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

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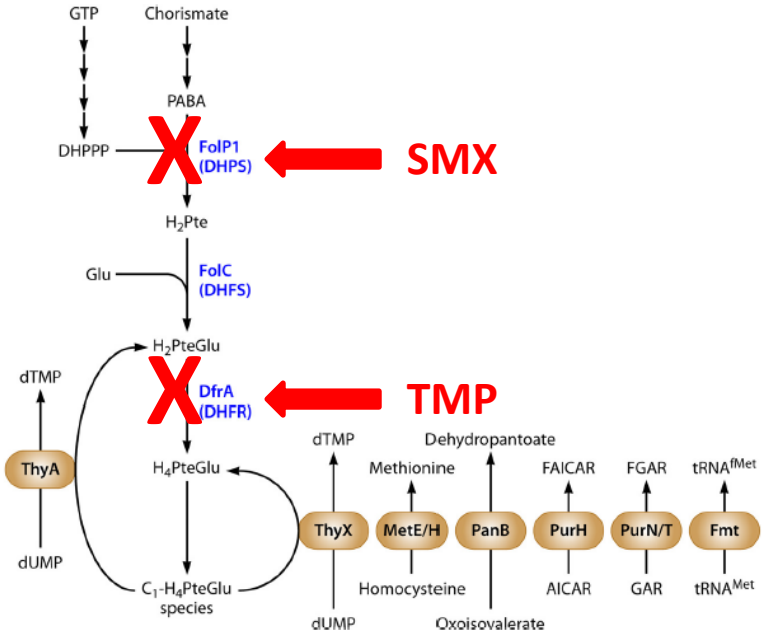
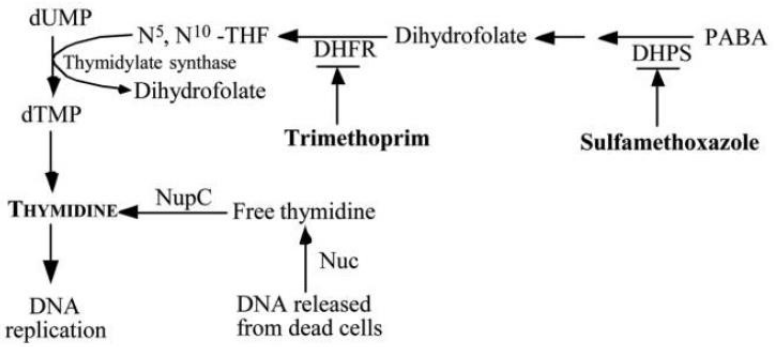
Trimethoprim-sulfamethoxazole (TMP-SMX)

- 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[1]
- MIC_{90} of SMX vs. *M. tb* = 10-20 µg/ml
- TMP not active at attainable concentrations[2]
- Mechanism of action is largely static (esp. in presence of thymidine or thymine)[3]

