Lysin Exebacase (CF-301) Exhibits Potent Bactericidal Activity in Human Synovial Fluid (HSF) Against Biofilm-Forming *Staphylococcus epidermidis* (*S. epidermidis*) Isolates

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Introduction

Exebacase (CF-301) is a novel, recombinantly-produced, bacteriophage-derived lysin (cell wall hydrolase) being examined in a Phase 2 study for the treatment of *S. aureus* bacteremia, including endocarditis, used in addition to conventional antibiotics. Hallmark features supporting clinical development of exebacase include rapid bacteriolytic effects, synergy with antibiotics, a low propensity for resistance, an extended postantibiotic effect, and, significantly, anti-biofilm activity (1-3).

The potent ability of exebacase to eradicate *Staphylococcus aureus* (*S. aureus)* biofilms has been shown: a) in vitro, on abiotic surfaces (1,2); b) in vivo, on rabbit cardiac valves (3); and c) on an infected hemodialysis catheter from a patient with *S. aureus* bacteremia (4). We furthermore demonstrated exebacase activity against *S. aureus* in human synovial fluid (2). *S. aureus* and *Staphylococcus epidermidis (S. epidermidis)* are a important causes of joint infections, and can form biofilms in synovial fluid which facilitate adherence to prosthetic materials, making prosthetic joint infections (PJIs) particularly difficult to treat with conventional antibiotics (5-7).

To extend our study of exebacase in human synovial fluid (HSF), we now report the analysis of antimicrobial activity against biofilm-forming clinical *S. epidermidis* isolates in HSF. Findings from this study may have important implications with respect to further evaluation of the potential therapeutic use of exebacase for joint infections.

Objective

To determine the susceptibility of clinical *S. epidermidis* isolates to exebacase in HSF and to observe the disruption of aggregates and biofilms formed in HSF.

Methods

Exebacase MICs were determined by broth microdilution (BMD) following CLSI methodology (M07-A11) using a non-standard medium (caMHB supplemented to 25% with horse serum and 0.5 mM with DTT; caMHB-HSD) approved for use by the CLSI in antimicrobial susceptibility testing with exebacase (8). Activity in HSF (Discovery Life Sciences) was similarly determined by BMD in caMHB with 50% HSF (caMHB-HSF). The caMHB-HSF supports growth and biofilm formation of both *S. epidermidis* and *S. aureus*. Fifty-three *S. epidermidis* clinical isolates and two MRSA strains were chosen for study; each isolate was previously demonstrated to form biofilms (2). For macroscopic analysis of exebacase activity on biofilms formed in HSF in the manner described (5-7), 10^8 CFUs of *S. epidermidis* isolate NRS6 were incubated for 24 hours at 37°C in 24-well plates, treated with 0.1 or 1 µg/mL exebacase for 2 hours, stained with ethidium bromide (EtBr) and visualized by UV fluorescent imaging. Untreated controls were included. Biofilms were similarly formed and treated in HSF, prior to staining with Alex Fluor^{488}-WGA (showing cells in green) and PI (showing biofilm matrix in red) and visualization by fluoresence microscopy. Scanning electron microscopy (SEM) was used to demonstrate biofilm formation in HSF and elimination after 2 hour treatments with exebacase at concentrations of 0.01, 0.1 and 1 µg/mL.

References

1. Schuch et al. (2014) JID, 209:1469–1478
2. Schuch et al. (2017) AAC, 61:e02666-16
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4. Oh et al. (2018) ECCMID poster P1435
5. Perez and Patel (2015) JID 212:335-6
6. Dastgheyb et al. (2015) JID 211:641-50.
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Antimicrobial Activity of Exebacase Against *S. epidermidis* in HSF

- **MICs were determined in caMHB-HSF to access activity in conditions supporting biofilm formation**
- **For comparison, MICs were also determined in exebacase AST medium (caMHB-HSD); values are also included for *S. aureus***

