Bactericidal Activity of the Combination of Levofloxacin with Rifampin in Experimental Prosthetic Knee Infection in Rabbits Due to Methicillin-Susceptible

*Staphylococcus aureus*

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The combination of levofloxacin and rifampin has been recommended for the treatment of staphylococcal prosthetic infection. In a rabbit model of prosthetic knee infection due to a susceptible clinical strain of *Staphylococcus aureus*, the combination of levofloxacin and rifampin was bactericidal, significantly reduced bacterial titers in bone compared with levels for rifampin and controls (*P* < 0.05), sterilized 6 of 12 animals, and prevented the selection of resistant mutants that was observed with rifampin alone, validating clinical recommendations.

Because of its pharmacodynamic and pharmacokinetic properties, rifampin is considered a cornerstone of antibiotic treatment of staphylococcal prosthesis joint infections (14, 19). Indeed, rifampin is highly active in vitro against staphylococci, has good diffusion in bone, is active against small-colony variants sequestered into phagocytic cells (16), and retains bactericidal activity against nongrowing staphylococci, such as those that adhere to foreign bodies (11, 19). Rifampin has been shown to be effective in experimental studies (8, 14). However, this drug should not be given alone, due to the frequent development of resistant mutants (19). Thus, the combination of rifampin with fluoroquinolones that share pharmacodynamic and pharmacokinetic properties with rifampin has been shown to be the most effective treatment for such infections (4, 8, 14, 19). Levofloxacin, the *L*-isomer of the racemate ofloxacin, has good diffusion in bone, is active against small-colony variants sequestered into phagocytic cells (16), and retains bactericidal activity against nongrowing staphylococci, such as those that adhere to foreign bodies (11, 19). Rifampin has been shown to be effective in experimental studies (8, 14). However, this drug should not be given alone, due to the frequent development of resistant mutants (19). Thus, the combination of rifampin with fluoroquinolones that share pharmacodynamic and pharmacokinetic properties with rifampin has been shown to be the most effective treatment for such infections (4, 8, 14, 19). Levofloxacin, the *L*-isomer of the racemate ofloxacin, has a high level of in vitro activity against *Staphylococcus aureus* (7) and demonstrated good activity in foreign-body experimental models (13, 17) and good penetration in bone samples from patients undergoing arthroplasty (12). Levofloxacin has been recommended for treatment of staphylococcal prosthetic joint infections in adults (20). However, its specific activity in prostatic joint infections has not been clearly established. We therefore evaluated the efficacy of levofloxacin, alone or in combination with rifampin, for treatment of rabbit experimental prosthetic knee infections due to *S. aureus*.

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*S. aureus* 17848, isolated from a patient with an infected knee prosthesis, was used for all experiments. The MICs of levofloxacin and rifampin were determined by using the Etest method (AB Biodisk, Solna, Sweden) as recommended by the manufacturer. The mutant prevention concentration (MPC), recorded as the lowest antibiotic concentration completely inhibiting bacterial growth after incubation at 37°C for 72 h, was determined for rifampin and levofloxacin, as described previously (2). Time-kill curve studies were used to determine the bactericidal activities of levofloxacin and rifampin alone and in combination. Exponential-phase cultures were diluted in 10 ml of fresh Mueller-Hinton broth to yield a final inoculum of 10^6 CFU/ml. The concentrations of antibiotic used were equivalent to 1× MIC and 4× MIC for levofloxacin and 2× MIC for rifampin. After 0, 3, 6, and 24 h of incubation in a shaking water bath at 37°C, serial dilutions of 0.1-ml samples were subcultured onto trypticase soy agar plates and incubated at 37°C for 24 h before CFU were counted. Bactericidal effect was defined by a ≥3-log_{10} decrease of the initial inoculum. Synergy was defined by a decrease of at least 2-log_{10} CFU/ml between the combination and its most active constituent after 24 h of incubation.

New Zealand White rabbits, each weighing between 2.5 and 3 kg, were experimentally infected. Animal experiments were performed in accordance with prevailing regulations in the European Commission (9). This model has been described in detail elsewhere (1, 5). Briefly, a silicone-elastomer implant, commonly used in arthroplasty of the first metatarsophalangeal joint (Silastic HP great toe implant; Swanson Design, Dow-Corning [provided by Ortho Technique, Créteil, France]) was implanted as a tibial prosthetic component under general anesthesia. Immediately after surgery, animals were inoculated with of 10^7 CFU of *S. aureus* in a final volume of 0.5 ml in phosphate-buffered saline, injected into the knee close to the prosthesis.

