Pharmacokinetics and Pulmonary Disposition of Tedizolid and Linezolid in a Murine Pneumonia Model under Variable Conditions

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In vivo pharmacokinetics are often evaluated in only one variation of an infection model, and the resulting exposures are assumed to be similar in each model. We evaluated and compared the effect of lung infection and immune status on the murine pharmacokinetics and pulmonary disposition of tedizolid and linezolid. Both factors resulted in differing blood and pulmonary exposure profiles, with similar trends for tedizolid and linezolid. These data highlight the importance of pharmacokinetic confirmation in each model.

Tedizolid (formally torezolid), the active moiety of tedizolid phosphate, is a novel oxazolidinone with activity against Gram-positive pathogens (6, 9), including methicillin-resistant Staphylococcus aureus. Since linezolid is the only FDA-approved oxazolidinone, there has been much interest in comparing the in vitro and in vivo efficacy of these two oxazolidinones against clinically relevant pathogens. When assessing the in vivo pharmacodynamics of antimicrobials, neutropenic and immunocompetent infection models are often used to evaluate the degree of antibacterial activity of a given regimen, as well as the impact of the host immune system on bacterial clearance. Frequently, the drug exposures are evaluated in only one variation of the infection model and it is assumed that the pharmacokinetic profile is similar in each of these potential variations. Without pharmacokinetic confirmation, exposure disparities rather than the host immune system may actually be responsible for any differences in efficacy, leading to inaccurate comparisons between models and/or the compounds under investigation. In this study, we sought to evaluate and compare the effect of lung infection and immune status on the pharmacokinetics and pulmonary disposition of tedizolid and linezolid.

Analytical grade tedizolid phosphate (lot 9AK0017E; Albany Molecular Research, Inc., Albany, NY) and linezolid (lot 0014; Pfizer, Inc., Groton, CT) were used for the in vivo analyses. Immediately prior to each in vivo experiment, each antimicrobial was weighed, reconstituted, and further diluted in appropriate diluents to achieve the desired concentration. Each solution was stored under refrigeration and discarded 24 h after reconstitution. Specific-pathogen-free, female BALB/c mice weighing approximately 20 g each were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN), and utilized throughout these experiments. The study was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee. Animals were maintained and used in accordance with National Research Council recommendations and provided food and water ad libitum.

The neutropenic pneumonia model has been well described previously (2, 3, 4). Briefly, mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide (Baxter, Deerfield, IL) 250 mg/kg and 100 mg/kg given 4 days and 1 day, respectively, prior to inoculation. Six hours prior to the initiation of antimicrobial therapy, isoflurane-anesthetized mice were held upright and orally inoculated with 0.05 ml of a 10⁷ CFU/ml suspension of S. aureus 156 in 3% mucin (Sigma-Aldrich, St. Louis, MO). Inocula were administered directly into the buccal cavity of the mice, and their nares were blocked to induce aspiration. Mice utilized in the immunocompetent studies underwent the same procedure as neutropenic mice but without the use of cyclophosphamide prior to inoculation with an inoculum of 10⁶ CFU/ml. For the uninfected mice, no procedures were performed prior to dose administration.

Single doses of tedizolid 8.4 mg/kg or linezolid 60 mg/kg were administered to the mice, as these doses have been shown to sim-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Model</th>
<th>Blood ( \text{fAUC}^b )</th>
<th>ELF AUC</th>
<th>ELF penetration ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tedizolid</td>
<td>Immunocompetent</td>
<td>4.7 (0.1)c</td>
<td>43.9 (2.8)c</td>
<td>9.34</td>
</tr>
<tr>
<td></td>
<td>Neutropenic</td>
<td>3.35 (0.1)c</td>
<td>35.6 (0.2)c</td>
<td>10.63</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>2.77 (0.1)c</td>
<td>17.0 (0.1)c</td>
<td>6.14</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Immunocompetent</td>
<td>115.9 (44.0)d</td>
<td>155.8 (14.2)d</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>Neutropenic</td>
<td>53.4 (4.7)</td>
<td>61.4 (17.9)</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>36.3 (0.9)</td>
<td>62.5 (17.7)</td>
<td>1.72</td>
</tr>
</tbody>
</table>

a Dosages were 8.4 mg/kg for tedizolid and 60 mg/kg for linezolid. AUC (area under the concentration-time profile) data are reported as means, with standard deviations in parentheses. ELF, epithelial lining fluid.

b For linezolid, the \( \text{fAUC} \) (area under the free-drug concentration-time profile) from 0 to 12 h was determined, and for tedizolid, the \( \text{fAUC} \) from 0 to 24 h was determined.

c The AUC was significantly different than in other models (\( P < 0.001 \)).

d The AUC in the immunocompetent model was significantly different than in the neutropenic and uninfected models (\( P < 0.001 \)).