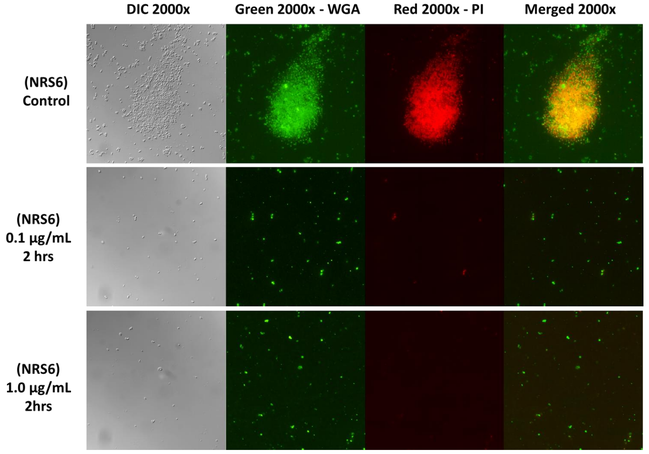
	Exebacase MIC (µg/mL)					
	caMHB-HSF			caMHB-HSD		
Organism (# of isolates)	**MIC_{50}**	**MIC_{90}**	**Range**	**MIC_{50}**	**MIC_{90}**	**Range**
S. epidermidis (N=53)	0.015	0.125	0.0078-2	0.5	0.5	0.25-2

	CF-301 MIC (µg/mL)	
***S. aureus* strain**	**caMHB-HSF**	**caMHB-HSD**
MW2	0.03	0.5
ATCC BAA-42	0.03	0.5

- **Exebacase demonstrated potent activity against *S. epidermidis* in HSF with an $MIC_{50/90}$ of 0.015/0.125 µg/mL and a range of 0.0078-2 µg/mL**
- **The activity against *S. epidermidis* was similar to that observed against *S. aureus***

Disruption of *S. epidermidis* Biofilms by Exebacase in HSF

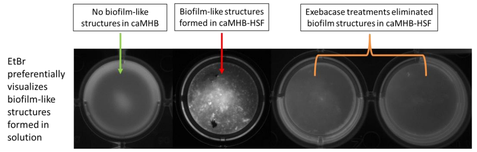
- ***S. epidermidis* (strain NRS6) biofilms were formed in HSF for 24 hours prior to treatment for 24 hours with exebacase at 0.1 and 1 µg/mL for 2 hours**
- **DIC and fluorescence images are shown for untreated and treated samples**
- **Alexa Flour^{488}-WGA strained exopolysaccharide in biofilms (and individual bacteria), while PI stained the entire biofilm, merged images show the colocalization**



- **Two hours of treatment with exebacase (at 0.1 and 1 µg/mL) eliminated *S. epidermidis* biofilms formed in HSF**

Macroscopic Imaging of Anti-Biofilm Activity

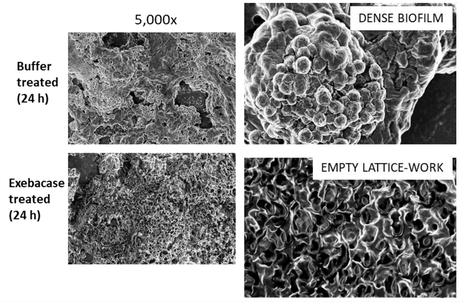
- **The impact of treatment on EtBr staining of biofilm structures formed by NRS6 in HSF were examined**



- **Biofilm-like structures formed in the presence of HSF**
- **Exebacase eliminated the biofilm structures within 2 hrs**

SEM Analysis of Biofilm Disruption in HSF

- **We are undertaking an ultrastructural analysis of exebacase treatment of biofilms formed by either *S. aureus* (strain ATCC BAA-42) or *S. epidermidis* (NRS6) in HSF**
- **The analysis of *S. aureus* biofilms formed over 24 hrs in HSF and treated with exebacase (1 µg/mL) for 24 hours is shown:**



- ***S. aureus* is cleared from biofilms formed in HSF, leaving a matrix of (likely) synovial fluid-derived material**
- **The analysis of *S. epidermidis* is currently in progress**

Conclusions

- **Exebacase has potent in vitro bactericidal and anti-biofilm activities against *S. epidermidis* and *S. aureus* in HSF**
- **Exebacase may have the potential to be developed as a treatment for *S. epidermidis* in joint infections, particularly PJIs, which are complicated by biofilms against which antibiotics are generally ineffective**