Seven days after inoculation (day 7), rabbits were randomly assigned to an untreated control group (*n* = 10) or a group receiving treatment with levofloxacin alone (*n* = 12), rifampin
alone \( (n=11) \), or levofloxacin combined with rifampin \( (n=12) \). Each regimen was administered for 7 days. Antibiotic dosing regimens were chosen in order to reproduce plasma concentrations in humans. For rifampin, a dosing regimen of 10 mg/kg of body weight twice a day, given intramuscularly, was used, as it produced peak levels of 9.3 ± 0.5 \( \mu \)g/ml in plasma 2 h after injection, which were close to the levels obtained for humans after a 10-mg/kg dose (18). The levofloxacin dosing regimen was determined in pilot studies in order to obtain peak concentrations and areas under the concentration-time curve over 24 h (AUC\(_{0-24}\) levels) comparable to those achieved for humans with a dose of 750 mg per day, given intravenously (i.v.), i.e., peak levels of 11 to 12 \( \mu \)g/ml and AUC\(_{0-24}\) levels of 90 \( \mu \)g · h/ml (3, 7). The dosing regimen of levofloxacin tested which best reproduced human levels was 25 mg/kg twice a day, given i.v., which produced peak levels ranging from 12.8 to 14.4 \( \mu \)g/ml and AUC\(_{0-24}\) levels ranging from 58 to 78 \( \mu \)g · h/ml 15 min after injection.

All the animals were killed by i.v. injection of pentobarbital 3 days after the end of therapy (day 17). The upper 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 then crushed in an autopulverizer (Spex 6700 freeze; Mill Industries, Inc., NJ). The pulverized bone was suspended into 10 ml of sterile saline. Serial dilutions were made, and 0.1 ml of the dilution was plated on trypticase soy agar and horse blood agar plates. After 48 h of incubation at 37°C, the number of viable organisms was determined. The lowest detectable bacterial counts according to bone weight were 1.53 to 1.83 log\(_{10}\) CFU/g bone. Mutant-resistant \textit{S. aureus} isolates were detected in bone samples from six untreated rabbits randomly chosen and in all the treated rabbits. Portions (0.1 ml) of undiluted bone homogenate were plated onto Mueller-Hinton agar containing levofloxacin at a concentration of twofold the MIC or rifampin at a concentration of eightfold the MIC. After 72 h of incubation at 37°C, colonies were detected and MICs of levofloxacin and rifampin were measured.

Peak and trough levofloxacin plasma levels were determined for six infected rabbits 10 min (peak) and 12 h (trough) after i.v. injection on the last day of therapy for animals treated with levofloxacin alone. The levofloxacin and rifampin concentrations in bone samples from all treated animals were determined using 1-ml portions of the pulverized bone. Levofloxacin
TABLE 1. Effects of 7-day treatment regimens of levofloxacin or rifampin, alone or in combination, in rabbits with experimental prosthetic knee infections due to *S. aureus* 17848

<table>
<thead>
<tr>
<th>Treatment agent (dose)</th>
<th>No. of rabbits with sterile bone/no. tested</th>
<th>Log_{10} no. of CFU/g of bone (mean ± SD)</th>
<th>No. of rabbits with mutants resistant to rifampin/no. tested$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0/10</td>
<td>6.36 ± 1.33</td>
<td>2/6</td>
</tr>
<tr>
<td>Levofloxacin (25 mg/kg b.i.d. i.v.)</td>
<td>5/12</td>
<td>2.92 ± 1.33$^b$</td>
<td>0/12</td>
</tr>
<tr>
<td>Rifampin (10 mg/kg b.i.d. i.m.)</td>
<td>5/11</td>
<td>3.20 ± 2.12$^b$</td>
<td>4/11</td>
</tr>
<tr>
<td>Levofloxacin-rifampin</td>
<td>6/12</td>
<td>1.99 ± 0.52$^{b,c}$</td>
<td>0/12</td>
</tr>
</tbody>
</table>

$^a$ b.i.d., twice a day; i.m., intramuscularly.
$^b$ Significantly different from the level for untreated controls ($P < 0.05$).
$^c$ Significantly different from the level for rifampin alone ($P < 0.05$).
$^d$ No mutants resistant to levofloxacin were found in rabbits.

and rifampin concentrations were measured by high-performance liquid chromatography.

