Combination therapy for the treatment of melioidosis

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Introduction

Burkholderia pseudomallei is intrinsically resistant to many antimicrobial agents, most apparent in acidic conditions that may be encountered *in vivo.* Finafloxacin has been shown to have activity in these environments, where other fluoroquinolones are less active. It has been shown to be safe in human clinical trials and, furthermore, to offer protection against *B. pseudomallei* in murine models of infection. In an attempt to improve efficacy (and prevent relapse of infection) a combination therapy approach was investigated. The activity of finafloxacin against *B. pseudomallei* in combination with a panel of antibiotics was determined *in vitro*.

Methods

1. The *in vitro* activity of finafloxacin was evaluated in combination with azithromycin, doxycycline, co-trimoxazole and meropenem by checkerboard assay.
2. The fractional inhibitory concentrations (ΣFIC) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone, and FIC B is the MIC of drug B in the combination/MIC of drug B alone.
3. A combination is synergistic when the ΣFIC is ≤0.5, indifferent when the ΣFIC is >0.5 to <4, and antagonistic when the ΣFIC is ≥4.
3. Time kill assays were performed at multiples of the MIC to determine whether the combinations were synergistic (2 log reduction) or antagonistic (2 log increase).
4. *B. pseudomallei* strain K96243 was used in all assays, grown in CAMBH adjusted to pH 5 or pH 7.

Results

- Combining finafloxacin with co-trimoxazole or doxycycline at either pH resulted in an ΣFIC that were >0.5 and <2, therefore the activity was classed as indifferent (Table 1).
- None of the concentrations evaluated resulted in a ΣFIC that would be classed as antagonistic.
- Finafloxacin combined with azithromycin or meropenem resulted in activity that was classed as indifferent or synergistic (dependent on the concentration and pH).

pH 5			
Finafloxacin (µg/ml)	**Azithromycin (µg/ml)**	**ΣFIC**	**Interpretation**
0.25	0.5-64	0.3-0.4	**Synergy**
0.06-1	128-512	0.8-1.5	Indifference
	Meropenem (µg/ml)		
0.06-0.5	0.12-2	0.6-1.1	Indifference
	Co-trimoxazole (µg/ml)		
0.06-1	1-16	1.1-1.5	Indifference
	Doxycycline (µg/ml)		
0.06-1	0.25-1	0.8-1.3	Indifference
pH 7			
Finafloxacin (µg/ml)	**Azithromycin (µg/ml)**	**ΣFIC**	**Interpretation**
1	2	0.5	Synergy
0.06-1	4-64	0.6-1.1	Indifference
	Meropenem (µg/ml)		
0.5	0.25	0.5	Synergy
0.06-0.5	0.5-1	0.6-1.1	Indifference
	Co-trimoxazole (µg/ml)		
0.06-1	1-16	1.1-1.5	Indifference
	Doxycycline (µg/ml)		
0.06-0.5	0.25-1	0.8-1.3	Indifference

Table 1. Checkerboard assays with *B. pseudomallei.*

Combinations at 2 X MIC

- At pH 5, synergy was observed for finafloxacin used in combination with doxycycline ($p<0.0001$) (Figure 1).
- At pH 5, finafloxacin and doxycycline combined are more active than finafloxacin combined either co-trimoxazole or azithromycin ($p<0.05$).
- At pH 7, antagonism was observed for finafloxacin used in combination with co-trimoxazole ($p<0.0001$).

