Trovafloxacin Treatment of Viridans Group Streptococcus Experimental Endocarditis

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The activity of trovafloxacin was compared with those of vancomycin and penicillin in a model of Streptococcus sanguis species group (trovafloxacin MIC, 0.125 µg/ml) and Streptococcus mitis species group (trovafloxacin MIC, 0.125 µg/ml) experimental endocarditis. Rabbits with catheter-induced aortic valve vegetations were given no treatment, trovafloxacin at 15 mg/kg of body weight three times a day (t.i.d.), vancomycin at 15 mg/kg twice a day, or penicillin at 1.2 × 10^6 IU t.i.d. After 3 days of treatment, the animals were sacrificed; cardiac valve vegetations were aseptically removed and cultured quantitatively. Penicillin was as active as vancomycin as measured by in vivo clearance of bacteria. Trovafloxacin was less active (P < 0.05) than vancomycin or penicillin against S. sanguis species group infection but had similar efficacy against S. mitis species group infection. Quinolones, despite MICs in the susceptible range, may not be active for serious infections caused by some viridans group streptococci.

Trovafloxacin, a fluoroquinolone antimicrobial agent, has activity in vitro against many gram-positive cocci which is superior to that of several other quinolone antimicrobial agents (2). This activity includes activity against viridans group streptococci (2). Viridans group streptococci are the etiological agents in 30 to 40% of cases of native valve endocarditis (7). Patients with viridans group streptococcal endocarditis receive parenteral therapy with either penicillin G or ceftriaxone with or without an aminoglycoside, or with vancomycin administered alone (8). The potential role of trovafloxacin and of other new orally administered fluoroquinolones for the treatment of viridans group streptococcal endocarditis or other serious viridans group streptococcal infection is unknown. An oral antimicrobial regimen for the treatment of such infections in compliant patients would be convenient, would eliminate potential complications related to the use of intravenous therapy, and would likely be cost-effective.

The purpose of our study was to investigate whether the activity in vitro of trovafloxacin against viridans group streptococci is predictive of activity in vivo in a rabbit model of experimental endocarditis.

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Trovafloxacin was obtained from Pfizer (Groton, Conn.), vancomycin hydrochloride was obtained from Lederle (Wayne, N.J.), and penicillin was obtained from Wyeth-Ayerst (Philadelphia, Pa.).

MICs for 50 viridans group streptococcal isolates obtained from patients with infection, including 30 from patients with infective endocarditis, were determined by a standard microtube dilution assay, and minimum bactericidal concentrations (MBCs) were determined with a 0.1-ml subculture onto blood agar plates (6).

The trovafloxacin MIC at which 90% of the isolates were inhibited (MIC<sub>90</sub>) was 0.25 µg/ml (range, ≤0.125 to 0.6 µg/ml), and the trovafloxacin MBC at which 90% of the isolates were killed (MBC<sub>90</sub>) was 1 µg/ml (range, ≤0.125 to 4 µg/ml). The vancomycin MIC<sub>90</sub> was 1 µg/ml (range, 0.25 to 4 µg/ml), and the vancomycin MBC<sub>90</sub> was >128 µg/ml (range, 0.5 to >128 µg/ml). The penicillin MIC<sub>90</sub> was 0.25 µg/ml (range, >0.125 to 2 µg/ml), and the penicillin MBC<sub>90</sub> was 1 µg/ml (range, ≤0.125 to 64 µg/ml). A Streptococcus sanguis species group isolate and a Streptococcus mitis species group isolate recovered from patients with endocarditis were chosen for study in experimental endocarditis. MICs for the S. sanguis species group of trovafloxacin, vancomycin, and penicillin were 0.125, 1, and 0.25 µg/ml, respectively; corresponding MBCs were 1, >128, and 64 µg/ml, respectively. For the S. mitis species group trovafloxacin, vancomycin, and penicillin were 0.125, 1, and ≤0.125 µg/ml, respectively, and MBCs were 0.25, 1, and ≤0.125 µg/ml, respectively. When the determination was repeated using an inoculum of 10<sup>8</sup> CFU/ml, MICs for the S. sanguis species group were found to be 16, 2, and 0.25, respectively, and MBCs were found to be >128, >128, and 64 µg/ml, respectively; corresponding MICs for the S. mitis species group (i.e., 10<sup>8</sup>-CFU/ml inoculum) were 16, 1, and ≤0.125 µg/ml, respectively, and corresponding MBCs were 16, 1, and ≤0.125 µg/ml, respectively.

