Susceptibility of *Mycobacterium tuberculosis* to sulfamethoxazole, trimethoprim and their combination over a 12 year period in Taiwan

Tsi-Shu Huang1–3, Calvin M. Kunin4,5, Bo-Shiun Yan6, Yao-Shen Chen7,8, Susan Shin-Jung Lee7 and Wan-Jr Syu9*

1Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; 2Section of Microbiology, Department of Pathology and Laboratory Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 3Department of Medical Technology, Fooyin University, Kaohsiung, Taiwan; 4Department of Internal Medicine, Ohio State University, Columbus, OH, USA; 5Department of Internal Medicine, University of Arizona, Tucson, AZ, USA; 6Institute of Biochemistry and Molecular Biology, National Taiwan University, Taipei, Taiwan; 7Department of Infectious Diseases, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; 8Graduate Institute of Environmental Education, National Kaohsiung Normal University, Kaohsiung, Taiwan; 9Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan

*Corresponding author. Tel: +886-2-28267003; Fax: +886-2-28212880; E-mail: wjsyu@ym.edu.tw

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Objectives: This study was designed to determine the susceptibility of clinical isolates of multidrug-resistant (MDR) and non-MDR *Mycobacterium tuberculosis* to sulfamethoxazole, trimethoprim and trimethoprim/sulfamethoxazole over a 12 year period in Taiwan.

Patients and methods: We examined a total of 117 clinical isolates of *M. tuberculosis* collected from Southern Taiwan, 116 from 1995 to 2006 and an extensively drug-resistant (XDR) isolate in 2009. These included 28 isolates susceptible to all four first-line agents, 52 MDR isolates and 36 isolates with a mixed combination of drug resistance patterns other than MDR and 1 XDR isolate.

Results: Sulfamethoxazole inhibited 80% growth of all 117 isolates regardless of their susceptibility to the first-line agents at an MIC90 of 9.5 mg/L. The concentration required to inhibit 99% growth was 38 mg/L. There were no significant changes in the MIC50 or MIC90 of sulfamethoxazole over a 12 year period. All 117 isolates were resistant to trimethoprim at 8 mg/L. The combination of trimethoprim/sulfamethoxazole at a ratio of 1:19 had no additive or synergistic effects.

Conclusions: Sulfamethoxazole inhibited the growth of clinical isolates of *M. tuberculosis* at achievable concentrations in plasma after oral administration. Susceptibility to sulfamethoxazole remained constant over a 12 year period. Trimethoprim was inactive against *M. tuberculosis* and trimethoprim/sulfamethoxazole provided no additional activity. Although the current and prior studies demonstrate that sulfamethoxazole is active against *M. tuberculosis* the search needs to continue for more active, lipid-soluble sulphonamides that are better absorbed into tissues and have improved therapeutic efficacy.

Keywords: sulphones, sulphonamides, treatment

Introduction

Tuberculosis (TB) is one of the major causes of death worldwide. Treatment is complicated by the need for prolonged therapy, multidrug regimens and the need for good compliance. The global emergence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB has made treatment even more difficult. Therefore, there is a continuing need for novel, minimally toxic drugs that are effective against tubercle bacteria.1,2

In addition to developing new compounds, expanding the indications of established drugs would lead to a fast avenue for the treatment of tuberculosis. Previously licensed drugs have the advantage of known pharmacological properties, safety and potential for drug interactions. For example, the combination of meropenem and clavulanate has been shown to be effective against drug-susceptible and drug-resistant XDR clinical isolates, and persistent bacteria.3 However, its use is limited to severe cases of tuberculosis because of the need for parenteral administration.

Sulphones and sulphonamides were used for the treatment of tuberculosis in the early 1950s. Extensive data are available for the old sulphonamide and sulphone compounds in the treatment of *Mycobacterium tuberculosis* (MTB) in vitro and in vivo, including data that were generated from experiments...
in animals and humans. These early-phase drugs were abandoned for the treatment of tuberculosis mostly because of their toxicity and the availability of potent streptomycin, isoniazid and rifampicin at that time.

