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Pharmacodynamic Assessment of Lysin Exebacase (CF-301) in Addition to Daptomycin against *Staphylococcus aureus* in the Neutropenic Murine Thigh Infection Model

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ABSTRACT (revised)

Background: Exebacase is a novel bacteriophage-derived lysin displaying rapid *Staphylococcus aureus*-specific bacteriolysis. Exebacase is being developed for the treatment of methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA) bacteremia and endocarditis, used in addition to, standard of care antibiotics including daptomycin (DAP). We evaluated the *in vivo* efficacy of exebacase alone and in addition to DAP against *S. aureus* strains using a murine thigh infection model.

Methods: Eight *S. aureus* isolates (1 MSSA and 7 MRSA) were studied. Exebacase MICs of the isolates determined in human and ICR mice serums (100%) ranged from 0.5-2 mg/L and 16-128 mg/L, respectively, while DAP broth microdilution MICs were 0.25-0.5 mg/L. ICR mice were rendered neutropenic via cyclophosphamide then the thighs were inoculated with bacterial suspensions (10^7 CFU/mL). Mice were administered one of three monotherapy regimens subcutaneously: i) DAP doses with sufficient dynamic range to evaluate synergy that yielded stasis or growth of the isolates at 24h, providing an area under the free drug concentration–time curve ($fAUC_{0-24}$) equivalent to 5-8% of that achieved in humans receiving DAP 6 mg/kg/day, ii) exebacase 15 mg/kg, or iii) exebacase 90 mg/kg/day. Combination regimens of sub-therapeutic DAP co-administered with 6 escalating exebacase doses ranging from 15 to 90 mg/kg/day were also examined. Control mice were vehicle-dosed. Efficacy was measured as the change in thigh bacterial density at 24h relative to 0h controls.

Results: On average, bacterial density in controls increased from 5.77 ± 0.25 at 0h to 8.28 ± 0.47 $\log_{10}$ CFU/thigh at 24h. As monotherapy, DAP resulted in mean growth of 0.39 ± 1.19 $\log_{10}$ CFU/thigh. exebacase 15 mg/kg alone resulted in a mean growth of 0.76 ± 1.24 $\log_{10}$ CFU/thigh while exebacase 90 mg/kg alone resulted in -0.26 ± 1.25 $\log_{10}$ CFU/thigh reduction. When administered with DAP, exebacase 15 mg/kg resulted in -1.03 ± 0.75 $\log_{10}$ CFU/thigh reduction, and higher exebacase doses did not yield further killing, with a mean $\log_{10}$ CFU/thigh reduction of -1.03 ± 0.72 (Range: -0.77 ± 0.98 to -1.20 ± 0.59 $\log_{10}$ CFU/thigh reduction) across the range of doses (exebacase 15 mg/kg to 90 mg/kg).

Conclusion: Exebacase administered with sub-therapeutic DAP exposure resulted in further bacterial killing compared with either agent alone highlighting a potential role for exebacase in enhancing the efficacy of DAP against *S. aureus* infections. Translational application of these *in vivo* data generated using a murine model to predict the efficacy in humans should consider the difference in exebacase activity between human and murine serums.

INTRODUCTION

- Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a major public health problem, and in the United States approximately 100,000 patients are hospitalized annually with invasive MRSA infections resulting in >18,000 deaths.[1]
- Exebacase is a novel anti-staphylococcal lysin that is being developed as an antimicrobial agent to lyse pathogenic bacteria by hydrolyzing peptidoglycan from outside the cell.[2]
- Exebacase is in clinical development as an adjunctive treatment to improve clinical cure rates of *S. aureus* bacteremia and endocarditis, used in addition to standard of care antibiotics including daptomycin (DAP).[2]

OBJECTIVES

- To asses the *in vivo* efficacy of exebacase alone and in addition to DAP against *S. aureus* strains using a murine thigh infection model.

METHODS

Antimicrobial Test Agents

- Exebacase (11 mg/mL; Lot # NBA0467-08, ContraFect Corporation, Yonkers, NY) was used for *in vivo* testing.
- DAP 500 mg commercial intravenous-use vials (Lot # 931096, TEVA, North Wales, PA) and analytical-grade daptomycin (Lot # NHU042015, Cubist Pharmaceuticals, MA) were used for *in vivo and in vitro* testing, respectively.

