SATURDAY - 260

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. It 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 recommended prophylaxis, have been associated with treatment failure and disease relapse[2].

Finafloxacin is a novel fluoroquinolone which has demonstrated improved antibacterial activity at acidic pH[3], therefore may offer an advantage in the treatment of intracellular infections, where the local site of infection is acidic[4]. The window of opportunity for finafloxacin against *F. tularensis* strain SchuS4 was compared to ciprofloxacin in a Balb/c mouse model of infection.

Methods

- *F. tularensis* strain SchuS4 was prepared by adding 10 µL of a frozen stock to 10 mL of phosphate buffered saline (PBS). This was adjusted to an OD of 0.1 at 590_{nm} to obtain an inoculum of approximately 10^8 cfu/ml. A serial dilution was performed in PBS to obtain the required challenge dose of 100 cfu. These dilutions were also plated onto blood cysteine glucose agar (BCGA) to calculate the actual dose delivered.
- Female BALB/c mice (8-10 week old) were anaesthetised with isoflurane (Isocare®, Animal Care Limited, York) and once sedated, 50 µL of the bacteria was delivered to both nares via a micropipette.
- Treatment with ciprofloxacin (30 mg/kg in 300 µl), PBS (300 µl), finafloxacin (37.5 mg/kg in 50 µl) or diluent (50 µl) was delivered to groups of 10 mice at 24, 48, 72 and 96 hours post challenge (pc). Therapy was delivered for 7 days, and administered twice daily by the intraperitoneal route (ciprofloxacin) or three times a day orally (finafloxacin). Mice were observed for 35 days post-challenge when the experiment was terminated.

Results

The intranasal dose received by the mice was calculated as 101 cfu. All control mice succumbed to infection by day 5 pc. All treatment regimens significantly improved survival compared to the controls ($p<0.001$). All mice receiving finafloxacin or ciprofloxacin initiated at 24 or 48 hours pc were protected from infection with *F. tularensis* (Figure1A). At 72 hours pc, mice did not display any overt signs of disease; however, upon cessation of therapy, 1 finafloxacin mouse and 4 ciprofloxacin mice succumbed to infection (Figure 1B). At 96 hours pc mice were displaying severe signs of infection. Three mice that received ciprofloxacin initiated at 96 hours pc survived until the end of the study, compared to five mice treated with finafloxacin (Figure 1B). Stratified analysis of the 72 and 96 hour data combined together demonstrates a benefit of finafloxacin therapy compared to ciprofloxacin ($p=0.042$) (Figure 1C).

References
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3. Lemaire S, Van Bambeke F and Tulkens P. (2011) 38: 52-59.
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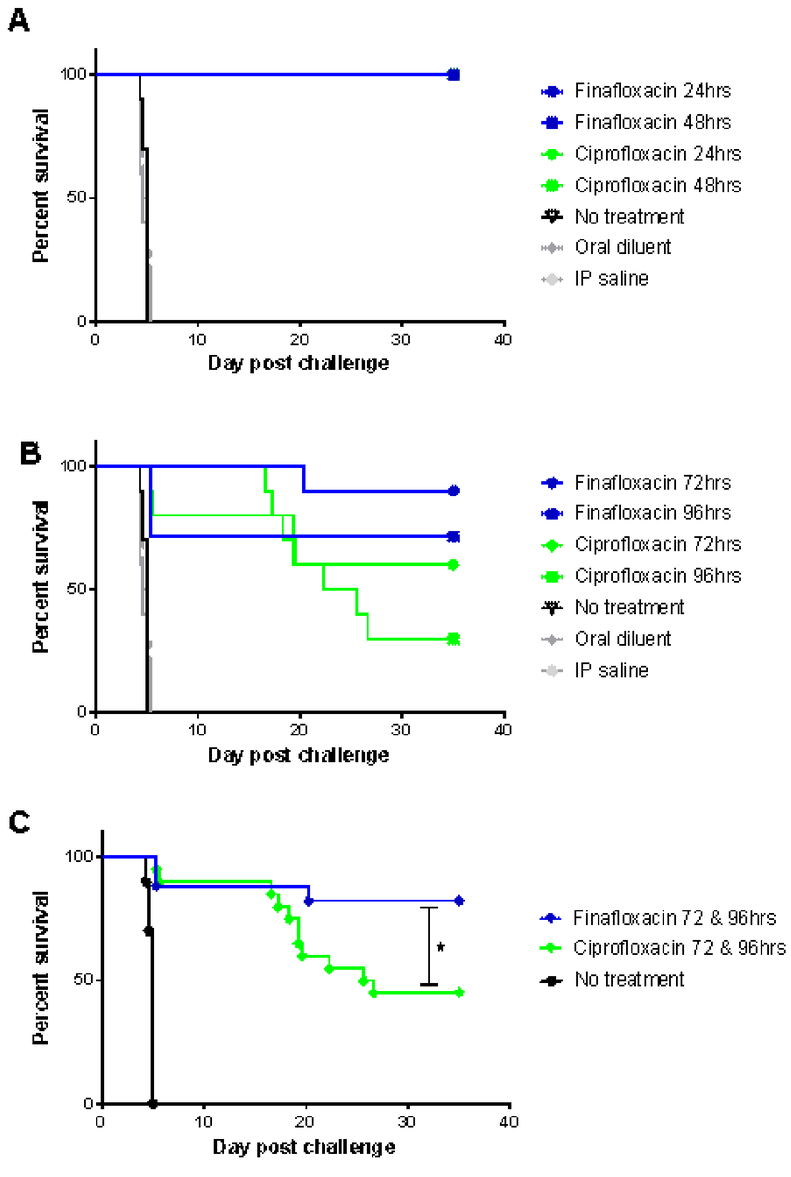

