Activities of Several Novel Oxazolidinones against Mycobacterium tuberculosis in a Murine Model

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Oxazolidinones are a new class of antibacterial protein synthesis inhibitors. The activities of selected oxazolidinones in vitro against gram-positive organisms, including methicillin-resistant Staphylococcus aureus, penicillin-resistant Streptococcus pneumoniae, and multiple drug-resistant enterococci have been reported previously (2, 4, 8, 15). The oxazolidinones have also been demonstrated to have activity in murine models of systemic infections caused by these organisms (6).

The activities of selected oxazolidinones in vitro have been observed against susceptible and resistant Mycobacterium tuberculosis (1, 3, 15). Ashokhar et al. (1) reported on the activity of DuP-721, an oxazolidinone, administered orally to mice infected with M. tuberculosis H37Rv (strain B-216) (1). A dose-dependent prolongation in the survival time of infected mice was observed. DuP-721 was not as active as isoniazid (INH) or rifampin (RIF) in the murine model.

Newly synthesized oxazolidinones were evaluated for their in vitro activities against M. tuberculosis, and subsequently, a murine model was used to evaluate the in vivo activities of the most active compounds. We report on the promising antituberculosis activities of two novel oxazolidinones.

MATERIALS AND METHODS

Drugs. Linezolid (PNU-100480, linezolid, INH, and RIF were determined by a broth dilution method (14) and were 1, 0.5, 0.25, 0.03, and 0.06 μg/ml, respectively. The MICs of linezolid, PNU-100480, and RIF were determined by a broth dilution method (14) and were 1, 0.5, 0.25, 0.03, and 0.06 μg/ml, respectively.

Medium. The organism was grown in modified 7H10 broth with 10% OADC enrichment and 0.05% Tween 80 on a rotary shaker for 5 days. The culture suspension was diluted in modified 7H10 broth to yield 100 Klett units/ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.) or approximately 5 × 10^7 CFU/ml. The size of the inoculum was determined by titration and counting from triplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% OADC enrichment. The plates were incubated at 37°C in ambient air for 4 weeks before counting of the colonies.

Infection study. Four-week-old female outbred CD-1 mice (Charles River, Wilmington, Mass.) were infected intravenously through a caudal vein. Each mouse received approximately 10^7 viable organisms suspended in 0.2 ml of modified 7H10 broth. There were eight mice per group.

Treatment for the initial study was started 1 day after infection. In the other studies, treatment began 1 week after infection. Therapy was given 5 days per week for 4 weeks. All agents were administered by gavage: the oxazolidinones were dosed at 100 mg/kg of body weight, INH was dosed at 25 mg/kg of body weight, and RIF was dosed at 20 mg/kg of body weight. Control groups of infected but untreated mice were killed at the initiation of therapy (early controls) or at the end of the treatment period (late controls). Mice were killed by cervical dislocation 3 to 5 days after administration of the last dose of drug. The spleens and right lungs were aseptically removed and were ground in a tissue homogenizer. The number of viable organisms was determined by titration on 7H10 agar plates. The plates were incubated at 37°C in ambient air for 4 weeks prior to counting of the colonies.

Statistical evaluations. The viable cell counts were converted to logarithms, which were then evaluated by one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by the Tukey honestly significance difference test (9) to make pairwise comparisons among means.

RESULTS

Comparison of PNU-100480, linezolid, and INH. The inoculum in this study was 7.0 × 10^7 viable mycobacteria. Treatment with PNU-100480, linezolid, and INH reduced the cell counts in spleens and lungs compared with those in the spleens and lungs of late controls (P < 0.01 for all agents) (Table 1). Eperozeld had little activity. The differences in organ cell counts between groups receiving PNU-100480 and INH were not significant (P > 0.05). Although linezolid was less active than PNU-100480 or INH (P < 0.01 for both), it had considerable activity in the murine system in this 4-week treatment study.
Dose-response study. The inoculum in this study was $2.2 \times 10^7$ viable mycobacteria. PNU-100480 and linezolid at doses ranging from 25 to 100 mg/kg were effective against organisms in the lungs and spleens ($P < 0.01$ for both) when the counts were compared to those in the lungs and spleens of the respective early control groups (Table 2). PNU-100480 at the 100-mg/kg dose was more active than linezolid against organisms in the spleens and lungs ($P < 0.01$). At the two lower doses the activity of PNU-100480 was not significantly different from that of linezolid ($P > 0.05$) except for its activity in the spleens when it was administered at 50 mg/kg ($P < 0.01$). At the 25-mg/kg dose, in spite of the similarity in organ cell counts for the two agents, it is clear that PNU-100480 is more active on the basis of the four deaths in the linezolid group.

PNU-100480 combination study. The inoculum in this study was $2.0 \times 10^7$ viable mycobacteria. PNU-100480, RIF, and INH alone and in two-drug combinations had comparable activities against M. tuberculosis in M. tuberculosis-infected mice (Table 3). There was no significant difference between the results for these treatment groups; however, the results for each group were significantly different than those for the early control group ($P < 0.01$).