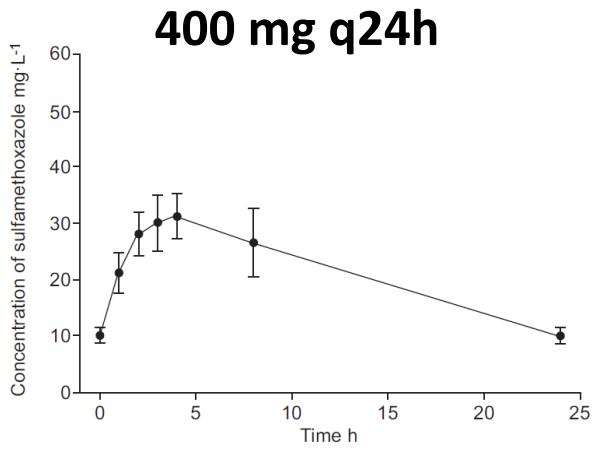


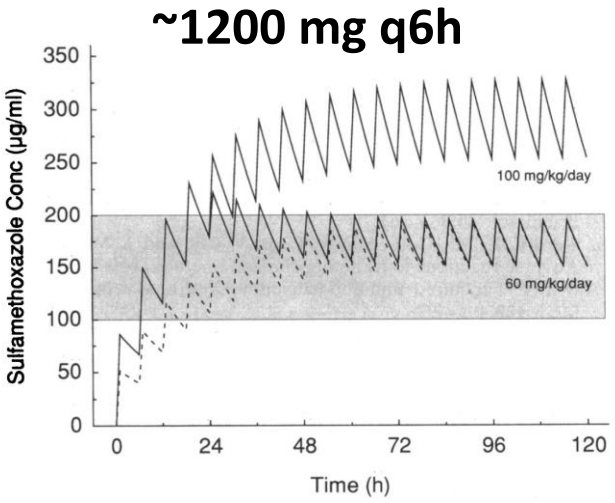


1. Minato et al, AAC 2015; 59:5097
2. Macingwana et al, JAC 2012; 67:2908
3. Proctor, CID 2008; 46:584

Recommended SMX dose range and PK

Subjects	SMX dose (mg)	Cmax (mg/L)	AUC (mg-h/L)	Ref
TB pts	400mg qd	~30	371.5	1
	800mg qd		882.5	1
Healthy	1200mg qd	88.4	1,437	2
Healthy (SS)	~1200mg q6h	247	4,871	3





1. Alsaad et al, ERJ 2013; 42:504
2. Li et al, Eur J Drug Metab PK 2012; 37:263
3. Stevens et al, AAC 1993; 37:448

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

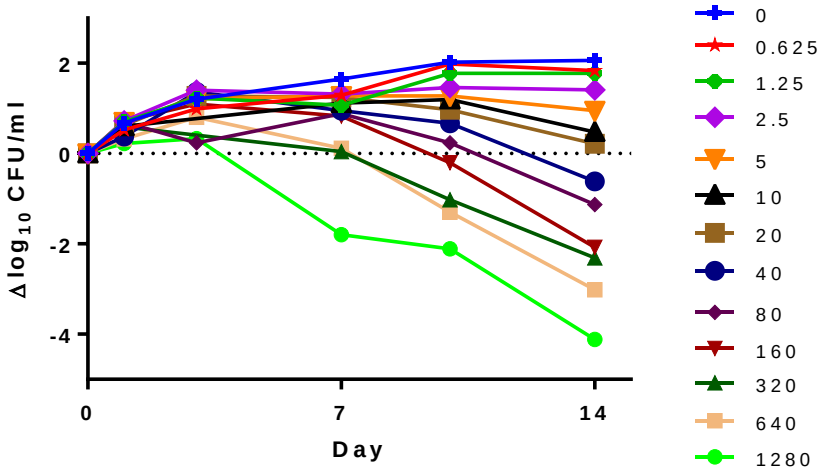
Alsaad et al, ERJ 2013; 42:504
Davies-Forsman et al, AAC 2014; 58:7557

Objectives

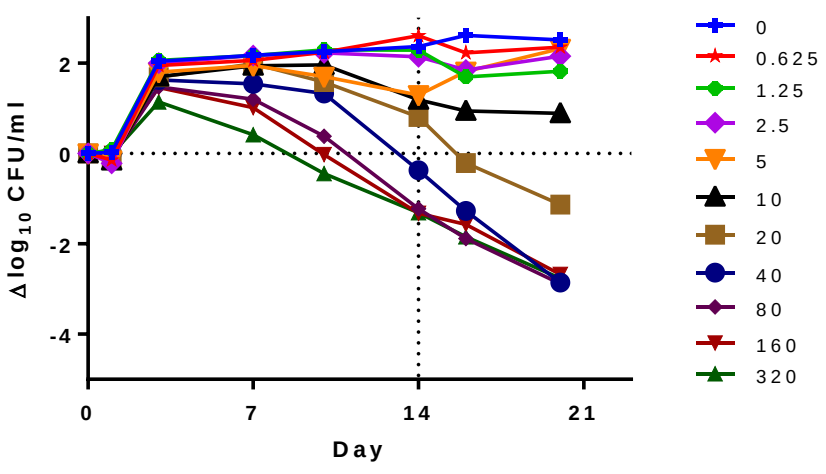
- Ascertain potential of TMP-SMX for treatment of TB through:
  - Time-kill studies of SMX in static *in vitro* culture models
    - Extracellular (7H9 broth)
    - Intracellular (J774 macrophages)
  - Dose fractionation studies of SMX in a dynamic *in vitro* culture model
  - Dose-ranging efficacy studies 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

