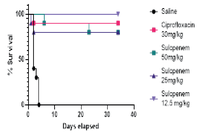
Murine Efficacy Studies of Sulopenem Against *Bacillus anthracis*

Sailaja Puttagunta, MD[1], Steven I. Aronin, MD[1], Stephanie A. Halasohoris[2], Ashley L. Babyak[2], M. Kelly Hourihan, PhD[2], and J. Matthew Meinig, PhD[2]
[1]Iterum Therapeutics, Old Saybrook, CT 06475; [2]US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702.

ABSTRACT

Background: Sulopenem is a thiopenem β-lactam antibiotic being developed for the treatment of infections caused by multi-drug resistant bacteria. Sulopenem is available as intravenous and oral pro-drug formulations, and its activity aligns with the most urgent drug-resistant antimicrobial threats defined by the CDC. Sulopenem possesses potent activity against species of the Enterobacterales that encode ESBLs or AmpC-type β-lactamases that confer resistance to third generation cephalosporins. It has also demonstrated good *in vitro* microbiological activity against a range of bacterial pathogens including penicillin resistant *S. pneumoniae,* β-lactamase-producing *H. influenza,* and *M. catarrhalis*. Sulopenem is also active *in vitro* against a number of bio-threat pathogens at concentrations likely to be achieved after oral dosing in humans and meets criteria to be tested in the murine models of *Bacillus anthracis, Yersinia pestis, Burkholderia mallei,* and *Burkholderia pseudomallei*. The development of novel medical countermeasures (MCMs) is critical to biodefense preparedness for both military and public health.
Methods: Female BALB/c mice were challenged with *B. anthracis* Ames spores by whole-body aerosol, with an average challenge of 15 x LD_{50} and randomly divided into cohorts of 10 mice per group. At 24h post-exposure prophylaxis (PEP), which represents therapy before onset of clinical symptoms, mice were treated QID for 14 days with vehicle (saline, IP), ciprofloxacin (30mg/kg, IP) or sulopenem etzadroxil (50, 25, or 12.5 mg/kg, PO). Mice were monitored for a total of 30 days and data analyzed to determine the effects of sulopenem etzadroxil on survival as compared to the positive treatment control, ciprofloxacin, using Log-Ranks tests for the pair wise comparisons with SAS software.
Results:

Figure 1: Effects of Sulopenem PEP on Survival in a Mouse model of Inhalational Anthrax



Conclusion: Sulopenem is active *in vivo* in the murine model of *B. anthracis.* Survival in the sulopenem treated groups was not statistically inferior to the ciprofloxacin positive control, a standard-of-care for PEP of *B. anthracis*. These results support further development of sulopenem for treating *B. anthracis* as a novel broad-spectrum and orally available MCM.

INTRODUCTION

- The threat of bioterrorism has increased in the past 20-25 years.
- The Centers for Disease Control and Prevention (CDC) has classified bioterrorism agents into three categories, based on ease of dissemination, potential to cause severe disease, and predicted mortality rate:

Category	Characteristics
A	Highest risk to national security and public health: • Easily disseminated or transmitted from person to person • High mortality rates and potential for major public health impact • Might cause public panic and social disruption • Require special action for public health preparedness
B	Second highest priority: • Moderately easy to disseminate • Moderate morbidity rates and low mortality rates • Require specific enhancements for diagnostic capacity and disease surveillance
C	Third highest priority, includes emerging pathogens that could be engineered for mass dissemination in the future because of: • Availability • Ease of production and dissemination • Potential for high morbidity and mortality rates and major health impact

- Current antibiotic treatment options against Category A and B biothreat pathogens are limited.
- Current agents are at risk for engineered antibiotic resistance.
- New therapeutic options are therefore needed for prophylaxis and treatment of the diseases caused by these pathogens.
- Sulopenem is a thiopenem β-lactam antibiotic being developed for the treatment of infections caused by multi-drug resistant bacteria.
- Sulopenem's activity aligns with the most urgent drug-resistant antimicrobial threats defined by the CDC, including potent activity against species of the Enterobacterales that encode ESBLs or AmpC-type β-lactamases that confer resistance to third generation cephalosporins.
- Sulopenem has demonstrated good *in vitro* microbiological activity against a range of bacterial pathogens including penicillin resistant *S. pneumoniae,* β-lactamase-producing *Haemophilus influenzae* and *Moraxella catarrhalis*.
- Sulopenem has also demonstrated good *in vitro* microbiological activity against a number of bio-threat pathogens at concentrations likely to be achieved after oral dosing in humans and meets criteria to be further tested in murine models of infection
- Sulopenem is available as intravenous and oral pro-drug formulations.

