Efficacy of linezolid in a staphylococcal endocarditis rabbit model

Martha P. Oramas-Shirey, Lewis V. Buchanan*, Christine L. Dileo-Fang, Charlene F. Dailey, Charles W. Ford, Donald H. Batts and John K. Gibson

Pharmacology 7250-209-205, 301 Henrietta Street, Pharmacia Corporation, Kalamazoo, MI 49001, USA

A rabbit endocarditis model was used to test the efficacy of oral linezolid and iv vancomycin. Twenty-four hours after catheter placement across the aortic valve, rabbits were infected with $3.5 \times 10^6$ cfu of *Staphylococcus aureus* (UC-9258). Two days after infection, control rabbits were killed, and treated rabbits were given 5 days of therapy with linezolid at 8 h intervals (tds) using either 25, 50 or 75 mg/kg/dose, or vancomycin at 12 h intervals (bd) using 25 mg/kg/dose. Linezolid at 75 and 50 mg/kg, and vancomycin significantly reduced *S. aureus* in aortic valve vegetations compared with the control. Linezolid at 25 mg/kg was ineffective. The efficacy of 75 and 50 mg/kg linezolid was related to maintenance of plasma drug levels near or above the linezolid MIC for UC-9258 (2 mg/L).

**Introduction**

The oxazolidinones are a new class of synthetic antibacterial agents and are chemically unrelated to any commercially available agent.\(^1\) In vivo and in vitro studies have demonstrated that the oxazolidinone, linezolid, has significant antimicrobial activity against multiresistant Gram-positive pathogens such as coagulase-negative staphylococcal species, *Staphylococcus aureus*, vancomycin-resistant enterococci and *Streptococcus pneumoniae*.\(^2\)

The mechanism of action is inhibition of the initiation phase of translation by blocking formation of the 70S subunit initiation complex.\(^3\) This mechanism of action is distinctive of the oxazolidinones, and consequently no inherent cross-resistance has been found in bacterial strains resistant to other protein synthesis inhibitors or other antimicrobial agents, including multidrug-resistant strains.\(^1,2\)

A rabbit model of aortic valve endocarditis developed by Garrison & Freedman\(^4\) continues to be the predominant tool to define the experimental effects of antibiotic agents for treatment of this disease. Bacterial endocarditis is a lethal infection that requires the administration of high levels of bactericidal antibiotics for prolonged periods of time for cure. A significant percentage of patients with endocarditis fail therapy or suffer relapse, either because resistance develops or because not all of the infection is cleared.\(^5\)

The efficacy of linezolid in treating serious *S. aureus* infections such as endocarditis has not been determined. To address this issue, we compared the therapeutic activities of a range of oral linezolid doses from 25 to 75 mg/kg tds, and a standard 25 mg/kg iv bd vancomycin dose in a rabbit model of aortic valve endocarditis.

**Materials and methods**

**In vivo tests**

All procedures in this study were in compliance with the Animal Welfare Act Regulations (9 CFR parts 1, 2 and 3) and with the Guide for the Care and Use of Laboratory Animals (ILAR 1996).

Experiments were performed on male, specific pathogen-free (SPF) New Zealand White rabbits weighing 2.0–2.5 kg. Employing sterile surgical technique under intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) anaesthesia, a polyethylene catheter (PE-50) was inserted into the right carotid artery and advanced across the aortic valve into the left ventricle. The catheter was sutured in place for the duration of the study. Only data from animals with correct catheter placement upon autopsy were included. Rabbits were infected 24 h after surgery with $3.5 \times 10^6$ cfu of *S. aureus* UC-9258. Forty-eight hours after infection, control rabbits were killed, and linezolid or vancomycin treatment was initiated. Rabbits were killed 8 h after the final dose of linezolid or 12 h after the final dose of vancomycin. Aortic valve vegetations, blood and ventricular myocardium were removed and homogenized, and quantitative

\*Corresponding author. Tel: +1-616-833-4467; Fax: +1-616-833-9763; E-mail: lvbuchan@am.pnu.com

© 2001 The British Society for Antimicrobial Chemotherapy
bacterial counts were determined by serial dilution and expressed as \( \log_{10} \text{cfu/g} \) of tissue. Culture-negative samples were assigned a value equal to the lowest level of detection based on tissue weight and one colony in an undiluted sample.

