Pharmacodynamics of sulfamethoxazole in *in vitro* and *in vivo* models of tuberculosis

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Trimethoprim-sulfamethoxazole (TMP-SMX)

- Safe, well tolerated, oral agents widely used to treat & prevent lung infections
- Efficacy of PAS provides validation for folate synthesis pathway as target for anti-TB therapy
- MIC$_{90}$ of SMX vs. *M. tb* = 10-20 µg/ml
- TMP not active at attainable concentrations
- Mechanism of action is largely static (esp. in presence of thymidine or thymine)

1. Minato et al, AAC 2015; 59:5097
2. Macingwana et al, JAC 2012; 67:2908
3. Proctor, CID 2008; 46:584
# Recommended SMX dose range and PK

<table>
<thead>
<tr>
<th>Subjects</th>
<th>SMX dose (mg)</th>
<th>Cmax (mg/L)</th>
<th>AUC (mg·h/L)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB pts</td>
<td>400mg qd</td>
<td>~30</td>
<td>371.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>800mg qd</td>
<td></td>
<td>882.5</td>
<td>1</td>
</tr>
<tr>
<td>Healthy</td>
<td>1200mg qd</td>
<td>88.4</td>
<td>1,437</td>
<td>2</td>
</tr>
<tr>
<td>Healthy (SS)</td>
<td>~1200mg q6h</td>
<td>247</td>
<td>4,871</td>
<td>3</td>
</tr>
</tbody>
</table>

1. Alsaad et al, ERJ 2013; 42:504
2. Li et al, Eur J Drug Metab PK 2012; 37:263
What dose of SMX for TB?

• Large dose range possible, although safety/tolerability concerns increase with dose
• PD parameter best correlated with SMX activity remains questionable
• Using a target fAUC/MIC ratio >25 has been suggested (as for melioidosis where, unlike TB, synergism exists between TMP and SMX)
• Using exploratory fAUC/MIC targets of 25, 50 and 75, cumulative fractional response ≥90% was reached at ≥2400, 3600 and 7200 mg/d, respectively

Alsaad et al, ERJ 2013; 42:504
Davies-Forsman et al, AAC 2014; 58:7557
Objectives

• Ascertain potential of TMP-SMX for treatment of TB through:
  – Time-kill studies of SMX in static *in vitro* culture models
    • Extracellular (7H9 broth)
    • Intracellular (J774 macrophages)
  – Dose fractionation studies of SMX in a dynamic *in vitro* culture model
  – Dose-ranging efficacy studies in acute & chronic mouse infection models
    • BALB/c (acute & chronic)
    • C3HeB/FeJ (chronic)
• Estimate exposures needed for efficacy in patients
Time-kill in 7H9 broth

- All concentrations enabled growth over the first 2-3 days
- Net stasis at 10-20 mg/L
- A bactericidal effect ensued after 3-7 days at concentrations ≥20 mg/L
Although H37Ra did not grow as vigorously, time-kill curves were generally similar.
Time-kill in 7H9 broth vs. J774 macrophages

- Concentrations ≥640 mg/L were toxic to cells
- Limited intracellular bacteriostatic effect evident after Day 7 at [SMX] ≥80 mg/L
  - consistent with intracellular MIC of 76 mg/L (4x higher than extracellular MIC)¹
  - but may be 32x less potent cidal effect against intracellular bacilli

1. Davies-Forsman et al, AAC 2014; 58:7557
Dose fractionation results (I)

Continuous infusion

Bolus or extended infusion

- Net 2 log10 kill with at continuous infusions of 60-120 \(\mu g/mL\)
- At similar AUC values, continuous infusion is more active than bolus or extended infusion
- Longer time above 3-4x MIC appears beneficial at a given AUC
Dose fractionation results (II)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cmax</th>
<th>AUC</th>
<th>AUC/MIC</th>
<th>T&gt; MIC</th>
<th>T&gt; 3-4xMIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>6h@120</td>
<td>120</td>
<td>1392</td>
<td>69.6</td>
<td>63%</td>
<td>33%</td>
</tr>
<tr>
<td>12h@120</td>
<td>120</td>
<td>2035</td>
<td>101.7</td>
<td>88%</td>
<td>67%</td>
</tr>
<tr>
<td>CI -60</td>
<td>60</td>
<td>1440</td>
<td>72.0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>CI -80</td>
<td>80</td>
<td>1920</td>
<td>96.0</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Dose fractionation results (III)