The MICs of levofloxacin and rifampin were 0.125 and 0.016 μg/ml, respectively, and the MPCs were 1 and 256 μg/ml, respectively. The results for the time-kill studies are presented in Fig. 1. Rifampin alone at a concentration of 2× MIC was associated with a bacterial regrowth at 24 h after an initial bacterial killing. Levofloxacin alone exhibited a concentration-dependent killing, with a bacteriostatic effect followed by a regrowth after 24 h of incubation at 1× MIC, compared with a rapid bactericidal activity at a concentration of 4× MIC. The combination of rifampin and levofloxacin prevented the regrowth observed with rifampin alone at both concentrations of levofloxacin tested. The combination of rifampin with levofloxacin (1× MIC) was synergistic. In contrast, with a higher concentration of levofloxacin (4× MIC), the bactericidal activity of the combination tended to be reduced compared with that of levofloxacin alone after 3 and 6 h of exposure, without achieving antagonism.

The levofloxacin concentrations in plasma from infected rabbits obtained 15 min and 12 h after the end of the infusion were 17.3 ± 3.3 μg/ml and 0.29 ± 0.13 μg/ml, respectively. In bone samples, 72 h after the end of therapy, the concentrations of levofloxacin were 0.18 ± 0.06 μg/g (range, 0.09 to 0.29 μg/g), whereas the concentrations of rifampin were undetectable in seven animals and ranged from 0.15 to 7.95 μg/g in the remaining 16 rabbits.

The results obtained with the different therapeutic regimens tested are shown in Table 1. Rifampin alone significantly reduced bacterial titers in bone compared with levels for untreated controls ($P < 0.05$) and sterilized 5 of 11 animals. As expected, four of the six animals treated with rifampin alone that were not sterilized retained mutant-resistant strains. This result can easily be explained by the high rifampin MPC (256 μg/ml) that could not be achieved by local concentrations of rifampin in bone (<8 μg/g in all cases). Levofloxacin alone was bactericidal, significantly reduced bacterial titers in bone compared with levels for untreated controls ($P < 0.05$), and sterilized 5 of 12 animals (42%). No mutants resistant to levofloxacin were detected in bone samples from animals that were not sterilized at the time of sacrifice.

The combination of levofloxacin and rifampin was bactericidal, significantly reduced bacterial titers in bone compared with levels for untreated controls and animals treated with rifampin alone ($P < 0.05$), and sterilized 6 of 12 animals. The bacterial counts in bone did not significantly differ from those for animals treated with levofloxacin alone. No mutants resistant to rifampin or levofloxacin were detected in animals that were not sterilized. The reason for the favorability of a combination of a fluoroquinolone and rifampin in vivo is complex (10). Bactericidal activity against staphylococci is not necessarily increased with the combination of rifampin and a fluoroquinolone, as confirmed by our in vitro and in vivo studies, and the benefit of the combination relies primarily on the prevention of emergence of resistance to rifampin (19).

Levofloxacin has been recommended for the treatment of staphylococcal prosthetic joint infections in adults at a dose of 750 mg every 24 h to 500 mg every 12 h, given orally (20). These dosing regimens correspond to AUC_{0-24} levels of 82 to 95 μg · h/ml (7), while the doses used in our study produced AUC_{0-24} levels ranging from 58 to 78 μg · h/ml, i.e., slightly lower than those recommended for humans (20). Taking into account the MIC of levofloxacin against the study strain (0.125 μg/ml) and the protein binding of levofloxacin in rabbits (45%) (6), the levofloxacin dosing regimen in our study was associated with free AUC_{0-24}/MIC ratios ranging from 209 to 280 μg · h/ml, far above the AUC_{0-24}/MIC ratios associated with efficacy in other foci of infection (15). Therefore, the use of levofloxacin in humans at the recommended doses should be associated with favorable outcomes for activity against staphylococcal strains fully susceptible to fluoroquinolones.

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REFERENCES