**Antimicrobial agents and treatment groups**

Linezolid was prepared as a 25 mg/mL oral suspension in Sterile Vehicle 122 (Pharmacia Corporation, Kalamazoo, MI, USA). Vancomycin (Sigma Chemical Co., St Louis, MO, USA) was dissolved in sterile saline at 25 mg/mL, and was administered intravenously via a marginal ear vein. Rabbits were assigned randomly to the following treatment groups: control (\( n = 14 \)); vancomycin 25 mg/kg bd for 5 days (\( n = 11 \)); linezolid 75 mg/kg tds for 5 days (\( n = 10 \)); linezolid 50 mg/kg tds for 5 days (\( n = 12 \)); and linezolid 25 mg/kg tds for 5 days (\( n = 5 \)). Two rabbits were excluded due to improper catheter placement. One rabbit receiving the 25 mg/kg dose of linezolid was killed early owing to illness and was therefore excluded from analysis.

**Bacterial strain**

A clinical isolate of *S. aureus* (UC-9258) from an endocarditis patient was used to produce infection in these studies. The MIC for the isolate was 2 mg/L for linezolid, <0.5 mg/L for vancomycin and 2 mg/L for methicillin [methicillin-susceptible *S. aureus* (MSSA)]. The mean bactericidal concentration (MBC) of linezolid was >64 mg/L for this strain.

UC-9258 was diluted in sterile saline and administered as a 1 mL intravenous bolus of \( 3.5 \times 10^6 \) cfu through a marginal ear vein.

**Plasma analysis**

Blood samples were obtained at 1 and 8 h after the initial linezolid dose, at 1 h after the penultimate linezolid dose and upon killing. Plasma was frozen until assayed for linezolid levels.

The plasma samples were analysed by high performance liquid chromatography/mass spectrometry/mass spectrometry (HPLC/MS/MS) using a PE SCIEX API 3000 triple quadruple mass spectrometer with a heated nebulizer ion source and a Hewlett Packard 1100 HPLC as the solvent delivery/injection system. The HPLC and mass spectrometer were controlled by PE SCIEX API MassChrom software version 1.1. HPLC mobile phase solutions used were 43% methanol with 2 mM ammonium acetate and 57% H2O with 2 mM ammonium acetate.

**Statistical analysis**

The results are reported as mean ± s.d. Comparisons of bacterial densities in blood, ventricular myocardium and aortic valve vegetation used the Kruskal–Wallis one-way analysis of variance on ranks followed by Dunn’s test for multiple comparisons. A \( P \) value ≤0.05 was considered statistically significant.

**Results and discussion**

Bacterial counts in aortic valve vegetations from rabbits treated with linezolid at 75 mg/kg/dose were significantly reduced to 3.1 ± 0.4 \( \log_{10} \text{cfu/g} \), compared with control (8.4 ± 1.0 \( \log_{10} \text{cfu/g} \) (Table I). Bacterial counts in the ventricular tissue were also significantly reduced compared with control. In rabbits receiving linezolid at 50 mg/kg, blood samples were culture negative and bacterial counts in both the ventricular tissue and the valve vegetations were significantly reduced when compared with control. The 25 mg/kg dose of linezolid was not effective in reducing valve vegetation, ventricular tissue or blood bacterial counts. In fact, the rabbits showed signs of substantial illness (weight loss, high temperature, lethargy and cessation of eating). Because of an obvious

### Table I. Bacterial counts in control and treated endocarditis rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bacterial counts (( \log_{10} \text{cfu/g} ))</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>blood</td>
<td>heart</td>
<td>valve</td>
<td>culture negative (valve/total)</td>
</tr>
<tr>
<td>Control (killed at 48 h)</td>
<td>2.4 ± 0.8</td>
<td>5.7 ± 1.0</td>
<td>8.4 ± 1.0</td>
<td>0/14</td>
</tr>
<tr>
<td>Linezolid 75 mg/kg tds po</td>
<td>1.5 ± 0</td>
<td>2.1 ± 0.05(^b)</td>
<td>3.1 ± 0.4(^b)</td>
<td>8/10</td>
</tr>
<tr>
<td>Linezolid 50 mg/kg tds po</td>
<td>1.5 ± 0</td>
<td>2.7 ± 1.3(^b)</td>
<td>4.3 ± 1.7(^b)</td>
<td>6/12</td>
</tr>
<tr>
<td>Linezolid 25 mg/kg tds po</td>
<td>1.8 ± 0.7</td>
<td>6.5 ± 1.1</td>
<td>9.6 ± 0.2</td>
<td>0/5</td>
</tr>
<tr>
<td>Vancomycin 25 mg/kg bd iv</td>
<td>1.5 ± 0</td>
<td>2.6 ± 1.7(^b)</td>
<td>4.0 ± 2.1(^b)</td>
<td>8/11</td>
</tr>
</tbody>
</table>

\(^a\)Values are mean ± s.d.

