Linezolid Therapy of Vancomycin-Resistant Enterococcus faecium Experimental Endocarditis

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We compared the activities of linezolid (25 mg/kg of body weight, administered intraperitoneally every 8 h) and of vancomycin (25 mg/kg of body weight, administered intraperitoneally every 8 h) in a rat model of vanA vancomycin-resistant Enterococcus faecium experimental endocarditis. Results were expressed as median log10 CFU per gram of vegetation after 3 days of treatment. The median log10 CFU per gram of vegetation was 10.1 among 7 untreated control animals, 10.2 among 9 vancomycin-treated animals, and 7.9 among 10 linezolid-treated animals. Linezolid treatment was more active (P < 0.05) than vancomycin treatment or no treatment. Oxazolidinones are a new class of synthetic antimicrobial agents which inhibit initiation of protein synthesis. Linezolid is the first oxazolidinone to be extensively developed and studied and is inhibitory against vancomycin-resistant enterococci in vitro (11).

Although quinupristin-dalfopristin is approved by the U.S. Food and Drug Administration for the treatment of patients with serious or life-threatening infections associated with vancomycin-resistant Enterococcus faecium bacteremia, and linezolid is approved by the U.S. Food and Drug Administration for the treatment of patients with vancomycin-resistant E. faecium infections, including those with concurrent bacteremia, the optimal management for patients with endocarditis caused by vancomycin-resistant E. faecium is unknown. The purpose of this study was to examine the activity of linezolid in a rat model of vancomycin-resistant E. faecium experimental endocarditis.

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The vancomycin resistance genotype of the E. faecium isolate studied was determined, using a previously described multiplex PCR-restriction fragment length polymorphism assay (12), to be vanA.

Susceptibility testing was performed using a broth macrodilution technique as described by the National Committee for Clinical Laboratory Standards (7, 8). The linezolid MIC and minimal bactericidal concentration were 2 and 128 μg/ml, respectively. The vancomycin MIC was >128 μg/ml. Time-kill experiments using the vancomycin-resistant E. faecium isolate were performed with 1, 10, and 20 μg of linezolid per ml and an initial inoculum of 10^5 CFU/ml in accordance with current guidelines (8). After 4 h, 6.9, 6.2, 5.0, and 4.7 log10 CFU of vancomycin-resistant E. faecium per ml were present in broths containing 0, 1, 10, and 20 μg of linezolid per ml, respectively.

After 24 h, 8.1, 7.8, 5.1, and 4.4 log10 CFU of vancomycin-resistant E. faecium per ml were present in broths containing 0, 1, 10, and 20 μg of linezolid per ml, respectively.

Experimental aortic valve bacterial endocarditis was established in 26 adult male Wistar rats. The animals were anesthetized with a combination of ketamine and xylazine, and the right carotid artery was exposed. The artery was ligated distally, and a sterile polyethylene catheter was inserted into the artery through a small incision and was advanced proximally. The distal end of the catheter was attached to a pressure-sensitive monitoring device to ensure proper placement of the catheter across the aortic valve in the left ventricle. The distal end of the catheter was sealed and the wound was closed over the catheter with surgical clips. Twenty-four hours after catheter placement, the animals were again anesthetized, and the distal end of the catheter was exposed. A 0.2-ml dose of saline containing 5 × 10^6 CFU of vancomycin-resistant E. faecium was injected into the cardiac catheter; the catheter was flushed with 0.5 ml of sterile saline and was sealed closed. The catheter was left in place for the duration of the experiment. The inocula were prepared by diluting a stationary-phase broth culture 1:15 in saline.

Antimicrobial therapy was initiated 24 h after bacterial challenge. After 3 days of treatment and 10 h after administration of the last dose of antimicrobial agent, the rats were sacrificed with a lethal dose of pentobarbital. The aortic valve leaflets and attached vegetations were aseptically removed and weighed. The tissues were homogenized in 2 ml of nutrient broth and serially diluted in nutrient broth. Aliquots (0.1 ml) of each dilution were plated onto the surfaces of blood agar plates and incubated for 48 h at 35°C in 5% CO₂. The plates were examined for purity and colony morphology. The colonies were counted and the log10 CFU of enterococci per gram of vegetation was algebraically calculated.

Linezolid (Pharmacia and Upjohn, Kalamazoo, Mich.) was dissolved in sterile water and administered intraperitoneally at a dose of 25 mg/kg of body weight three times daily. Vancomycin (Abbott Laboratories, North Chicago, Ill.) was administered intraperitoneally at a dose of 25 mg/kg of body weight three times daily. Untreated control rats were included in each experiment.

