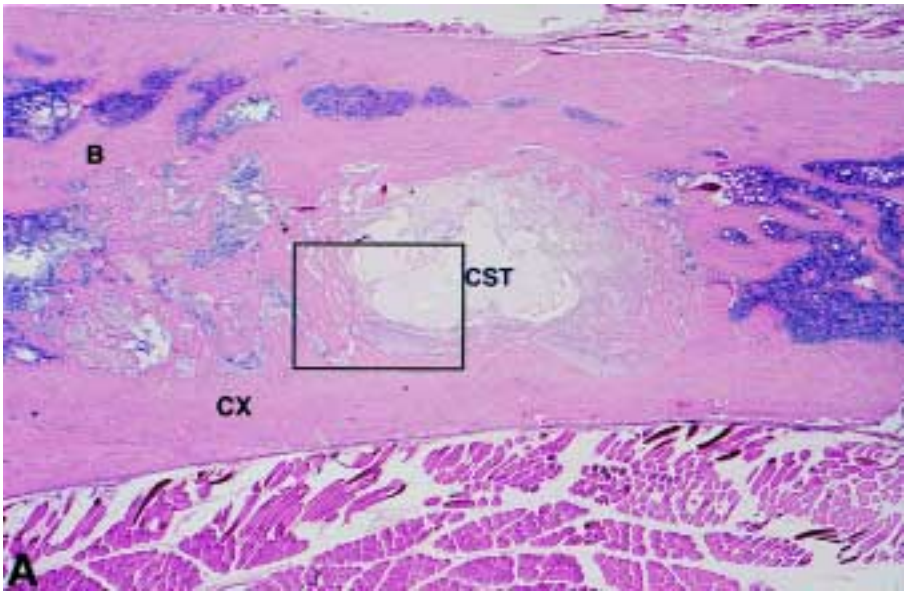

FIG. 6-A

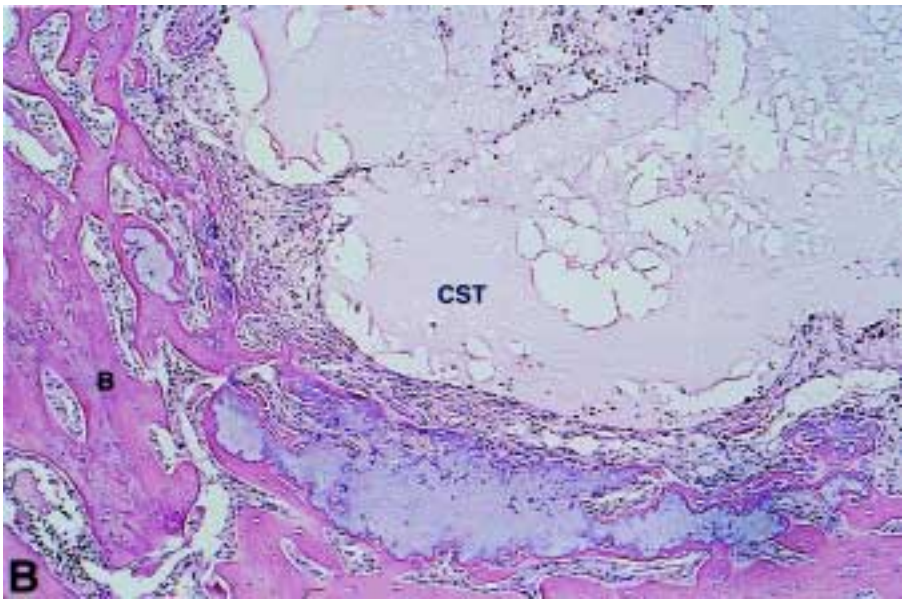

FIG. 6-B

Histological appearance of areas of cystic degeneration seen at a distance from the fracture callus in specimens from the ciprofloxacin-treated rats. CST = cyst, small B = intramedullary bone, and CX = cortex (original magnification × 25 [Fig. 6-A] and × 125 [Fig. 6-B]).