### TABLE 1. Activities of PNU-100480, linezolid, and epererezolid compared to that of INH in murine model of tuberculosis

<table>
<thead>
<tr>
<th>Treatment groupa</th>
<th>Log$_{10}$ CFU/organ (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td>Late Controlsb</td>
<td>7.63 ± 0.20 (5)c</td>
</tr>
<tr>
<td>INHc</td>
<td>4.36 ± 0.31 (6)</td>
</tr>
<tr>
<td>PNU-100480d</td>
<td>4.61 ± 0.26 (6)</td>
</tr>
<tr>
<td>Linezolidc</td>
<td>5.24 ± 0.32 (8)</td>
</tr>
<tr>
<td>Epererezolidf</td>
<td>7.07 ± 0.49 (7)</td>
</tr>
</tbody>
</table>

- a Treatment was started 1 day after the mice received $7 \times 10^6$ viable mycobacteria.
- b Three mice died (days 12, 18, and 22).
- c Values in parentheses are numbers of mice per group.
- d Two mice died (days 3 and 9).
- e Two mice died (days 9 and 31); data for lungs are based on data for only four mice due to contamination.
- f Eight mice were killed; however, for one spleen and four lungs the mycobacteria on the titer plates were too numerous to count.

### TABLE 2. Dose-response study of PNU-100480 and linezolid against M. tuberculosis ATCC 35801 in mice

<table>
<thead>
<tr>
<th>Treatment group, dose (mg/kg)b</th>
<th>Log$_{10}$ CFU/organ (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td>Early controls (7)b</td>
<td>8.06 ± 0.07c</td>
</tr>
<tr>
<td>Late controls (7)</td>
<td>6.70 ± 0.65</td>
</tr>
<tr>
<td>Linezolid, 25 (4)</td>
<td>5.85 ± 0.82</td>
</tr>
<tr>
<td>Linezolid, 50 (7)</td>
<td>6.11 ± 0.11</td>
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<tr>
<td>Linezolid, 100 (6)</td>
<td>5.34 ± 0.30</td>
</tr>
<tr>
<td>PNU-100480, 25 (7)</td>
<td>6.21 ± 0.44</td>
</tr>
<tr>
<td>PNU-100480, 50 (7)</td>
<td>5.40 ± 0.32</td>
</tr>
<tr>
<td>PNU-100480, 100 (8)</td>
<td>4.59 ± 0.31</td>
</tr>
</tbody>
</table>

- a Treatment was started 1 week after the mice received $2 \times 10^7$ viable mycobacteria.
- b Values in parentheses are numbers of mice per group; initially, there were eight mice/group; the lower numbers are due to death presumably secondary to tuberculosis for all mice except one mouse in the group receiving linezolid at 100 mg/kg. That mouse died because of a technical error.

### DISCUSSION

The oxazolidinones represent a novel class of antibacterial agents whose mechanism of action appears to be inhibition of an early step in the initiation phase of protein synthesis (5). Lin et al. (11) concluded that the oxazolidinones inhibit protein synthesis by binding to the 50S ribosomal subunit at a site close to the site(s) to which chloramphenicol and lincomycin bind but that the oxazolidinones are mechanistically distinct from these two antimicrobial agents. These agents have promising in vitro and in vivo activities against staphylococci, streptococci, enterococci, and Corynebacterium spp. (6, 15). Linezolid is currently in clinical trials for the treatment of skin and soft tissue infections, pneumonia, and bacteremia.

Two oxazolidinones, PNU-100480 and linezolid, have promising anti-M. tuberculosis activities in the murine test system. In the murine test system eperezolid was much less active than either PNU-100480 or linezolid. Preliminary pharmacokinetic data for PNU-100480 show that the drug appears to be well absorbed, with a mean plasma half-life following oral administration of 0.66 h in rats (7). PNU-100480 is rapidly and substantially converted to the sulfone metabolite and, to a lesser extent, the sulfone metabolite (7). The sulfone metabolite, which has potent anti-M. tuberculosis activity (3), achieves a peak level in the serum of rats of about 7 mg/ml after the administration of a 50-mg/kg dose (7). Pharmacokinetic and safety data for linezolid indicate that it is also well absorbed and appears to be well tolerated in 4-week toxicity studies with rats and dogs (10). Peak concentrations in the serum of rats of 17.7 and 36.0 µg/ml were observed following the administration of single oral doses of 50 and 125 mg/kg, respectively (10). CD-1 female mice given a single dose of 50 mg/kg of [14C]linezolid achieved a plasma radioactivity concentration of 37.5 µg-eq/g. The levels of radioactivity at 4, 8, and 10 h were 8.1, and 0.5 µg-eq/g, respectively. Radioactivity in plasma was primarily composed of parent drug (12).

The activity of PNU-100480 at 100 mg/kg was comparable to that of INH at 25 mg/kg in the murine test system. Linezolid was somewhat less active than PNU-100480 and INH. PNU-100480 has sufficient activity in the murine model to warrant its consideration as a candidate for clinical evaluation in humans. Linezolid is less active than PNU-100480; however, it is now undergoing clinical evaluation for the treatment of bacterial infections. Linezolid should be further studied with mice alone (at doses higher than 100 mg/kg) and in combination with
other anti-\textit{M. tuberculosis} agents in the murine system to better evaluate its potential for clinical development as an anti-\textit{M. tuberculosis} agent.

ACKNOWLEDGMENT

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REFERENCES


12. Slatter, J. G. Personal communication.

