In Vitro Activities of Linezolid against Clinical Isolates of *Mycobacterium tuberculosis* Complex Isolated in Taiwan over 10 Years[▽]

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Significant increases in the MIC₉₀s of linezolid in multidrug-resistant *Mycobacterium tuberculosis* isolates were seen between the baseline period of 2001 to 2003 (0.5 µg/ml) and 2004 (2 µg/ml). The MICs were 4 µg/ml in three strains. Both fluoroquinolones (except levofloxacin) and kanamycin were found to have statistically significant degrees of concordance with linezolid.

Tuberculosis (TB) is one of the major causes of death worldwide. The global prevalence of mycobacterial infection has been estimated to be 32% (1.9 billion people), with 8 million new cases of TB diagnosed annually and an average case fatality rate of 23% (11). In the year 2003, the incidence and mortality rate of TB in Taiwan were reported at 62.38 and 5.80 per 100,000 people, respectively (2), and TB is considered a more serious public health problem in southern than in northern Taiwan.

Although TB can be cured with chemotherapy, the treatment is exceedingly lengthy and results in poor patient compliance, which is a frequent cause of selection of drug-resistant and even multidrug-resistant (MDR) *Mycobacterium tuberculosis* complexes. If the treatment fails as a result of drug resistance, treatment with second-line drugs is necessary. In Taiwan, the overall rates of MDR *M. tuberculosis* among new cases and previously treated cases ranged from 1% to 3% and 15% to 46%, respectively (5). In our previous report, increases in the MIC₉₀s and rates of resistance to ciprofloxacin, ofloxacin, and levofloxacin were noted in the MDR group (6). Therefore, there is an increasing need for new antimicrobial agents against MDR *M. tuberculosis*.

Linezolid, the first oxazolidinone developed and approved for clinical use, is an inhibitor of bacterial ribosomal protein synthesis. It prevents the formation of a 70S initiation complex by binding to a site on the 50S ribosomal subunit near its interface with the 30S unit. This unique mechanism of action is believed to preclude its cross-resistance to currently available agents. In addition, as linezolid is a totally synthetic antimicrobial agent, there are no preexisting specific resistance genes among gram-positive bacteria (8). Linezolid was introduced to

Taiwan recently, in 2002, for treatment of gram-positive-bacterial diseases. However, in vitro activity of linezolid against *Mycobacterium tuberculosis* complex strains is still scarce and has not been reported to occur in Asia.

We evaluated the in vitro activities of linezolid against *M. tuberculosis* by using the standard agar proportion method against 199 strains isolated from 1995 to 2004. These included 88 susceptible isolates, defined as fully susceptible to the four first-line drugs (isoniazid, rifampin, streptomycin, and ethambutol); 57 MDR isolates, resistant to rifampin and isoniazid; and 54 combination isolates, defined as isolates with other combinations of resistance patterns. We also examined the correlations of MICs between linezolid and other second-line drugs.

Antibiotic preparations used in this study, obtained as pure substances from their manufacturers, included ciprofloxacin and moxifloxacin (Bayer, Wuppertal, Germany), levofloxacin (Daiichi Pharmaceutical Taiwan, Ltd.), rifabutin (Pharmacia Gruppo Pfizer, Inc.), and linezolid (Pfizer Taiwan). Kanamycin, ofloxacin, streptomycin, ethionamide, and para-aminosalicylic acid were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO).

Quality control was carried out by concomitant determination of the MICs of the same antimicrobial agents against *M. tuberculosis* H37Rv and an MDR *M. tuberculosis* clinical isolate.

Linezolid has excellent in vitro activity against all of the *M. tuberculosis* isolates tested, including the MDR *M. tuberculosis* isolates. The MICs range from <0.125 to 4 µg/ml, with both MIC₅₀ and MIC₉₀ as 0.5 µg/ml. The MIC against *M. tuberculosis* H37Rv was 0.5 µg/ml and was in agreement with the published value (7). Three strains showed MICs of 4 µg/ml. One of them was also resistant to fluoroquinolones and rifabutin. Its fluoroquinolone resistance is associated with the D94A mutation in the *gyrA* gene.