The synergistic activity of a combination of sulfamethoxazole and trimethoprim against a wide variety of Gram-positive and Gram-negative bacteria has been well established. Recently, there have been two examinations of the susceptibility of M. tuberculosis to trimethoprim/sulfamethoxazole and to sulfamethoxazole alone. Forcags et al. found that 98% of their 44 isolates were susceptible to the trimethoprim/sulfamethoxazole combination at an MIC of ≤19 mg/L. These included six MDR-TB isolates. Ong et al. using the Mycobacterium Growth Indicator Tube (MGIT; Becton Dickinson (BD)) broth dilution drug susceptibility test system, found that 12 isolates susceptible to the first-line agents were susceptible to sulfamethoxazole at a concentration ≤38 mg/L. They also showed that sulfamethoxazole was bacteriostatic against M. tuberculosis. This prompted us to examine the activity of sulfamethoxazole, trimethoprim and their combination against isolates of MTB collected over a 12 year period in Southern Taiwan.

Materials and methods

M. tuberculosis isolates

The collection consisted of a total of 117 clinical strains: 116 isolated at the Kaoshiung Veterans General Hospital in Southern Taiwan over a 12 year period (1995–2006) and one XDR-TB strain isolated in 2009. M. tuberculosis complex strains isolated before 2000 were cultured in a BACTEC 460 instrument (BD Diagnostic Instrument Systems, Towson, MD, USA). All inoculated Bactec 12B vials were tested twice a week for growth relative to the drug-free quadrant, which contained between 100 and 500 colonies. Since there are no standardized guidelines for susceptibility testing of M. tuberculosis against sulfamethoxazole and trimethoprim, we adopted the CLSI (formerly NCCLS) criteria for susceptibility of Mycobacterium kansasii and Mycobacterium marinum to these drugs. Resistance to sulfamethoxazole and trimethoprim were defined as MICs of >38.2 mg/L and ≥2.0 mg/L, respectively. These cut-offs also correspond to the achievable concentration of sulfamethoxazole following a single oral dose of 800 mg (160 mg for trimethoprim). Quality control for trimethoprim was performed by broth microdilution testing with Staphylococcus aureus ATCC 29213 according to the CLSI M7-A7 methodology. The endpoints were read as 80% or greater reduction in growth as compared with the control. The expected MIC range for the quality control strain was 1–4 mg/L according to the CLSI tables.

Results

Clinical isolates of M. tuberculosis

The distribution of the 117 isolates of M. tuberculosis recovered in Southern Taiwan (116 isolates from 1995–2006 and an XDR strain isolated in 2009) are shown in Table 1; they are grouped into the following categories: non-resistant, resistant to isoniazid and rifampicin, XDR, resistant to at least one injectable drug, resistant to at least rifampicin, isoniazid, any fluoroquinolone and to at least one of three injectable drugs used in anti-TB treatment (capreomycin, kanamycin and amikacin).

Susceptibility testing

MICs were determined using the standard agar dilution method. Briefly, 7H11 agar-containing medium was prepared from a dehydrated base as recommended by the manufacturer. After autoclaving and cooling to 50–56°C, oleic acid/albumin/dextrose/catalase (OADC) supplement (BD Biosciences) and appropriate drug working solutions were aseptically added to the medium mixtures in 2-fold dilutions. The final medium mixture (4 mL each) was pipetted into individual plastic quadrant Petri plates. One quadrant in each plate receiving no drug in the medium mixture was used to serve as a control for the bacterial growth while the other quadrants had a series of 2-fold-diluted antibiotics in the same medium. To prepare the bacteria, M. tuberculosis colonies were suspended in 7H9 broth (BD Biosciences). The mixtures were vortexed and allowed to settle for 20 min to remove any debris. The supernatant fluid was then transferred to a second tube. This process was repeated twice. Then, the cells were diluted in 10 mL of 50 mM sodium phosphate buffer (pH 7.0) and adjusted with a colorimeter (Vitek Systems, Inc., Hazelwood, MO, USA) to yield 1 McFarland unit. The suspension contained approximately 10⁸ cfu/mL. The broth was diluted 1:1000 to provide inocula of 10³ cfu to each of the four quadrants. The plates were incubated for 3 weeks at 35°C. MICs were defined as the lowest concentrations that inhibited a defined percentage (80% or 99%) of the bacterial growth relative to the drug-free quadrant, which contained between 100 and 500 colonies.