Bacterial isolates and susceptibility testing

- Eight *S. aureus* isolates (1 MSSA and 7 MRSA) were used in this study (**Table 1**).
- MICs of DAP were determined using broth microdilution in triplicate as outlined by CLSI.[3]
- Exebacase MICs determined in human and ICR mouse serum (100%) were provided by ContraFect Corp.

Neutropenic Thigh Infection Model

- Pathogen-free, female ICR mice (20-22g) were obtained from Envigo RMS, Inc. (Indianapolis, IN). All studies were in accordance with the Institutional Animal Care and Use Committee at Hartford Hospital.
- Mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide (150 mg/kg on Day-4, 100 mg/kg on Day-1). Uranyl nitrate (5 mg/kg on Day-3) was administered to produce a controlled degree of renal impairment to help simulate daptomycin human exposures.
- Isolates were transferred twice on TSA II™ plates (BD BioSciences, Sparks, MD) and incubated at 37°C. After 18 to 24h incubation of the isolate second transfer, a bacterial suspension of approximately 10^7 CFU/mL was made for inoculation.
- Thigh infection was produced by intramuscular inoculation of 0.1 mL of inoculum 2h prior to therapy initiation.

Human-simulated Daptomycin Exposure Pharmacokinetic Studies

- Total DAP concentrations were determined using a validated HPLC method and free DAP plasma concentrations were calculated by multiplying total concentrations by 8.5%.
- DAP PK parameters were determined in the model with single doses of DAP from 5 to 20 mg/kg.
- PK simulations (Phoenix version 6.3; Pharsight Corp., Mountain View, CA) were performed to generate dosing regimens in mice targeting similar $fAUC_{0-24}$ values to those achieved with doses of 6 mg/kg at steady-state in healthy volunteers.[4]
- DAP doses providing an area under the free drug concentration–time curve ($fAUC_{0-24}$) equivalent to 5-8% of that achieved in humans receiving DAP 6 mg/kg/day were utilized.

Dose Ranging Studies

- For each isolate tested, 3 untreated mice (six thighs) were used as 0h controls, 3 additional mice (receiving normal saline) as 24h controls, and 3 mice per each treatment dosing regimens was utilized.
- Treatments were initiated 2h post inoculation and continued for 24h.
- Mice were administered one of the following regimens subcutaneously:
  - **(i)** DAP doses with sufficient dynamic range to evaluate synergy that yielded stasis or growth of the isolates (provided an area under the free drug concentration–time curve ($fAUC_{0-24}$) equivalent to 5-8% of that achieved in humans receiving DAP 6 mg/kg/day)
  - **(ii)** exebacase 15 mg/kg/day or exebacase 90 mg/kg/day
  - **(iii)** combination regimens of sub-therapeutic DAP co-administered with 6 escalating exebacase doses ranging from 15 to 90 mg/kg/day were also examined
- Efficacy was measured as the change in thigh bacterial density at 24h relative to 0h controls.

METHODS

Dose fractionation Studies

- For each isolate tested, two total daily doses (TDD) of exebacase were evaluated: 75 and 90 mg/kg/day. Each of the exebacase TDDs was fractionated into two regimens i.e. one third of the entire dose was administered three times (q8h), or the entire dose was administered once (q24h) over a 24h period for a total of 2 groups per each TDD.