The calluses from the ciprofloxacin-treated animals contained a smaller number of hypertrophic chondrocytes at the endochondral ossification front than did the calluses from the control animals (0.77×10^4 compared with 1.3×10^4 cells per square millimeter, $p = 0.004$) (Table III). On the average, the cells from the ciprofloxacin-treated animals were larger than those from the control animals (17.0×10^{-5} compared with 6.4×10^{-5} square millimeters). There was no significant difference between the calluses from the control and ciprofloxacin-treated animals with regard to the percentage of the endochondral ossification front occupied by newly formed bone (58.3 compared with 56.9 percent, $p = 0.84$), but the thickness of the trabecular bone in the calluses from the ciprofloxacin-treated animals was decreased (66.8 compared with 84.6 micrometers) (Table III). These differences resulted from the smaller number of bone trabeculae in the control animals. Decreased trabecular thickness in the ciprofloxacin-treated animals was associated with a decrease in the percentage of the trabecula that was bone (39.0 percent compared with 48.2 percent in the controls). The differences in chondrocyte size and trabecular bone structure were trends but were not significant.

The low-magnification histological examination also showed cystic abnormalities, at a distance from the fracture site, in the bones from the ciprofloxacin-treated animals (Figs. 6-A and 6-B). These cysts were com-

TABLE III
HISTOMORPHOMETRIC ANALYSIS OF ENDOCHONDRAL BONE FORMATION*

	Control Group (N = 4)	Ciprofloxacin-Treated Group† (N = 3)
Hypertrophic cartilage		
Size of chondrocytes *(mm^2)*	$6.4 \pm 2.2 \times 10^{-5}$	$17.0 \pm 7.2 \times 10^{-5}$ (p = 0.12)
No. of chondrocytes *(cells per mm^2)*	$1.3 \pm 0.13 \times 10^4$	$0.77 \pm 0.11 \times 10^4$ (p = 0.004)
Endochondral bone		
Trabecular area *(percent)*	58.3 ± 10.5	56.9 ± 7.2 (p = 0.84)
Trabecular thickness *(μm)*	84.6 ± 8.9	66.8 ± 13.8 (p = 0.14)
Trabecular composition		
Percent bone	48.2 ± 7.2	39.0 ± 3.5 (p = 0.08)
Percent cartilage	10.1 ± 4.4	17.9 ± 4.7 (p = 0.07)

*The values are given as the mean and the standard deviation.
†The p values represent the significance of the difference compared with the control group.

posed of an acellular matrix surrounded by a poorly defined fibrous layer. The number of cysts was not quantitated, but one or more cysts were seen in all femora from the ciprofloxacin-treated animals.

Ultrastructural Analysis

Examination of the experimental fracture calluses with electron microscopy revealed differences among the treatment groups. Ultrastructural changes in the chondrocytes of the ciprofloxacin-treated group consisted of swelling of nuclei, intracellular organelles, cytoplasmic vacuolization, disruption of the plasma and nuclear membranes, and chondrocyte death (Fig. 7-B). Necrotic chondrocytes had pyknotic nuclei, disrupted plasma membranes, and fragmented cytoplasmic organelles dispersed into the extracellular matrix. Abnormal arrangement of the collagen fibers in the pericellular area and matrix was also observed. These pathological changes were not seen in the control group (Fig. 7-A).

Discussion

Ciprofloxacin has been approved for the treatment of soft-tissue, urinary tract, and other common infections and is currently the most widely prescribed quinolone antibiotic. Clinically, this antibiotic is often administered for infection remote from a fracture site; however, the fracture site is exposed to serum concentrations of the fluoroquinolone. The adverse effect of fluoroquinolones on developing cartilage has been extensively investigated[3,7,19,32]. The fracture repair process, manifested as fracture callus, is a rapidly growing cartilaginous complex, which undergoes intramembranous and endochondral ossification — mechanisms similar to those of maturing cartilage of young animals. The toxic effects of fluoroquinolones on cartilage in fracture callus have not been previously investigated, to our knowledge. In this study, ciprofloxacin was administered in a manner that was designed to simulate the effect of systemic exposure of a healing fracture to fluoroquinolones in a patient being treated for a remote infection such as a urinary tract infection. We purposely avoided the investigation of fracture-healing in the presence of infection at the site of the fracture callus.

