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Efficacy of Sparfloxacin and Autoradiographic Diffusion Pattern of [^{14}C]Sparfloxacin in Experimental *Staphylococcus aureus* Joint Prosthesis Infection

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Using a new rabbit model of methicillin-susceptible *Staphylococcus aureus* knee prosthesis infection, we compared the efficacies of sparfloxacin (50 mg/kg of body weight subcutaneously, twice a day) and pefloxacin (50 mg/kg subcutaneously, twice a day). A partial knee replacement was performed with a silicone implant fitted into the intramedullary canal of the tibia, and 5×10^7 CFU of methicillin-susceptible *S. aureus* was injected into the knee. The 7-day treatment regimen was started 15 days later. The MICs and MBCs of sparfloxacin and pefloxacin were, respectively, 0.06 and 0.25 μg/ml (MIC) and 0.25 and 1 μg/ml (MBC). The peak levels of sparfloxacin and pefloxacin in serum were 3.6 and 21 μg/ml, respectively. Three weeks after the end of treatment, animals were sacrificed and tibias were removed, pulverized, and quantitatively cultured. In contrast to pefloxacin (3.61 ± 1.64 $\log_{10}$ CFU/g of bone), sparfloxacin significantly reduced the bacterial density (2.12 ± 1.1 $\log_{10}$ CFU/g of bone) ($P = 0.01$) in comparison with the level in controls (4.59 ± 1.21 $\log_{10}$ CFU/g of bone), without selection of resistant variants. Sparfloxacin was significantly more effective than pefloxacin ($P = 0.025$). The autoradiographic pattern of [^{14}C]sparfloxacin diffusion was studied in noninfected animals with prostheses and in infected animals 15 days after inoculation. Sixty minutes after completion of infusion of 250 μCi of [^{14}C]sparfloxacin, in infected animals the highest levels of radioactivity were detected around the prosthesis, in femoral cartilage, and in articular ligaments. Radioactivity was slightly less intense in bone marrow and muscles and was very weak in compact bone. The distribution of sparfloxacin in uninfected rabbits was similar. Thus, sparfloxacin may represent a valid alternative therapy in these infections provided that it is carefully monitored for potential side effects.

Infection of orthopedic implants is a severe and worrying hospital-acquired infection. Despite advances in prophylactic measures and surgical technique, the overall incidence of bacterial infections complicating primary arthroplasty is 0.5 to 2% (25). The difficulty of curing these infections is related to the difficulty in eradicating bacteria on the surface of implants and also in bone infections in general with antibiotics (3). The impaired diffusion of antibiotics into infected bone tissues and abscesses surrounding the prosthesis (28) may represent a factor limiting the efficacy of these drugs. Thus, surgical debridement, removal of all foreign material, and prolonged, high-dose intravenous (i.v.) antimicrobial therapy are necessary for a successful outcome.

As randomized prospective trials are difficult to design because of the heterogeneity of the disease, the choice of antibiotics relies mostly on theoretical considerations and experimental data on osteomyelitis (23) or from the subcutaneous tissue cage model of infection (29, 31). At present, no experimental therapeutic study has been conducted with a joint prosthesis model closely approximating the human infection (24).

New fluoroquinolones offer an alternative strategy for antibiotic treatment of staphylococcal joint prosthesis infection. Indeed, these compounds show good activity against methicillin-susceptible staphylococci, can be administered orally, and have a low rate of adverse effects. Fluoroquinolones, in monotherapy or in combination with rifampin, have been shown to be effective in experimental *Staphylococcus aureus* osteomyelitis (10, 18) and in osteomyelitis in humans (15, 16). Ciprofloxacin or ofloxacin in combination with rifampin has also been used successfully in *Staphylococcus*-infected orthopedic implants (8, 30).

Using a new model of experimental knee prosthesis infection that closely mimics human infection and was described elsewhere (1), we compared the efficacies of sparfloxacin, a new fluoroquinolone, and pefloxacin. As the pattern of antibiotic distribution in infected bone tissue and close to the infected prosthesis could represent an important factor conditioning in vivo activity, we studied the diffusion of [^{14}C] sparfloxacin in this model. Autoradiography was used because our previous studies of experimental cardiac vegetations (4–6) showed that the method allows precise analysis of the pattern of antibiotic diffusion into the various areas of infected tissue.

