A-497 Pharmacodynamics of minocycline plus rifampicin against *S.aureus* studied in an in vitro model of infection

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Abstract

Background: Minocycline (min) is widely used as oral therapy for MRSA infection in countries where the dominant MRSA clones remain tetracycline susceptible. Min is often combined in clinical practice with rifampicin (rif). There is minimal pre-clinical pharmacodynamic (PD) data to support this practice. The aim of this study was to assess the combination of min+rif in terms of reducing bacterial load and risk of emergence of resistance (eor) using an in vitro pharmacokinetic (PK) model.
Methods: A single compartment dilutional in vitro model was used. The simulated PK was based on 100mg 12hrly min (fCmax 0.6mg/L, t½ 12h) and 300mg 12hly rif (fCmax 1.0mg/L, t½ 6h). Five strains of S.aureus (4 MRSA, 1 MSSA), min MICs 0.12-1.0mg/L were used at an inoculum of 106CFU/ml. Simulations were performed over 72h and bacterial load assessed by viable count and risk of eor by changes in population profiles in 7 day long experiments.
Results: Addition of rif to min in experiments simulating standard human doses reduced the bacterial load compared to min alone at 24h, 48h and 72h (72h viable count min alone 5.7±2.4 log CFU/ml; 72h viable count min+rif 3.4±1.5 log CFU/ml). In min dose ranging studies the addition of rif reduced the fAUC/MIC for bacteriostatic effect at 72h from 21.5±11.2 for min alone to 8.3±8.6 for min+rif. There was no eor to min in any experiment over 72h. In 7 day experiments growth was observed on MICx4 rifampicin plates in 100% of experiments (10/10) when the min fAUC/MIC was in the range 0.1-2; log count resistant mutants >7 log10. If the min fAUC/MIC was >48 rifampicin resistant mutants occurred in 14% of experiments (1/7).
Conclusion: Addition of rif to min decreases bacterial load compared to min alone and reduces the min fAUC/MIC exposures needed for anti-staphylococcal bacteriostatic and cidal effects. Min resistance was not detected in S.aureus but rif resistance occurs much more readily. Addition of rif to min increases antibacterial effect at the risk of increased rif resistance.

Introduction

- Minocycline (MIN) is widely used as oral therapy for MRSA infection in countries where the dominant MRSA clones remain tetracycline susceptible. MIN is often combined with rifampicin (RIF).
- There is minimal pre-clinical pharmacodynamic (PD) data to support this practice.

- As part of the EU 7th Framework Programme (FP7) project AIDA (preserving old antibiotics for the future) we assessed the combination of MIN +RIF in terms of reducing bacterial load and minimising emergence of resistance (EoR).

Materials and methods

- A dilutional single compartment *in vitro* pK model was used to simulate a range of *ƒ*AUC/MIC MIN serum concentrations based on the pK of 100 mg q12h (fCmax 0.6mg/L, t1/2 12h) and RIF (600mg q12 (fCmax 1.0mg/L, t1/2 6h).
- Due to the difference in half-lives between MIN and RIF), the model was supplemented with MIN throughout each dosing period via a separate dosing chamber to achieve the required profile.
- Five strains of S.aureus were used (4 MRSA and one MSSA).
- The inoculum was 10^6 CFU/mL.

Materials and methods cont

- Antibacterial effect (ABE) was measured by log change in viable count at 24h, 48h and 72h relative to the starting inocula (logCFU/mL).
- A sigmoid Emax model was used to relate T>MIC with ABE using the Boltzmann equation using Graph Pad Prism™.
- EoR was assessed by changes in population profiles over 7 day experiments

Results

- The addition of RIF to MIN in standard human doses reduced the bacterial load compared to MIN alone at 24, 48, and 72h (Figure 1).
At 72h the addition of RIF reduced the bacterial count from 5.7 ± 2.4 for MIN alone to 3.4 ± 1.5 log cfu/mL when given in combination.
- In the dose ranging studies the addition of RIF reduced the fAUC/MIC for a static effect , -1 and -2 log drop at 24 and 72h (Tables 1 and 2)
- In 7day experiments growth was observed on MIC x4 RIF plates in 100% of experiments (10/10) (Table 2).
- If the MIN fAUC/MIC was >48 then RIF resistant mutants occurred in 14% of experiments (1/7).

