Sulopenem is Efficacious in a Rabbit Model of Inhalational Anthrax

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ABSTRACT

Background

Sulopenem (SUL) is a penem β-lactam antibiotic in development for the treatment of resistant bacterial infections. It is available as intravenous and oral prodrug formulations, and its activity aligns with the most urgent drug-resistant antimicrobial threats defined by the CDC. SUL possesses potent activity against Enterobacterales species that encode ESBLs or AmpC-type β-lactamases that confer resistance to 3rd generation cephalosporins. SUL has also demonstrated potent *in vitro* activity against numerous biothreat pathogens, including *Bacillus anthracis* at concentrations likely to be achieved after oral dosing in humans. *B. anthracis* is a CDC Tier 1 Select Agent, and remains of considerable concern for biodefense. The potential for mass casualties resulting from a biothreat incident combined with the risk of antibiotic resistance necessitates development of novel medical countermeasures (MCM) for *B. anthracis*. Orally administered SUL was previously shown to be protective in the murine model of inhalational anthrax and provided proof-of-concept to advance the drug into the well-established rabbit model.

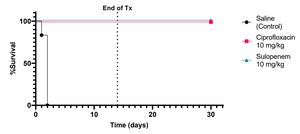
Methods

Female New Zealand White rabbits were challenged with a lethal dose of *B. anthracis* Ames spores via the inhalational route. Post-exposure prophylaxis (PEP) was initiated at 24 ±1 h postchallenge with cohorts (N=6) of animals receiving vehicle (saline, SC), ciprofloxacin (CIP) 10 mg/kg, SC or SUL 10 mg/kg, SC. This dosing regimen was informed by pharmacokinetic models to establish a human-equivalent dose. Treatment was continued BID for 14 days. Clinical progression and survival were assessed up to 30 days postchallenge.

Results

The untreated saline control group demonstrated 100% lethality with a median time to death of 48 h. Both the SUL and CIP groups demonstrated 100% survival through study termination on day 30.

Figure: Survival in the three experimental cohorts following a lethal inhalational anthrax challenge.



Treatment is listed as total daily doses, which were delivered in two equal doses at q12h for 14 days

Conclusions

As a MCM for inhalational anthrax, SUL would provide considerable advantages as an addition to the slate of current standards of care. Combined with positive efficacy results in two preclinical models of inhalational anthrax and advantages offered by an orally available penem antibiotic, SUL remains a promising candidate for advanced development for treating *B. anthracis*.

INTRODUCTION

Bacillus anthracis is a highly virulent gram-positive, spore-forming bacteria and the etiological agent of anthrax. *B. anthracis* is also a CDC Tier 1 select agent and poses a significant threat to both military and public health due to the potential for intentional release. Novel therapies are needed to combat this biological threat. Sulopenem is a penem β-lactam antibiotic in development for the treatment of resistant bacterial infections. Orally administered sulopenem was previously shown to be protective in the murine model of inhalational anthrax and provided proof-of-concept to advance the drug into the well-established rabbit model. Here we report the pharmacokinetics of sulopenem in New Zealand white (NZW) rabbits and efficacy results in the anthrax model.

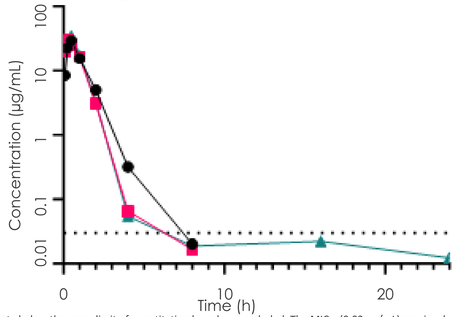
METHODS

Pharmacokinetics Study: Sulopenem (70 mg/animal) was given as a bolus SC injection into special-pathogen free female NZW rabbits (N=3, 3.5 kg); blood samples were taken post dose (0.083, 0.25, 0.5, 1, 2, 4, 8, 16, and 24h). Sulopenem was quantified in rabbit plasma using an in-house LC/MS-MS method. The resulting pharmacokinetic data were analyzed by a noncompartmental model and average parameters from the three subjects were calculated. Compartmental models of sulopenem pharmacokinetics in rabbits were developed to allow for dosing regimen simulations.