(This work was presented in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy [4a].)

MATERIALS AND METHODS

Test strain. A methicillin-susceptible strain of *S. aureus* (MSSA) was used in this study. This strain was isolated from a patient with an infected knee prosthesis treated at Bichat Hospital, Paris, France. Its virulence was maintained by intraperitoneal injection into mice.

In vitro antibiotic susceptibility test. The MICs and MBCs were determined in Mueller-Hinton broth (Diagnostics Pasteur, Marne-la-Coquette, France) by the tube dilution method (22). The antibiotics tested were sparfloxacin (Rhône DPC-Europe Laboratories, Paris, France) and pefloxacin (Roger-Bellon Laboratories, Neuilly-sur-Seine, France). Each tube contained a twofold dilution of

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the antibiotic and a final bacterial inoculum of 10^6 CFU/ml. The tubes were incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of antibiotic that prevented turbidity in the test tube after incubation. The MBC was defined as the lowest concentration of antibiotic that killed at least 99.9% of the organisms after incubation, as determined by plating 0.1 ml from each clear-broth tube onto Trypticase soy agar and incubating the plates for 18 h at 37°C.

Experimental prosthesis infection. Twenty-four New Zealand White rabbits, each weighing between 2.5 and 3 kg, were used (20 rabbits for therapeutic studies and 4 rabbits for autoradiographic studies). They were housed in individual cages with a natural light-dark cycle. The experimental protocol was in keeping with French legislation on animal experimentation.

This model has been described in detail elsewhere (1). Briefly, an orthopedic surgeon partially replaced the rabbit's right knee with a tibial component. The operation was performed under general anesthesia induced by intramuscular injection of ketamine (25 mg/kg of body weight) and then continuous inhalation of 1% isoflurane. We utilized a silicone-elastomer implant commonly used in arthroplasty of the first metatarsophalangeal joint (Silastic; great toe implant HP; Swanson Design, Dow-Corning France, S.A.). This device was implanted as a tibial prosthetic component. The stem of the nail-shaped silicone implant (14 mm long) was inserted into the intramedullary canal of the tibia, with the implant head (15 by 5 mm) replacing the tibial plateaus. The skin of the animal's right leg was shaved 24 h before the operation. Prior to surgery, the skin was cleaned with an iodine solution. A longitudinal skin incision was made, and the knee was exposed. After dislocation of the tibia, the epiphysial plates were removed. The metaphysis was exposed, the cancellous bone of the medullary cavity of the proximal metaphysis was reamed (15 mm) and filled with the prosthesis, and then the deep fascia and the skin were closed.

Immediately after surgery, animals were infected by injection of 5×10^7 CFU of MSSA, in a 0.5-ml volume, into the knee close to the prosthesis. Each rabbit was given oral acetaminophen (80 mg/day) as postoperative analgesia for 2 days.

Therapeutic studies. (i) Treatment and evaluation of therapy. Fifteen days after inoculation (day 15), subcutaneous (s.c.) injections of sparfloxacin (50 mg/kg of body weight twice a day [b.i.d.]) and pefloxacin (50 mg/kg, b.i.d.) were started. Each regimen was administered for 7 days. Animals were killed by i.v. injection of pentobarbital 3 weeks after the end of therapy (day 35) in order to take into account regrowth of bacteria after the end of therapy while avoiding having results influenced by the persistence of residual antibiotics in bone. Untreated control rabbits were also sacrificed on day 35. At that time, the right hind leg was dissected and the tibia and the femur were separated from the surrounding soft tissues. A smear of the prosthesis was made on a blood agar plate. For quantitative bacterial counts, the upper one-third (length, 3 cm) of the tibia, including compact bone and marrow, was isolated, split with a bone crusher, weighed, cut into little pieces, frozen in liquid nitrogen, and crushed in an autopulverizer (Spex 6700; Freezer/Mill Industries Inc., Metechen, N.J.). The pulverized bone was suspended in 10 ml of sterile saline, and serial dilutions were made and plated on Trypticase soy agar. After overnight incubation at 37°C, the number of viable organisms was determined. The results are expressed as means ± standard deviations (SD) of the $\log_{10}$ CFU/g of bone.

