

Short Communication

In Vitro Activity of Linezolid against Clinical Isolates of *Mycobacterium tuberculosis*, including Multidrug-Resistant and Extensively Drug-Resistant Strains from Beijing, China

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SUMMARY: The in vitro activity of linezolid was evaluated against 84 clinical isolates of *Mycobacterium tuberculosis*, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, isolated from the center for tuberculosis research and treatment of the Chinese army. Linezolid showed excellent activity, with minimum inhibitory concentrations (MICs) of 0.125–0.5 µg/mL against all tested isolates. There were no differences in the MIC₅₀ and MIC₉₀ of linezolid between susceptible, isoniazid-resistant, MDR, and XDR. Indeed, all of the groups displayed identical MIC₉₀ values of 0.25 µg/mL, which is lower than previously reported in similar studies. We conclude that linezolid may be a more effective drug against *M. tuberculosis* and may play an important role in treating drug-resistant tuberculosis in China.

Tuberculosis (TB) is considered one of the most fatal infectious diseases in developing countries (1,2). Its recommended treatment includes no less than 4 effective drugs (3), but the soaring incidence of drug resistance has caused difficulty in its treatment, leading to higher costs and longer treatment cycles. Multidrug-resistant (MDR) TB refers to strains of *Mycobacterium tuberculosis* that are resistant to at least isoniazid (INH) and rifampin (RFP). Treatment for patients with MDR-TB requires the use of second-line drugs for more than 24 months (4). Recently, TB resistance to an increasing number of second-line drugs and drugs specifically used to treat MDR-TB has hindered the prevention and treatment of TB (5). Extensively drug-resistant (XDR) TB refers to MDR-TB strains that are also resistant to at least 1 of the 3 injectable second-line drugs, amikacin (AM), kanamycin (KN), or capreomycin, and a fluoroquinolone. As such, XDR-TB is susceptible to fewer than 3 of the remaining classes of second-line drugs, making it difficult for treatment to meet the international standards. Patients with XDR-TB have a higher risk of death and treatment failure than those with MDR-TB (6).

China has the second highest prevalence of TB in the world. In a previous study, we found troublingly high rates of MDR and XDR in PLA 309 Hospital, which is the center for TB research and treatment of the Chinese army located in Beijing (7). We also found very poor treatment outcomes among patients with MDR- and XDR-TB (unpublished data). Therefore, new anti-TB

drugs are urgently needed to treat MDR- and XDR-TB in China.

Linezolid was the first oxazolidinone compound licensed for clinical use. It has been suggested as an alternative treatment for patients infected with MDR *M. tuberculosis* isolates (8,9). However, there are few in vitro studies of linezolid's activity against clinical *M. tuberculosis* isolates from China, and there are none on linezolid's activity against XDR isolates from China. In this work, we tested the in vitro activity of linezolid against clinical strains of *M. tuberculosis* isolated from patients recruited by the Chinese PLA 309 Hospital from January 2007 to December 2009.

A total of 84 clinical isolates were included in this work along with the strain H37Rv (7). We previously investigated the antibiotic resistance patterns of 4 first-line drugs (INH, RFP, streptomycin [SM], ethambutol [EMB]) and 4 second-line drugs (ofloxacin [OFL], KN, AM, *p*-aminosalicylic acid [PAS]). All strains were cultured on Lowenstein-Jensen (LJ) media before use. Linezolid was obtained from Sigma (CAS:165800-03-3; St. Louis, Mo., USA) and was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 50 mg/mL. The minimum inhibitory concentration (MIC) was defined as the lowest antimicrobial concentration that inhibited more than 99% of bacterial growth. The MIC was determined by serially diluting each compound 2-fold on Middlebrook 7H10 agar media supplemented with oleic acid, albumin, dextrose, and catalase (OADC) according to the Clinical and Laboratory Standards Institute (formerly named National Committee for Clinical Laboratory Standards) (10). An inoculum of 10⁴ colony-forming units (CFU) of each *M. tuberculosis* strain was added to each tube. The tubes were incubated at 36.5°C; after 3 weeks, bacterial growth was examined by counting CFU.

Antimycobacterial susceptibility patterns of the clini-

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Table 1. In vitro activity of linezolid against 84 clinical isolates of *M. tuberculosis*¹⁾

Isolate	No. of isolates	Resistance phenotype	MIC ($\mu\text{g/ml}$)		
			Range	MIC ₅₀	MIC ₉₀
H37Rv	1	Susceptible			
(all susceptible INH-resistant)	15	Susceptible	0.125–0.5	0.25	0.25
	2	INH, SM			
	6	INH, EMB			
Total	8		0.125–0.25	0.25	0.25
MDR	8	RFP, INH			
	9	RFP, INH, SM			
	12	RFP, INH, EMB			
	2	RFP, INH, OFL			
	14	RFP, INH, EMB, SM			
Total	45		0.125–0.5	0.25	0.25
XDR	5	RFP, INH, EMB, SM, OFL, KN, PAS			
	11	RFP, INH, EMB, SM, OFL, AM, PAS			
Total	16		0.125–0.5	0.25	0.25

¹⁾ The MIC of strain H37Rv (ATCC 27294) was 0.5 $\mu\text{g/ml}$.