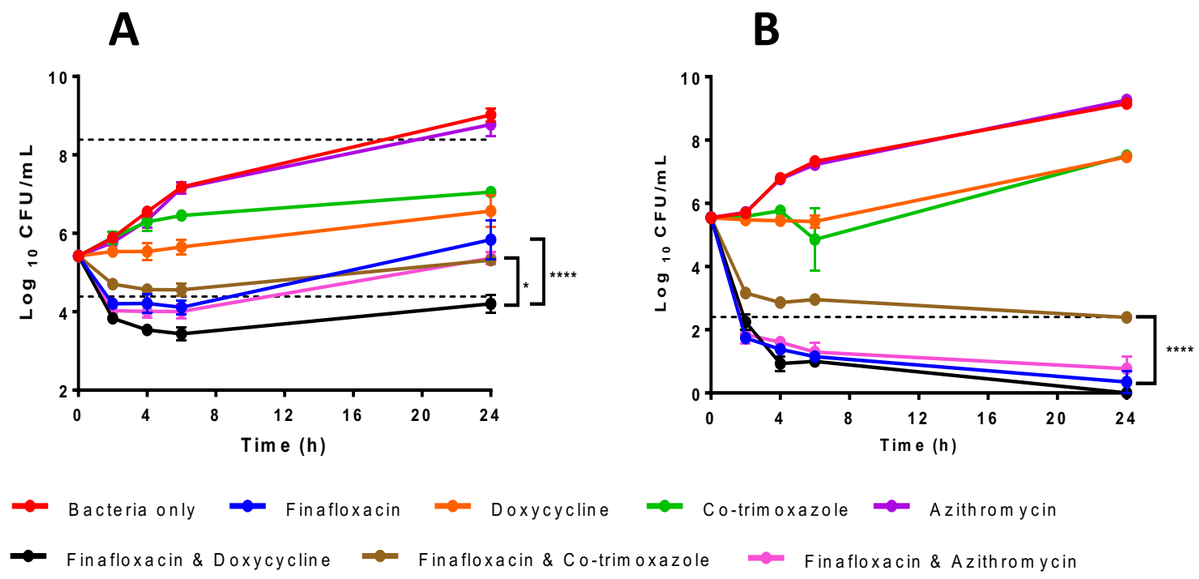

Figure 1. Time kill assays for *B. pseudomallei.* (A) pH 5, finafloxacin (2 µg/ml), doxycycline (2 µg/ml), co-trimoxazole (32 µg/ml). pH 7, finafloxacin (8 µg/ml), doxycycline (1 µg/ml), co-trimoxazole (32 µg/ml). Azithromycin was used at a concentration in the checkerboard that demonstrated synergy (2 µg/ml). The error bars represent the SEM of 2 biological replicates. The dotted line represents a 2 $\log_{10}$ reduction/increase in CFU/ml from the most active antibiotic on its own.

Combinations at 4 X MIC

- At pH 5, the activity of finafloxacin combined with co-trimoxazole is reduced when compared to finafloxacin ($p<0.05$), finafloxacin combined with azithromycin ($p<0.0001$) and finafloxacin combined with doxycycline ($p<0.01$) (Figure 2).
- At pH 7, the activity of finafloxacin combined with co-trimoxazole is reduced when compared to finafloxacin on its own ($p<0.001$), finafloxacin combined with azithromycin ($p<0.0001$) and finafloxacin combined with doxycycline ($p<0.001$).

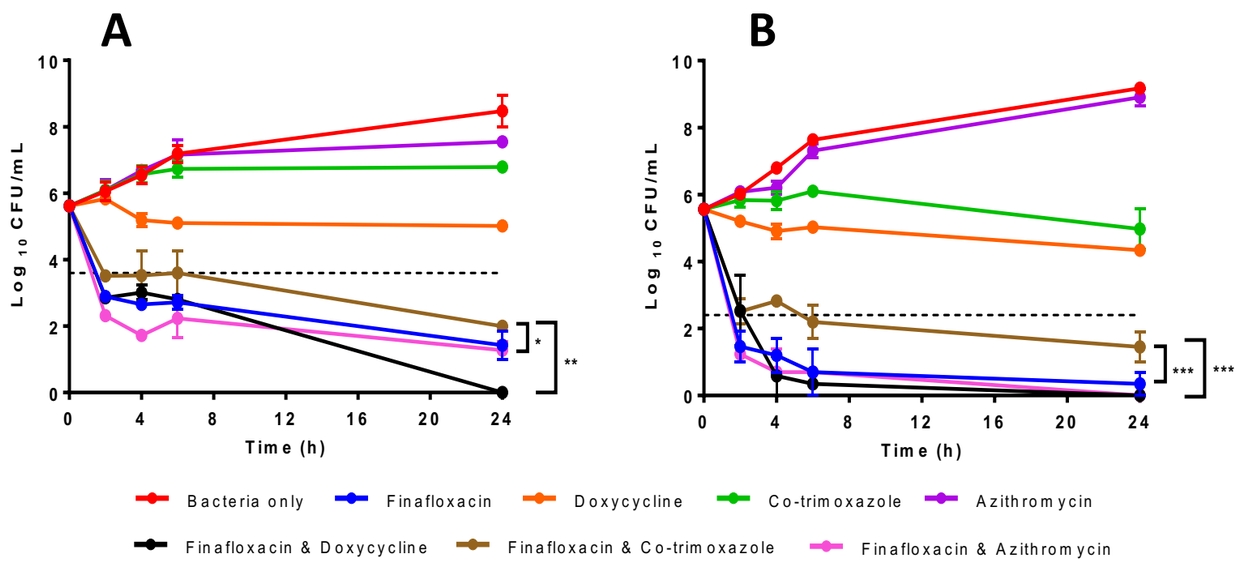

Figure 2. The time kill assays for *B. pseudomallei.* (A) pH 5, finafloxacin (4 µg/ml), doxycycline (4 µg/ml), co-trimoxazole (64 µg/ml). pH 7, finafloxacin (16 µg/ml), doxycycline (2 µg/ml), co-trimoxazole (64 µg/ml). Azithromycin was used at a concentration in the checkerboard that demonstrated synergy (2 µg/ml). The error bars represent the SEM of 2 biological replicates. The dotted line represents a 2 $\log_{10}$ reduction/increase in CFU/ml from the most active antibiotic on its own.

Discussion

Finafloxacin has demonstrated a significant protective benefit in a mouse model of melioidosis, however some mice relapse with infection. This *in vitro* data has identified combinations of antibiotics that result in differences when compared to antibiotics used individually. Finafloxacin, when used in combination with doxycycline, has a synergistic effect compared with finafloxacin used with co-trimoxazole which is antagonistic. Some of these combinations will be taken forwards into PK studies which will lead to efficacy studies in the future.

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