- Over a range of higher AUC values (i.e., 1500-9000 mg-h/L), the continuous infusion is more active than a single bolus dose.
- A beneficial effect of higher Cmax values during the first wk cannot be excluded.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cmax</th>
<th>AUC</th>
<th>AUC/MIC</th>
<th>T&gt; MIC</th>
<th>T&gt; 4xMIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q24h</td>
<td>328.2</td>
<td>2928</td>
<td>146.4</td>
<td>66%</td>
<td>~55%</td>
</tr>
<tr>
<td>Q12h</td>
<td>220.6</td>
<td>3009</td>
<td>150.4</td>
<td>100%</td>
<td>88%</td>
</tr>
<tr>
<td>CI120</td>
<td>120.0</td>
<td>2880</td>
<td>144.0</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Cmax</th>
<th>AUC</th>
<th>AUC/MIC</th>
<th>T&gt; MIC</th>
<th>T&gt; 4xMIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q24h</td>
<td>1425</td>
<td>8928</td>
<td>437.9</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>CI365</td>
<td>365.0</td>
<td>8928</td>
<td>437.9</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Summary of *in vitro* observations

• Largely time-dependent bactericidal activity, with maximum rate of kill attained at 4xMIC
  – For a given AUC, higher T>4xMIC correlates with greater effect
• Net 1 log reduction at or above AUC/MIC of 25
Activity of SMX in an acute mouse infection model of TB

- Oral dosing of TMP/SMX (5d/wk) x 4 wk, beginning 3 d after aerosol infection

Daily doses ≤1000 mg/kg did not prevent death, although 300-1000 mg/kg extended survived compared to no treatment.

<table>
<thead>
<tr>
<th>SMX dose (mg/kg)</th>
<th>C_max (µg/ml)</th>
<th>AUC_last (µg*h/ml)</th>
<th>fAUC_last/MIC</th>
<th>fT&gt;4xMIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1158</td>
<td>5450</td>
<td>68</td>
<td>~33%</td>
</tr>
<tr>
<td>3000</td>
<td>1503</td>
<td>12649</td>
<td>158</td>
<td>~55%</td>
</tr>
</tbody>
</table>
Activity of SMX in a chronic BALB/c mouse infection model

- Oral dosing (5d/wk) x 4 wk, beginning 4 wk after aerosol infection w/ Mtbi H37Rv

Daily doses >1000 mg/kg bid were toxic

<table>
<thead>
<tr>
<th>SMX dose (mg/kg)</th>
<th>$C_{\text{max}}$ ($\mu$g/ml)</th>
<th>AUC$_{\text{last}}$ ($\mu$g*h/ml)</th>
</tr>
</thead>
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<tr>
<td>1000</td>
<td>1158</td>
<td>5450</td>
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<tr>
<td>3000</td>
<td>1503</td>
<td>12649</td>
</tr>
</tbody>
</table>
Activity of SMX in chronic C3HeB/FeJ mouse infection models

- Oral dosing (5d/wk) x 4 wk, beginning 4-6 wk after aerosol infection w/ Mtb H37Rv

**BALB/c**

Daily doses of 3000 mg/kg bid were toxic.

**C3HeB/FeJ**

Daily doses ≥ 3000 mg/kg bid were toxic.
Conclusions

• SMX exhibits a curious time-kill curve with a slow onset bactericidal effect that is increased by prolonged exposures

• Potency is reduced at least 4-8x against intracellular bacilli, in line with previous observations regarding intracellular [SMX] and MIC comparisons

• Bactericidal effect in the dynamic in vitro system is time-dependent; for a given AUC/MIC, time above 3-4xMIC (ie, 60-80) increases the effect

• Despite pushing doses to the murine toxicity limit, exceeding the plasma exposures safely attainable in patients and producing 100% fT$_{>4xMIC}$ in plasma, no bactericidal effect was evident in BALB/c or C3HeB/FeJ after 4 wks of dosing
Limitations of mouse studies

• Monotherapy of short duration does not exclude possible contribution to combination therapy
• Although C3HeB/FeJ mice develop caseous lesions, the timing and extent of lesion development is heterogeneous; in some mice, intracellular bacilli still predominate
• [SMX] in caseum or ELF have not been examined
• Compared to humans, mice have higher blood and tissue [thymidine] that may rescue bacteria from the SMX effect on folate biosynthesis\textsuperscript{1-3}
• Access to Gly, Met and Ser in RPMI and \textit{in vivo}, or \textit{S}-adenosyl-methionine \textit{in vivo}, are others ways \textit{Mtb} may bypass inhibition of folate synthetic pathway\textsuperscript{4}

1. Minato et al, AAC 2015; 59:5097
2. Proctor, CID 2008; 46:584
3. Nottebrock & Then, Biochemical Pharmacol 1977; 26:2175
Acknowledgements

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  – Faculty and staff of the JHU Center for TB Research
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