Finafloxacin prophylaxis protects against acute inhalational murine melioidosis

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Introduction

Melioidosis, caused by *Burkholderia pseudomallei*, is intrinsically resistant to many antimicrobial agents, particularly in conditions that may be encountered *in vivo*[1]. Treatment is divided into two phases; the acute phase aimed at preventing death from overwhelming sepsis and the oral eradication phase aimed at killing residual bacteria, minimising the risk of relapse. The acute phase typically involves parenteral ceftazadime or meropenem for 10-14 days[1]. The current recommended treatment for the oral eradication phase is co-trimoxazole for a minimum of 12-20 weeks[2].

Finafloxacin is a novel C-8-cyano-fluoroquinolone containing a unique chiral C7 substituent; this unique property confers enhanced activity under acidic conditions, where other fluoroquinolones, including ciprofloxacin, are inactivated. Therefore finafloxacin may exhibit advantages over other fluoroquinolones in acidic sites of infection, including *B. pseudomallei*, which typically resides within acidic cellular organelles such as phagosomes and phagolysosomes. Finafloxacin is currently under development for clinical treatment of infections with acidic foci by MerLion Pharmaceuticals GmbH Ltd[3]. Several studies have demonstrated finafloxacin has increased bactericidal activity at acidic pH against a spectrum of Gram-negative and Gram-positive bacteria[4,5].

The aim of this study is to investigate the efficacy of finafloxacin in comparison to ciprofloxacin and co-trimoxazole when used as post exposure prophylaxis in a murine model inhalational of melioidosis.

Methods

- Groups of 20 female Balb/c mice were restrained in a nose only exposure chamber and exposed to a dynamic aerosol of *B. pseudomallei* K96243 for 10 minutes resulting in a retained dose of approximately 113 CFU.
- Therapy was initiated at 6 hours post challenge to groups of 20 mice as shown in table 1. All treatment regimens were continued for 14 days.
- At 24 hours and 14 days post challenge 5 mice from each group were culled and the spleen, liver and lungs were harvested for bacterial load analysis.
- At day 63 all surviving mice were culled and the spleen, liver and lungs were harvested for bacterial load analysis.

Treatment	Route of administration	Dose mg/kg	Frequency of dosing
Finafloxacin	Oral	37.5	Every 8 hours
Finafloxacin	Intraperitoneal	30	Every 12 hours
Ciprofloxacin	Intraperitoneal	30	Every 12 hours
Co-trimoxazole	Oral	240	Every 12 hours

Table 1 Antibiotic regimens

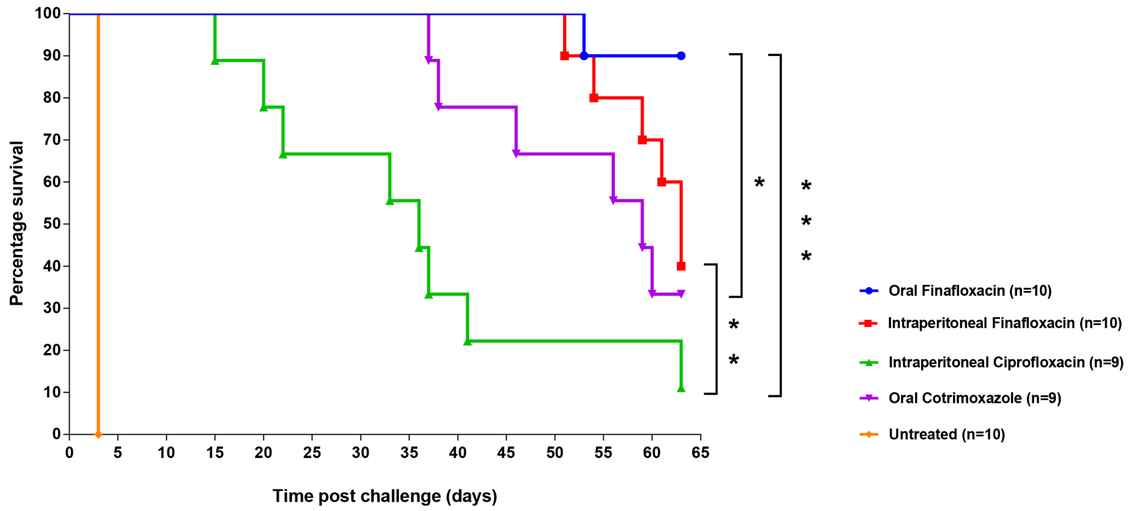

Figure 1. Survival of mice following challenge with aerosolised *B. pseudomallei* K96243. Mice were challenged with approximately 113 CFU of *B. pseudomallei* K96243 by the inhalational route and treated with oral finafloxacin (37.5 mg/kg) every 8 hours, intraperitoneal finafloxacin (30 mg/kg) every 12 hours, intraperitoneal ciprofloxacin (30 mg/kg) every 12 hours or oral co-trimoxazole (240 mg/kg) every 12 hours. All regimens were initiated at 6 hours post challenge and continued for 14 days. Untreated controls received sterile water. Statistical analysis was performed using the Mantel-Haenszel log rank test (*p<0.5, **p<0.01, ***p<0.001).