Expt 1 (H37Rv)



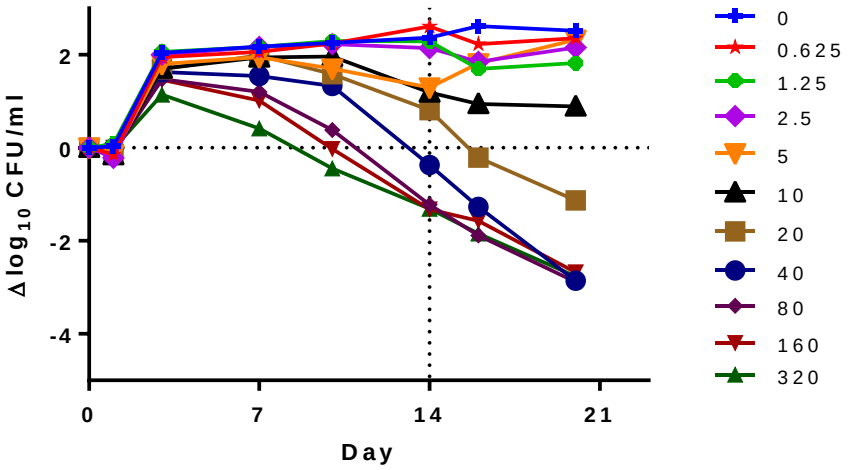
Expt 2 (H37Rv)



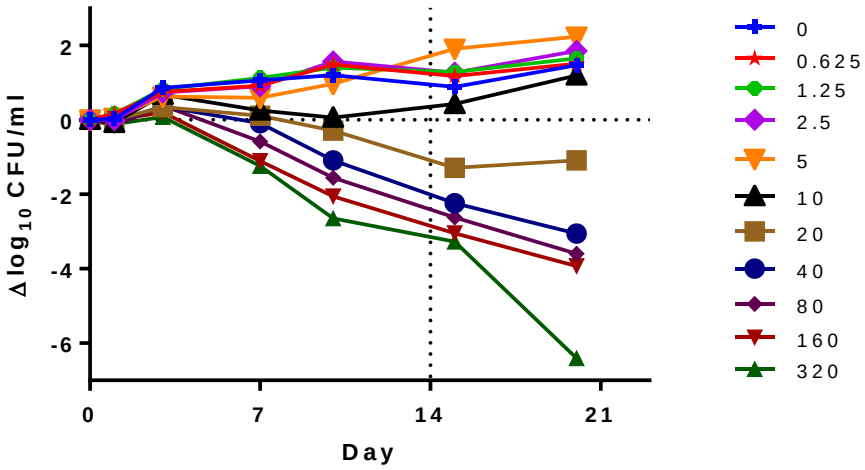
- All concentrations enabled growth over the first 2-3 days
- Net stasis at 10-20 mg/L
- A bactericidal effect ensued after 3-7 days at concentrations ≥20 mg/L

Time-kill in 7H9 broth

Expt 2 (H37Rv)



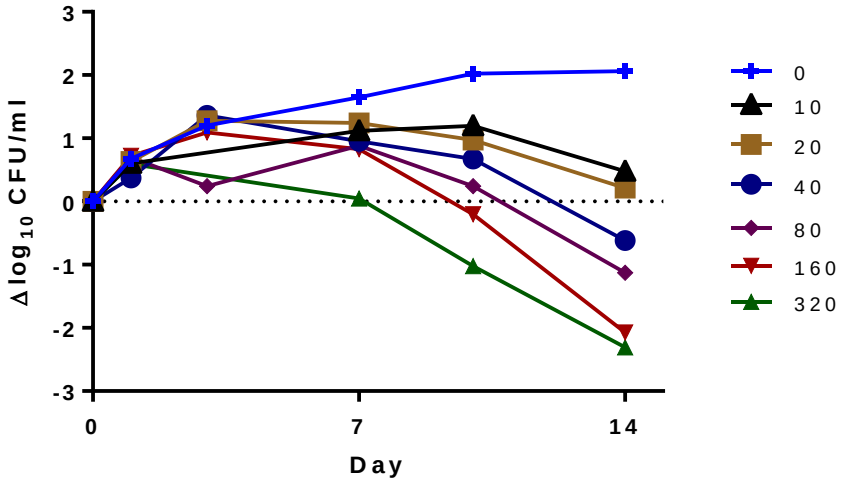
Expt 3 (H37Ra)



