Oral Temafloxacin versus Vancomycin for Therapy of Experimental Endocarditis Caused by Methicillin-Resistant Staphylococcus aureus

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Staphylococcus aureus is a common cause of infective endocarditis, and cases of endocarditis caused by methicillin-resistant S. aureus (MRSA) are increasing in frequency, especially among intravenous drug users (8). Treatment of these infections requires prolonged use of intravenous vancomycin alone or in combination with rifampin or gentamicin (11, 12). This usually necessitates a long hospital stay, and, in intravenous drug users with limited venous access, may require placement of a Hickman catheter or similar device. An effective drug that could be administered orally would be of great benefit to these patients. Temafloxacin is a new fluoroquinolone compound that has good activity against staphylococci including MRSA (5, 9). According to data from the manufacturers (Abbott Laboratories, North Chicago, Ill.), oral administration of the drug in humans results in levels in serum and tissue above the MBCs for these organisms (9). The purpose of this study was to compare orally administered temafloxacin with parenteral vancomycin, each with and without rifampin, for the treatment of endocarditis caused by MRSA in rats.

MATERIALS AND METHODS

Organism. The strain of MRSA used in this study was isolated from a patient with endocarditis. It was shown to be methicillin resistant by growth on Mueller-Hinton agar containing 20 μg of methicillin per ml. Stock cultures were prepared by incubating the organism in Mueller-Hinton broth (MHB) for 18 h at 37°C and freezing 1-ml samples at −70°C. At the time of each experiment, a frozen sample was subcultured into MHB and incubated overnight at 37°C.

In vitro studies. The MICs and MBCs of temafloxacin, vancomycin, and rifampin were determined by standard serial dilution techniques with inocula of 10⁵ CFU/ml (7). The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation. The MBC was defined as the lowest concentration that killed 99.9% of the organisms after 24 h, as determined by quantitative culture of each nonturbid MIC dilution.

The survival of 3 × 10⁶ CFU of MRSA per ml was studied in flasks of MHB with the following antimicrobial agents: temafloxacin (3 μg/ml), vancomycin (25 μg/ml), rifampin (1 μg/ml), temafloxacin (3 μg/ml) plus rifampin (1 μg/ml), and vancomycin (25 μg/ml) plus rifampin (1 μg/ml). The antimicrobial concentrations were based on levels achievable in human serum. Kill curves were constructed by quantitative culture of a sample from each flask at 0, 3, 6, and 24 h (2). Additional kill curves were constructed by using starting inocula of 5 × 10⁶ or 10⁸ CFU/ml in flasks containing temafloxacin (6 μg/ml) or vancomycin (25 μg/ml) each in MHB and in rat serum. Plates were incubated at 37°C for 48 h.

Pharmacokinetic studies. At 18 h after infection, rats were given temafloxacin (100 mg/kg) by gastric lavage and were sacrificed at 1, 2, 3, 4, 6, 8, and 10 h following the administration of the dose. Antibiotic concentrations were determined in serum and vegetation by a disk diffusion method with Klebsiella pneumoniae as the test organism (1). Therapeutic studies. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Altamonte, Vt.) weighing 200 to 300 g were anesthetized. The right carotid artery of each was cannulated, and the catheter was advanced across the aortic valve (10). After 24 h, each animal was inoculated with 10⁶ CFU of MRSA in 1 ml of MHB via the tail vein. This inoculum produced endocarditis in all rats.

At 18 h after infection, the following treatment regimens were begun: temafloxacin (100 mg/kg) by gavage every 12 h; vancomycin (60 mg/kg) intramuscularly every 12 h; rifampin (6 mg/kg) intramuscularly every 12 h; temafloxacin (100 mg/kg) by gavage plus rifampin (6 mg/kg) intramuscularly every 12 h; vancomycin (60 mg/kg) intramuscularly plus rifampin (6 mg/kg) intramuscularly every 12 h. Each treatment regimen was administered for 5 days, and survivors were sacrificed 12 h after the last dose. Groups of untreated controls were sacrificed at the time of the onset of antimicrobial therapy and 12 h after the last dose of antimicrobial therapy (18 and 150 h after infection). The doses of vancomycin and temafloxacin were those required to result in peak concentrations similar to those achieved in humans (6; unpublished data from Abbott Laboratories).

At the time of sacrifice, all valvular vegetations were

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FIG. 1. Survival of *S. aureus* in MHB (control) and MHB containing vancomycin (25 μg/ml), temafloxacin (3 μg/ml), rifampin (1 μg/ml), vancomycin (25 μg/ml) plus rifampin (1 μg/ml), or temafloxacin (3 μg/ml) plus rifampin (1 μg/ml).

excised, pooled, and weighed. Vegetations from each animal were homogenized in 0.5 ml of MHB and were quantitatively cultured. Plates were incubated for 48 h at 37°C. Therefore, in sterile vegetations, the number of CFU was recorded as 2 or 3 log units, CFU/g.

**Antimicrobial concentrations.** Blood for peak concentrations in serum was obtained from tail veins 60 min after the administration of temafloxacin and vancomycin on the third day of therapy. Blood for trough levels was obtained from the inferior vena cava at the time of sacrifice. Concentrations of temafloxacin and vancomycin were measured by a paper disk agar diffusion method (1) with *Bacillus subtilis* as the test organism for vancomycin and *K. pneumoniae* as the test organism for temafloxacin.

