Efficacy of Finafloxacin against *Francisella tularensis* strain SchuS4

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Introduction

Francisella tularensis is the causative agent of tularemia, a severe and potentially life-threatening disease. In the USA *F. tularensis* has been classed as a category A select agent as it has a very low infectious dose by the inhalational route, is highly virulent and has previously been weaponised[1]. There is no licensed vaccine and the currently recommend prophylaxis, orally delivered ciprofloxacin or doxycycline, have been associated with treatment failure and disease relapse[2].

Finafloxacin is a novel fluoroquinolone shown to have increased antibacterial activity at acidic pH[3]. Other fluoroquinolones have reduced antibacterial activity at lower pH and therefore finafloxacin may offer an advantage in the treatment of intracellular infections, where the local site of infection is acidic[4]. The aim of this study was to evaluate the *in vitro* and *in vivo* activity of finafloxacin against *F. tularensis* in comparison to ciprofloxacin.

Methods and Results

Minimum inhibitory concentrations (MIC's) and minimum bactericidal concentrations (MBC's)

- The MIC and MBC of finafloxacin (supplied by MerLion Pharmaceuticals Pte Ltd) and ciprofloxacin (Sigma-Aldrich) were determined for *F. tularensis* strains SchuS4 and HN63, using the broth micro dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines[5]. Assays were performed in 96 well plates in Modified Cysteine Partial Hydrolysate (MCPH) broth and incubated at 37°C for 24 hours. The minimum bactericidal concentrations (MBCs) for finafloxacin and ciprofloxacin were determined by plating 100 µl aliquots from the MIC dilutions showing no visible bacterial growth onto Blood, cysteine, glucose agar (BCGA), in triplicate. The plates were incubated for 72 hours at 37°C. The MIC was recorded as the lowest concentration of an antibiotic that inhibited the visible growth of the bacteria after overnight incubation.The MBC was recorded as the lowest concentration of antibiotic that killed 99.9% of the bacteria in the original inoculum.

	Minimum inhibitory concentrations (µg/mL)		Minimum bactericidal concentrations (µg/mL)	
	Finafloxacin	Ciprofloxacin	Finafloxacin	Ciprofloxacin
F. tularensis SchuS4	0.016	0.03	0.5	1
F. tularensis HN63	0.03	0.03	0.25	1

Table 1. MIC's (µg/mL) and MBC's (µg/mL) for finafloxacin and ciprofloxacin for F. tularensis strains SchuS4 and HN63.

- Finafloxacin had comparable inhibitory activity to ciprofloxacin against both strains of *F. tularensis*, however finafloxacin had increased bactericidal activity in comparison to ciprofloxacin (Table 1).

Macrophage assays

- Intracellular kill assays were performed in J774 murine macrophages infected with *F. tularensis* strain SchuS4 at an MOI of 1 for 30 minutes. Following a PBS wash step, ciprofloxacin and finafloxacin were added at 10 times the MIC. At 2, 4, 6 and 24 hr macrophages were lysed and the intracellular bacterial load was enumerated by plating onto BCGA plates.
- At 10 x MIC both ciprofloxacin and finafloxacin showed significantly reduced bacterial load in comparison to the untreated control ($p<0.0001$). Ciprofloxacin showed a significantly reduced bacterial load in comparison to finafloxacin ($p<0.05$) (Figure 1).

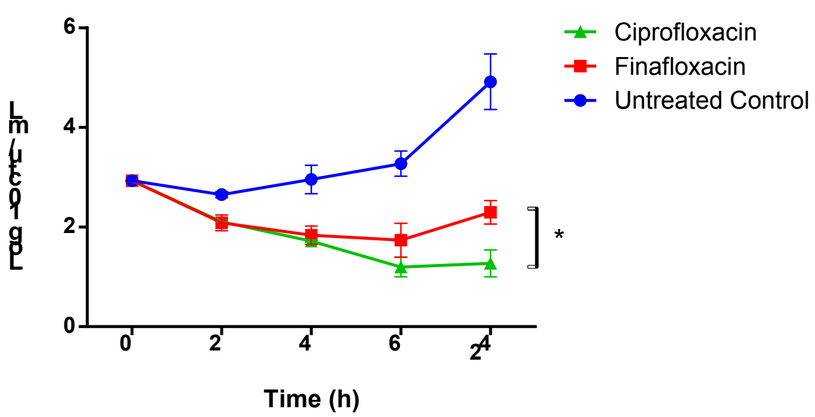

Figure 1. The intracellular activity of finafloxacin and ciprofloxacin for F. tularensis strain SchuS4 .