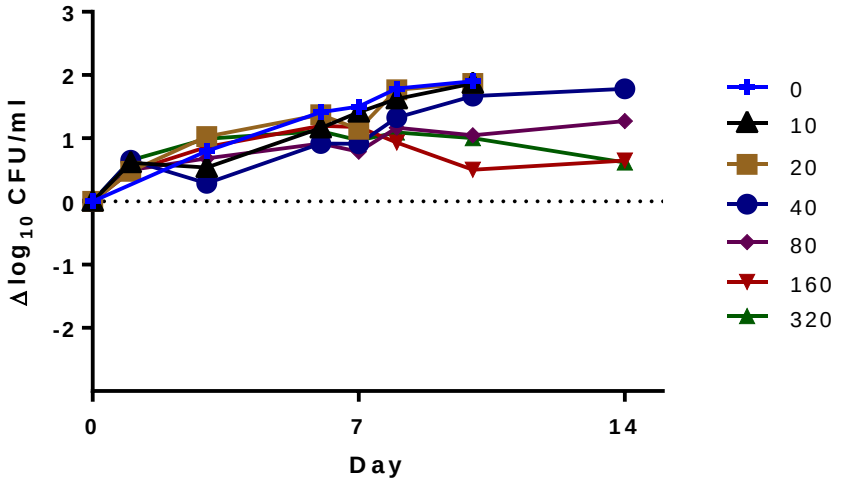
- Although H37Ra did not grow as vigorously, time-kill curves were generally similar

Time-kill in 7H9 broth vs. J774 macrophages

7H9 broth



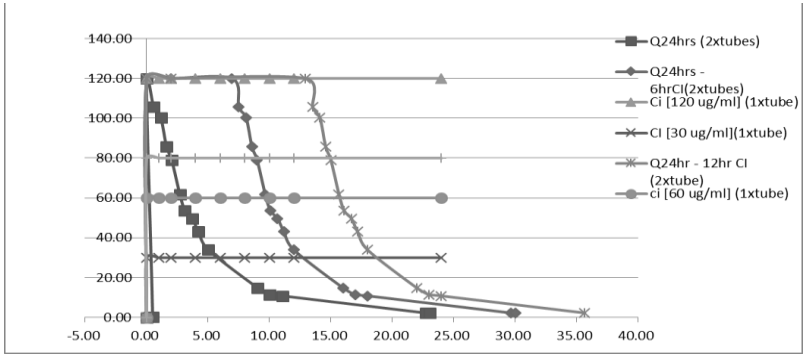
J774 macrophages

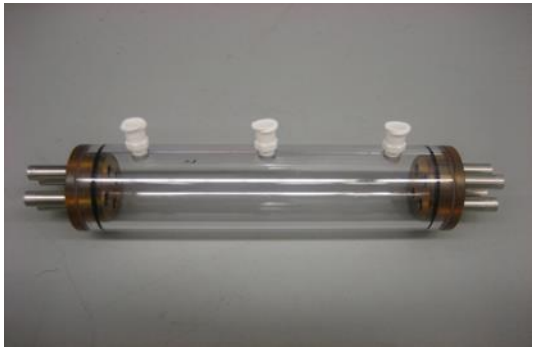


- Concentrations ≥640 mg/L were toxic to cells
- Limited intracellular bacteriostatic effect evident after Day 7 at [SMX] ≥80 mg/L
  - consistent with intracellular MIC of 76 mg/L (4x higher than extracellular MIC)[1]
  - but may be 32x less potent cidal effect against intracellular bacilli