**Development of resistance.** Emergence of resistance to temafloxacin during therapy was evaluated by quantitative culture of a 0.1-ml sample of homogenized vegetation on agar plates containing 5 μg of temafloxacin per ml of agar and by then incubating for 48 h at 37°C.

**Statistics.** Mean vegetation bacterial counts were compared by using one-way analysis of variance with the Newman-Keuls test for multiple comparisons.

## RESULTS

**In vitro studies.** The temafloxacin, vancomycin, and rifampin MICs and MBCs, respectively, were 0.78 and 1.36, 1.56 and 3.13, and <0.024 and 0.78 μg/ml. Representative kill curves at an inoculum of 3 × 10^6 CFU/ml in MHB are shown in Fig. 1. Temafloxacin was more rapidly bactericidal than was vancomycin. Addition of rifampin to either temafloxacin or vancomycin decreased the bactericidal rate, thus demonstrating antagonism. The use of rat serum as the growth medium increased the bactericidal rate with vancomycin and to a slight degree with temafloxacin (Fig. 2).

**Pharmacokinetic studies.** The pharmacokinetic study results with temafloxacin are shown in Table 1. Levels in both serum and vegetation at 10 h were well above the MICs for the organism.

**Therapeutic studies.** There was no significant difference in survival between groups receiving vancomycin or temafloxacin (Table 2). However, only temafloxacin-containing regimens resulted in a significant reduction in bacterial counts in vegetations after 5 days (*P < 0.001*). This difference occurred despite the fact that levels of vancomycin in serum were above the MBCs for the organism throughout the dosing interval. The addition of rifampin to either vancomycin or temafloxacin did not cause a significant difference in either survival or vegetation counts.

There was no growth on temafloxacin-containing agar from homogenized vegetations of any animal, i.e., no emergence of resistance was demonstrated.

## DISCUSSION

Experimental endocarditis is a rigorous test of efficacy for an antimicrobial agent, as host defenses play little role and cure of infection rests entirely on the agent (4). This study shows the efficacy of oral temafloxacin, a new antimicrobial agent of the quinolone class, in treating experimental MRSA endocarditis in rats. In this study, temafloxacin was superior to vancomycin, which is considered the drug of choice for this infection. While vegetations from vancomycin-treated rats had bacterial counts similar to those of untreated controls, temafloxacin caused a significant drop in bacterial counts. Other investigators have also reported poor results with vancomycin in the treatment of MRSA endocarditis in experimental animals (3). The reasons for the lack of efficacy

<table>
<thead>
<tr>
<th>h after administration</th>
<th>Mean levels in serum (μg/ml)</th>
<th>Mean levels in vegetation (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.32</td>
<td>&lt;0.32</td>
</tr>
<tr>
<td>1</td>
<td>4.1</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>4.3</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>8</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>10</td>
<td>2.6</td>
<td>2.9</td>
</tr>
</tbody>
</table>

*Three to four rats in each group.*
of vancomycin in our study are unclear. The levels of vancomycin in serum at both peak and trough were above the MBCs for the organism. Vancomycin is not highly protein bound, and the in vitro studies in serum showed an increased rate of bactericidal activity. Studies from this laboratory demonstrated high levels of vancomycin in homogenized rat vegetations (6). However, recent studies with radiolabeled teicoplanin, a glycopeptide similar to vancomycin, showed good penetration of teicoplanin superficially into cardiac vegetations but poor and uneven penetration deep into the vegetations (A. C. Cremieux, J. M. Vallois, B. Massiere, M. Ottoviani, A. Bouvet, and C. Carbon, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 922, 1987). Perhaps the poor results with vancomycin in this study can be explained by similar poor penetration deep into the vegetations or by strain differences in staphylococci.

Vancomycin is successful in the majority of cases of MRSA endocarditis, and wide experience with it dictates its continued use as the drug of choice for such infections (12). However, vancomycin is inconvenient to administer because it must be given intravenously and some patients need alternate therapy either because of lack of venous access or intolerance of the drug. The findings of this study support further evaluation of temafloxacin for the treatment of staphylococcal infections.

TABLE 2. Results of in vivo experiments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals/total</th>
<th>Mean vegetation bacterial counts (log_{10} CFU/g)</th>
<th>Levels in serum (5-day peak/trough) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surviving</td>
<td>With sterile vegetations</td>
<td>Day 0</td>
</tr>
<tr>
<td>Controls</td>
<td>9/9</td>
<td>0/9</td>
<td>9.5</td>
</tr>
<tr>
<td>Controls</td>
<td>5/21</td>
<td>0/5</td>
<td>9.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1/6</td>
<td>0/1</td>
<td>10.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>11/14</td>
<td>0/11</td>
<td>8.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin plus rifampin</td>
<td>5/6</td>
<td>0/5</td>
<td>9.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Temafloxacin</td>
<td>16/20</td>
<td>5/16</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Temafloxacin plus rifampin</td>
<td>5/7</td>
<td>1/5</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not significant versus controls, rifampin, vancomycin, and vancomycin plus rifampin.

<sup>b</sup> P < 0.001 versus controls, rifampin, vancomycin, and vancomycin plus rifampin.

ACKNOWLEDGMENT

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LITERATURE CITED


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