Efficacy Study: Special pathogen-free female NZW rabbits were challenged in a head-only aerosol chamber with *B. anthracis* (BA) spores (Ames). Spores were heat shocked prior to aerosolization. The aerosol was generated using a three-jet collision nebulizer. Integrated air samples were obtained from the chamber during each exposure using an all-glass impinger (AGI). AGI samples were serially diluted and plated on sheep blood agar plates and grown for 24 h at 37°C. The inhaled dose of BA spores, represented as colony-forming units (CFU)/rabbit, was calculated for each animal. The average challenge dose was 41.3 x LD50 (LD50 = 105,000 cfu) with a range of 17.4-68.9 x LD50 (target = 28.6 x LD50, 3E6 cfu/animal). Rabbits were randomized into cohorts (N=6) based on order of aerosol exposure. Treatment with ciprofloxacin, sulopenem, or saline commenced 24 h post challenge and continued q12h for a total of 14 days (Table 4). Animals were observed at least twice daily during the treatment period and clinical scores were used to determine humane endpoints if appropriate. Following treatment, animals were observed at least once daily until study termination at Day 30. At the study termination, all surviving animals were euthanized by barbiturate overdose. Terminal whole blood samples were drawn and plated to confirm sterility.

RESULTS

Figure 1: Plasma Concentration Over Time in Naïve NZW Rabbits Dosed With a Single Subcutaneous Injection of Sulopenem at 20 mg/kg

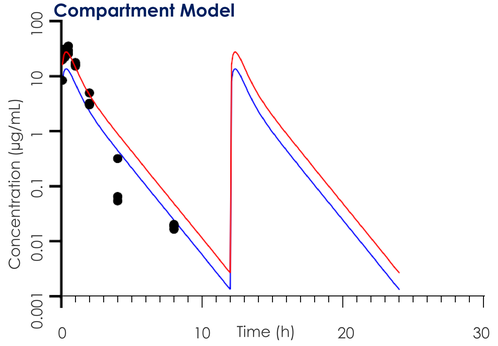


Data below the assay limit of quantitation have been excluded. The MIC_{90} (0.03 µg/mL) previously determined for a 30-strain panel of *B. anthracis* is shown by the horizontal dotted line. Each color represents a different animal. Mean concentrations were subsequently used to create a model.

Table 1: Noncompartmental Analysis of a Single Dose of Sulopenem in Rabbits

	Animal #		
	1	2	3
Dose (mg/animal)	70		
C_{max} (µg/mL)	29.2	19.9	35.4
T_{max} (h)	0.5	0.25	0.5
AUC_{last} (µg*h/mL)	36.5	35.4	41.1
$AUC_{0\text{-}inf}$ (µg*h/mL)	36.5	35.4	41.1
$t_{1/2}$ (h)	0.78	0.91	0.91
V_D/F (mL)	2159	2592	2243
Cl/F (mL/h)	1915	1975	1703

Figure 2: Simulated Plasma Levels of Sulopenem Based on One-Compartment Model



The simulated data is based on 35 mg/animal (blue) or 70 mg/animal (red) subcutaneous dosing at q12h intervals. Overlayed with the simulated data is the experimental data for a single bolus 20 mg/kg dose of sulopenem to 8h post dose.