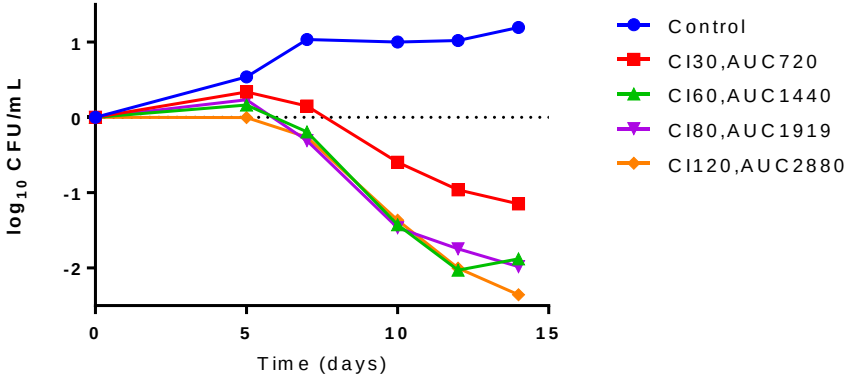
1. Davies-Forsman et al, AAC 2014; 58:7557

Dose fractionation results (I)

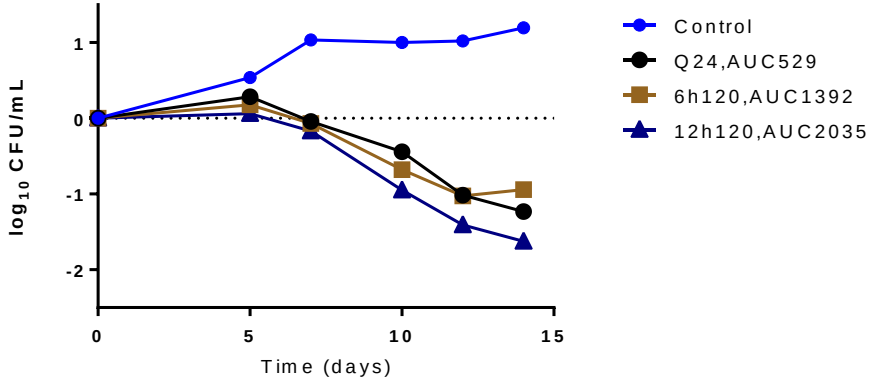




Continuous infusion

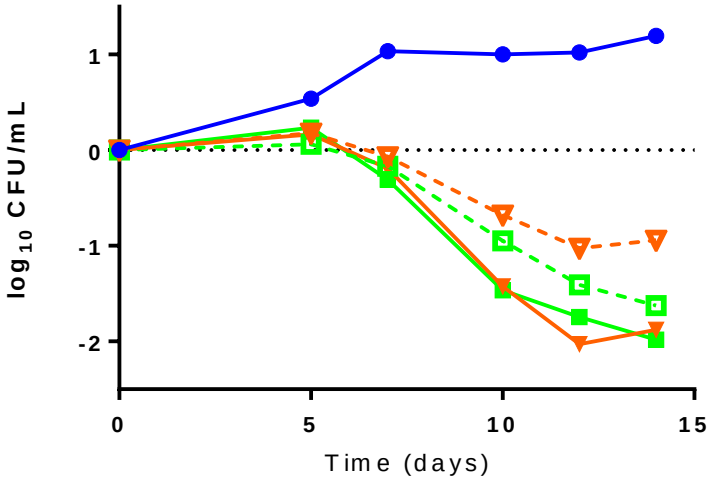


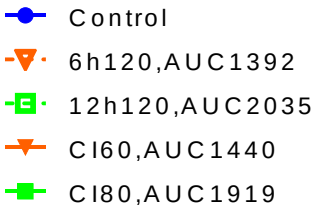
Bolus or extended infusion



- Net 2 log10 kill with at continuous infusions of 60-120 ug/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

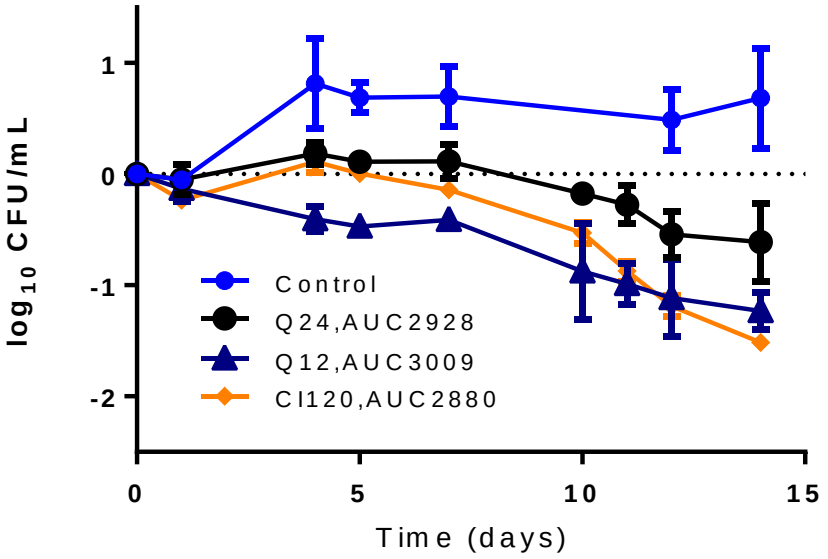
Dose fractionation results (II)