(ii) Serum antibiotic levels. Antibiotic levels in serum were determined with uninfected rabbits. An s.c. injection of pefloxacin or sparfloxacin (50 mg/kg) was administered to six rabbits (three in each group). Blood was drawn 1, 1.5, 2, 2.5, 3, and 5 h after injection. The samples were stored 1 month at −20°C until assayed. Sparfloxacin and pefloxacin concentrations were measured by high-performance liquid chromatography. Plasma calibration curves ranged from 0 to 5 mg/liter for sparfloxacin and from 0 to 20 mg/liter for pefloxacin. The responses were linear over the concentration ranges tested. Within-day coefficients of variation were always <4% for sparfloxacin and <0.5% for pefloxacin. Between-day reproducibility was evaluated by using the coefficients of variation of the quality controls. These were, respectively, 6.8 and 4.4% for 0.25 and 1.25 mg of sparfloxacin per liter and 2.5 and 0.7% for 2.5 and 15 mg of pefloxacin per liter. The lower limits of detection were 0.1 mg/liter for sparfloxacin and 0.5 mg/liter for pefloxacin.

Areas under the concentration-time curves were calculated by using the trapezoidal rule.

Quantitative autoradiography of [^{14}C]sparfloxacin diffusion into infected bone. Fifteen days after inoculation, 250 μCi of [^{14}C]sparfloxacin was injected i.v. over 30 min into two infected and two uninfected rabbits. Animals were sacrificed 60 min (one infected rabbit and one uninfected rabbit) or 180 min (one infected rabbit and one uninfected rabbit) after the end of the infusion.

To evaluate the influence of duration of infection on the autoradiographic pattern of diffusion, an additional infected rabbit was injected with [^{14}C]sparfloxacin 30 days after infection and sacrificed 60 min after the end of the infusion.

After being skinned, both hind limbs of each animal were frozen in liquid nitrogen and stored at −70°C. Sections (50 μm thick) of the limb (including the prosthesis, the bones, the ligaments, and part of the surrounding muscles) were cut at −25°C on an LKB macrocryostat and collected on adhesive tape. Autoradiographic films (Hyper-film β-Max; Amersham, Les Ulis, France) exposed to the leg sections for 6 weeks were developed, and autoradiographic images were quantified. For each hind limb, four different sections were quantified by computed densitometry (Autorad; Imstar, Paris, France). The results are expressed as the mean ± SD tissue-to-blood radioactivity concentration ratios. The radioactivity level in blood was determined with the heart ventricles of the animals by using the same autoradiographic imaging technique.

Statistics. Bacterial densities in bone were compared between the experimental groups by using the nonparametric Mann-Whitney U test. Results are expressed as means ± SD. A P value of <0.05 was considered significant.

TABLE 1. Effect of antibiotic treatment on experimental *S. aureus* prosthesis infection in rabbits

Treatment[a]	No. of rabbits		$\log_{10}$ CFU/g of bone (mean ± SD)
	Total	With sterile bone	
None	6	0	4.59 ± 1.21
Sparfloxacin	7	4	2.12 ± 1.16[b]
Pefloxacin	7	1	3.61 ± 1.64

[a] Rabbits were treated s.c. for 7 days with sparfloxacin (50 mg/kg, b.i.d.) or pefloxacin (50 mg/kg, b.i.d.).

[b] Significantly different from values for untreated controls ($P = 0.01$) and the pefloxacin-treated group ($P = 0.025$).

RESULTS

In vitro studies. The MICs and MBCs of sparfloxacin and pefloxacin were 0.06 and 0.25 μg/ml (MIC) and 0.25 and 1 μg/ml (MBC), respectively.

Serum antibiotic levels in uninfected animals. Administration of sparfloxacin (50 mg/kg, s.c.) to uninfected animals resulted in a mean ± SD peak concentration in serum of 3.60 ± 1.44 μg/ml 1 h after injection. After an injection of pefloxacin (50 mg/kg, s.c.), the mean ± SD peak concentration in serum was 21.5 ± 2.45 μg/ml.

Areas under the concentration-time curves were 129 ± 25 mg · h · liter^{-1} for pefloxacin and 39 ± 8.8 mg · h · liter^{-1} for sparfloxacin.

