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EXPERIMENTAL THERAPEUTICS

In Vitro and *In Vivo* Activities of Contezolid (MRX-I) against *Mycobacterium tuberculosis*

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ABSTRACT The *in vitro* activity of contezolid (MRX-I) against clinical isolates of *Mycobacterium tuberculosis* was evaluated using a microtiter broth dilution assay. MRX-I was as effective as linezolid (LZD) *in vitro*. MRX-I and LZD were subsequently studied in BALB/c mice infected intranasally with *M. tuberculosis* Erdman. MRX-I and LZD at 100 mg/kg significantly reduced the bacterial load in lungs compared to the untreated early and late controls.

KEYWORDS *Mycobacterium tuberculosis*, *in vitro*, mouse model, oxazolidinones

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* has severely hampered the control of tuberculosis (TB) infection worldwide. Improved regimens to treat these types of infections are needed. Oxazolidinones were found to have promising *in vitro* activity against *M. tuberculosis* soon after their discovery (1, 2). Linezolid (LZD), the first oxazolidinone to be used in humans, was observed to have promising *in vitro* (3) and *in vivo* (4) activities against *M. tuberculosis*. LZD has been found to be a useful agent in regimens for the therapy of drug-resistant TB in humans (5); however, its use has been limited in part due to toxicity issues, which include myelosuppression and peripheral and optic neuropathy (5–7). Based on phase I clinical trials, contezolid (MRX-I), a new oxazolidinone developed to treat Gram-positive infections, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp., was shown to have decreased toxicity compared to LZD (6, 8). MRX-I may lead to a dramatic improvement in ease of use in patients with drug-resistant TB. In this study, we evaluated the *in vitro* and *in vivo* activities of MRX-I compared to LZD against *M. tuberculosis*.

Isoniazid (INH) was purchased from the Sigma-Aldrich Chemical Company (St. Louis, MO). Sutezolid (SZD) and tedizolid (TZD) were obtained from the National Institute of Allergy and Infectious Diseases (NIAID; Bethesda, MD) and Trius Pharmaceutical (San Diego, CA), respectively. LZD and MRX-I were provided by MicuRx Pharmaceuticals (Hayward, CA). For *in vitro* testing, all drugs were dissolved in 100% dimethyl sulfoxide (DMSO) at 5 mg/ml and were frozen at −20°C. Drugs were diluted in modified 7H10 broth (pH 6.6; 7H10 agar formulation with agar and malachite green omitted) with 10% oleic acid-albumin-dextrose-catalase (OADC) enrichment (BBL Microbiology Systems, Cockeysville, MD) and 0.05% Tween 80.

M. tuberculosis Erdman (ATCC 35801) and *M. tuberculosis* H37Rv (ATCC 27294) were purchased from the American Type Culture Collection (Manassas, VA). Clinical *M. tuberculosis* isolates were received from SUNY Upstate Medical University, the University of Stellenbosch, South Africa (Tommie Victor), the National Center of TB and Lung Disease of Georgia (Republic of Georgia, Natalia Shabladze), National Jewish Health (Leonid Heifets), and the Public Health Research Institute (Barry Kreiswirth). The isolates were grown in modified 7H10 broth with 10% OADC enrichment and 0.05% Tween 80

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TABLE 1 MICs of MRX-I compared to INH, LZD, SZD, and TZD against 22 isolates of *Mycobacterium tuberculosis*[a]