In vivo efficacy study

- *F. tularensis* SchuS4 was prepared for the animal challenge by growing in MCPH broth shaking at 37°C for 48 hours. Prior to use the OD_{600nm} of the culture was adjusted to 0.1, which equates to approximately $1x10^9$ CFU/ml. Bacterial numbers were determined by enumeration of serially diluted bacteria on agar plates. All experiments with *F. tularensis* SchuS4 were carried out in a Class III microbiological safety cabinet complying with British Standard 5726.

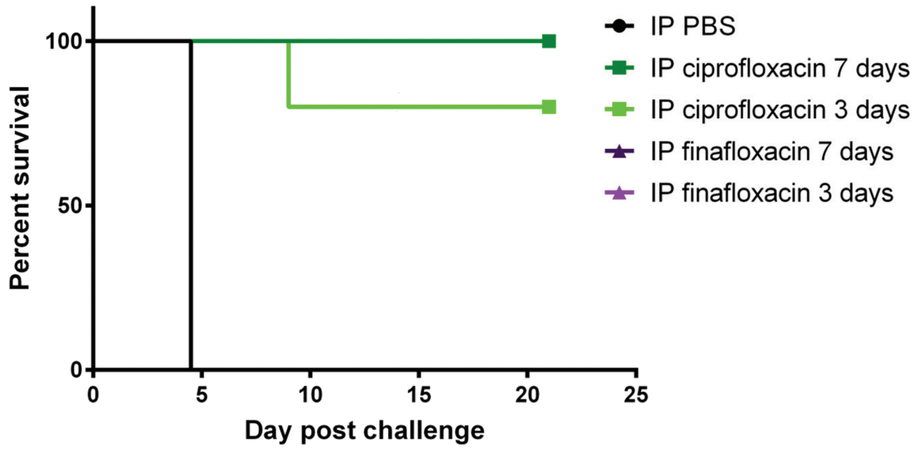

Figure 2. The protective efficacy of finafloxacin and ciprofloxacin against F. tularensis strain SchuS4 in vivo

- Female BALB/c mice (8-10 week old) were obtained from a licensed UK supplier. Mice were caged within an ACDP (UK) level 3 animal containment isolator, complying with British Standard 5726, and allowed to acclimatise for five days before challenge. All experiments with mice were carried out in accordance with the UK Animal (Scientific Procedures) Act (1986).
- Groups of 5 Balb/c mice were restrained in a nose only exposure chamber and exposed to a dynamic aerosol of *F. tularensis* SchuS4 for 10 minutes resulting in a retained dose of approximately 35 CFU.
- Therapy with ciprofloxacin (30 mg/kg in 300 µl), PBS (300 µl) or finafloxacin (50 µl) was delivered to groups of 5 mice at 24 hours post-challenge. Therapy was delivered for 3 or 7 days, and administered twice daily by the intraperitoneal route. Mice were observed for 3 weeks post-challenge when the experiment was terminated.
- Finafloxacin offered 100% survival against *F. tularensis* SchuS4 when delivered intraperitoneally for 3 or 7 days compared to ciprofloxacin which offered 80% and 100% respectively (Figure 2).

Conclusions

- Finafloxacin showed a similar level of antibacterial activity to ciprofloxacin against *F. tularensis* SchuS4 and HN63 *in vitro*
- Finafloxacin is an alternative treatment for Balb/c mice infected with inhalational tularaemia.
- A reduced number of doses are required compared to ciprofloxacin to achieve 100% protection *in vivo*
- Finafloxacin is a promising medical countermeasure for the treatment of infections caused by *F. tularensis* and further *in vivo* work is ongoing.

References

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