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Efficacy of Delafloxacin Treatment in an Inhalational Mouse Model of Tularemia.

Marjorie Torres[1], Sandra McCurdy[2], Stephanie A. Halasohoris[1], Ashley Babyak[1], Annette M. Gray[1], J. Matthew Meinig[1], Mary K. Hourihan[1]

[1]Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; [2]Melinta Therapeutics, Parsippany, NJ

Abstract

Francisella tularensis (FT) is a zoonotic pathogen that is the etiologic agent of tularemia. FT is endemic in the Northern Hemisphere and its low infectivity, broad availability in animal reservoirs, and ease of aerosolization raise concerns as a potential biothreat agent. Developing novel broad-spectrum medical countermeasures (MCM) to treat tularemia and other bacterial infections caused by bacterial biothreat agents is important to combat agents with naturally acquired antimicrobial resistance (AMR) or through man-made manipulation to bypass current therapies. Delafloxacin is an anionic fluoroquinolone marketed by Melinta Therapeutics that is approved in the United States and Europe as a therapy for community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections. Previous studies demonstrated that delafloxacin is protective in mouse models of inhalational anthrax and melioidosis. Mouse model tularemia studies were performed by the Bacterial Therapeutics Core Branch (BTCB) at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). In this study the mice were challenged with FT strain SChuS4-1 via whole-body aerosol, with an average challenge of 36.4 x LD_{50}. Following challenge with FT, mice received saline (BID, IP), ciprofloxacin (30 mg/kg, BID, IP), gentamicin (24 mg/kg, BID, SC), or delafloxacin (96.5 mg/kg, BID, SC) at 24 or 48 hours (h), representing postexposure prophylaxis (PEP) or treatment (TX), respectively. The untreated saline control group demonstrated a median time to death (MTD) of 7 days with 100% lethality by day 8. Delafloxacin-treated cohorts demonstrated survival that was not statistically inferior to ciprofloxacin in PEP (70% vs 60%) or TX (60% vs 80%) cohorts. The delafloxacin response was intermediate between ciprofloxacin and gentamicin with gentamicin survival trending higher, but not statistically different than delafloxacin in PEP (70% vs 100%) or TX (80% vs 90%) cohorts. These data indicated that delafloxacin was similar to treatment controls in clearing bacteria. By matching efficacy observed with two strong standard-of-care antibiotics, delafloxacin demonstrates promise for future development as an MCM against inhalational tularemia.

Introduction

- Developing medical countermeasures (MCM) against biothreat agents is essential to protecting the Warfighter, as well as the general population, from devastating morbidity and mortality following a bioterror event.
- Fluoroquinolones, including ciprofloxacin, are standard-of-care for multiple bacterial agents, including biothreat agents, however:
  - Emerging or engineered resistance to standard-of-care therapies are a threat to Force Health Protection and Public Health
  - Many bacterial biothreat agents reside in lower pH environments during pathogenesis (respiratory tract, intracellular)
- Delafloxacin:
  - Broad-spectrum anionic fluoroquinolone
  - Oral and intravenous formulations are available
  - Approved therapy by FDA (Baxdela) and EMA (Quofenix) for treatment in adults with:
    - Acute bacterial skin and skin structure infections (ABSSSI)
    - Community-acquired bacterial pneumonia (CABP)
  - CABP indication "critical" for FDA to approve biothreat agents under the Animal Rule
  - Delafloxacin possesses unique physicochemical properties that allow for enhanced antimicrobial activity at lower pH
  - Less susceptible to on-target mutations conferring resistance to certain fluoroquinolones
  - Studies have shown that more on-target mutations are required for delafloxacin resistance

Methods

Animal aerosol challenge. Pathogen free, female, 6- to 8-week-old BALB/c mice (approx. 20g) were challenged against FT strain SchuS4-1 via whole-body aerosolization to induce mouse models of tularemia .

Mice were treated for 14 days post-challenge in FT cohorts at 24 h (PEP) or 48 h (TX) with saline (disease control), gentamicin or ciprofloxacin as standard-of-care treatment controls, or an estimated HED of delafloxacin using the dosing regimen depicted in Table 1.