Therapeutic studies. All control animals had positive prosthesis smear cultures with a mean bacterial count of 4.59 ± 1.21 $\log_{10}$ CFU/g of bone (Table 1). In the sparfloxacin-treated group, four of seven animals had sterile bone; their mean bone bacterial density was significantly reduced compared with that of control animals ($P = 0.01$). In the group of animals treated with pefloxacin, only one of the seven animals had sterile bone and the mean bacterial density in bone was 1 log unit lower than that of control animals, but the difference was not significant. Sparfloxacin was significantly more effective than pefloxacin ($P = 0.025$). No sparfloxacin- or pefloxacin-resistant strain emerged in the bones of treated animals.

[^{14}C]sparfloxacin diffusion. The distribution of [^{14}C]sparfloxacin 60 min after the end of an infusion given 15 days after MSSA infection is visualized and quantified, respectively, in Fig. 1A and B and Fig. 2A. The diffusion of [^{14}C]sparfloxacin into the different tissues of the left leg (without a prosthesis) was similar to that in the right leg (with a prosthesis). The highest levels of radioactivity were detected in the tibial epiphysial disk cartilage, around the prosthesis, and in femoral articular cartilage, articular ligaments, and muscle, and these levels were higher than those in blood. In bone marrow, the [^{14}C]sparfloxacin level was similar to the level in blood. Cortical bone was practically devoid of radioactivity.

Autoradiography of the knee 180 min after the end of [^{14}C]sparfloxacin injection clearly showed the same pattern of diffusion (data not shown). The duration of infection did not influence the distribution pattern of [^{14}C]sparfloxacin either, as autoradiographs of animal leg sections obtained 30 days after inoculation were not different from those obtained after 15 days of infection (data not shown).

In uninfected animals (Fig. 1C and D and 2B), the sparfloxacin distribution was similar to that observed in infected ani-