Mycobacterium tuberculosis isolate, MIC_{50}, or MIC_{90}	MIC (μg/ml)				
	INH	LZD	SZD	TZD	MRX-I
Erdman	0.06	1	1	0.25	1
H37Rv	0.06	0.5	0.25	0.125	0.5
532	0.06	0.5	0.5	0.125	1
277	0.06	1	0.25	0.125	1
365	0.06	0.5	0.25	0.125	1
764	0.06	0.5	0.25	0.125	1
AH517	0.03	1	0.5	0.25	1
HN878	0.06	1	0.5	0.5	2
676	0.06	1	0.5	0.5	2
BW9	0.06	1	0.5	0.5	1
487	0.03	0.5	0.5	0.125	1
C913	0.06	0.5	0.5	0.125	0.5
CDC1551	0.03	0.5	0.25	0.125	0.5
S1863	0.03	0.5	0.25	0.125	0.5
5	1	0.5	0.5	0.125	0.5
56	2	0.5	0.5	0.125	0.5
265	1	0.5	0.5	0.25	0.5
258	2	0.5	0.5	0.125	0.5
352	2	0.5	0.25	0.125	0.5
C-31	4	0.25	0.125	0.125	0.25
5037	>8	1	0.5	0.25	1
S982	8	1	0.5	0.25	1
MIC_{50}	0.06	0.5	0.5	0.125	0.5
MIC_{90}	4	1	0.5	0.5	1

[a]MRX-I, contezolid; INH, isoniazid; LZD, linezolid; SZD, sutezolid; TZD, tedizolid. The MIC_{50} and MIC_{90} are defined as the concentrations at which 50% and 90% of the clinical isolates tested were inhibited, respectively.

on a rotary shaker at 37°C for 7 to 10 days. The cultures were diluted to 100 Klett units (equivalent to 5×10^7 CFU/ml, Photoelectric colorimeter; Manostat Corp., New York, NY).

Polystyrene 96-well round-bottom plates (Corning, Inc., Corning, NY) were prepared with 50 μl of modified 7H10 broth per well. Drugs were diluted in modified 7H10 broth to four times the maximum concentration tested (64 μg/ml for the oxazolidinones and 8 μg/ml for INH), and 50 μl was added to the first well and serially diluted, leaving the last well with broth only (positive growth control). Organisms were diluted in 7H10 broth to a final concentration of approximately 1×10^5 CFU/ml (inoculum range, 6×10^4 to 2.4×10^6 CFU/ml). Fifty microliters of the inoculum was added to each well. Plates were sealed and incubated at 37°C in ambient air for 14 to 21 days prior to reading. The MIC was defined as the lowest concentration of drug required to visually inhibit the growth of *M. tuberculosis*. The MIC assays were run in duplicate. The MIC_{50} and MIC_{90} are defined as the concentrations at which 50% and 90% of the clinical isolates tested were inhibited, respectively.

The MICs of MRX-I compared to the three other oxazolidinones and INH against *M. tuberculosis* are presented in Table 1. We tested the compounds against both susceptible and MDR or XDR *M. tuberculosis* isolates. Eight *M. tuberculosis* isolates were resistant to INH (MIC > 1 μg/ml). MRX-I was as effective as LZD against all *M. tuberculosis* isolates. There was no appreciable difference in MIC_{90} (range, 0.5 μg/ml to 1 μg/ml) between the oxazolidinones evaluated. The MIC_{50} and MIC_{90} for MRX-I, LZD, SZD, and TZD were 0.5 μg/ml and 1 μg/ml, 0.5 μg/ml and 1 μg/ml, 0.5 μg/ml and 0.5 μg/ml, and 0.125 μg/ml and 0.5 μg/ml, respectively.

Based on the promising *in vitro* results, the drug was evaluated *in vivo*. Six-week-old female BALB/c mice were purchased from Charles River Laboratories (Wilmington, DE) and maintained in the Syracuse Veterans Affairs Medical Center Veterinary Medical Unit in an animal biosafety level 3 facility. Mice were housed six to a cage in microisolator

TABLE 2 Log_{10} CFU of *Mycobacterium tuberculosis* in the lungs of mice infected and treated with LZD and MRX-I[a]

Group	No. of mice	Log CFU ± SD
Early control	6	4.67 ± 0.17
Late control	6	6.03 ± 0.31
LZD 100 mg/kg	6	3.61 ± 0.42
MRX-I 100 mg/kg	6	3.76 ± 0.47
MRX-I 50 mg/kg twice daily	5	4.55 ± 0.33
MRX-I 25 mg/kg twice daily	6	5.07 ± 0.26