Results

- All untreated mice succumbed to infection by 72 hours post challenge. All antibiotics offered better protection than no treatment (p<0.001). Oral finafloxacin provided better protection than intraperitoneal ciprofloxacin (p<0.001) and oral co-trimoxazole (p=0.012); intraperitoneal finafloxacin also provided better protection than intraperitoneal ciprofloxacin (p=0.009) but afforded no improvement over co-trimoxazole treatment (p=0.287); additionally there was no difference between finafloxacin administered orally or by intraperitoneal injection (p=0.069).
- In untreated control animals, bacteria had disseminated from the lungs to the spleen and liver within 24 hours. All treatments reduced the bacterial load in the liver in comparison to untreated controls (p<0.05), with no bacteria detected in the ciprofloxacin or finafloxacin treatment groups. In the spleen both co-trimoxazole and ciprofloxacin provided no control of bacterial load compared to no treatment (p>0.05; (Figure 2); however, both routes of administration of finafloxacin afforded a reduction in bacterial load with no bacteria recovered from this organ.
- At 14 days post challenge oral and intraperitoneal finafloxacin and co-trimoxazole-treated mice had no detectable *B. pseudomallei* organisms in the spleen, liver or lungs (Figure 3). Four out of 5 mice treated with intraperitoneal ciprofloxacin had detectable *B. pseudomallei* in all organs and all mice had bacteria in the lungs ranging from $1x10^3$ to $3x10^4$ CFU/g, higher than in other antibiotic treatment groups (p<0.01).
- At the end of the experiment all surviving mice in the co-trimoxazole and finafloxacin groups had detectable *B. pseudomallei* in at least one organ.

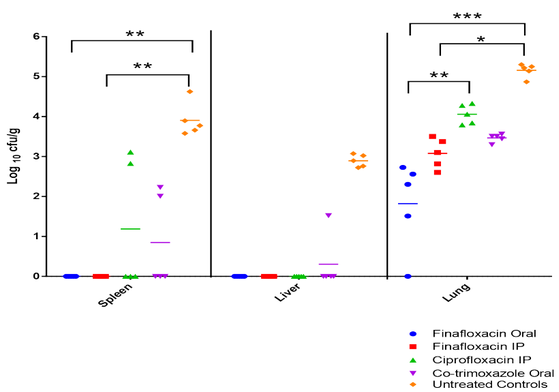

Figure 2.
Bacterial load in organs at 24 hours post challenge with aerosolised *B. pseudomallei* K96243. Bacterial counts (CFU/g) in the spleen, liver and lungs of 5 mice per group at 24 hours post challenge with approximately 113 CFU of inhalational *B. pseudomallei* K96243. Statistical analysis was performed using two-way ANOVA with Dunn's multiple comparison test (*p<0.05, **p<0.01, ***p<0.001).

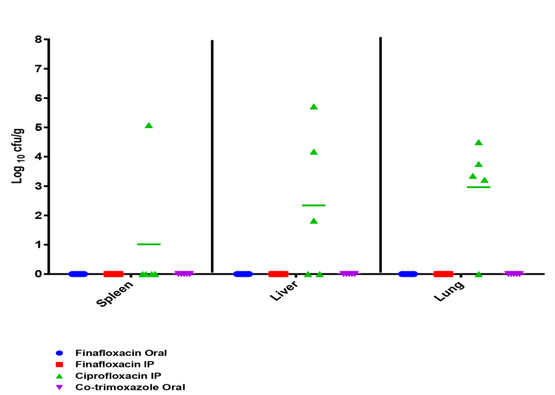

Figure 3.
Bacterial load in organs at 14 days post challenge with aerosolised *B. pseudomallei* K96243. Bacterial counts (CFU/g) in the spleen, liver and lungs of 5 mice per group at 14 days post challenge, and treated with oral finafloxacin (37.5 mg/kg) every 8 hours, intraperitoneal finafloxacin (30 mg/kg) every 12 hours, intraperitoneal ciprofloxacin (30 mg/kg) every 12 hours or oral co-trimoxazole (240 mg/kg) every 12 hours. All regimens were initiated at 6 hours post challenge and continued for 14 days.

Conclusions

- Finafloxacin has rapid bactericidal activity against *B. pseudomallei* in acidic conditions and this confers to in vivo protection when delivered as oral PEP in a murine model of inhalational melioidosis, offering significantly better protection than ciprofloxacin or co-trimoxazole.
- All surviving mice were colonised with *B. pseudomallei* indicating that the antibiotics had failed to prevent the establishment of a chronic infection.
- This is very promising data showing that this novel fluoroquinolone could provide an alternative treatment or PEP option for melioidosis. Further studies are warranted to investigate finafloxacin further.

References
1. Dance D. Int J Antimicrob Agents 2014; 43: 310-318.
2. Chetchotisakd P, Wirongrong C, Chaowagul W, Anunnatsiri S, Phimda K, Mootsikapun P et al. The Lancet 2014; 383: 807-814.
3. Emrich NC, Heisig A, Stubbings W, Labischinski H and Heisig P. Journal of Antimicrobial Chemotherapy 2010; 65: 2530-2533.
4. Higgins PG, Stubbings W, Wisplinghoff H and Seifert H. Antimicrob Agents Chemother 2010; 54: 1613-1615.
5. Stubbings W, Leow P, Chee Yong G, Goh F, Korber-Irrgang B, Kreksken M et al. at 14 days post challenge Antimicrob. Agents Chemother 2011; 55: 4394-4397.