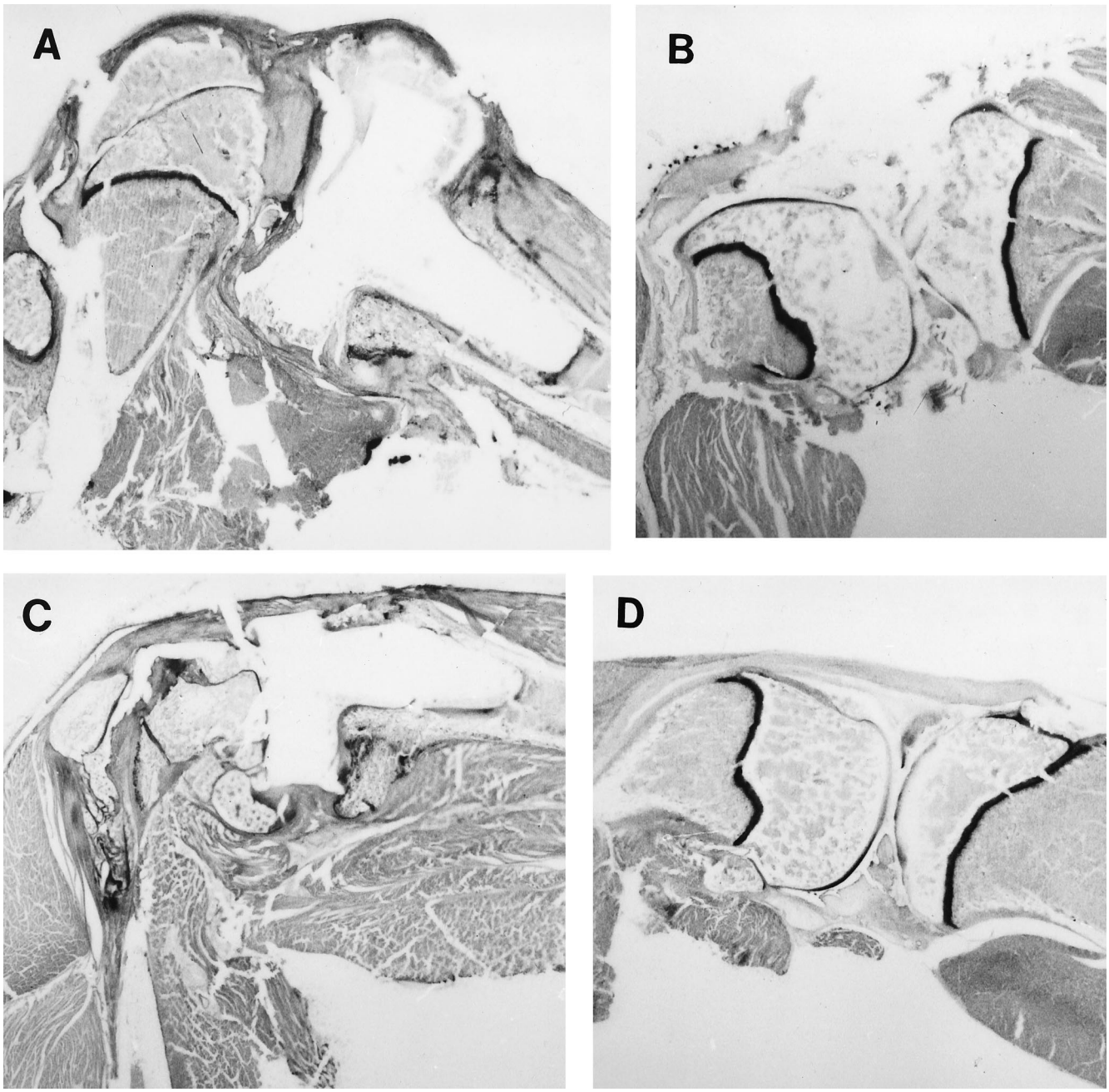

FIG. 1. Autoradiography of the right knee (with prosthesis) (A and C) and the left knee (B and D) 15 days after injection of MSSA (A and B) or saline (C and D). Autoradiographs of legs of [^{14}C]sparfloxacin-treated rabbits were taken 60 min after the end of i.v. infusion. Radioactivity appears as dark grains. The gradient of darkness indicates the concentration scale, with black representing the highest concentration. Magnification, ×1.5.

mals. The difference noted in the epiphysial disk cartilage radioactivity concentration ratio was not significant.

DISCUSSION

Foreign-body infections are a major cause of morbidity and implant failure. Among these infections, infection of a joint prosthesis is a major problem, representing a calamity for the patient because it leads to prolonged hospitalization and often repeated surgical procedures.

Although surgical management has progressed (13, 17), the type and duration of antimicrobial therapy effective in the treatment of these infections remain controversial. Because of the heterogeneity of the disease, randomized prospective trials of antimicrobial therapy are difficult to devise. Thus, the choice of antibiotics relies mostly on theoretical considerations, such as in vitro activity of antibiotics, presumed penetration into bone, potential toxicities and experimental data obtained in animal models of osteomyelitis (23), or subcutaneous tissue cage models of infection (31). In spite of the description by Southwood et al. of infection in experimental hip arthroplasties in rabbits (27), at present, no experimental therapeutic study has been conducted with a model of prosthetic joint infection close to the human situation (24). Our prosthetic