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ulate humanized plasma exposures (1, 7, 8). Blood and bron-
chialveolar lavage (BAL) fluid were collected from groups of six 
mice at 1, 2, 4, 8, and 12 h after the dose for both compounds with 
the additional time point of 24 h for tedizolid in each of the 
murine models. Plasma (tedizolid) or serum (linezolid) samples, 
hereinafter referred to as blood, were separated by centrifugation 
and stored at −80°C until analysis. Concentrations were analyzed 
using validated liquid chromatography-tandem mass spectrometry 
(LC–MS–MS) and high performance liquid chromatography 
(HPLC) assays for tedizolid and linezolid, respectively. The pro-
tein binding values for tedizolid and linezolid were 85% and 30% 
(1, 5, 8), respectively, and the area under the free-drug concen-
tration–time curve (fAUC) for both regimens was calculated using 
the trapezoidal rule. Differences in exposures were compared us-
ing a one-way analysis of variance test followed by the Tukey mul-
tiple-comparison post hoc test. Portions of blood and BAL fluid 
were tested for their urea concentrations by a commercially avail-
able urea assay (TecoDiagnostics, Anaheim CA). The drug con-
centrations in epithelial lining fluid (ELF) were calculated from 
the following formula: ELF concentration = BAL fluid concentra-
tion ÷ (blood urea concentration/BAL fluid urea concentration) 
(4, 10).

Pharmacokinetic exposures and relative penetration ratios are 
presented in Table 1. The concentration–time profiles in blood for 
each agent for all model conditions are shown in Fig. 1. The blood 
pharmacokinetic exposures for both tedizolid and linezolid were 
consistently the highest in the immunocompetent model and the 
lowest in the uninfected model. Similarly, ELF exposures for both 
drugs were highest in the immunocompetent animals. Overall, 
tedizolid had enhanced ELF penetration for all three models in 
comparison with linezolid. Additionally, the presence of infection 
improved penetration for tedizolid (penetration ratio, 9.32 to 
10.62 for the infected versus 6.13 for the uninfected model), 
whereas linezolid had enhanced penetration in the uninfected an-
imals (penetration ratio, 0.87 to 1.28 for the infected versus 1.72 
for the uninfected model).

While these dosing regimens of tedizolid and linezolid may 
achieve similar blood exposures to humans in the neutropenic 
BALB/c mouse, substantially higher exposures were attained in 
the immunocompetent model and lower exposures in uninfected 
mice. For tedizolid, exposures in the immunocompetent and un-
infected models were 41% higher and 17% lower than what was 
observed in the neutropenic model. As for linezolid, more pro-
nounced trends were noted, with exposures increased by 117% in 
the immunocompetent model and decreased by 32% in the uninf-
infected model.

While it is not evident mechanistically why these exposure dif-
fferences occurred between the models, the trend was consistent 
for both agents. As seen in Fig. 1, the differences among the mod-
els were seemingly due to a change in the rate of elimination rather 
than changes in the volume of distribution. If this change in elim-
ination were predicted by the presence of infection, one would 
expect the immunocompetent and neutropenic models to have 
similar blood exposures; if it were due to a drug interaction with 
cy clophosphamide, similar exposures would be expected in im-
munocompetent and uninfected mice. Since neither of these ob-
vious scenarios occurred, other unidentified factors or a combina-
tion of these factors appear responsible for these exposure dispari-
ties.

Immune status and the presence of lung infection resulted in 
discordances in the blood and pulmonary profiles of tedizolid and 
linezolid. Any assumptions made regarding similar pharmaco-
kINETIC exposures and drug disposition between variations of the 
same model would lead to inaccurate efficacy comparisons if these 
dosing regimens were employed in these models. These substan-
tional differences in exposures due to the murine model used em-
phasize the importance of pharmacokinetic confirmation in each 
murine model utilized.

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