Comparative Evaluation of A-56619, A-56620, and Nafcillin in the Treatment of Experimental Staphylococcus aureus Osteomyelitis

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A rabbit model for Staphylococcus aureus osteomyelitis was used to compare the results of treatment with A-56619 and A-56620, two new aryl-fluoroquinolones, and nafcillin. A-56619 (15 mg/kg) and A-56620 (20 mg/kg), both used for 28 days of treatment, were injected subcutaneously every 12 h, and nafcillin (40 mg/kg) was injected every 6 h. After treatment, S. aureus was found on bone marrow cultures from 19 of 20 control rabbits, 6 of 20 treated with A-56619, 14 of 20 treated with A-56620, and 8 of 20 treated with nafcillin. Drug concentrations in serum and infected bone were measured 1 h after A-56619 and A-56620 injection and 30 min after nafcillin injection in a group of rabbits that had been infected for 3 to 4 weeks. The concentrations in infected bone were similar for all three drugs and were significantly higher than in uninfected bone. The results of this study showed that A-56619 had a high rate of eradication of S. aureus from infected bone and compared favorably to nafcillin.

Quinolone antibacterial agents have been used to treat various kinds of bacterial infections, including osteomyelitis. This class of antibacterial agents has been shown to be effective in vitro against a broad spectrum of bacterial organisms, including Staphylococcus aureus and Pseudomonas aeruginosa. Ciprofloxacin, a new carboxyquinolone, has been shown to be extremely effective as monotherapy in sterilizing experimental P. aeruginosa osteomyelitis (10). Although P. aeruginosa is often recovered from patients with posttraumatic osteomyelitis, S. aureus is the most common organism isolated (16).

Two new aryl-fluoroquinolones, A-56619 and A-56620, developed by Abbott Laboratories have been shown to be potent broad-spectrum antibacterial agents (1, 13, 14). To be a useful antibacterial agent in the treatment of osteomyelitis, the drug must be effective in vivo against S. aureus. Both quinolones were effective in eradicating systemic S. aureus infection in mice when given subcutaneously (P. B. Fernandes, N. Shipkowitz, D. Chu, L. Coen, N. Ramer, and G. R. Granneman, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 79, 1984). The purpose of the following study was to compare the effectiveness of the two quinolones with that of nafcillin, a standard drug effective against S. aureus, as therapy for experimental S. aureus osteomyelitis.

MATERIALS AND METHODS

Induction of osteomyelitis infection. The original strain of S. aureus was obtained from Carl Norden, Montefiore Hospital, Pittsburgh, Pa. The organism was passed through a mouse to maintain its virulence and stored in defibrinated sheep blood at −70°C. Tube dilution sensitivities of this S. aureus strain (10 CFU) were measured for A-56619, A-56620, and nafcillin in cation-supplemented Mueller-Hinton broth (CSMHB). Both MICs and MBCs (2) were determined for each drug.

New Zealand White rabbits, weighing approximately 2 kg, were used. A 16-gauge needle was inserted percutaneously through the lateral aspect of the left tibial metaphysis into the intramedullary cavity. A 0.1-ml volume of 5% sodium morrhuate (Eli Lilly & Co., Indianapolis, Ind.). 0.1 ml of a suspension of S. aureus (7 × 10^6 CFU/ml), and 0.1 ml of sterile saline were injected sequentially (7). The needle was removed, and the rabbit was returned to the cage.

Therapeutic trials. The rabbits were randomized into four groups at the time of infection (day zero), and treatment was begun 14 days later. At least two infected but untreated controls (group 1) were included with each treatment series. Group 2 received A-56619 (15 mg/kg) every 12 h, group 3 received A-56620 (20 mg/kg) every 12 h, and group 4 received nafcillin (Bristol Laboratories, Syracuse, N.Y.) (40 mg/kg) every 6 h. The antibacterial agents were given from day 14 through day 42 (28 days). Injections were given subcutaneously into the back of the neck. After completion of the treatment regimen, the rabbits were observed for 2 weeks before sacrifice.

Both A-56619 and A-56620 were prepared weekly by Abbott Laboratories and shipped in solution to our institution on the same day they were prepared. The prepared drugs were reportedly stable for up to 7 days after preparation. A-56619 and A-56620 were used in our studies within 7 days of preparation.

Roentgenograms of both tibiae were taken at drug initiation (day 14), at termination of drug treatment (day 42), and before sacrifice (day 56). Severity of the infection by roentgenographic appearance was graded by a rating system previously reported (5). The rabbits were weighed before infection and once weekly until they died or were sacrificed.