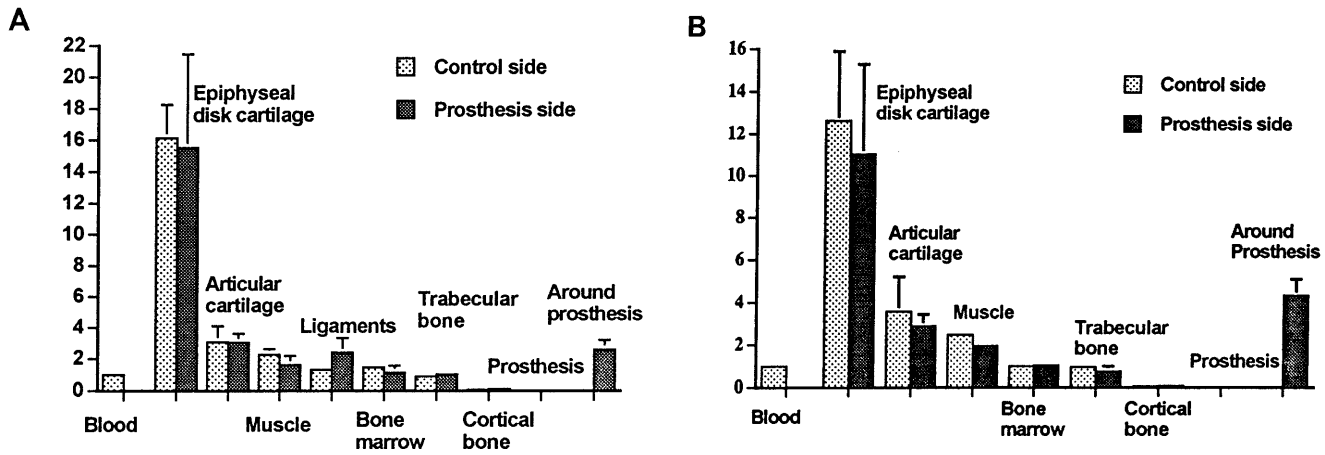

FIG. 2. Mean tissue/blood radioactivity concentration ratios (±SD) (error bars) in the right (prosthesis side) and the left (control side) legs of rabbits 15 days after MSSA inoculation (A) or injection with saline (B). The extent of dispersion or SD refers to the measurement of radioactivity on different sections of tissue from the same animal.

knee infection model is easily reproducible and causes infection in 100% of the inoculees when the inoculum of 5×10^7 CFU is properly injected into the operative site near the prosthesis. It closely reproduces acute postoperative infection in humans with infection of the artificial joint space, prosthesis, and adjacent bone, as demonstrated by histological examinations (1). The bone bacterial density in control animals 6 weeks after inoculation was 4.59 ± 1.21 $\log_{10}$ CFU/g of bone, a value close to that obtained in experimental staphylococcal osteomyelitis (18, 20). As in humans, infection in rabbits progresses with a subacute course with long-term survival of animals and progressive destructive bone lesions. This experimental model is thus adequate to comparatively evaluate the efficacies of different antibiotic regimens.

Bone drug levels have been measured repeatedly for newer antimicrobial agents. However, as underlined by Norden (23), the significance of these measurements is at best dubious and there appears to be no correlation between levels of antimicrobial agents in bone and success of therapy in experimental models. The difficulty of interpreting these values is attributed to the heterogeneous diffusion of antibiotics. We previously studied antibiotic diffusion in infected cardiac vegetations (4–6) and found that the antibiotic distribution pattern may represent an important factor conditioning in vivo activity. Autoradiography allows both a quantitative approach to antibiotic diffusion and a detailed anatomical localization of the radiolabeled drug.

In our study, the efficacy of sparfloxacin, a new fluoroquinolone, was compared with that of pefloxacin. Indeed, fluoroquinolones have been shown to be as effective as conventional therapy in experimental models of osteomyelitis (10, 18) and in clinical trials in patients with osteomyelitis (7, 14, 15, 21). Pefloxacin, like ciprofloxacin or ofloxacin, is often given orally, alone or in combination with rifampin, to treat bone and joint infections, including staphylococcal orthopedic implant-related infections (8, 30). In our model, sparfloxacin significantly reduced the bacterial counts relative to levels in control animals. In contrast, this effect was not significant with pefloxacin. Sparfloxacin was significantly more effective than pefloxacin. The significantly better effect of sparfloxacin could be attributable to its higher area under the concentration-time curve/MIC ratio (9). Sparfloxacin and pefloxacin levels in serum were higher than those obtained in humans given standard doses. However, in light of the impaired diffusion of most antibiotics into bones, high doses are usually recommended to cure bone infections.

This therapeutic effect of sparfloxacin is in accordance with the high degree of penetration of [^{14}C]sparfloxacin into the purulent exudate in the artificial joint space close to the prosthesis and into the bone marrow nearby. These radioactivity levels were at least equal to or higher than levels in blood, suggesting concentrations far above the MIC and MBC for the organisms, at least at peak serum drug levels. In contrast, as previously noted when conventional methods were used to measure the concentrations of other antibiotics, sparfloxacin penetrated very poorly into compact bone. This could explain the well-known difficulty in sterilizing bone infections with antibiotics. These findings must be interpreted with caution because values were usually obtained at only one time point. Performing pharmacokinetic analyses at different sampling times after injection of the drug into animals by our autoradiographic method was hardly feasible for economic reasons. However, the distribution pattern obtained 180 min after the end of the infusion was comparable to that observed 2 h earlier, suggesting that, despite the concentration decrease with time, the diffusion pattern remains similar and thus the same tissue/blood radioactivity concentration ratios can be measured.