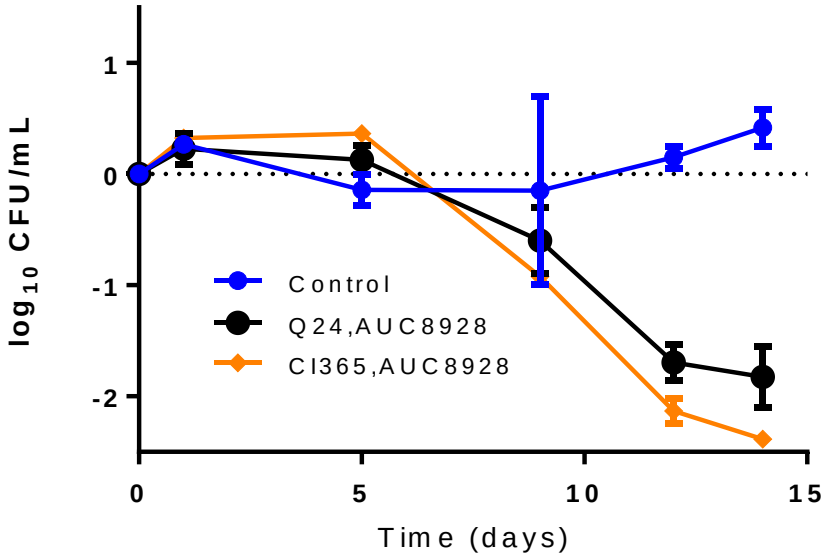


Group	Cmax	AUC	AUC/MIC	T> MIC	T> 3-4xMIC
6h@120	120	1392	69.6	63%	33%
12h@120	120	2035	101.7	88%	67%
CI -60	60	1440	72.0	100%	100%
CI -80	80	1920	96.0	100%	100%

Dose fractionation results (III)



Group	Cmax	AUC	AUC/MIC	T> MIC	T> 4xMIC
Q24h	328.2	2928	146.4	66%	~55%
Q12h	220.6	3009	150.4	100%	88%
CI -120	120.0	2880	144.0	100%	100%



Group	Cmax	AUC	AUC/MIC	T> MIC	T> 4xMIC
Q24h	1425	8928	437.9	100%	50%
CI -365	365.0	8928	437.9	100%	100%

- Over a range of higher AUC values (ie, 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

