ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1999, p. 1189–1191 Vol. 43, No. 5
0066-4804/99/$04.00+0

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

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Received 20 October 1998/Returned for modification 29 December 1998/Accepted 25 February 1999

The activities of linezolid, eperezolid, and PNU-100480 were evaluated in a murine model of tuberculosis. Approximately 10^7 viable *Mycobacterium tuberculosis* ATCC 35801 organisms were given intravenously to 4-week-old outbred CD-1 mice. In the first study, treatment was started 1 day postinfection and was given by gavage for 4 weeks. Viable cell counts were determined from homogenates of spleens and lungs. PNU-100480 was as active as isoniazid. Linezolid was somewhat less active than PNU-100480 and isoniazid. Eperezolid had little activity in this model. In the next two studies, treatment was started 1 week postinfection. A dose-response study was performed with PNU-100480 and linezolid (both at 25, 50, and 100 mg/kg of body weight). PNU-100480 was more active than linezolid, and its efficacy increased with an escalation of the dose. Subsequently, the activity of PNU-100480 alone and in combination with rifampin or isoniazid was evaluated and was compared to that of isoniazid-rifampin. The activity of PNU-100480 was similar to that of isoniazid and/or rifampin in the various combinations tested. Further evaluation of these oxazolidinones in the murine test system would be useful prior to the development of clinical studies with humans.

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). Ashtekar 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-100766; formerly U-100766), eperezolid (PNU-100592; formerly U-100592), and PNU-100480 (formerly U-100480) were provided by Pharmacia & Upjohn, Inc., Kalamazoo, Mich. INH and RIF were purchased from Sigma Chemical Co., St. Louis, Mo. The oxazolidinones and RIF were dissolved in dimethyl sulfoxide and were subsequently diluted in distilled water. INH was dissolved in distilled water. The drugs were prepared each morning, prior to administration.

Isolate. *M. tuberculosis* ATCC 35801 (strain Erdman) was obtained from the American Type Culture Collection (ATCC), Rockville, Md. The MICs of all antimicrobial agents were determined in modified 7H10 broth (7H10 agar formulation with agar and malachite green omitted; pH 6.6) supplemented with 10% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco Laboratories, Detroit, Mich.) and 0.05% Tween 80 (13). The MICs of PNU-100480, linezolid, eperezolid, 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.

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 7.0×10^6 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). Eperezolid 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.

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

Treatment group[a]	Log_{10} CFU/organ (mean ± SD)	
	Spleen	Lung
Late Controls[b]	7.63 ± 0.20 (5)[c]	8.47 ± 0.51 (5)
INH[d]	4.36 ± 0.31 (6)	3.81 ± 0.17 (6)
PNU-100480[e]	4.61 ± 0.26 (6)	3.59 ± 0.33 (4)
Linezolid	5.24 ± 0.32 (8)	5.03 ± 0.45 (8)
Eperezolid[f]	7.07 ± 0.49 (7)	7.77 ± 0.11 (4)

[a] Treatment was started 1 day after the mice received 7×10^6 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.

Dose-response study. The inoculum in this study was 2.2×10^7 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 2.0×10^7 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 2. Dose-response study of PNU-100480 and linezolid against *M. tuberculosis* ATCC 35801 in mice

Treatment group, dose (mg/kg)[a]	Log_{10} CFU/organ (mean ± SD)	
	Spleen	Lung
Early controls (8)[b]	8.06 ± 0.07[c]	8.17 ± 0.17
Late controls (7)	6.70 ± 0.65	7.39 ± 0.39
Linezolid, 25 (4)	5.85 ± 0.82	6.64 ± 0.46
Linezolid, 50 (7)	6.11 ± 0.11	6.26 ± 0.30
Linezolid, 100 (6)	5.34 ± 0.30	5.52 ± 0.20
PNU-100480, 25 (7)	6.21 ± 0.44	6.96 ± 0.22
PNU-100480, 50 (7)	5.40 ± 0.32	5.95 ± 0.64
PNU-100480, 100 (8)	4.59 ± 0.31	4.49 ± 0.50

[a] Treatment was started 1 week after the mice received 2×10^7 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.

TABLE 3. Activities of PNU-100480 in combination with INH or RIF against *M. tuberculosis* ATCC 35801

Treatment group[a]	Log_{10} CFU/organ (mean ± SD)	
	Spleen	Lung
Early controls (7)[b]	7.62 ± 0.21	5.96 ± 0.30
Late controls[c]		
PNU-100480 (8)	3.87 ± 0.38	2.75 ± 0.44
INH (7)[d]	3.95 ± 0.38	3.11 ± 0.30
RIF (7)[d]	3.79 ± 0.71	3.35 ± 0.43
INH-RIF (7)[d]	3.19 ± 0.56	3.05 ± 0.66
PNU-100480–INH (7)[d]	3.93 ± 0.20	2.90 ± 0.45
PNU-100480–RIF (7)[e]	3.24 ± 0.59	2.55 ± 0.40

[a] Treatment was started 1 week after the mice received 2×10^7 viable mycobacteria.
[b] Values in parentheses are numbers of mice per group.
[c] Five mice were dead 14 days after infection. Technical problems occurred with the other mice; therefore, organ counts are not available.
[d] One mouse in each group died between day 9 and day 11 after infection.
[e] Organ cultures for one mouse were contaminated.

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 sulfoxide metabolite and, to a lesser extent, the sulfone metabolite (7). The sulfoxide metabolite, which has potent anti-*M. tuberculosis* activity (3), achieves a peak level in the serum of rats of about 7 μg/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 [^{14}C]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-*M. tuberculosis* agents in the murine system to better evaluate its potential for clinical development as an anti-*M. tuberculosis* agent.

ACKNOWLEDGMENT

This study was supported by a grant from Pharmacia & Upjohn, Inc.

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