MIC, minimum inhibitory concentration; MIC₅₀ and MIC₉₀, MICs for 50% and 90% of the organisms, respectively; INH, isoniazid; SM, streptomycin; EMB, ethambutol; RFP, rifampin; OFL, ofloxacin; KN, kanamycin; PAS, *p*-aminosalicylic acid; AM, amikacin.

cal isolates are summarized in Table 1. Of the 84 cases included in this study, 15 strains were susceptible to all of the tested drugs, and 8 INH-resistant isolates were resistant to INH as well as EMB or SM. The number of MDR (excluding XDR) and XDR strains were 45 and 16, respectively. Of the MDR strains, 8 isolates were resistant to only INH and RFP; 23 were resistant to 1 additional drug, and 14 were resistant to all of the tested first-line drugs. All 16 XDR isolates were resistant to INH, RFP, and OFL, and 5 were resistant to KN, while the other 11 were resistant to AM.

The susceptibility to linezolid for all of the *M. tuberculosis* isolates examined is shown in Table 1. The MICs for linezolid ranged from 0.125 to 0.5 $\mu\text{g/ml}$ for all tested isolates. The MIC against *M. tuberculosis* H37Rv was 0.5 $\mu\text{g/ml}$, in accordance with previously published data (11). There were no differences in linezolid MIC₅₀ (MICs for 50% of the organisms) and MIC₉₀ values among susceptible, INH-resistant, MDR, and XDR groups. All groups displayed identical MIC₉₀ values of 0.25 $\mu\text{g/ml}$ (Table 1).

The soaring incidence of drug resistance has made treating TB increasingly difficult. Compared with patients with pansusceptible TB, patients with MDR- and XDR-TB have significantly worse short- and long-term outcomes due to the lack of effective anti-TB drugs, the longer course of treatment, and adverse side effects that are difficult to tolerate. In China, drug resistance to both first- and second-line anti-TB drugs represents a serious public health concern. The excessive use, possibly even overuse, of second-line drugs may explain the increased resistance to these medications. Therefore, the treatment of TB is expected to become increasingly difficult, and new antimicrobial agents against MDR- and XDR-TB are urgently needed.

Several studies have previously measured the in vitro activity of linezolid against *M. tuberculosis* isolates.

Richter et al. evaluated the activity of linezolid against 210 MDR-TB strains and found the first strain that could not be inhibited by concentration of linezolid < 8 $\mu\text{g/ml}$ (12). Prammananan et al. investigated a large number of MDR-TB strains, including 9 XDR-TB isolates, in Thailand and found that 2 strains that were not inhibited by a concentration of linezolid < 6 $\mu\text{g/ml}$ (13). Ermertcan et al. reported good activity of linezolid against 67 strains of *M. tuberculosis* (33 MDR and 34 non-MDR isolates) in western Turkey, with MICs of 0.06–1 $\mu\text{g/ml}$ (14). These data suggest that linezolid's activity against TB varies across different geographic areas. To the best of our knowledge, few studies have measured the in vitro activity of linezolid against *M. tuberculosis* strains isolated in China, and no reports have specifically included XDR cases. In the present study, linezolid showed excellent in vitro activity against all *M. tuberculosis* isolates tested and inhibited both MDR and XDR isolates with MICs of 0.125–0.5 $\mu\text{g/ml}$. Linezolid was approved for marketing in 2007 by the Chinese government. However, this drug has not been recommended as one of the anti-TB drugs in China. In addition, it is in limited supply in many hospitals in China. This could represent why the MICs in the present study are lower than those previously reported in similar studies.

Previous studies have reported that some strains are resistant to both linezolid and fluoroquinolones, suggesting that there might be cross-resistance (15). Cross-resistance between linezolid and other second-line anti-TB drugs has not yet been reported. However, the present study did not observe any cross-resistance between linezolid and other drugs. Because the peak concentration obtained after oral administration of 600 mg twice a day is 21.2 ± 5.8 mg/L with a half-life of 5.4 h, it was recommended that 8 mg/L be the cutoff point for linezolid. Therefore, our data suggest that linezolid

could be an effective treatment for all patients infected with the isolates in our study, even those with MDR- and XDR-TB.

Over recent years, many studies have focused on evaluating the in vivo efficacy of linezolid in the treatment of MDR- and XDR-TB (8,9). Published clinical trials have shown that linezolid plays an important role as part of an optimized MDR- or XDR-TB regimen (16,17). In these studies, linezolid was used in combination with other anti-TB drugs; accordingly, its antibacterial activity was not directly measured. In China, 1 clinical report indicated that the clinical symptoms of patients with pulmonary TB (1 confirmed and 2 suspected) improved within 2 days after a single use of linezolid (18). Since all strains in our study found were susceptible to linezolid, we conclude that linezolid may be more effective and may play an important role in treating drug-resistant TB in China.

In summary, we examined the in vitro anti-TB activity of linezolid. Our results suggest that linezolid has excellent activity against *M. tuberculosis* including XDR strains. Further in vitro and in vivo studies are needed to investigate the efficacy of linezolid, especially in China.

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Conflict of interest None to declare.

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