RESULTS

Table 2: Simulated PK and PK/PD Parameters for the Final Mouse and Rabbit Doses Compared to the Human IV Dose of Sulopenem

	Human (1 g, QD, 3h INF)	Mouse (50 mpk, QID, PO)	Rabbit (35 mg, BID, SC)
C_{max} (µg/mL)	9.15	8.5	13.7
%*f*T>MIC = 1 µg/mL	>21	13	24
%*f*T>MIC = 0.015 µg/mL		72	72

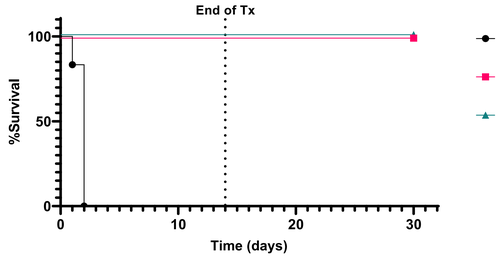
Table 3: Log-Rank Tests for Pair Wise Comparison of Sulopenem vs Ciprofloxacin

	Dead / Total	Median Time to Death (days)	vs. Saline Log-Rank (p-value)	vs. Ciprofloxacin Log-Rank (p-value)
Saline	6/6	2		
Ciprofloxacin	0/6	Undefined	0.0012	
Sulopenem	0/6	Undefined	0.0012	1

Table 4: Study Treatment Cohorts

Cohort	N per Group	Drug	Dose (mg/animal)	Start Time (h)	Route	Frequency
1	6	Saline	n/a	24	SC	BID
2		Ciprofloxacin	35			
3		Sulopenem	35			

Figure 3: Survival in the Three Experimental Cohorts Following a Lethal Inhalational Anthrax Challenge



Treatment is listed as total dose per animal per dose, which were delivered in two equal doses at q12h for 14 days. This dose (35 mg/dose or 70 mg/day) is equivalent to total daily dose of 20 mg/kg/day for a 3.5 kg animal.

CONCLUSIONS

- 100% survival was observed in both the ciprofloxacin positive control group as well as the sulopenem group
  - All surviving animals had no signs of disease during the treatment phase, and no detectable bacteremia at study termination
- These findings suggest that sulopenem would be effective as a post-exposure prophylaxis for inhalational anthrax at doses currently used in clinical trials
- As an MCM for inhalational anthrax, sulopenem would provide considerable advantages as an addition to the slate of current standards of care:
  - Sulopenem's broad-spectrum activity would likely bypass natural or engineered resistance targeting the most common antibiotics used for anthrax (i.e. fluoroquinolones and tetracyclines).
  - Sulopenem has both intravenous and oral administration options. While the intravenous route would be appropriate in critical care scenarios, the oral route would be highly advantageous in Warfighter deployment in austere environments in a post-exposure prophylaxis scenario
- Combined with positive efficacy results in two preclinical models of inhalational anthrax and advantages offered by an orally available penem antibiotic, sulopenem remains a promising candidate for advanced

REFERENCES

- Cavallo, J. D.; Ramisse, F.; Girardet, M.; Vaissaire, J.; Mock, M.; Hernandez, E., Antibiotic susceptibilities of 96 isolates of Bacillus anthracis isolated in France between 1994 and 2000. Antimicrob Agents Chemother 2002, 46 (7), 2307-9.
- Corey, A.; Migone, T. S.; Bolmer, S.; Fiscella, M.; Ward, C.; Chen, C.; Meister, G., Bacillus anthracis protective antigen kinetics in inhalation spore-challenged untreated or levofloxacin/ raxibacumab-treated New Zealand white rabbits. Toxins (Basel) 2013, 5 (1), 120-38.
- Dunne, M. W., S. I. Aronin, A. F. Das, K. Akinapelli, J. Breen, M. T. Zelasky, and S. Puttagunta. "Sulopenem for the Treatment of Complicated Urinary Tract Infections Including Pyelonephritis: A Phase 3, Randomized Trial." Clin Infect Dis 76, no. 1 (Jan 6 2023): 78-88.
- Guyton, A. C. "Measurement of the Respiratory Volumes of Laboratory Animals." Am J Physiol 150, no. 1 (Jul 1 1947): 70-7.

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