The bactericidal activities in vitro of trovafloxacin against the two strains studied in the experimental-endocarditis model were determined using time-kill studies. Time-kill studies were performed using an inoculum of 10<sup>5</sup> to 10<sup>6</sup> CFU in 25 ml of Mueller-Hinton broth. Cultures contained no antimicrobial or various concentrations of trovafloxacin, vancomycin, or penicillin. After 0, 6, and 24 h of incubation at 35°C in room air, 0.1-ml aliquots were removed from each culture and serially diluted; 0.1-ml aliquots of each dilution were plated on blood agar plates and incubated for 48 h at 35°C in 5% CO<sub>2</sub>. The bacterial count was expressed as log<sub>10</sub> CFU per milliliter; bacterial killing was expressed as the bacterial count following incubation minus the initial inoculum. A bactericidal effect was defined as a ≥3-log<sub>10</sub> CFU/ml reduction of the initial inoculum.

Time-kill studies with the S. sanguis species group isolate demonstrated less than a 2-log<sub>10</sub> CFU/ml reduction of the
The lack of in vivo activity of trovafloxacin in experimental endocarditis demonstrated potent killing at 24 h by trovafloxacin, vancomycin, and penicillin. Time-kill studies with the S. mitis species group isolate used in experimental endocarditis demonstrated potent killing at 24 h by trovafloxacin.

Antimicrobial concentrations in serum were determined in triplicate by a microbiological assay (5). Bioassays were performed on Mueller-Hinton agar seeded with Klebsiella pneumoniae ATCC 10031 as the indicator organism for trovafloxacin, Bacillus subtilis ATCC 6633 for vancomycin, and Micrococcus lutea ATCC 9341 for penicillin. Paper disks with 20 μl of serum were placed on the bioassay plates, which were then incubated for 16 to 18 h in room air at 35°C for K. pneumoniae or 30°C for B. subtilis and M. lutea. The zone sizes were measured with calipers, and concentrations were calculated against a five-point standard curve by linear regression.

Mean concentrations in the sera of five uninfected rabbits obtained at timed intervals after a single dose of the study antimicrobial are shown in Table 2 (5). Antimicrobial dosages for in vivo study were chosen to approximate expected peak concentrations in human serum after the administration of fixed dosages. Peak concentrations in serum were achieved within 30 min after administration of trovafloxacin and vancomycin and within 2 h after penicillin administration. The area under the concentration-time curve from 0 to 24 h (AUC0–24) for trovafloxacin was 32.7 μg · h/ml; the ratio of the AUC0–24 for trovafloxacin to the trovafloxacin MIC for the S. sanguis and S. mitis species group isolates was 262.

Aortic valve experimental bacterial endocarditis was established in New Zealand White rabbits (weight, 2.5 to 3.0 kg) by a modification of the technique described by Garrison and Freeman (4). After surgical anesthesia was induced, a midline incision was made in the neck and the right carotid artery was exposed. The artery was ligated distally and clamped proximally; a sterile polyethylene catheter was inserted into the artery through a small incision and advanced proximally into the left ventricle. The distal end of the catheter was sealed and ligated in place and left in place for the duration of the experiment. The surgical wound was closed over the catheter with sutures and surgical clips. Twenty-four hours after catheter placement, rabbits were infected by intravenous (i.v.) injection of 10^7 CFU of viridans group streptococci per ml. Twenty-four hours after bacterial challenge, a blood culture was obtained to ensure the presence of endocarditis; antimicrobial therapy was initiated at this time and continued for 3 days. Rabbits were either treated (controls) or were treated with trovafloxacin at 15 mg/kg of body weight (2 ml of a 7.5-μg/ml preparation) i.v. three times daily, vancomycin at 15 mg/kg i.v. twice daily, or procaine penicillin at 1.2 × 10^6 IU intramuscularly three times daily. For each antimicrobial agent, concentrations in serum were assayed 30 min after administration of the first dose on the second day of treatment for therapeutic monitoring (5). The mean 30-min concentrations in serum of animals infected with S. sanguis species group were 5.2 μg/ml for trovafloxacin, 40 μg/ml for vancomycin, and 16 μg/ml for penicillin. Twelve hours after administration of the last dose of the antimicrobial, the animals were sacrificed with 100 mg of i.v. pentobarbital/kg. The chest cavity was opened, the heart was excised and opened, and the aortic valves were aseptically removed. The vegetations were weighed and homogenized with 2 ml of Todd-Hewitt broth in a Stomacher 80 (Tekmar Co., Cincinnati, Ohio). The homogenate was cultured quantitatively for viridans group streptococci. Serial 10-fold dilutions in Todd-Hewitt broth were plated on blood agar plates (0.1-ml aliquot per plate) and incubated for 48 h at 35°C in 5% CO2. Tissue culture results were expressed as log10 CFU of streptococci per gram of valve vegetation. Statistical analysis was performed by rank sum analysis.