METHODS

- *In vivo* antibacterial activity of sulopenem was evaluated against *Bacillus anthracis*.
- Female BALB/c mice, 6-8 weeks of age, were challenged with *B. anthracis* Ames spores by whole-body aerosol generated by using a three-jet collision nebulizer
- The average challenge was 15.3 x LD_{50} (2 separate sprays: 13.6 and 16.9 x LD_{50})
- Integrated air samples were obtained from the chamber during each exposure using an all-glass AGI-30 impinger
- Aerosol bacterial concentrations were serially diluted and plated on blood agar plates
- The inhaled dose of *B. anthracis*, represented as CFU/mouse, was estimated using Guyton's formula
- Mice were equally distributed into test groups upon the conclusion of each aerosol run
- Cohort sizes for statistical evaluation were 10 mice per group
- At 24h post-exposure prophylaxis, which represents therapy before onset of clinical symptoms, mice were treated for 14 days with vehicle (saline), ciprofloxacin, or sulopenem etzadroxil (Table 1)
- The dose formulation was calculated based on an average body weight of 20 grams for 6–8-week-old female BALB/c mice
- Mice were monitored for a total of 34 days following challenge; survival was assessed at least twice daily during treatment (days 1-14), and at least once daily thereafter
- At the conclusion of the observation (34 days), all surviving animals were humanely euthanized and spleen and lung from three surviving animals in each cohort were harvested for determination of animal bacterial load
- Mean time to death (MTD) were evaluated using Student's t-test; Kaplan-Meier survival analysis was used to calculate survival curves as well as MTD; resulting survival curves were compared by Log-rank tests
- Data were analyzed to determine the effects of sulopenem etzadroxil on survival as compared to the positive treatment control, ciprofloxacin, using Log-Ranks tests for the pair wise comparisons with SAS software.

RESULTS

Table 1: Study Treatment Cohorts

Cohort	Drug	Dose (mg/kg)	Route of Administration	Frequency of Administration
1	Saline	n/a	IP	BID
2	Ciprofloxacin	30	IP	BID
3	Sulopenem etzadroxil	50	PO	QID
4	Sulopenem etzadroxil	25	PO	QID
5	Sulopenem etzadroxil	12.5	PO	QID

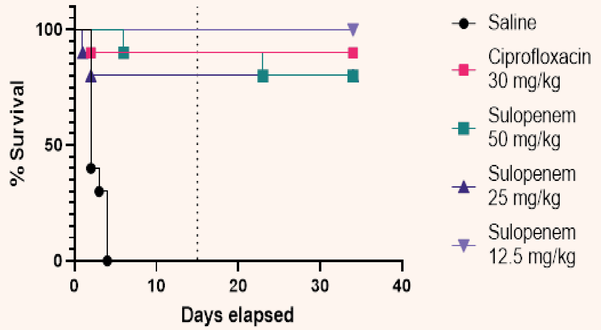
Table 2: Effects of Sulopenem and Comparators on Survival

Drug	Dose (mg/kg)	Dead / Total	Mean Time to Death (days)	Vs. Ciprofloxacin Log-Rank (p-value)[1]
Saline	n/a	10/10	2	- -
Ciprofloxacin	30	1/10	Undefined	- -
Sulopenem etzadroxil	50	2/10	Undefined	0.2994
Sulopenem etzadroxil	25	2/10	Undefined	0.5151
Sulopenem etzadroxil	12.5	0/10	Undefined	0.3173

[1]Log-rank comparison of survival curves

RESULTS

Figure 1: Effects of Sulopenem Post-exposure Prophylaxis on Survival in a Mouse Model of Inhalational Anthrax



Dotted line depicts last day of therapy

Table 3: Spleen Colony-Forming Units, Surviving Groups

Group	Treatment	Mouse #	Spleen Weight (g)	Undiluted Homogenate (CFU/g)
KH002	Ciprofloxacin 30 mg/kg	1	0.074	0
		2	0.11	0
		3	0.079	0
KH007	Sulopenem etzadroxil 50 mg/kg	1	0.101	0
		2	0.08	0
		3	0.084	0
KH008	Sulopenem etzadroxil 25 mg/kg	1	0.084	0
		2	0.092	0
		3	0.084	0
KH009	Sulopenem etzadroxil 12.5 mg/kg	1	0.083	0
		2	0.075	0
		3	0.081	0

Table 4: Lung Colony-Forming Units, Surviving Groups

Group	Treatment	Mouse #	Lung Weight (g)	Undiluted Homogenate (CFU/g)
KH002	Ciprofloxacin 30 mg/kg	1	0.328	101
		2	0.276	181
		3	0.251	167
KH007	Sulopenem etzadroxil 50 mg/kg	1	0.328	125
		2	0.276	Contaminated
		3	0.251	155
KH008	Sulopenem etzadroxil 25 mg/kg	1	0.22	241
		2	0.238	303
		3	0.342	32
KH009	Sulopenem etzadroxil 12.5 mg/kg	1	0.315	124
		2	0.285	211
		3	0.309	94

CONCLUSIONS

- Sulopenem is active *in vivo* in the murine model of *B. anthracis*
- Effect of sulopenem on survival in this animal model was similar to ciprofloxacin, a standard-of-care for post-exposure prophylaxis of *B. anthracis*
- All spleen homogenates analyzed at the study termination were sterile in the sulopenem and ciprofloxacin treatment groups, and low and similar levels of *B. anthracis* persisted in lungs at the study termination in both treatment groups, which is expected due to ungerminated spore persistence
- By demonstrating equivalence to the standard-of-care ciprofloxacin, sulopenem shows promise as an effective post-exposure prophylaxis measure in the treatment of inhalational anthrax.
- These results support further development of sulopenem for treating *B. anthracis* as a novel broad-spectrum and orally available medical countermeasure.
- Sulopenem has many advantages as a new generation penem including having both IV and oral (the prodrug) formulations.

REFERENCES

1. Guyton AC (1947). Measurement of the respiratory volumes of laboratory animals. *Am J Physiol*. 150(1):70-77.
2. Hartings JM, Roy CJ (2004). The automated bioaerosol exposure system: preclinical platform development and a respiratory dosimetry application with nonhuman primates. *J Pharmacol Toxicol Methods* 49(1):39-55.
3. May, K.R. (1973). The Collison Nebulizer: Description, Performance and Application. *Journal of Aerosol Science*, 4, 235-238.

Disclaimer: Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.