Table 1. Dosing regimen for delafloxacin and comparator antibiotics

Compound	Dose (mg/kg)	Admin. BID	Start Time (h)	Therapy
Saline	n/a	IP	24	PEP and TX
Ciprofloxacin	30	IP	24	PEP
Gentamicin	24	SC	24	PEP
Delafloxacin	96.5	SC	24	PEP
Ciprofloxacin	30	IP	48	TX
Gentamicin	24	SC	48	TX
Delafloxacin	96.5	SC	48	TX

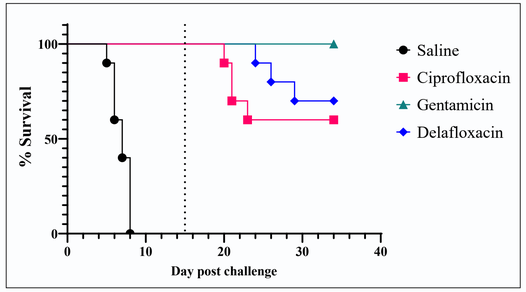
BID – twice daily, h – hours, IP- intraperitoneal, SC - subcutaneous

Results

In vivo Results

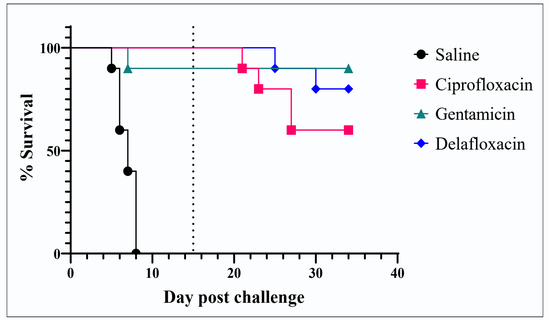
Figure 1. Efficacy of Delafloxacin PEP Therapy in an Inhalational Model of Tularemia

Following PEP Therapy (24 h)



Dotted line depicts last day of therapy.

Following TX Therapy (48 h)



Dotted line depicts last day of therapy.

Survival Comparisons

Table 2. Effects of Delafloxacin PEP Therapy on Survival following FT Challenge

Following PEP Therapy (24 h)

	Dead / Total	vs. Ciprofloxacin Log-Rank (p-value)	vs. Gentamicin Log-Rank (p-value)
Saline	10/10		
Ciprofloxacin	4/10		
Gentamicin	0/10	0.0291	
Delafloxacin	3/10	0.5222	0.0671

Following TX Therapy (48 h)

	Dead / Total	vs. Ciprofloxacin Log-Rank (p-value)	vs. Gentamicin Log-Rank (p-value)
Saline	10/10		
Ciprofloxacin	4/10		
Gentamicin	1/10	0.1647	
Delafloxacin	2/10	0.3034	0.5842

Log-Rank Tests for Pairwise Comparison of Delafloxacin compared to Ciprofloxacin and Gentamicin.

Summary and Conclusion

Efficacy of Delafloxacin in an Inhalation Model of Tularemia:

- The untreated saline control group demonstrated a MTD of 7 days (100% lethality).
- Delafloxacin-treated cohorts demonstrated survival that was statistically non-inferior to ciprofloxacin in PEP (70% vs 60%) or TX (80% vs 60%) cohorts.
- Delafloxacin was statistically non-inferior to gentamicin in PEP (70% vs 100%) and TX (80% vs 90%) cohorts.

Conclusions:

These results at the HED regimen complement and improve upon previous reports evaluating delafloxacin against FT at lower doses. By evaluating an HED regimen and demonstrating statistical equivalence to two standard-of-care antibiotics, the current study supports further investigation of delafloxacin as a therapy for inhalational FT. Due to its broad spectrum of activity, delafloxacin offers a promising solution for antimicrobial therapy in far-forward operations where therapies are limited, and diagnostics are unavailable. Additionally, these results support the use of delafloxacin as a therapeutic against bacterial bioterrorism attacks of unknown origin.

For More Information:

Multiple mutations are required for fluoroquinolone resistance
McCurdy S, et al. *Antimicrob Agents Chemother*. **2017** 24;61(9).
https://doi.org/10.1128/aac.00772-17

Delafloxacin is efficacious against a mouse model of melioidosis
McCurdy S, et al. *Antimicrob Agents Chemother.* **2021** 17;65(10).
https://doi.org/10.1128/aac.00736-21

Delafloxacin is efficacious against a mouse model of anthrax
McCurdy S, et al.. *J Antimicrob Chemother*. **2023** 2;78(3).
https://doi.org/10.1093/jac/dkad015

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Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense Health Agency.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 2011. The facility where this research was conducted is fully Accredited by the Association for Assessment and Accreditation Of Laboratory Animal Care International.

POINT OF CONTACT

Matt Meinig

james.m.meinig.civ@health.mil