Bone cultures. At the conclusion of the study, rabbits were sacrificed by intracardiac injection of sodium pentobarbital. The bones were cultured by the method previously reported (5), in which the proximal and distal ends of the tibia were swabbed and streaked to check for any growth. Also, 1 ml of sterile tryptic soy broth was flushed through the marrow cavity, and 0.5 ml of the tryptic soy broth recovered was cultured quantitatively by serial dilution techniques. The MICs and MBCs (2) were determined for the S. aureus recovered from the marrow.

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Hematologic measurements. The sedimentation rate (modified Wintrobe), hematocrit, and blood leukocyte count were measured before infection, at drug initiation, at drug termination, and before the rabbits were sacrificed.

Drug kinetics in serum and simultaneous level measurements in serum and bone. A group of uninfected rabbits and a group that was selected 3 to 4 weeks after induction of infection were given a single subcutaneous injection of one of the three antibacterial agents. Concentrations of A-56619 and A-56620 in serum were determined from blood drawn 1, 2, 6, and 12 h after injection; concentrations of nafcillin were measured 0.5, 1, 2, 3, and 6 h after injection. Bone assay for drug concentrations was obtained 1 h after injection of A-56619 and A-56620 and 0.5 h after injection of nafcillin.

Drug assays in serum and bone. An agar disk diffusion bioassay was used to measure drug concentrations in both serum and bone eluates. Bacillus subtilis spores (Difco Laboratories, Detroit, Mich.) served as the test organism for A-56619 and A-56620. The lower limit of detection was 0.39 μg/ml for A-56619 and 0.20 μg/ml for A-56620 in serum. Saccharomyces lutea (ATCC 9341) was used as the test organism for nafcillin. The lower limit of detection of nafcillin in serum was 0.20 μg/ml.

Infected or uninfected bone was prepared for assay by grinding drug in an IKA Werk bone mill (Janke & Kunkel, Inc., Staufen im Breisgau, Federal Republic of Germany). The drug was eluted in a mixture of equal parts of normal rabbit serum and 0.1 M phosphate buffer (pH 7.5). Twice as much buffer-serum solution was used as the weight of bone powder. Standard solutions of the drugs were prepared by adding normal uninfected bone to buffer-serum solution containing known amounts of drug. The lower limit of detection of the assay was 0.25 μg/g for A-56619, 0.13 μg/g for A-56620, and 0.10 μg/g for nafcillin.

Growth and kill curves. In vitro effectiveness of each antibacterial agent against S. aureus was determined under both aerobic and anaerobic conditions. Growth and kill studies were performed in a series of 50-ml plastic conical centrifuge tubes (Becton Dickinson and Co., Cockeysville, Md.) connected by thin rubber Tygon tubing inserted into the plastic tube caps. One day before the assay, 19 ml of sterile CSMHB was pipetted into each sterile tube. In the aerobic assays, filtered ambient air was allowed to flow through the tube system for 12 to 24 h before the start of the assay. For anaerobic assays, filtered nitrogen was allowed to pass through the tube system in a similar manner. The tubes were placed in an incubator at 37°C, and their contents were gently mixed over a stirrer. An oxygen transducer was sealed into the bottom of the tube that was farthest from the air or nitrogen source, and the tube was allowed to equilibrate overnight. For aerobic assays, the oxygen tension was 155 mm Hg (100 mm Hg = 13.322 Pa), and for anaerobic assays it was less than 10 mm Hg.

S. aureus was grown overnight in CSMHB (aerobic) or nitrogen-reduced CSMHB (anaerobic). This strain of S. aureus grows overnight to approximately 5 × 10⁸ CFU/ml. For each assay, the overnight culture was diluted to contain 2 × 10⁵ CFU/ml in either aerobic or anaerobic CSMHB. A 0.1-ml volume of the S. aureus suspension was added through the sample tubing protruding from each assay centrifuge tube. The sample tubing was clamped when not in use. A-56619 and A-56620 were added in a quantity that yielded a final concentration of 1.5 μg/ml in 20 ml, and nafcillin was added to yield a final concentration of 2.0 μg/ml in 20 ml. The drugs were added in the same manner to at least six tubes per run which served as the kill tubes. The antibiotic concentrations selected approximated the mean concentrations of these antibiotics assayed from osteomyelitic bone. At least three tubes in each run contained no antibiotic and served as growth tubes. CSMHB was added through the sample tubing of each centrifuge tube to a final volume of 20 ml in each tube, with a concentration of 2.0 × 10⁵ CFU of S. aureus per ml. The final inoculum approximated the mean concentration of bacteria recovered from the bone washings of untreated rabbits in the in vivo therapeutic trials. A sample was immediately removed from each growth tube and each kill tube for quantitation of S. aureus, and additional samples were removed at 1, 3, 6, and 24 h. The number of viable S. aureus cells was determined at each assay time by preparing serial 10-fold dilutions in sterile 0.85% saline and streaking them onto blood agar.