Richter et al. (9) claimed that they had found the first linezolid-resistant strains with MICs of 8 µg/ml, 1 dilution higher

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TABLE 1. Trends of susceptibility of the *M. tuberculosis* complex to linezolid

Yr(s) of isolation	No. of isolates	MIC ($\mu\text{g/ml}$)			MIC ₅₀ ($\mu\text{g/ml}$) ^a			MIC ₉₀ ($\mu\text{g/ml}$) ^a		
		Range	50%	90%	Susceptible	MDR	Combination	Susceptible	MDR	Combination
1995–1997	34	≤0.125–2	0.25	0.5	0.5	0.25	0.25	0.5	0.5	0.5
1998–2000	37	≤0.125–1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2001–2003	44	≤0.125–4	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5
2004 ^a	84	≤0.125–4	0.5	2	0.5	0.5	0.5	0.5	2	0.5

^a Susceptible, fully susceptible to isoniazid, rifampin, streptomycin, and ethambutol; MDR, resistant to isoniazid and rifampin; Combination, other combination of resistance patterns.

than ours. However, the MIC of strain H37Rv was 1 $\mu\text{g/ml}$, also 1 dilution higher. In addition, three strains with MICs greater than 16 $\mu\text{g/ml}$ were reported by Rodriguez et al. in 2002 (10).

The MIC₅₀ and MIC₉₀ results are similar to previous reports (1, 10). No difference was noted in different susceptibility groups or different periods of time.

The trends in the MIC₉₀s of linezolid in the susceptible, MDR, and combination groups during the 3-year periods are shown in Table 1. The only significant increase in MIC₉₀ was seen for the MDR strains. This was noted between the baseline period of 2001 to 2003 and 2004 when analyzed by the Mann-Whitney test on SPSS version 12 software (SPSS, Inc., Chicago, IL) ($P = 0.016$). The MIC₉₀s were 0.5 $\mu\text{g/ml}$ in 2001 to 2003 and 2 $\mu\text{g/ml}$ in 2004.

All the patients with high linezolid MICs had not been previously treated with linezolid. Therefore, the trend of increasing linezolid MIC is not due to previous exposure to linezolid for treatment of other bacterial diseases in the community.

We examined the correlations of MICs with linezolid MIC as an independent variable by calculating Goodman and Kruskal's measure (4), which is a commonly used measure of ordinal association in the two-way contingency tables in the SPSS version 12.0 software program. With this measure, linezolid showed significant concordance between both fluoroquinolones (except levofloxacin) and kanamycin when the fluoroquinolone MIC was used to predict linezolid MIC, with

values of 0.057 ($P = 0.000$), 0.054 ($P < 0.05$), 0.059 ($P < 0.05$), and 0.053 ($P < 0.05$) for ciprofloxacin, ofloxacin, moxifloxacin, and kanamycin, respectively (Table 2).

It is interesting but unclear how these distinct mechanisms correlate with each other. The effect of efflux pumps remains controversial (3, 10). Future studies are needed to determine if a relation to an elevated linezolid MIC exists and to elucidate the possible mechanism involved in this process.

In the current study, we found that the MICs of linezolid were increased in MDR *M. tuberculosis* isolates in patients with no prior exposure to this antimicrobial agent. The correlation between fluoroquinolones (except levofloxacin) and linezolid may be relevant to the trend of increasing fluoroquinolone resistance (6). Although the increase of MIC does not confer linezolid resistance, it may act additively with another mechanism to achieve clinically significant linezolid resistance through stepwise accumulation of resistance elements and mutations. The correlation between kanamycin and linezolid has not been reported previously.

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TABLE 2. Analysis of correlation of *M. tuberculosis* MIC with linezolid MIC as an independent variable

Antimicrobial agent	MIC range	Result with linezolid MIC as independent variable	
		Goodman and Kruskal tau	<i>P</i>
Fluoroquinolones			
Ciprofloxacin	≤0.25->4	0.057	0.000
Ofloxacin	≤0.25->4	0.054	0.001
Levofloxacin	≤0.25->4	0.025	0.453
Moxifloxacin	0.03->4	0.059	0.030
Aminoglycosides			
Streptomycin	<0.25->8	0.040	0.325
Kanamycin	<0.375->48	0.053	0.027
Other drugs			
Rifabutin	<0.25->32	0.051	0.123
Ethionamide	≤1.25->80	0.046	0.112
PAS ^a	<0.03->64	0.063	0.107

^a PAS, para-aminosalicylic acid.