Development of *Francisella tularensis* Schu S4 Post Exposure Prophylaxis and Delayed Treatment Models Using a 14-day Ciprofloxacin and Doxycycline Dosing Schedule

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ABSTRACT

Objective: An aerosol model of infection for *Francisella tularensis* SchuS4 was developed to evaluate therapeutic post-exposure prophylaxis treatment (PEP) and delayed treatment strategies, with both using 14-day ciprofloxacin and doxycycline dosing schedules. The relapse potential after antibiotic treatment was also evaluated during a post-dosing recovery evaluation period.

Results: The inhaled dose was $6.56x10^2$ CFU/mouse. For PEP therapy groups, 100% and 90% survival was observed with ciprofloxacin or doxycycline dosed mice, respectively, after the 14 day dosing period. After completion of the PEP recovery evaluation period, 100% and 60% survival of the ciprofloxacin and doxycycline dosed mice was observed, respectively. For the delayed treatment groups (dosing start was 72 hours post-challenge), survival was 100% and 90% for ciprofloxacin and doxycycline, respectively, at the end of the dosing period. After the completion of the delayed treatment recovery evaluation period, survival was 100% and 80% for ciprofloxacin and doxycycline, respectively. No control mice survived beyond day 6.

Conclusions: Ciprofloxacin demonstrated complete protection against aerosolized *F. tularensis* through the recovery phase for both PEP and delayed treatment groups, while doxycycline demonstrated partial protection during the dosing and recovery phases in both PEP and delayed treatment groups.

INTRODUCTION

Francisella tularensis, a Gram-type negative facultative intracellular coccobacillus, is the causative agent for tularemia. The primary mechanism of virulence appears to be the ability to enter and proliferate in macrophages and tissues thereby inducing a host inflammatory response (Oyston PCF, 2008). *F. tularensis* is also considered one of the most infectious pathogenic bacteria due to an extraordinarily low inoculum (<10 CFU) that is required for infection both through inhalation or inoculation (Dennis et al., 2001). As a result, *F. tularensis* is considered a potential bioterrorism agent if released via an aerosol.

Our goals for this study were three-fold: 1) to develop and characterize a BALB/c murine model for pneumonic tularemia using an IITRI-developed, nose-only bioaerosolization system; 2) to determine the aerosol LD_{50} of a *F. tularensis* subsp. tularensis strain SCHU S4 using BALB/c mice; and 3) to evaluate and compare the efficacy of the bactericidal ciprofloxacin and the bacteriostatic doxycycline antibiotic on BALB/c mice exposed to a 50-LD_{50} aerosol challenge to *F. tularensis*.

METHODS

***F. tularensis* Challenge Material:** *F. tularensis* subsp. tularensis strain SCHU S4 culture (NR-643) was obtained from *bei* resources. Frozen working cell bank *F. tularensis* SCHU S4 stocks, stored at ≤ -65°C, were thawed and 50 mL of the culture was inoculated into 100 mL Brain Heart Infusion Broth with IsoVitaleX (BHI w/Iso). The culture was grown with 5% CO_2 at 37°C ± 2°C with shaking at approximately 200 rpm for 16 hours until an optical density of 1.0 ± 0.05 at 600 nm (OD_{600}) was achieved (approximately 1.0×10^{10} CFU/mL). Purity was assessed by colony morphology.

***F. tularensis* Aerosol Generation:** The culture was serially diluted to desired concentrations in 1X phosphate-buffered saline (PBS) for inhalation exposure to minimize foaming during the aerosolization process. *F. tularensis* aerosols were generated using the IITRI bioaerosol system which utilizes a 64-port, flow-past, nose-only inhalation exposure chamber (Lab Products Inc., Seaford, DE) and six Pari LC Plus jet nebulizers (Pari, Germany). With 20 PSI of pressurized air, each nebulizer has a flow rate of approximately 5 LPM each with a cumulative total of approximately 30 LPM flow rate with all six nebulizers operating concurrently. All exhaust air was passed through a series of HEPA filters prior to exiting the facility. Viable aerosol material from the breathing zone of the exposure chamber was determined by two all glass impingers (AGI) connected in series with each containing 10 mL of phosphate buffered saline solution. Additionally, 3 mm glass beads were included in the upstream impinger to improve collection efficiency. Aerosol particle size distribution was measured by a viable 6-stage Andersen cascade impactor (Tisch Environmental Inc. OH). Cysteine Heart Agar with defibrinated rabbit's blood (CHAB) was used as the collection medium in the Andersen cascade impactor. Time of exposure for a typical run was 10 minutes. All titers of *F. tularensis* were determined on chocolate agar (CHOC) and incubated with 5% CO_2 at 37°C ± 2°C for approximately 48 hours.

LD_{50} Determination: Animals used were 9 week old female BALB/c mice weighing on average 18g to 20g. Each experimental group had 10 mice.

Antibiotic Administration: Ciprofloxacin (50 mg/kg) and doxycycline (40 mg/kg) were administered twice a day via the intraperitoneal dosing route. Dosing schedule was initiated 24 hours after exposure for the post exposure prophylaxis assessment or 72 hours after exposure for the delayed treatment assessment. After completion of the dosing phase, animals were monitored for an additional 14 days each. After the dosing period, relapse potential was evaluated for 21 additional days.