RESULTS

Of 94 rabbits that were infected for the study (Table 1), 14 (15%) died before treatment, which began on day 14, and were not included in the data analysis.

Bone cultures. Cultures from 19 (95%) of 20 untreated infected (control) rabbits yielded S. aureus. Cultures were positive for S. aureus in 6 (30%) of 20 rabbits treated with A-56619, 14 (70%) of 20 rabbits treated with A-56620, and 8 (40%) of 20 rabbits treated with nafcillin. The difference between each drug treatment group and the control group was significant at P < 0.05. The differences between A-56619 and A-56620 treatment groups were also significant (P < 0.05). No significant difference in the proportion of rabbits with positive bone culture was found between the groups treated with nafcillin and those treated with A-56620.

The MICs and MBCs of both A-56619 and A-56620 for this S. aureus strain were 0.39 μg/ml. The MIC of nafcillin for this S. aureus strain was also 0.39 μg/ml, and the MBC was 0.78 μg/ml. The MICs and MBCs of the three drugs for the S. aureus isolates recovered from rabbits in which treatment failed were all within 2 dilutions of the corresponding values for the S. aureus isolates used to infect the rabbits. The number of viable bacteria (mean ± the standard error of the

<table>
<thead>
<tr>
<th>Drug (dose [mg/kg])</th>
<th>No. of positive bone cultures/total (%)</th>
<th>Mean ± (SEM) CFU/µl log₁₀ S. aureus</th>
<th>No. of deaths/total (%)</th>
<th>Mean ± (SEM) wt increase during:</th>
<th>Mean ± (SEM) radiographic severity of infection:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>Treatment (wk 2-6)</td>
<td>Before treatment (wk 2)</td>
</tr>
<tr>
<td>None (controls)</td>
<td>19/20 (95)</td>
<td>4.14 ± 0.23</td>
<td>1/20 (5)</td>
<td>0.74 ± 0.03</td>
<td>3.60 ± 0.17</td>
</tr>
<tr>
<td>A-56619 (15)</td>
<td>6/20 (30)</td>
<td>1.60 ± 0.22</td>
<td>1/20 (5)</td>
<td>0.69 ± 0.05</td>
<td>3.55 ± 0.17</td>
</tr>
<tr>
<td>A-56620 (20)</td>
<td>14/20 (70)</td>
<td>2.93 ± 0.47</td>
<td>1/20 (5)</td>
<td>0.65 ± 0.04</td>
<td>3.60 ± 0.17</td>
</tr>
<tr>
<td>Nafcillin (40)</td>
<td>8/20 (40)</td>
<td>1.32 ± 0.15</td>
<td>3/20 (15)</td>
<td>0.38 ± 0.07</td>
<td>3.55 ± 0.14</td>
</tr>
</tbody>
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* The rating system used is described in reference 5.

TABLE 1. Effects of A-56619, A-56620, and nafcillin on experimental S. aureus osteomyelitis

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mean \( \log_{10} \) found in the flushing of the left tibias for infected control rabbits (4.14 \( \pm \) 0.43) was significantly higher than the values for both the A-56619-treated (1.60 \( \pm \) 0.22) and nafcillin-treated (1.32 \( \pm \) 0.15) groups \( (P < 0.01) \). The mean number of bacteria found in the left tibial flushing for the A-56620-treated rabbits (2.93 \( \pm \) 0.47) was also significantly higher than the values for the A-56619- and nafcillin-treated groups \( (P < 0.5) \), but no significant difference was found between A-56620 and the control group.

**Weight changes.** The mean weight for all four groups of rabbits decreased from induction of infection (day zero) to the beginning of therapy (week 2) but then increased until the conclusion of the study. Of all the rabbits treated, the ones receiving A-56619 gained the most weight (0.95 kg) during the study \( (P < 0.025) \).

**Roentgenographic severity.** The roentgenographic severity score of infected bones measured before therapy (week 2) were similar for the control and all three treatment groups (Table 1). The severity scores showed a significant improvement \( (P < 0.05) \) from weeks 2 to 8 of the study in rabbits treated with A-56619 or nafcillin. There was no roentgenographic improvement in the A-56620 treatment group. The control rabbits had nearly identical mean severity scores throughout the study. The majority of the rabbits had sequestra.

**Concentrations of antibiotic in serum and bone.** Concentrations of A-56619, A-56620, and nafcillin in the sera of infected and uninfected rabbits after single subcutaneous injections of the respective drugs are shown in Table 2. The profiles in infected and uninfected rabbits are identical for each drug, except for A-56619, whose concentration in infected rabbits was lower at 6 and 12 h \( (P < 0.05) \). The calculated half-lives for A-56619 and A-56620 were 8.3 and 5.7 h, respectively.

