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Efficacy of Delafloxacin Treatment in an Inhalational Mouse Model of Plague

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Abstract

Yersinia pestis, the etiologic agent of plague, is a zoonotic pathogen that is endemic throughout the world and poses a potential risk as a biothreat weapon. Developing novel broad-spectrum medical countermeasures (MCM) to treat plague 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 by the FDA and EMA as a therapy for community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI). Previous studies have demonstrated that delafloxacin is protective in mouse models of inhalational anthrax and melioidosis. The current study was conducted to evaluate the efficacy of delafloxacin in the prophylaxis and treatment of inhalational plague in mice. Briefly, female BALB/c mice were challenged with *Y. pestis* strain CO92 via whole-body aerosol, with an average challenge of 32.8 x LD50. Following challenge with CO92, 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 42 hours (h), representing postexposure prophylaxis (PEP) or treatment (TX) , respectively. Animals were treated for 7 days and observed for a total of 33 days following challenge. The untreated saline control group demonstrated a median time-to-death (MTD) of 2 days, and as expected, 100% lethality was observed by Day 2 in the untreated controls. Survival rates for the delafloxacin cohorts were compared to the positive treatment controls, ciprofloxacin and gentamicin, using a Log-Rank test. Within the PEP cohorts, survival was 100% with delafloxacin, which was not statistically different to ciprofloxacin (100%) and gentamicin (90%). Survival in the delafloxacin group (60%) was also not significantly different from ciprofloxacin (80%) or gentamicin (70%) after 42h TX. In vitro minimum inhibitory concentration (MIC) studies were also conducted to determine the activity of delafloxacin in *Y. pestis* strains demonstrating AMR. In these studies, delafloxacin retained low MIC values in AMR strains compared to decreased activity against ciprofloxacin, gentamicin, and/or doxycycline. Overall, these proof-of-concept studies support the continued investigation of delafloxacin as a MCM in the treatment of inhalational plague.

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

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

Methods

***In vitro* MIC studies.** MICs were performed following CLSI guidelines. Delafloxacin and comparator antibiotics were tested on a 10-strain panel of BSL-2 *Y. pestis* surrogate strains, including 9 AMR strains and a CO92 surrogate (CO92 pgm$^-$).

Animal aerosol challenge. Pathogen free, female, 6- to 8-week-old BALB/c mice (approx. 20g) were challenged against *Y. pestis* (YP) strain CO92 via whole-body aerosolization. Antibiotics were administered at human-equivalent doses (HEDs) in order to mimic clinical drug exposures following FDA-approved doses.

Mice were treated for 7 days post-challenge at 24 h (PEP) or 42 h (TX) in YP cohorts 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	42	TX
Gentamicin	24	SC	42	TX
Delafloxacin	96.5	SC	42	TX

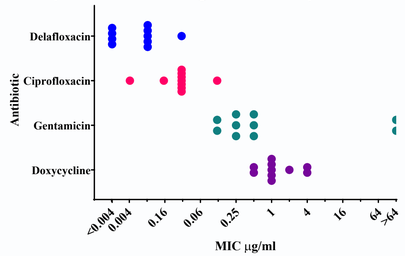
BID – twice daily, h – hours, IP- intraperitoneal, SC - subcutaneous

Results

In vitro AMR Results

Delafloxacin MICs trend substantially lower compared to standard-of-care antibiotics for plague when evaluated against a 10-strain panel of BSL-2 surrogate strains of *Y. pestis*.
These results mimic what has previously been observed for delafloxacin, particularly against Gram-positive pathogens.

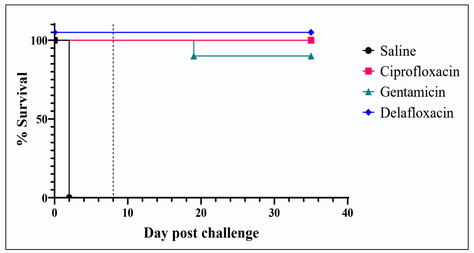
Figure 1. Efficacy of Delafloxacin Against AMR *Y. pestis* Strains



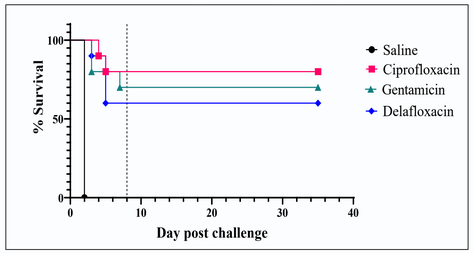
In vivo Results

Figure 2. Efficacy of Delafloxacin in an Inhalational Model of Plague

Following PEP Therapy (24 h)



Following TX Therapy (42 h)



Dotted line depicts last day of therapy.

Survival Comparisons

Table 2. Efficacy of Delafloxacin in an Inhalational Model of Plague

Following PEP Therapy (24 h)

	Dead / Total	vs. Ciprofloxacin Log-Rank (p-value)	vs. Gentamicin Log-Rank (p-value)
Saline	10/10		
Ciprofloxacin	0/10		
Gentamicin	1/10	0.3173	
Delafloxacin	0/10	1	0.3173

Following TX Therapy (42 h)

	Dead / Total	vs. Ciprofloxacin Log-Rank (p-value)	vs. Gentamicin Log-Rank (p-value)
Saline	10/10		
Ciprofloxacin	2/10		
Gentamicin	3/10	0.5963	
Delafloxacin	4/10	0.3722	0.6998

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

Summary and Conclusion

Efficacy of Delafloxacin in an Inhalational Model of Plague

- The untreated saline control group demonstrated an MTD of 2 days (100% lethality).
- Delafloxacin-treated cohorts demonstrated survival that was statistically non-inferior to ciprofloxacin in PEP (100% vs 100%) or TX (60% vs 80%) cohorts.
- The delafloxacin response demonstrated survival that was statistically not different to gentamicin survival in PEP (100% vs 90%) or TX (60% vs 70%) cohorts.

Efficacy of Delafloxacin in AMR *Y. pestis* Strains

- *Y. pestis* strains (BSL2 surrogates) with AMR mutations showed decreased susceptibility against standard-of-care drugs used to treat plague.
- Delafloxacin retained very low MICs (high potency) against this panel of AMR *Y. pestis* strains

These studies demonstrate that delafloxacin is an effective therapy in the inhalational murine challenge model of plague when used at the HED approved for CABP treatment. Furthermore, the positive susceptibility results against the *Y. pestis* BSL2 AMR strains supports the continued investigation of delafloxacin against mechanisms of resistance in *Y. pestis*. Together, these studies support further investigation of delafloxacin as a therapy for inhalational plague, as well as its use as a broad-spectrum therapeutic against bacterial bioterrorism attacks of unknown origin.

ACKNOWLEDGEMENTS AND DISCLAIMERS
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.

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