Poster #4556 AAR-08 | ASM Microbe | 6-18-2023, Houston, TX.

Efficacy of BWC0977 Therapy in an Inhalational Mouse Model of Tularemia

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Abstract

Francisella tularensis (FT) is a zoonotic pathogen that is the etiologic agent of tularemia. FT's low infectivity, availability in animal reservoirs, and ease of aerosolization raise concerns for its use as a biothreat agent. Developing novel broad-spectrum medical countermeasures to treat tularemia and other bacterial infections is important to combat agents with naturally acquired or with engineered antimicrobial resistance (AMR). BWC0977 is a novel bacterial gyrase/TopoIV inhibitor developed by Bugworks Research, Inc., that acts at a site and mechanism distinct from that of fluoroquinolones. Previous *in vitro* studies have shown that BWC0977 demonstrates broad-spectrum activity against FT strains, including attenuated surrogate strains harboring AMR phenotypes. In this study, the efficacy of BWC0977 was evaluated in the treatment of inhalational murine tularemia. Female BALB/c mice were challenged with FT strain SCHUS4-1 via whole-body aerosol. Treatment with the comparator control antibiotics ciprofloxacin (30 mg/kg, BID, IP) or gentamicin (24 mg/kg, BID, SC), saline or test article vehicles (BID, PO), or with BWC0977 (150 mg/kg, BID, PO) were initiated 24 h or 48 h after pathogen challenge to mimic post-exposure prophylaxis (PEP) or treatment (TX) after onset of clinical symptoms, respectively. All cohorts were treated for 14 days and observed until day 33 following challenge. The untreated saline and vehicle control groups demonstrated 100% lethality, with median time-to-death (MTD) of 7 and 5 days, respectively. In contrast, BWC0977 was statistically superior to ciprofloxacin, with 100% survival in the BWC0977 cohorts and 60% in the ciprofloxacin cohorts at both 24 h and 48 h. BWC0977 efficacy was not significantly inferior to gentamicin treatment at 24 h (100% survival in both groups) and at 48 h (BWC0977 - 100% vs gentamicin - 90%). In addition, no bacteria (measured as CFUs/mouse) were isolated from spleens of three representative surviving mice in the ciprofloxacin, gentamicin, and BWC0977 cohorts. These data indicate that BWC0977 was similar to treatment controls in clearing bacteria. By meeting or exceeding the efficacy observed with two strong standard-of-care antibiotics, BWC0977 demonstrates exceptional promise for future development in the treatment of 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.
- Ciprofloxacin and gentamicin are standard-of-care antimicrobials against multiple bacterial agents, including biothreat agents; however, emerging or engineered resistance to standard-of-care therapies are a threat to force health protection and to public health.
- BWC0977 is a novel clinical-stage gyrase + TopoIV inhibitor
  - No overlap with quinolone on-target resistance
  - Oral and intravenous administration potential
  - Proposed indications: hospital-acquired pneumonia & complicated urinary tract infections
- Broad-spectrum *in vitro* activity across biothreat and public health pathogens

Table 1: In Vitro Activity of BWC0977 Against Bacterial Biothreat Agents

	BWC0977			Ciprofloxacin		
	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
Bacillus anthracis	0.001-0.015	0.004	0.004	0.03-0.12	0.06	0.12
Yersinia pestis	0.002-0.015	0.008	0.015	<0.004-0.06	0.03	0.06
Francisella tularensis	0.001-0.03	0.004	0.015	0.008-0.5	0.03	0.06
	BWC0977			Ceftazidime		
	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
Burkholderia pseudomallei	0.12-2	0.5	1	0.5->64	4	4
	BWC0977			Azithromycin		
	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
Burkholderia mallei	0.008-8	2	8	0.25-4	1	2

* All minimum inhibitory concentrations (MICs) expressed as µg/mL.

- *In vivo* experiments demonstrate that BWC0977 therapy is non-inferior to ciprofloxacin in a mouse model of inhalational anthrax.
- BWC0977 is currently in a Phase 1 clinical trial to determine safety and tolerability in healthy volunteers.