Simultaneous concentrations of A-56619, A-56620, and nafcillin in serum, uninfected bone, and infected bone are compared in Fig. 1. The respective mean concentrations in infected bone were significantly higher for the three drugs than the values for uninfected bone \( (P < 0.05) \).

**Growth and kill curves.** The growth curves of \( S. aureus \) were similar under aerobic and anaerobic conditions (Fig. 2). Furthermore, there was no difference in the results of kill

![Graph showing antibiotic concentration in sera and bones](http://aac.asm.org/)

**FIG. 1.** Concentrations of A-56619 (15 mg/kg), A-56620 (20 mg/kg), and nafcillin (40 mg/kg) in sera and osteomyelitic and normal bones of infected rabbits after single subcutaneous injections. The bar graph with half brackets indicates the mean \( \pm \) the SEM for 6 to 8 animals.
curves determined under either aerobic or anaerobic conditions for any of the three drug treatments. The kill rate was greater for both quinolones than for nafcillin at 1, 3, and 6 h ($P < 0.05$).

DISCUSSION

In the experiments reported here, both A-56619 and nafcillin were effective in eradicating $S. aureus$ from experimental osteomyelitis. Seventy percent of A-56619-treated rabbits and 60% of nafcillin-treated rabbits had negative tibial cultures for $S. aureus$. Treatment with A-56620 was not as effective; only 30% of the treated rabbits had negative $S. aureus$ tibial cultures. $S. aureus$ isolated from the rabbits with positive tibial cultures showed no evidence of resistance to the treatment antibiotic. Both the A-56619 and nafcillin treatment groups had significantly lower counts of $S. aureus$ in the infected tibias than did the untreated controls.

Roentgenographic improvement was seen with A-56619 and nafcillin, but not with A-56620. Roentgenographic severity scores were similar at week 2 for all groups; therefore, we take it that the degree of osteomyelitis was approximately the same for all rabbits at the start of therapy.

Doses for the two quinolones were selected that would produce concentrations in serum which were at least eight times the MIC value for this $S. aureus$. The dose for nafcillin also produced concentrations in serum that exceeded eight times the MIC and yielded concentrations in serum similar to those reported in humans. A-56619 and A-56620 concentrations in serum were detectable for 12 h, whereas nafcillin concentrations were detectable for only 6 h. Both quinolones are reported to have long half-lives (Fernandes et al., 24th ICAAC).

The concentrations of A-56619 and A-56620 in infected bone were almost identical, and all three drugs had significantly higher concentrations in infected bone than in uninfected bone. The reason for the increased concentrations of these antibiotics in infected bone may relate to increased blood flow, serum trapping, increased penetration of the antibiotic into microabscesses, or some other mechanism. In an earlier experimental $S. aureus$ osteomyelitis study, we found no increase in blood flow in osteomyelitic bone by argon-wash-in and washout techniques (3).

Despite the importance of in vitro testing, antibiotics used to treat osteomyelitis must be evaluated in vivo. Infected bone has been shown to have a low intramedullary oxygen tension (3), and some antibiotics, notably the aminoglycosides and vancomycin, have been found to be less effective under anaerobic conditions (9, 12, 15). In our study, anaerobic kill curves were similar to aerobic kill curves for all three drugs. Thus, anaerobic conditions did not have a deleterious effect on the abilities of these antibacterial agents to kill this $S. aureus$ strain. Anaerobic kill curves, however, showed greater activities of A-56619 and A-56620 than of nafcillin. The reason for the similar results of treatment in vivo between A-56619 and nafcillin, despite better killing in vitro by A-56619, is unclear.

A-56620 was not as effective in eradicating $S. aureus$ from experimental osteomyelitis as were the other two antibiotics. Concentrations of A-56620 in bone and serum were similar to those of A-56619. Further studies must be performed to establish the precise reasons for the mediocre performance of A-56620 in experimental $S. aureus$ osteomyelitis.

The rabbit model has been used extensively to evaluate both single and combination therapy of $S. aureus$ osteomyelitis. In the rabbit model, the diffuse nature of the osteomyelitis and the small size of the animal preclude debridement surgery. The drug regimen must sterilize the bone without help from the surgical removal of necrotic bone. Both nafcillin and A-56619 were as effective as any single treatment regimen previously used in this model with this strain of $S. aureus$ (3, 4, 6, 8, 9, 11).

In general, it is more difficult to cure experimental osteomyelitis in rabbits than in humans, where extensive debridement surgery is performed. Thus, if results with the rabbit model can be extrapolated to humans, it appears that both nafcillin and A-56619 would be effective therapy for $S. aureus$ osteomyelitis when good MIC and MBC sensitivities are demonstrated by tube dilution testing. A-56619 is especially attractive since it has a long half-life, may be given orally, and has a broad spectrum of activity against other organisms.

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LITERATURE CITED