Figure 1: The ABE of MIN versus MIN+RIF standard doses

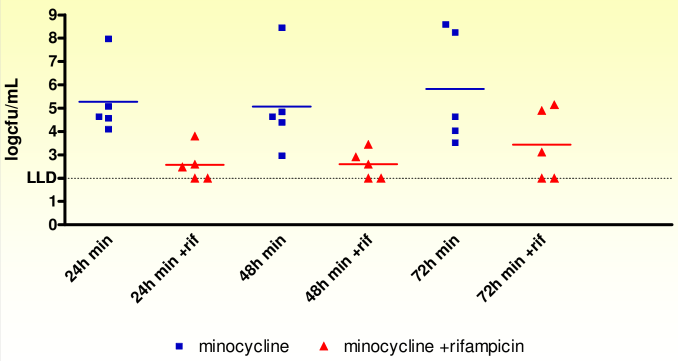

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Table 1:Comparison of fAUC/MIC for MIN and MIN+RIF at 24h for the individual S.aureus strains

24h AUC/MIC							
		Min			Min + Rif OD		
strain	MIC (mg/L)	static effect	-1 log drop	-2 log drop	static effect	-1 log drop	-2 log drop
ATCC 29213	0.19	9.0	17.4	51.2	0.4	0.5	0.6
45494	0.12	19.0	29.3	>200	1.7	2.5	3.2
43241	0.25	11.2	20.5	41.5	0.4	0.6	0.8
33827	0.5	3.2	7.3	>200	0.2	0.3	0.5
33922	1	19.2	38.2	>200	0.9	1.0	1.1
Mean +SD		12.3 ± 6.8	22.5 ± 11.8	-	0.7 ± 0.6	1.0 ± 0.9	1.2 ± 1.1

Table 2: Comparison of fAUC/MIC for MIN and MIN +RIF at 72h for the individual *S.aureus* strains

72h AUC/MIC							
		Min			Min + Rif OD		
strain	MIC (mg/L)	static effect	-1 log drop	-2 log drop	static effect	-1 log drop	-2 log drop
ATCC 29213	0.19	29.8	56.6	105	5.5	6.4	7.2
45494	0.12	36.5	47.7	>200	3.1	4.2	6.7
43241	0.25	13.9	20.1	31.7	7.9	12.5	20.7
33827	0.5	10.3	13.9	19.5	0.6	1.2	1.8
33922	1	17	27.8	55.6	3	3.3	3.4
Mean +SD		21.5±11.2	33.2±18.4	52.9±37.6*	4.0±2.8	5.5±4.3	8.0±7.5

Table 2: Growth on MIC recovery plates at 7days

growth on rifampicin MIC x4 recovery plates at 168h		
minocycline AUC/MIC	Exps with colonies recovered	count on MICx4 plates (log cfu/mL)
0	3/3	8.2 ± 0.1*
0.1 - 2.0	6/6	7.7 ± 1.6
2.1 - 4.0	4/4	7.1 ± 2.2
4.1 - 6.0	3/5	4.7 ± 3.2
6.1 - 12.0	4/4	4.6 ± 2.7
12.0 - 48.0	3/6	2.6
>48	1/7	3
Growth controls	0/4	<2.0

Conclusions

- Addition of RIF 600mg/day reduces bacterial load compared to MIN alone and reduces fAUC/MIC exposures need for anti-staphylococcal bacteriostatic and bactericidal effects.
- MIN resistance was not detected in S.aureus but RIF resistance occurs much more readily.
- Addition of RIF increases antibacterial effect but at the risk of increased RIF resistance.