Methods

- Pathogen free, female, 6- to 8-week-old BALB/c mice (approx. 20g) were challenged against the highly virulent FT SchuS4-1 strain via whole-body aerosolization in a mouse model of inhalational tularemia, with a mean inhaled dose of 36.4 x LD_{50}.
- Mice were treated with saline (negative control), oral vehicle, ciprofloxacin or gentamicin (both standard-of-care treatment controls), or BWC0977 using the dosing regimen depicted in Table 2.
- Treatments were initiated at 24 h (PEP) or 48 h (TX) post-challenge with FT. Mice were treated twice daily (BID) for 14 days, then observed until Day 33 following challenge.
- Spleens from three surviving mice from each cohort were harvested, homogenized, and plated undiluted on chocolate agar. Plates were incubated for 48 h at 35 ± 2 ºC. Bacterial burden was determined by analyzing CFUs/spleen.

Results

Figure 1: Efficacy of PEP Therapy with BWC0977 in an Inhalational Model of Tularemia

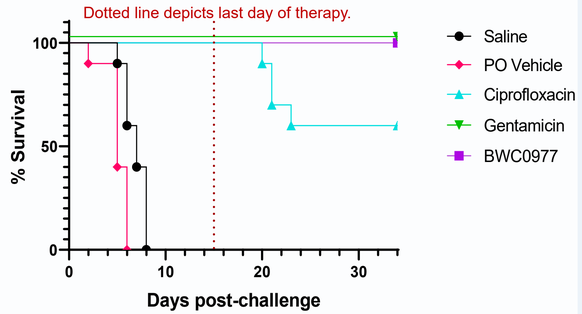

Figure 2: Efficacy of TX Therapy with BWC0977 in an Inhalational Model of Tularemia

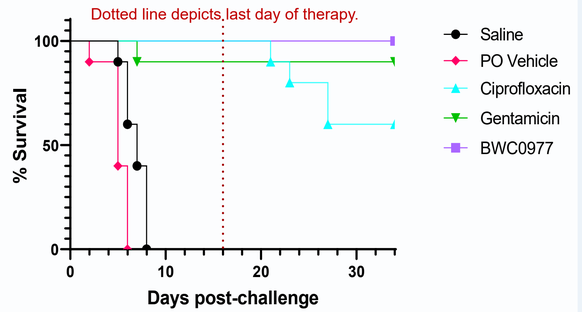

Table 2: Survival Results Following Therapy

Compound	Dose (mg/kg)	Route (BID)	Start time (h)	# Deaths	MTD	% Survival
Saline (disease control)	NA	IP	24	10	7	0
PO (oral vehicle control)	NA	PO	24	10	5	0
Ciprofloxacin	30	IP	24	4	UD	60
Gentamicin	24	SC	24	0	UD	100
BWC0977	150	PO	24	0	UD	100
Ciprofloxacin	30	IP	48	4	UD	80
Gentamicin	24	SC	48	1	UD	90
BWC0977	150	PO	48	0	UD	100

MTD - median time-to-death, NA - not applicable, BID - twice daily, h -hours, IP - intraperitoneal, PO - oral, SC – subcutaneous, UD - undefined.

Table 3: Survival Statistics Following Therapy with BWC0977

	Start Time (h)	Dead / Total	vs. Ciprofloxacin (p-value)[1]	vs. Gentamicin (p-value)[1]
Saline (disease control)	24	10/10		
PO (vehicle control)	24	10/10		
Ciprofloxacin	24	4/10		
Gentamicin	24	0/10	0.0291	
BWC0977	24	0/10	0.0291	1
Ciprofloxacin	48	4/10		0.1647
Gentamicin	48	1/10	0.1647	
BWC0977	48	0/10	0.0295	0.3173

[1] Log-rank comparison of BWC0977 to ciprofloxacin and gentamicin.

Summary

Efficacy of BWC0977 in an Inhalational Model of Tularemia:

- The untreated saline control cohort demonstrated an MTD of 7 days (100% lethality).
- The PO vehicle control cohort demonstrated an MTD of 5 days (100% lethality).
- Survival following BWC0977 therapy was statistically superior to ciprofloxacin in both PEP and TX cohorts, with 100% survival in all BWC0977-treated groups and 60% in all ciprofloxacin-treated groups.
- Survival following BWC0977 therapy was statistically non-inferior to gentamicin in both PEP or TX cohorts, with 100% survival in all groups.
- Spleens from three representative mice from each treatment group that survived until the end of the study were sterile, based on CFU determinations (data not shown).

Conclusions

These studies demonstrate statistical superiority or equivalence to two standards-of-care antibiotics for the treatment of inhalational tularemia support further investigation of BWC0977 as a broad-spectrum therapeutic against bacterial bioterrorism attacks of unknown origin.

ACKNOWLEDGEMENTS AND DISCLAIMERS

This work was sponsored by the Defence Threat Reduction Agency (CB10640).

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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