Figure 1. Percentage survival of mice following challenge with *F. tularensis* SchuS4 by the intranasal route. Mice were challenged with 101 CFU of *F. tularensis* and treated with oral finafloxacin (37.5 mg/kg) every 8 hours or intraperitoneal ciprofloxacin (30 mg/kg) every 12 hours. Regimens were initiated at 24 and 48 hours (A) and 72 and 96 hours (B) pc, and continued for 7 days. Survival data for the groups receiving therapy at 72 and 96 hours have been combined in C. Asterisks indicate a statistically significant difference in survival, * for $p=0.042$.

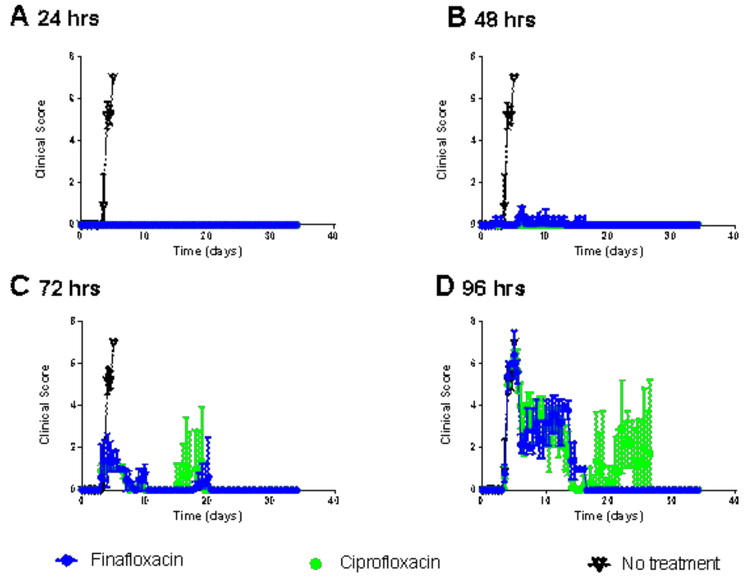

Figure 2. Clinical scores of *F. tularensis* infected mice following initiation of ciprofloxacin and finafloxacin therapy. Mice were challenged with 101 CFU of *F. tularensis* and treated with oral finafloxacin (37.5 mg/kg) every 8 hours or intraperitoneal ciprofloxacin (30 mg/kg) every 12 hours. The graphs show the cumulative clinical scores when antibiotics were initiated at 24 hours (A), 48 hours (B), 72 hours (C) and 96 hours (D) pc and continued for 7 days.

When therapy was initiated at 24 or 48 hours pc, mice treated with ciprofloxacin or finafloxacin developed mild, transient clinical signs of disease (Figure 2). Delaying the initiation of therapy to 72 hours also had a considerable benefit, the severity of clinical signs significantly reduced ($p < 0.01$) (Figure 2). At 96 hours mice displayed severe clinical signs; however, administering treatment at this late stage did have a positive effect. Those mice that survived 48 hours after initiation of therapy largely resolved their clinical signs (Figure 2). The data also suggests that more mice treated with ciprofloxacin relapsed with infection upon cessation of therapy, compared to those treated with finafloxacin (Figure 2).

Discussion

Finafloxacin offered similar levels of protection to ciprofloxacin at each time point. The stratified analysis of therapy initiated at 72 and 96 hours pc, suggests that finafloxacin offers a greater level of protection compared to ciprofloxacin. Furthermore, when therapy was delayed until 96 hours pc, none of the finafloxacin treated mice relapsed following cessation of therapy. This is particularly encouraging considering how severely ill these mice were when therapy was initiated. This suggests finafloxacin could be useful for prophylaxis or treatment of *F. tularensis* infection and further investigation of the efficacy is warranted.