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In previous experiments using a rat model of *Staphylococcus aureus* osteomyelitis, we determined the pharmacokinetics of linezolid and vancomycin when administered as outlined above. We observed a peak concentration of linezolid in serum of 21.4 μg/ml and an area under the concentration-time curve from 0 to 24 h of 185 μg · h/ml. The concentration of linezolid in serum was greater than the MIC for the study isolate for ≥75% of the dosing interval. The linezolid peak concentration and the area under the concentration-time curve were similar to those documented for humans. The peak concentration of vancomycin in serum was 47 μg/ml (9).

Results of treatment of vancomycin-resistant *E. faecium* experimental endocarditis are shown in Table 1. The log10 CFU of enterococci per gram of vegetation from three animals sacrificed at the start of therapy were 7.7, 8.3, and 8.9. Differences in the mean log10 CFU of *E. faecium* per gram of vegetation among the different treatment groups were analyzed using the Wilcoxon rank sum test. Vancomycin treatment results were not significantly different from those obtained with no treatment, and linezolid treatment was more active (*P* < 0.05) than vancomycin treatment or no treatment.

Our study indicates that linezolid displays significant in vivo activity in an experimental rat model of vancomycin-resistant *E. faecium* endocarditis. The reduction in CFU per gram of vegetation noted herein with linezolid is commensurate with that typically observed with ampicillin alone for ampicillin-susceptible experimental enterococcal endocarditis but is not as great as that typically observed with the synergistic bactericidal combination of ampicillin and gentamicin for ampicillin- and gentamicin-susceptible experimental enterococcal endocarditis (4). LY333328 is an investigational glycopeptide and is bactericidal against enterococci, whereas linezolid is bacteriostatic (10, 11). Despite this, the reduction in densities of CFU per gram of vegetation found in our study using linezolid (2.2 log10 CFU/g) was similar to those reported with LY333328 in rabbits with experimental aortic valve endocarditis due to *Enterococcus faecalis* with VanA (2.1 log10 CFU/g) and VanB (2.8 log10 CFU/g) phenotypes (13).

There are few effective options available for the treatment of humans with vancomycin-resistant (penicillin-resistant) enterococcal endocarditis. Quinupristin-dalfopristin treatment of vancomycin-resistant *E. faecium* endocarditis in humans has reportedly been successful in four of nine cases (6). Recently, successful treatment of a patient with vancomycin-resistant *E. faecium* endocarditis with the combination of quinupristin-dalfopristin, doxycycline, and rifampin has been reported (5). Development of in vitro resistance to quinupristin-dalfopristin during therapy for enterococcal bacteremia is cause for concern (2). Furthermore, quinupristin-dalfopristin is not active against *E. faecalis*.

Intraperitoneally administered linezolid has been shown to be active in a murine model of vancomycin-susceptible *E. faecalis* systemic infection, with a 50% effective dose (ED50) of 10.0 mg/kg, and oral linezolid treatment has been shown to be active in an immunocompromised murine model of vancomycin-resistant *E. faecium* systemic infection, with an ED50 of 24.0 mg/kg (3). In a murine model of vancomycin-susceptible *E. faecalis* soft tissue infection, oral linezolid had an ED50 of 11.0 mg/kg (3).

Schälin et al. recently examined the in vivo activity of linezolid against one strain each of vancomycin-susceptible *E. faecalis* and vancomycin-resistant *E. faecium* in a rat model of intra-abdominal abscess (14). At a dose of 25 mg/kg of body weight twice daily, intravenous or oral linezolid produced small but statistically significant reductions in abscess bacterial density for *E. faecalis* (14). At a dose of 100 mg/kg/day, intravenous linezolid treatment led to a decrease of approximately 2 log10 CFU/g of abscess (14). Against *E. faecium* infection, oral linezolid administered at a dose of 25 mg/kg of body weight twice daily reduced the bacterial density by approximately 2 log10 CFU/g of abscess (14).

Chien et al. recently reported the microbiologic cure with linezolid therapy of 10 of 15 humans infected with vancomycin-resistant enterococci (1). Their cases included two patients with endocarditis, one of whom was successfully treated with linezolid (1).

Our results indicate that linezolid is active in a rat model of experimental vancomycin-resistant *E. faecium* endocarditis. Further studies of linezolid for the treatment of vancomycin-resistant enterococcal endocarditis may be warranted; a bactericidal combination of linezolid with a second antimicrobial agent would be desirable.

### REFERENCES


### TABLE 1. Outcome of therapy of endocarditis due to vancomycin-resistant *E. faecium*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Median log10 CFU/g of vegetation (25th–75th percentile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>7</td>
<td>10.1 (10.0–10.1)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>10</td>
<td>7.9 (7.0–8.8)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>9</td>
<td>10.2 (9.7–10.7)</td>
</tr>
</tbody>
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*P* < 0.05 versus value for control animals or vancomycin-treated animals.