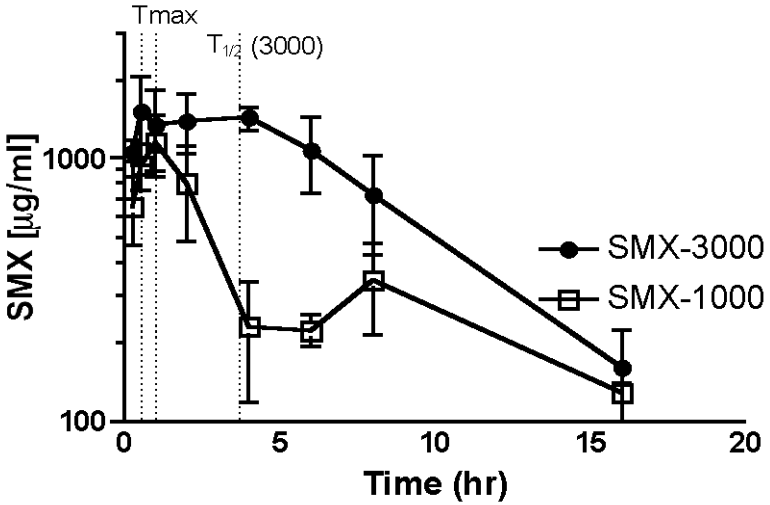
Summary of *in vitro* observations

- 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

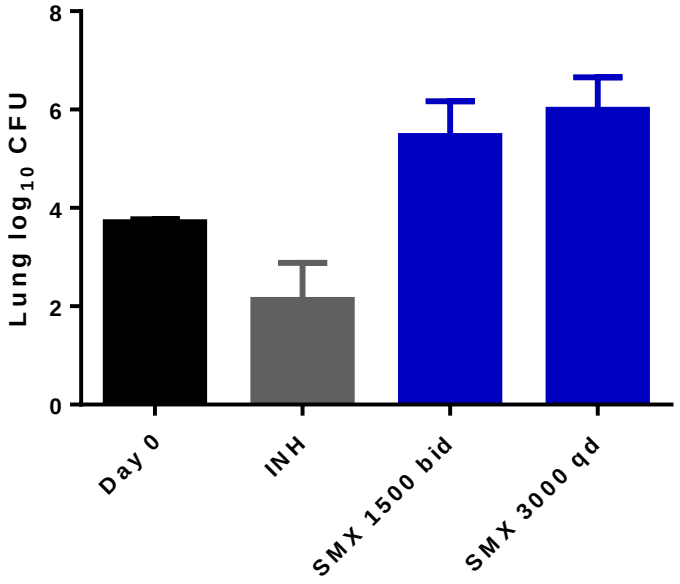
Activity of SMX in an acute mouse infection model of TB

- 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.



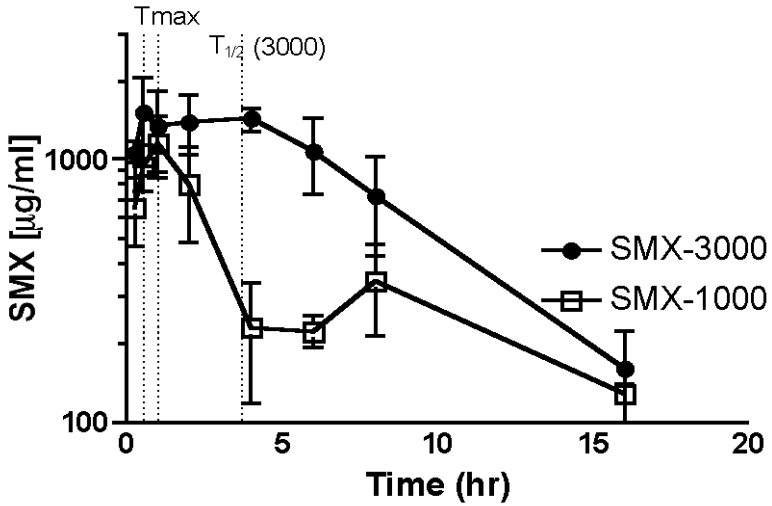
SMX dose (mg/kg)	C_{max} (µg/ml)	AUC_{last} (µg*h/ml)	$fAUC_{last}$/ MIC	fT> 4xMIC
1000	1158	5450	68	~33%
3000	1503	12649	158	~55%