\(^b\)\( P \leq 0.05 \) compared with control group.
lack of efficacy with this dose of linezolid, only five rabbits received this treatment regimen. Treatment with vancomycin at 25 mg/kg produced a significant reduction in the valve vegetation and ventricular tissue bacterial counts when compared with the control (Table I). These effects are similar to previous studies that have shown a therapeutic dose of vancomycin as being between 20 and 30 mg/kg administered intravenously bd in this model.

In the present study all linezolid doses produced blood levels that were above the MIC of UC-9258 at peak. Only the 75 and 50 mg/kg doses of linezolid produced blood levels that were above the MIC at trough (Table II), and only these doses produced significant antibacterial effects. This is consistent with endocarditis studies conducted by Carbon using other antimicrobial agents. Peak and trough levels of all linezolid doses on day 5 of dosing were higher compared with levels on day 1, indicating drug accumulation. We observed that peak blood levels of linezolid in this study were considerably higher than those achieved with approved dosing (600 mg bd) in humans. These high peak linezolid blood levels were a consequence of tds dosing and drug accumulation, and reflect differences in the bioavailability and pharmacokinetics of linezolid in rabbits versus humans. In humans, linezolid is well absorbed orally with absolute bioavailability of 100%.

However, in a preliminary rabbit study, the oral bioavailability of linezolid was only 31%. Therefore, to maintain linezolid trough blood levels above the MIC and achieve a therapeutic effect in the present study, tds dosing was necessary. Although this study cannot predict linezolid efficacy in human endocarditis, our results show antimicrobial effects in experimental endocarditis when linezolid trough blood levels are maintained above the MIC. In humans receiving the standard oral linezolid dose of 600 mg/kg bd, average blood levels for a 12 h trough were c. 6 mg/L, three times the MIC for the clinical isolate used in our studies.

Linezolid at concentrations of ≥4 mg/L inhibits most Gram-positive cocci, including methicillin-resistant staphylococci, vancomycin-resistant enterococci and penicillin-resistant pneumococci. Linezolid is bacteriostatic in vitro, producing <2 log reduction in bacterial counts at 24 h, with an MBC of >64 mg/L. In contrast to its in vitro effects, the results with linezolid in the present in vivo study (4 to 5 log reductions in bacterial counts after 5 days of therapy) clearly constitute more than a bacteriostatic effect.

In conclusion, this study shows that, like vancomycin, linezolid is effective for the treatment of experimental staphylococcal endocarditis in rabbits when plasma drug levels remain above the MIC, as has been demonstrated with other agents in this model. This study demonstrates the first evidence of the effectiveness of linezolid for the treatment of deep-seated experimental infections such as endocarditis.

Acknowledgements

We would like to thank Judy A. Lawson and Richelle J. LeMay for their helpful assistance in performing surgery and dosing animals, and Raymond J. Zielinski and Ming-Shang T. Kuo for the analysis of linezolid in blood.

References


Linezolid and experimental endocarditis

Table II. Concentration of linezolid in plasma (mg/L)

<table>
<thead>
<tr>
<th>Linezolid treatment</th>
<th>Day 1 of treatment</th>
<th>Day 5 of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dose 1 peak</td>
<td>dose 1 trough</td>
</tr>
<tr>
<td>75 mg/kg</td>
<td>25.2 ± 4.7</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>13.9 ± 5.6</td>
<td>0.26 ± 0.36</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>3.90 ± 1.6</td>
<td>0.005 ± 0.002</td>
</tr>
</tbody>
</table>

*Values are mean ± s.d., linezolid MIC 2 mg/L.


Received 26 June 2000; returned 21 September 2000; revised 31 October 2000; accepted 20 November 2000