In Vitro Activities of Linezolid against Clinical Isolates of 

*Mycobacterium tuberculosis* complex Isolated in Taiwan over Ten

Years

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Running title: Linezolid activity of *M. tuberculosis* complex in Taiwan

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Abstract

Significant increase in MIC$_{90}$ of linezolid in multidrug-resistant *Mycobacterium tuberculosis* was seen between the baseline period of 2001-2003 (0.5 µg/ml) and 2004 (2 µg/ml). The MIC was 4 µg/ml in three strains. A statistically significant degree of concordance was found between both fluoroquinolones (except levofloxacin) and kanamycin, and 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 tuberculosis diagnosed annually and an average case fatality rate of 23% (12). In the year 2003, the incidence and mortality rate of tuberculosis in Taiwan was reported at 62.38 and 5.80 per 100,000 people, respectively (2) and is considered as 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 for selection of drug resistant and even multi-drug resistant Mycobacterium tuberculosis complex (MDR-TB). If the treatment fails as a result of drug resistance, treatment with second-line drugs is necessary. In Taiwan, the overall rates of MDR-TB among new cases and previously treated cases ranged from 1% to 3% and 15% to 46%, respectively (5). In our previous report, an increase in the MIC\textsubscript{90} 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-TB.

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 with currently available agents. In addition, as 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 (MTB) strains is still scarce and has not been reported in Asia.

We evaluated the in vitro activities of linezolid of MTB using the standard agar proportion method against 199 strains isolated from 1995 to 2004. These included 88 susceptible, defined as fully susceptible to the four first line drugs (isoniazid, rifampin, streptomycin, and ethambutol); 57 MDR, resistant to rifampin and isoniazid; and 54 combinations, defined as isolates with other combinations of resistance patterns. We also examined the correlation of MICs between linezolid and other second-line drugs. Antibiotic preparations used in this study, obtained as pure substance from their manufacturers, included ciprofloxacin and moxifloxacin (Bayer, Wuppertal, Germany), levofloxacin (Daiichi Pharmaceutical Taiwan Ltd.), rifabutin (PHARMACN Gruppo Pfiuer Inc.), linezolid (Pfizer Taiwan). Kanamycin, ofloxacin, streptomycin, ethionamide, and para-aminosalicylic acid (PAS) were purchased from
Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, US).

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

Linezolid has excellent in vitro activity against all of the MTB tested, including MDR-TB. The MIC ranges from <0.125 to 4 µg/ml with both MIC<sub>50</sub> and MIC<sub>90</sub> 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 MIC of 4 µg/ml. One of them was also resistant to fluoroquinolones and rifatubin. Its fluoroquinolone-resistance is associated with the D94A mutation in *gyrA* gene.

Richter et. al. (9) claimed that they found first linezolid resistant strains with MIC of 8 µg/ml, one dilution higher than ours. However, the MIC of strain H37Rv was 1 µg/ml, also one dilution higher. In addition, three strains with MIC greater than 16 µg/ml were reported by Rodriguez et. al. in 2002 (10).

The MIC<sub>50</sub> and MIC<sub>90</sub> results are similar to previous reports. No difference was noted in different susceptibility groups or different period of time.

The trends in MIC<sub>90</sub> of the linezolid in the susceptible, MDR and combinations group during the three-year periods is shown in Table 1. The only significant increase in MIC<sub>90</sub> was seen for the MDR strains. This was noted between the baseline period
of 2001-2003 and 2004 when analyzed by Mann-Whitney test on SPSS Version 12 software (SPSS, Inc., Chicago, IL, USA) \((p=0.016)\). The MIC\(_{90}\) was 0.5 µg/ml in 2001-2003 and 2 µg/ml in 2004.

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

We examined the correlation of MICs with linezolid MIC as an independent variable by calculating Goodman and Kruskal’s measures \((4)\) which is a commonly used measure of ordinal association in two-way contingency tables on SPSS Version 12.0 software. It showed significant concordance between both fluoroquinolones (except levofloxacin) and kanamycin, and linezolid when using the fluoroquinolones MIC 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 know if its 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-
TB isolates in patients with no prior exposure to this antimicrobial agent. The correlation between fluoroquinolones (except levofloxacin) and linezolid may be relevant to the increasing trend of 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.

Acknowledgement

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Table 1. Trends of susceptibility of *M. tuberculosis* complex to linezolid.

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>MIC (µg/ml)</th>
<th>MIC(_{50}) (µg/ml)</th>
<th>MIC(_{90}) (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Range 50% 90%</td>
<td>Susceptible MDR Combinations</td>
<td>Susceptible MDR Combinations</td>
</tr>
<tr>
<td>1995-1997 (34)</td>
<td>≤0.125-2 0.25 0.5</td>
<td>0.5 0.25 0.25</td>
<td>0.5 0.5 0.5</td>
</tr>
<tr>
<td>1998-2000 (37)</td>
<td>≤0.125-1 0.5 0.5</td>
<td>0.5 0.5 0.5</td>
<td>0.5 0.5 0.5</td>
</tr>
<tr>
<td>2001-2003 (44)</td>
<td>≤0.125-4 0.5 0.5</td>
<td>0.25 0.5 0.5</td>
<td>0.5 0.5 0.5</td>
</tr>
<tr>
<td>2004* (84)</td>
<td>≤0.125-4 0.5 2</td>
<td>0.5 0.5 0.5</td>
<td>0.5 2 0.5</td>
</tr>
</tbody>
</table>
Table 2. Analysis of correlation of *M. tuberculosis* MIC with linezolid as independent variable.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC range</th>
<th>Goodman and Kurskal Tau</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluoroquinolones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.25-&gt;4</td>
<td>0.057</td>
<td>0.000</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>≤0.25-&gt;4</td>
<td>0.054</td>
<td>0.001</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤0.25-&gt;4</td>
<td>0.025</td>
<td>0.453</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.03-&gt;4</td>
<td>0.059</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>Aminoglycoside</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>≤0.25-&gt;8</td>
<td>0.040</td>
<td>0.325</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>&lt;0.375-&gt;48</td>
<td>0.053</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Other drugs</strong></td>
<td></td>
<td></td>
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<tr>
<td>Rifabutin</td>
<td>&lt;0.25-&gt;32</td>
<td>0.051</td>
<td>0.123</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>≤1.25-&gt;80</td>
<td>0.046</td>
<td>0.112</td>
</tr>
<tr>
<td>PAS</td>
<td>&lt;0.03-&gt;64</td>
<td>0.063</td>
<td>0.107</td>
</tr>
</tbody>
</table>

2 PAS: para-aminosalicylic acid
References