Antimicrobial agents

Sulfamethoxazole and trimethoprim were purchased from Sigma (Sigma–Aldrich Co., St. Louis, MO, USA). Sulfamethoxazole was dissolved in a few drops of 1 N NaOH. The stock solutions were stored as aliquots at −20°C. Working solutions were prepared by dilution with distilled water.
Table 2. Activities of sulfamethoxazole alone and in combination with trimethoprim against 117 clinical isolates of *M. tuberculosis*, grouped according to susceptibility to the first-line agents

<table>
<thead>
<tr>
<th>Year</th>
<th>Susceptiblea</th>
<th>MDRb</th>
<th>mixed patternsc</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995–97</td>
<td>7</td>
<td>15</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>1998–2000</td>
<td>7</td>
<td>12</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>2001–03</td>
<td>8</td>
<td>10</td>
<td>14</td>
<td>32</td>
</tr>
<tr>
<td>2004–06</td>
<td>6</td>
<td>15</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>2009</td>
<td>0</td>
<td>1</td>
<td>(XDR)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>53</td>
<td>36</td>
<td>117</td>
</tr>
</tbody>
</table>

SMX, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole.
\(^a\)Susceptible to isoniazid, rifampicin, streptomycin and ethambutol.
\(^b\)Resistant to isoniazid and rifampicin.
\(^c\)Resistance patterns other than those defined by MDR and XDR.

Table 3. MIC\(_{50}\) and MIC\(_{90}\) of sulfamethoxazole at 80% inhibition of growth for 116 isolates of *M. tuberculosis* isolated in Southern Taiwan and grouped according to the years recovered

<table>
<thead>
<tr>
<th>Year</th>
<th>No.</th>
<th>MIC range</th>
<th>MIC(_{50})</th>
<th>MIC(_{90})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995–97</td>
<td>31</td>
<td>1.19–19</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>2001–03</td>
<td>32</td>
<td>2.38–19</td>
<td>9.5</td>
<td>19(^a)</td>
</tr>
<tr>
<td>2004–06</td>
<td>28</td>
<td>(\leq1.19–19)</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>(\leq1.19–19)</td>
<td>9.5</td>
<td>9.5</td>
</tr>
</tbody>
</table>

\(^a\)Four strains susceptible to 19 mg/L sulfamethoxazole in this period.

12 year trend in susceptibility of *M. tuberculosis* to sulfamethoxazole

The MIC\(_{50}\) and MIC\(_{90}\) of sulfamethoxazole that inhibited 80% growth of *M. tuberculosis* by year of isolation are shown in Table 3. Susceptibility to sulfamethoxazole remained about the same during the study period except for 2001–03. The MIC\(_{90}\) for this period increased from 9.5 to 19 mg/L; this was due to a set of four strains isolated during this 3 year period having an MIC\(_{90}\) of 19 mg/L. However, this occurrence did not notably affect the overall trend.

Effect of the medium on susceptibility of *M. tuberculosis* to sulfamethoxazole and trimethoprim/sulfamethoxazole