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/ Mtb H37Rv

  Daily doses >1000 mg/kg bid were toxic



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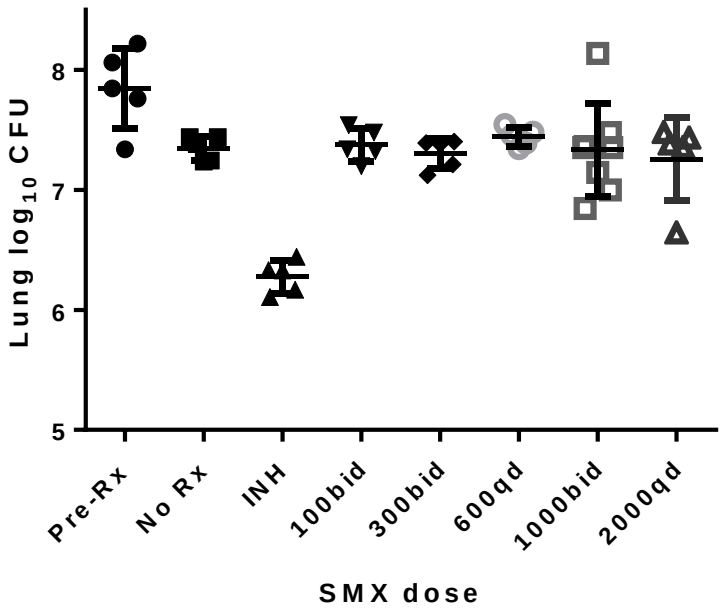
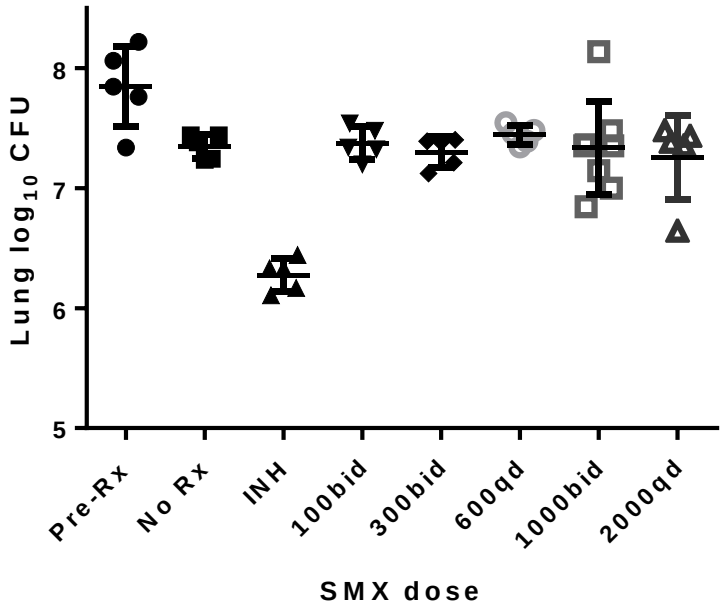


Activity of SMX in chronic C3HeB/FeJ mouse infection models

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

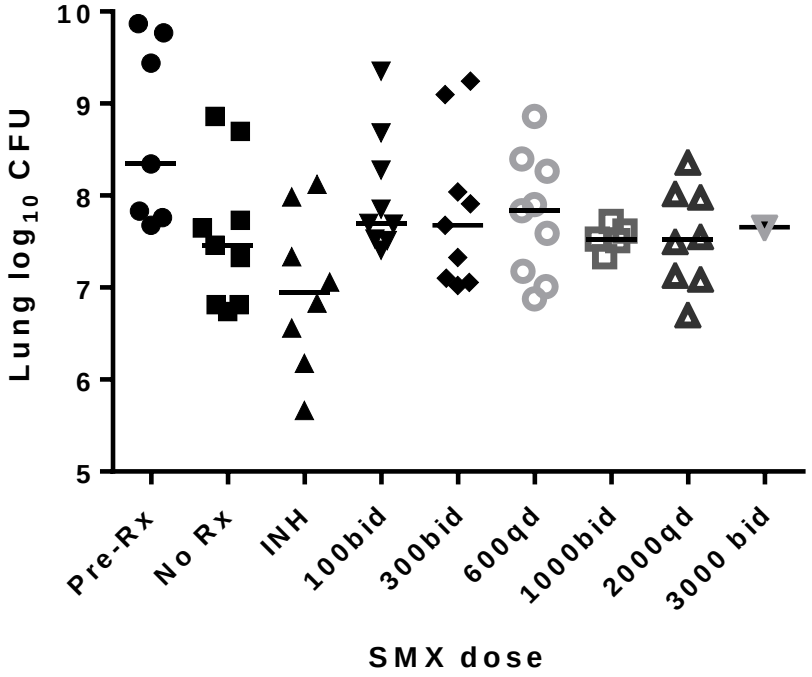
BALB/c

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[1-3]
- Access to Gly, Met and Ser in RPMI and *in vivo*, or *S*-adenosyl-methionine *in vivo*, are others ways *Mtb* may bypass inhibition of folate synthetic pathway[4]

1. Minato et al, AAC 2015; 59:5097
2. Proctor, CID 2008; 46:584
3. Nottebrock & Then, Biochemical Pharmacol 1977; 26:2175
4. Nixon et al, Cell Chem Biol 2014; 21:819

Acknowledgements

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