The present investigation revealed decreased callus strength at four weeks after fracture in rats treated with ciprofloxacin. This observation supports the hypothesis that exposure to ciprofloxacin affects early fracture-healing and alters the progression of normal fracture callus formation. Although histological examination of the fracture calluses from the ciprofloxacin-treated animals showed progressive formation of cartilage and subperiosteal bone and replacement of cartilage by endochondral ossification, histological abnormalities were apparent when the specimens were compared with the fracture calluses from the cefazolin-treated and control animals. These abnormalities included a persistent central region of fibrous tissue, a decreased size and number of chondrocytes at the endochondral ossification front, and abnormalities of trabecular bone formation. The decreased number and size of the chondrocytes in the fracture callus of the ciprofloxacin-treated animals suggest that ciprofloxacin may have a primary effect on chondrocyte maturation and development, possibly including a direct toxic effect on chondrocytes. There was striking electron microscopy evidence of chondrocyte death in fracture calluses exposed to ciprofloxacin. We suggest that the abnormal trabecular bone formation is linked to a direct effect of ciprofloxacin on the chondrocytes. This adverse effect on chondrocyte function then leads to an inefficient conversion of cartilage to bone, which is manifested by decreased mechanical properties of the fracture callus.

Previous studies have documented histological and

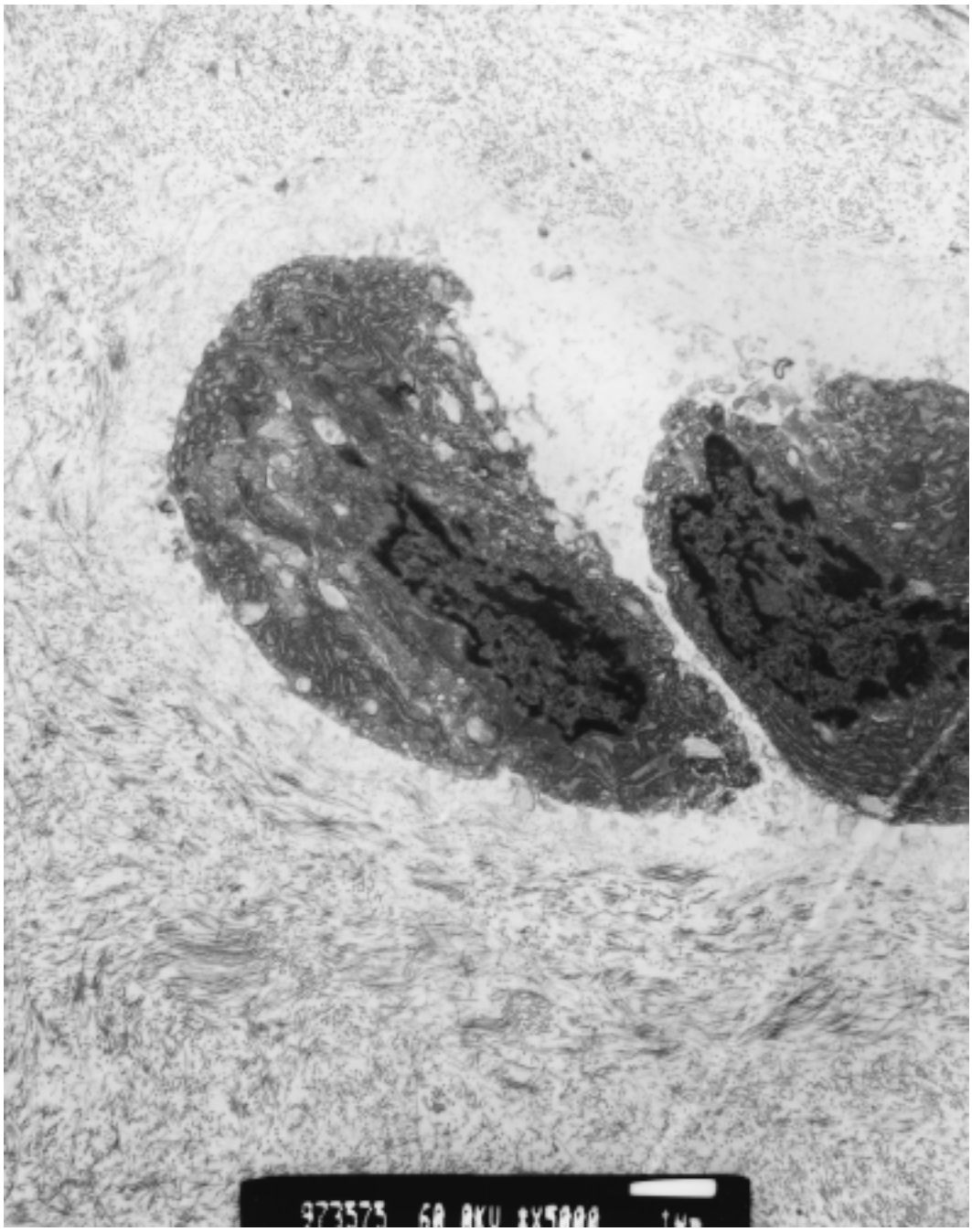

FIG. 7-A

Ultrastructural appearance of chondrocytes and surrounding matrix in the fracture callus of control (Fig. 7-A) and ciprofloxacin-treated (Fig. 7-B) rats after four weeks of healing. Chondrocytes from calluses in normal rats have intact membranes, dense nuclei, and well defined intracellular organelles. Prominent abnormalities in chondrocytes from calluses in ciprofloxacin-treated rats included pyknotic nuclei, disrupted plasma membranes, and fragmented cytoplasmic organelles (original magnification × 5000).

ultrastructural changes in articular cartilage of immature mammals exposed to fluoroquinolones[8,9,22]. The ultrastructural alterations in the chondrocytes and their surrounding matrix following exposure to ciprofloxacin in the present study are very similar to previous descriptions of necrotic chondrocytes and hypervascularization observed with exposure of articular cartilage to fluoroquinolone[7,16,28]. It is important to note that the changes in fracture-healing in the present study were detected in association with serum concentrations of ciprofloxacin analogous to typical therapeutic concentrations achieved in humans. Supratherapeutic serum levels were often used in earlier studies of quinolone toxicity[4].

Many mechanisms for toxic effects on developing articular cartilage have been proposed. The initial changes in immature articular cartilage after exposure to fluoroquinolone may occur in the chondrocytes or in the ma-