[a]LZD, linezolid; MRX-I, contezolid.

cages. The mice ingested water and Prolab RMH 3000 rodent chow (PMI Nutrition International, Brentwood, MO) *ad libitum* throughout the course of the studies. All animal protocols were approved by the Subcommittee for Animal Studies (SAS), Veterans Affairs Medical Center, Syracuse, NY. *M. tuberculosis* Erdman (ATCC 35801), grown in modified 7H10 broth with 10% OADC and 0.05% Tween 80, was diluted to 5×10^7 CFU/ml and frozen at −70°C. On the day of infection, the organism was thawed, sonicated, and further diluted to approximately 2.5×10^5 CFU/ml. The actual inoculum was determined by titration and plating in triplicate on 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 prior to counting.

Mice were anesthetized by intramuscular delivery of a Telazol (45 mg/kg)-xylazine (7.5 mg/kg) cocktail (Lederle Parenterals, Carolina, Puerto Rico, and Bayer Corp., Shawnee Mission, KS, respectively) and subsequently infected intranasally with 8.2×10^3 CFU of *M. tuberculosis* Erdman in a 20-μl volume. Mice were randomly assigned to one of the following 6 groups: untreated early controls (EC) for determination of the infection load at the initiation of therapy, late controls (LC) to determine the infection load at the completion of therapy, 100 mg/kg LZD, 100 mg/kg MRX-I once daily, 50 mg/kg MRX-I twice daily, or 25 mg/kg MRX-I twice daily. LZD was dissolved in 20% ethanol–80% double-distilled water (ddH_2O) to deliver 100 mg/kg in a 0.2-ml volume and was dosed once daily by gavage. MRX-I was dissolved in 20% DMSO–20% hydroxypropyl β-cyclodextrin–60% ddH_2O to deliver either 100 mg/kg, 50 mg/kg, or 25 mg/kg in a 0.2-ml volume. MRX-I was dosed at 100 mg/kg once daily, 50 mg/kg twice daily, or 25 mg/kg twice daily by gavage.

Treatment was initiated 1 week postinfection and was administered 5 days per week for 4 weeks. At the initiation of therapy, the EC group was euthanized by CO_2 asphyxiation, as were the mice at the completion of therapy. The right lung from each mouse was aseptically removed and ground in a sealed tissue homogenizer (IdeaWorks! Laboratory Devices, Syracuse, NY). The number of viable organisms was determined by serial dilution and titration on 7H10 agar plates supplemented with 10% OADC. Plates were incubated at 37°C in ambient air for 4 weeks prior to counting.

The viable cell counts per lung were converted to logarithms, which were then evaluated by analysis of variance (ANOVA). Statistically significant effects from the analysis of variance were further evaluated by Dunnett's multiple-comparison posttest.

Lungs from the early control mice had a bacterial load of approximately 4.67 ± 0.17 log_{10} CFU per lung (Table 2). The untreated late-control group had significantly more mycobacteria in the lungs than the early control group ($P < 0.05$). LZD and MRX-I dosed at 100 mg/kg once daily had significantly reduced the CFU recovered from the lungs compared to that in the early and late-control mice ($P < 0.05$). There was no significant difference between the reduction seen with LZD and once-daily MRX-I at 100 mg/kg ($P > 0.05$). Twice-daily MRX-I at 50 mg/kg and 25 mg/kg were significantly more effective than the late-control mice ($P < 0.05$). Once-daily MRX-I at 100 mg/kg was significantly more effective than twice-daily 50 mg/kg and 25 mg/kg MRX-I ($P < 0.05$). There was no statistical difference between the efficacies of twice-daily 50 mg/kg MRX-I and 25 mg/kg MRX-I ($P > 0.05$).