Media 7H10 and 7H11 differ by the absence or presence of digested casein, respectively. To assure that the lack of synergism with the combination of trimethoprim/sulfamethoxazole was not due to medium difference,\(^5\) MICs of sulfamethoxazole and trimethoprim/sulfamethoxazole were re-assessed with 20 representative MDR-TB isolates. The MICs of sulfamethoxazole and trimethoprim/sulfamethoxazole in 7H10 were 2-fold less than those seen for 7H11 with the isolates tested. Otherwise, the patterns were quite similar (Table 4).
Their findings for sulfamethoxazole alone are similar to those of Forsgås et al., who also used the agar dilution method but with 7H10 medium, instead of our 7H11. They reported that 98% of 44 isolates had MICs of trimethoprim/sulfamethoxazole of ≤19 mg/L. We found that at 80% inhibition of bacterial growth, all our 117 isolates had MICs of sulfamethoxazole of ≤19 mg/L. They reported that 11/44 (25%) of their isolates were susceptible at 1- to 2-fold lower concentrations of sulfamethoxazole when tested in combination with trimethoprim.5 We found that in our isolates the trimethoprim/sulfamethoxazole combination was not more active than sulfamethoxazole alone. However, we did find that in 7H10 all isolates seemed to be more susceptible to sulfamethoxazole (or trimethoprim/sulfamethoxazole) than in 7H11, since the MICs were 2-fold reduced. Therefore, the enzyme-digested casein-containing 7H11 agar may reduce the drug susceptibility although it enhances the growth of some recalcitrant isolates of drug-resistant M. tuberculosis.15

Ong et al., using the MGIT 960 system, have found that the MIC of sulfamethoxazole against a collection of 12 M. tuberculosis isolates was ≤38 mg/L. Wallace et al., using the 7H9 microdilution method, found that ≥90% of 10 isolates of MTB were susceptible to 8 mg/L sulfamethoxazole. We found that 109/117 (93.2%) of our strains were susceptible to 9.5 mg/L sulfamethoxazole and that the susceptibility to sulfamethoxazole was independent of the resistance to the first-line agents (Table 2). Taking these results together, we could generally conclude that many clinical isolates, if not all, remain susceptible to sulfamethoxazole.

All of the tested isolates of M. tuberculosis from Southern Taiwan in our 12 year collection were resistant to trimethoprim at >8 mg/L. This concentration cannot be achieved in plasma after oral administration of the trimethoprim/sulfamethoxazole standard dose. Our results are in accord with those of Suling et al.17 and Jacobs,18 who also found that M. tuberculosis is resistant to trimethoprim. It is not known so far whether the mechanism of trimethoprim resistance in M. tuberculosis is due to overexpression of dfrA as suggested by studies with M. smegmatis.19

Wiktor et al.16 reported that trimethoprim/sulfamethoxazole reduced mortality by 41% in African HIV-1-infected patients with tuberculosis. They attributed this phenomenon to better control of secondary infections. We agree with Köser et al.21 that ‘A clear understanding of the resistance mechanisms of trimethoprim/sulfamethoxazole in M. tuberculosis is urgently needed since the WHO has renewed its call for widespread use of this drug combination for the prophylactic treatment of patients with HIV.’ Excessive use of antimicrobial drugs, including trimethoprim/sulfamethoxazole, is known to be widespread in developing countries.22,23 Our finding that susceptibility to sulfamethoxazole of M. tuberculosis did not notably change over a 12 year period in Taiwan is somewhat reassuring in that strain resistance of M. tuberculosis to sulfamethoxazole has not been evident. However, we cannot predict whether it may emerge in the future.

In summary, we have confirmed that sulfamethoxazole exhibits in vitro activity against clinical isolates of M. tuberculosis and that inhibiting 80% of the growth of all 117 isolates could be achieved at 19 mg/L. To achieve 99% inhibition of growth for all isolates, 38 mg/L sulfamethoxazole is needed and this concentration is still achievable in plasma if orally administered at 800 mg. However, sulfamethoxazole has several unfavourable pharmacological properties; these include poor lipid solubility and a restricted distribution pattern, typically confined to the intravascular space. On the other hand, trimethoprim alone was not active against our isolates of M. tuberculosis and it did not augment the activity of sulfamethoxazole. Intriguingly, there were no significant changes in susceptibility to sulfamethoxazole over a 12 year period. Therefore, it is suggested that a search for more active, lipid-soluble compounds with improved tissue distribution might enhance the therapeutic value of sulphonamides/sulphones toward treating tuberculosis.

**Acknowledgements**

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Transparency declarations
None to declare.

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