RESULTS

Table 1. Summary of Aerosol Exposure Parameters for LD_{50} Determination in BALB/c Mice

		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
F. tularensis SCHU S4 Concentration	Post-challenge Titer (CFU/ml)	0	$2.52x10^4$	$8.2x10^4$	$1.3x10^6$	$2.46x10^6$	$9.8x10^6$
	All-glass Impinger Titer (CFU/ml)	0	18.3	40	3500	6700	41,600
	Presented Dose (CFU/animal)	0	7	17	97	250	780
Particle Size	Particle Diameter µm	NA	1-3 µm				
Spray Factor (Average $3.0x10^5$)		NA	$1.5x10^{-6}$	$1.1x10^{-5}$	$5.5x10^{-5}$	$2.8x10^{-5}$	$4.8x10^{-5}$

Table 2. Summary of Mean Time to Death and survival for LD_{50} Determination in BALB/c Mice

Group	Actual Challenge (CFU/animal)	Females MTD (Days)	Females Survival (%)
1	0	ND	100
2	7	6.0	70
3	17	6.4	30
4	97	5.8	10
5	250	5.0	0
6	780	4.7	0

The calculated LD50 (Reed-Muench) was determined to be 11.6 CFU/animal. ND= No death within the group

Figure 1. Survival of BALB/c Mice at Different Exposure Concentrations of *F. tularensis*

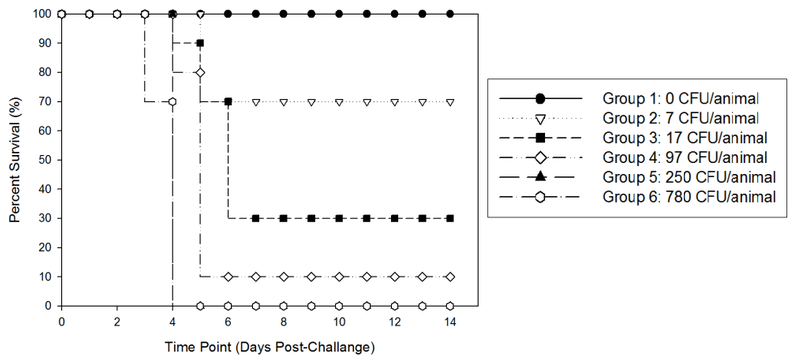


Table 3. Summary of Ciprofloxacin and Doxycycline Dose Results

Group	Sample	Inhaled Dose (CFU/animal)	MTD (Days)	Survival (%)
1	Ciprofloxacin (50 mg/kg)	$6.56x10^2$ (appx. 50-LD_{50})	ND	100
2	Doxycyclin (40 mg/kg)		18.0	60
3	None (saline)		5.3	0

Figure 2. Survival After Dosing with Ciproflxacin and Doxycycline 24 Hours Post-challenge

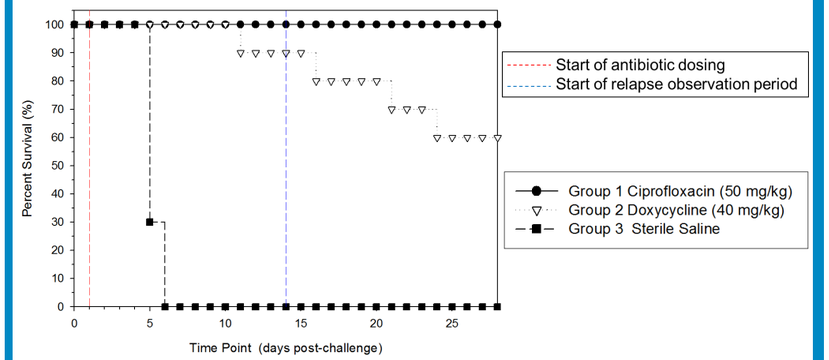
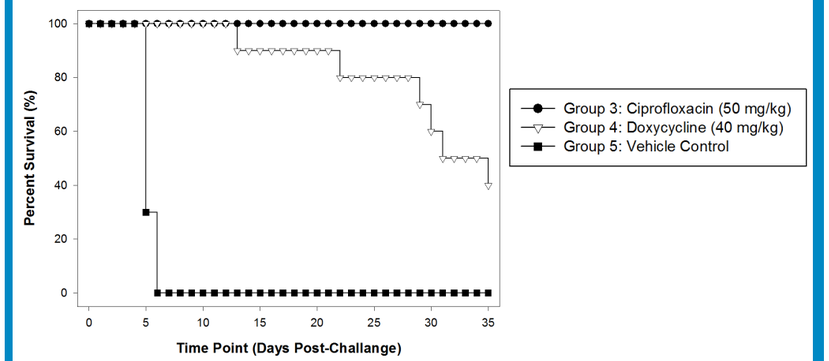


Figure 3. Survival After Dosing with Ciprofloxacin and Doxycycline 72 Hours Post-challenge



RESULTS AND CONCLUSIONS

- LD_{50} was determined to be 11.6 CFU/mouse.
- When challenged at 50-LD_{50} *F. tularensis*, all BALB/c mice survived with 50 mg/kg ciprofloxacin when administered within 24 hours post-exposure and dosed twice a day for 14 days. No relapse was observed during the 21 day post-dose exposure.
- When challenged at 50-LD50 *F. tularensis*, there was 90% survival with 40 mg/kg doxycycline administered at 24 hours post-exposure and dosed twice a day for 14 days. During the relapse period, 60% of the animals survived 4 additional days, thereafter.
- No survival was observed in mice that did not receive antibiotic. Mice died within 5-6 days post-exposure.
- While protection from *F. tularensis* was evident from both the bactericidal and bacteriostatic antibiotics (ciprofloxacin and doxycycline, respectively), this study demonstrates that bactericidal treatment for 14 days is effective in preventing relapse of the infections. However, treatment of bacteriostatic antibiotic such as doxycycline may require an extended period of treatment to prevent relapse of the infection.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Trudy Grossman, Ph.D (Tetraphase) and Anne Radcliff, Ph.D (CUBRC) for their valuable input towards the design of this study.