This study evaluated the *in vitro* and *in vivo* activities of MRX-I against *M. tuberculosis*.

The *in vitro* activity of MRX-I was similar to that of LZD against both drug-susceptible and drug-resistant *M. tuberculosis*. Its activity in a murine tuberculosis model was also similar to that of LZD. It would be interesting to determine if higher doses of MRX-I in mice result in enhanced activity.

It would also be of interest to determine if the addition of the less-toxic oxazolidinone MRX-I to current MDR TB therapy would result in a shorter course or perhaps would allow for the simplification of therapy. The present version of the Bangladeshi regimen (9) requires 9 months of treatment with moxifloxacin, clofazimine, ethambutol, and pyrazinamide supplemented with prothionamide, kanamycin, and high-dose INH during the 4-month initial intensive phase. Perhaps MRX-I could substitute for the aminoglycoside, thus avoiding the toxicity and administration issues associated with an aminoglycoside.

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REFERENCES

1. Ashtekar DR, Costa-Periera R, Shrinivasan T, Iyyer R, Vishvanathan N, Rittel W. 1991. Oxazolidinones, a new class of synthetic antituberculosis agent: *in vitro* and *in vivo* activities of DuP-721 against *Mycobacterium tuberculosis*. Diagn Microbiol Infect Dis 14:465–471. https://doi.org/10.1016/0732-8893(91)90002-W.
2. Slee AM, Wuonola MA, McRipley RJ, Zajac I, Zawada MJ, Bartholomew PT, Gregory WA, Forbes M. 1987. Ozaxolidinones, a new class of synthetic antibacterial agents: *in vitro* and *in vivo* activities of DuP 105 and DuP 721. Antimicrob Agents Chemother 31:1791–1797. https://doi.org/10.1128/AAC.31.11.1791.
3. Barbachyn MR, Hutchinson DK, Brickner SJ, Cynamon MH, Kilburn JO, Klemens SP, Glickman SE, Grega KC, Hendges SK, Toops DS, Ford CW, Zurenko GE. 1996. Identification of a novel oxazalidinone (U-100480) with potent antimycobacterial activity. J Med Chem 39:680–685. https://doi.org/10.1021/jm950956y.
4. Cynamon MH, Klemens SP, Sharpe CA, Chase S. 1999. Activities of several novel oxazolidinones against *Mycobacterium tuberculosis* in a murine model. Antimicrob Agents Chemother 43:1189–1191.
5. Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E, Jin B, Park H, Kwak H, Kim H, Jeon H-S, Jeong I, Joh JS, Chen RY, Olivier KN, Shaw PA, Follmann D, Song SD, Lee J-K, Lee D, Kim CT, Dartois V, Park S-K, Cho S-N, Barry CE, III. 2012. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med 367:1508–1518. https://doi.org/10.1056/NEJMoa1201964.
6. Gordeev MF, Yuan ZY. 2014. New potent antibacterial oxazolidinone (MRX-I) with an improved class safety profile. J Med Chem 57:4487–4497. https://doi.org/10.1021/jm401931e.
7. Mehta S, Das M, Laxmeshwar C, Jonckheere S, Thi SS, Isaakidis P. 2016. Linezolid-associated optic neuropathy in drug-resistant tuberculosis patients in Mumbai, India. PLoS One 11:e0162138. https://doi.org/10.1371/journal.pone.0162138.
8. Eckburg P, Ge Y, Hafkiin B. 2017. Single- and multiple-dose study to determine the safety, tolerability, pharmacokinetics, and food effect of oral MRX-I versus linezolid in healthy adult subjects. Antimicrob Agents Chemother. 61:e02181-16. https://doi.org/10.1128/AAC.02181-16.
9. Van Deun A, Maug AK, Salim MA, Das PK, Sarker MR, Daru P, Rieder HL. 2010. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med 182:684–692. https://doi.org/10.1164/rccm.201001-0077OC.