RESULTS

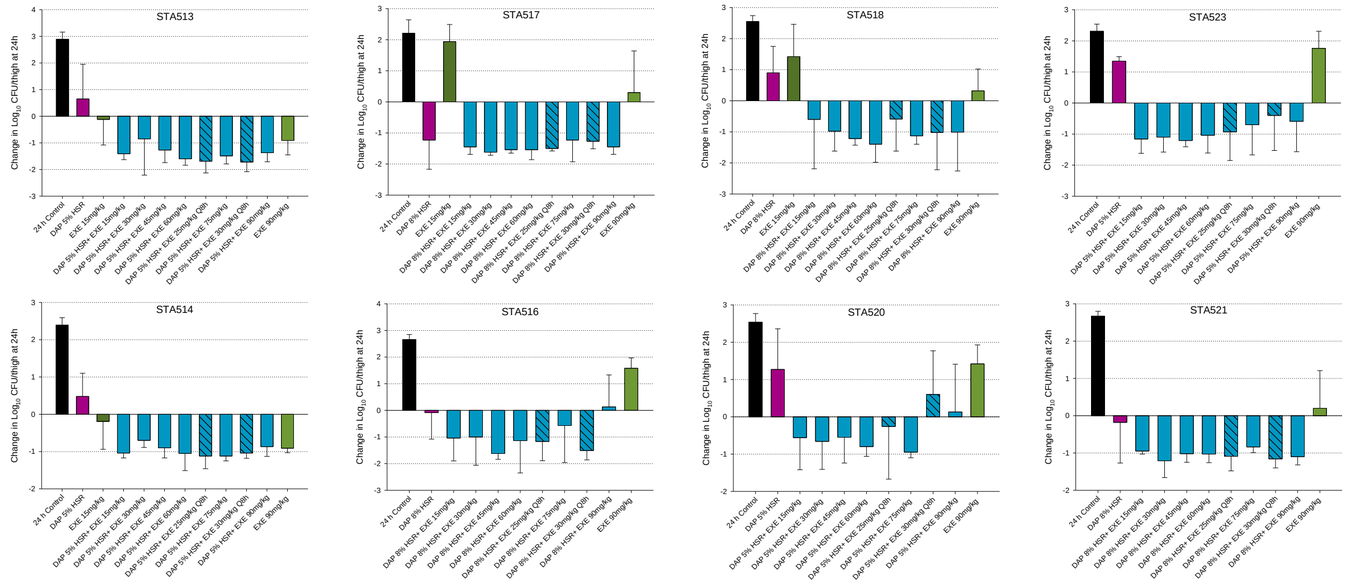
- On average, bacterial density in controls increased from 5.77 ± 0.25 at 0h to 8.28 ± 0.47 $\log_{10}$ CFU/thigh at 24h.
- As monotherapy, 5-8% DAP human simulated regimen (HSR) resulted in mean growth of 0.39 ± 1.19 $\log_{10}$ CFU/thigh.
- When administered with 5-8% DAP HSR, exebacase resulted in improved bacterial killing (Range: -0.77 ± 0.98 to -1.20 ± 0.59 $\log_{10}$ CFU/thigh reduction) across the range of exebacase doses (15 mg/kg to 90 mg/kg) compared to 5-8% DAP HSR alone.

Table 1. MICs of exebacase and DAP against MSSA and MRSA isolates

		MIC (µg/mL)		
Isolate ID	Bacterial Species	Exebacase[1]	Exebacase[2]	DAP
STA 513	MRSA/LRSA	0.5	64	0.5
STA 517	MRSA	2	32	0.5
STA 518	MRSA	0.5	64	0.25
STA 523	MRSA	0.5	128	0.5
STA 514	MRSA	0.5	16	0.5
STA 516	MRSA	1	128	0.5
STA 520	MRSA	0.5	128	0.5
STA 521	MSSA	0.5	128	0.5

LRSA, Linezolid-resistant *Staphylococcus aureus;* [1]*Determined in human serum (100%);* [2]*Determined in ICR mouse serum (100%)*

Figure 1. Efficacy of escalating exebacase (EXE) doses (15mg/kg – 90mg/kg) in addition to sub-therapeutic daptomycin humanized exposures compared with daptomycin and exebacase monotherapies. Bar graphs with diagonal lines represent exebacase dose fractionation (q8hr).



CONCLUSIONS

- Exebacase administered with sub-therapeutic DAP exposure resulted in further bacterial killing compared with either agent alone against all tested strains.
- For a given exebacase total daily dose, an increase in dosing frequency (fractionated dose) does not provide more bacterial kill compared with a single dose when given in addition to DAP.
- Data supports a potential role for exebacase in addition to DAP for treatment of infections due to MSSA and MRSA.
- Data generated was utilized in a global PK-PK study presented at ASM Microbe 2019 (S220).

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