The results of antimicrobial therapy of experimental endocarditis are shown in Table 3. For the S. sanguis species group isolate, the results of treatment with trovafloxacin were not significantly different from those for no treatment. Penicillin or vancomycin therapy was more effective (P < 0.05) than no treatment or trovafloxacin therapy. Penicillin therapy was as effective as that of vancomycin. Vancomycin, trovafloxacin, or penicillin therapy was more active against the S. mitis species group isolate than was such therapy for S. sanguis species group experimental endocarditis.

Reduction of viable bacteria in experimental endocarditis aortic valve vegetations is likely due exclusively to the bactericidal activity of the antimicrobial agent used for treatment. The lack of in vivo activity of trovafloxacin in S. sanguis exper-
TABLE 3. Results of treatment of viridans group streptococcus experimental endocarditis

<table>
<thead>
<tr>
<th>Species group</th>
<th>Treatment</th>
<th>No. of animals/</th>
<th>Log_{10} CFU/g of vegetation</th>
<th>Median</th>
<th>Range (25th–75th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no. sterile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sanguis</td>
<td>None</td>
<td>7/0</td>
<td>11.0</td>
<td>10.5–11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trovafloxacin</td>
<td>15/0</td>
<td>10.7</td>
<td>10.3–11.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>12/0</td>
<td>7.7</td>
<td>6.0–8.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>12/0</td>
<td>7.2</td>
<td>6.5–9.5</td>
<td></td>
</tr>
<tr>
<td>S. mitis</td>
<td>None</td>
<td>6/0</td>
<td>10.1</td>
<td>9.7–10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trovafloxacin</td>
<td>5/4</td>
<td>4.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4–5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>5/3</td>
<td>5.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7–5.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>6/4</td>
<td>4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3–5.6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Sterile cultures were assigned a value equal to the lower limit of detection.

Experimental endocarditis was not predicted by standard MIC susceptibility testing but did correlate with the results of time-kill studies, as well as with an elevated trovafloxacin MBC and tolerance of cell wall-active agents.

The extent to which other viridans group streptococci would mimic the in vivo results of our study is unknown. Entenza et al. (3) recently demonstrated that, in a rat model of viridans group streptococcal endocarditis, therapy with trovafloxacin was significantly less effective than therapy with ceftriaxone against a trovafloxacin-susceptible, penicillin-susceptible strain of <i>S. sanguis</i>. In this same study, trovafloxacin therapy was as effective as ceftriaxone therapy for experimental endocarditis caused by a penicillin-resistant strain of <i>S. mitis</i>. As in our study, trovafloxacin exhibited less in vivo activity against <i>S. sanguis</i> than against <i>S. mitis</i>.

Our results and the results of other studies suggest that trovafloxacin MICs may not correlate with in vivo activity. The reasons for this lack of activity in vivo are unclear. Emergence of resistance in vivo, although not specifically addressed in our experiments, is possible but seems unlikely considering the short duration of treatment.

Notably, vancomycin and penicillin were also less active in vivo against the <i>S. sanguis</i> isolate than against the <i>S. mitis</i> isolate, although to a less striking degree than trovafloxacin. The MBCs for the test agents against the <i>S. sanguis</i> isolate studied in vivo were all high, and this isolate was tolerant of both cell wall-active agents studied (penicillin and vancomycin). Tolerance of cell wall-active agents correlated not only with poor in vivo activity of both these agents but also with poor in vitro and in vivo activity of trovafloxacin.

Despite the recent concerns about hepatic toxicity associated with trovafloxacin use and the restriction imposed on trovafloxacin use by the Food and Drug Administration, our findings are important because similar results of viridans group streptococcal therapy with other fluoroquinolones may occur. For example, lack of efficacy of levofloxacin for viridans group streptococcal experimental endocarditis (1) suggests that the phenomenon observed in our study may be a general one and that fluoroquinolones, despite MICs in the susceptible range, may not be efficacious for serious infections caused by viridans group streptococci. Future studies with other animal models of infection and clinical trials are necessary to clarify this issue. Until such data are available, we suggest that the fluoroquinolones be used with caution for the treatment of serious viridans group streptococcal infections in humans.

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REFERENCES