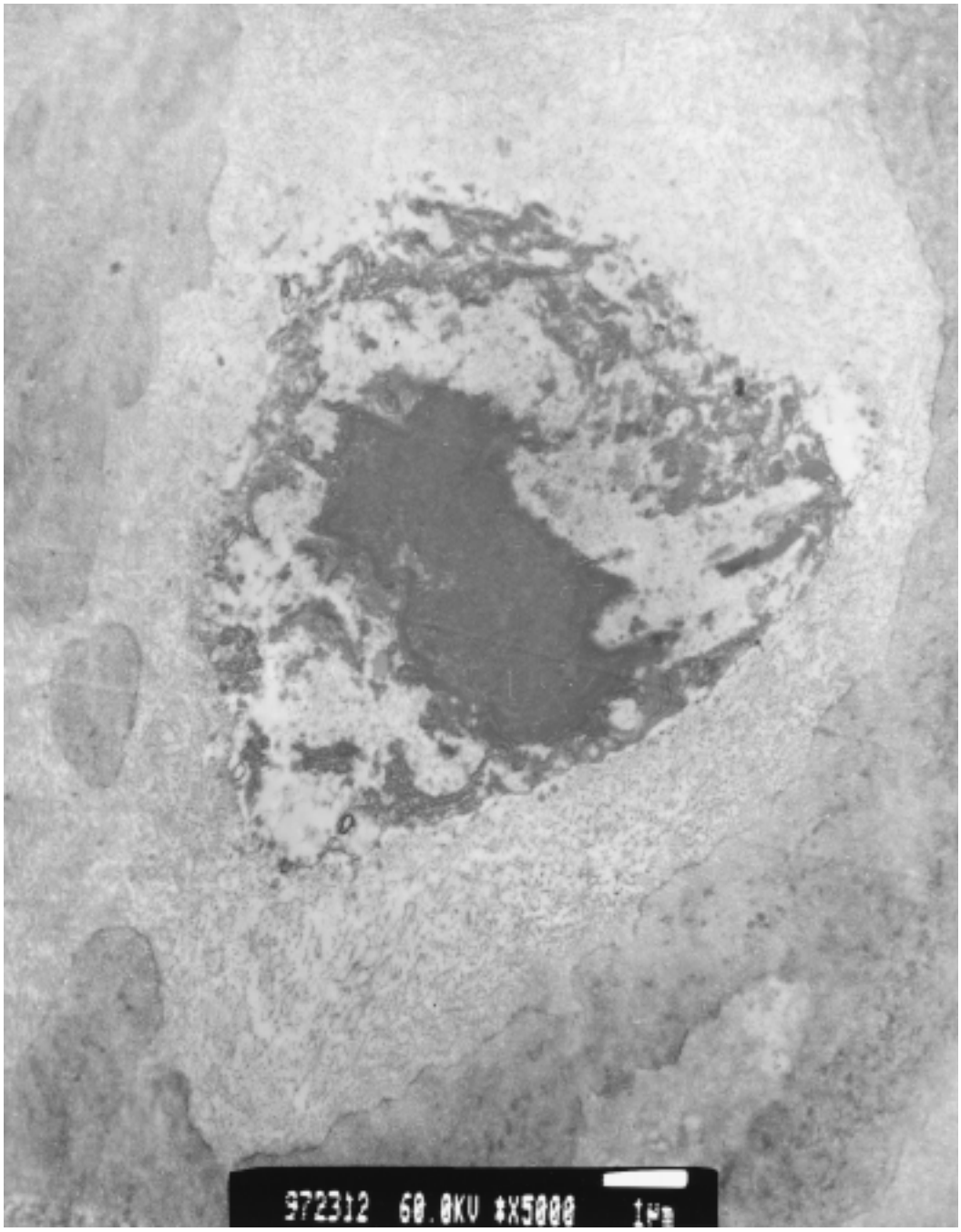

FIG. 7-B

trix and tend to occur in the larger weight-bearing joints such as the hip and knee[8,13,21]. The hypothesis that there is a direct toxic effect on chondrocytes has been supported by the observation of changes in chondrocytes within forty-eight hours after exposure. Degeneration of the cartilage matrix has also been observed following as few as two oral doses of ciprofloxacin[7]. Other possible mechanisms include action of ciprofloxacin as a DNA gyrase inhibitor. Bacterial DNA gyrase is the enzyme responsible for the compact wrapping, nicking, and rewrapping of bacterial DNA. The observation of impaired fracture-healing in rats treated with a bacterial DNA gyrase inhibitor supports the possibility of an important role of a mammalian analog for this enzyme in normal endochondral ossification. A proposed DNA analog in mammalian cells, known as mtDNA or mitochondrial DNA, has been shown *in vitro* to be sensitive to exposure to quinolones[2,10,14,23].

In addition to the inhibition of DNA, other possible mechanisms of quinolone chondrotoxicity include alterations in DNA synthesis or repair[6,18,34]. The most pronounced inhibitory effect is on the chondrocyte matrix secretory production of glycosaminoglycan and collagen components. This inhibition of human chondrocytes in cell culture occurred at levels corresponding to therapeutic serum levels of ciprofloxacin[29].

The data in the present study are consistent with the hypothesis that ciprofloxacin produces chondrotoxicity in experimental fracture callus as judged with histological, radiographic, and mechanical testing. A definitive statement regarding the mechanism of this chondrotoxicity cannot be made on the basis of the data in the present study, but further investigation of the effects of fluoroquinolones on fracture-healing seems warranted.

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