In our model, one remarkable autoradiographic result was the very high [^{14}C]sparfloxacin concentration in the artificial joint space in contact with the prosthesis. The same pattern was observed in infected and uninfected rabbits. In the latter the joint space was filled with a fibrinous exudate, whereas in infected rabbits the exudate was purulent. However, a nonspecific inflammatory process due to the presence of the prosthesis was seen in tissues close to the device, even in the absence of infection. This inflammatory reaction could lead to increased capillary blood flow and thus better antibiotic penetration. Since fluoroquinolones penetrate into inflammatory cells, sparfloxacin diffusion could also be favored by the flux of cells into the inflammatory site. The nonspecific inflammatory reaction may also explain why the radioactivity levels in bone marrow, cortical bone, femoral cartilage, and the tibial epiphysial disk were lower in the left leg (without a prosthesis) than in the right leg (with a prosthesis). The high-level intracellular penetration of fluoroquinolones, like that of rifampin or clindamycin, is also an explanation often evoked to explain the efficacy of these antibiotics in staphylococcal osteomyelitis

(23) and in device-related infections (8), since *S. aureus* can take up residence within cells and be protected from the action of antibiotics that do not penetrate cells well. Another explanation for the high [^{14}C]sparfloxacin levels in the joint space could be the penetration of this antibiotic into fibrin. Fibrin is an essential component of the inflammatory response. We previously investigated the autoradiographic patterns of sparfloxacin, pefloxacin, and temafloxacin diffusion into fibrin cardiac vegetations (5, 6) and found that the three quinolones were homogeneously distributed throughout the vegetations with a vegetation drug concentration approximately twofold higher than that in blood 30 min after the end of infusion of labeled antibiotic.

Autoradiography is a particularly well-adapted method for examining antibiotic distribution in infected tissues. Whole-body diffusion studies of labeled antibiotics in small animals are often performed for newer antimicrobial agents at an early stage of their development. Positron emission tomography has recently been used to evaluate the pharmacokinetics of ^{18}F-labeled fleroxacin in humans and rabbits with or without *Escherichia coli* thigh infections (11, 12). However, in the animal study, the low spatial resolution of this technique did not allow a precise analysis of antibiotic diffusion into infected foci. Autoradiography has only rarely been used to assess experimental infections in abscesses (28), endocarditis (4–6), and, more recently, an odontogenesis rabbit model (26) with [^{14}C]sparfloxacin. The [^{14}C]sparfloxacin levels in the mandibles of infected animals were lower than those in the serum (maximum concentration of drug, about 68% of the level in serum). Macroautoradiography revealed a more intensive diffusion of [^{14}C]sparfloxacin into the suppurative focus than in blood and more diffusion in the peripheral areas than in the core site of the suppurative lesion.

Another point of interest is the high levels of sparfloxacin measured in femoral cartilage, tibial epiphysial disk cartilage, and articular ligaments, especially the Achilles tendon (data not shown). Fluoroquinolones are known for their potential articular toxicity, and ligament rupture has been reported. Nalidixic acid and fluoroquinolones are contraindicated in children and pregnant women because of experimental articular toxicity observed in young animals (19). Binding of [^{3}H]nalidixic acid to articular cartilages and epiphysial disk cartilage has been described previously (2). Comparative studies of the distribution of fluoroquinolones in cartilage and an attempt to correlate the findings with histological modifications could contribute to prediction of adverse effects in humans.

In summary, sparfloxacin appears to be more effective than pefloxacin in our experimental joint prosthesis model and could be an attractive alternative therapy for infections of these implants, provided that therapy is carefully monitored for potential side effects. The detailed anatomical localization of the radiolabeled sparfloxacin obtained autoradiographically demonstrated its good penetration into the pus and around the prosthesis as well as its binding to ligaments. All these elements must be taken into consideration in determining the efficacy and potential toxicity of the drug, and the distribution pattern should be compared with those of other antibiotics.

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