Translational Study of the Antibiofilm Activity of Lysin CF-301 in an Infected Hemodialysis Catheter From a Patient with Suspected *S. aureus* Bacteremia

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Abstract

Background. CF-301 is a novel, recombinantly-produced, bacteriophage-derived lysin (cell wall hydrolase) which is in Phase 2 of clinical development for the treatment of *S. aureus* bacteremia including endocarditis used in addition to standard-of-care antibiotics. In contrast to conventional antibiotics, CF-301 has potent activity against staphylococcal biofilms in vitro and in animal models. To explore the activity of CF-301 against *S. aureus* biofilms associated with human infections, CF-301 was tested on a colonized explanted hemodialysis catheter from a patient with suspected *S. aureus* bacteremia. This is the first study to assess CF-301 activity on biofilm formed in the setting of human disease.

Materials/methods: An infected hemodialysis catheter was removed as part of clinical care. Segments of the catheter were bisected and allotted into different treatment groups (n=3 segments/group) with CF-301, daptomycin (DAP) or CF-301 + DAP. Clinically relevant concentrations of both CF-301 and DAP were included (i.e., 1 µg/mL). After 18 h, samples were homogenized for quantitative plating on Tryptic Soy Agar and Mannitol Salt Agar with 4 µg/mL oxacillin (OXA). Isolates (n=16) from the pretreatment group were examined by 16S rRNA amplicon sequencing and used to determine MIC values for CF-301, DAP, and OXA.

Results: CF-301 eradicated the biofilm at 1 µg/mL whereas DAP alone did not clear biofilm at 1 µg/mL. The addition of CF-301 and DAP resulted in clearance of the biofilm. The catheter biofilm included methicillin-resistant staphylococci (OXA MIC = 32-256 µg/mL), including *S. aureus*, *S. epidermidis*, and *S. capitis*.

Conclusions: A clinically relevant concentration of CF-301, alone and in addition to DAP, eradicated staphylococcal biofilm which formed inside a hemodialysis catheter in the setting of a human clinical infection, whereas an analogous concentration of DAP alone did not. These data provide important translation of the previously reported potent efficacy of CF-301 against biofilms formed in vitro and in animal models, to biofilms formed in the setting of human disease.

Introduction

The majority of catheter-related bloodstream infections (CRBSIs) are due to Gram-positive bacteria, including *Staphylococcus aureus* (*S. aureus*) (**1**). In the setting of CRBSIs, biofilm bacteria are encased in matrices comprised of bacterial proteoglycans and polysaccharides and human host components (such a fibrin and fibrinogen) that facilitate adherence and persistence on the catheter surface (**2**). Conventional antibiotics are ineffective at either penetrating or eradicating catheter biofilms, resulting in ≥1,000-fold increases in antibiotic tolerance compared to planktonic bacteria.

While there are currently no approved treatments to specifically eradicate biofilms, we have previously reported on the potent antibiofilm activity of lysins, a family of bacteriophage-encoded peptidoglycan hydrolases (**3,4**). CF-301 is a recombinant lysin which is in Phase 2 of clinical development for the treatment of *S. aureus* bacteremia including endocarditis. The ability of CF-301 to eradicate mature biofilms was demonstrated in vitro on a variety of abiotic surfaces, including polystyrene, PVC catheters, glass, and surgical mesh; furthermore, an $MBEC_{90}$ value of ≤0.25 µg/ml was determined using a set of 90 MSSA and MRSA strains. We also demonstrated the ability of CF-301, at clinically relevant concentrations, to eradicate biofilm-based staphylococcal infections in the context of both rabbit and rat infective endocarditis models (**5,6**) and a foreign-body murine infection model (data not shown).

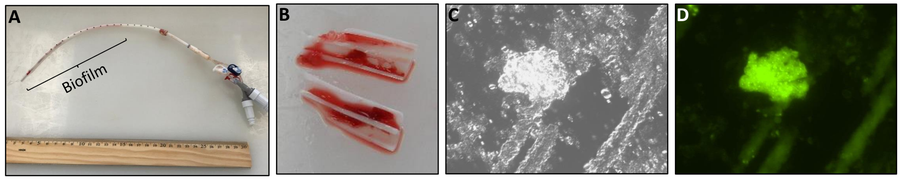
In this pilot study, we sought to determine whether the potent antibiofilm acitivity demonstrated by CF-301 in vitro and in animal infection models could be replicated against *S. aureus* biofilms formed in a central venous catheter in the setting of human disease. As such, we examined the ability of CF-301 alone and in addition to daptomycin (DAP) to eradicate biofilm found inside an hemodialysis catheter removed from a patient with a suspected CRBSI.

Methods

- Hemodialysis catheter was removed as part of clinical care of a patient with suspected *S. aureus* CRBSI
- Catheters were cut into equal length segments and bisected to expose the lumen
- Samples were stained with FilmTracer Calcein Green Biofilm Stain for fluorescence microscopy
- Segments were randomized into the 5 following groups (N=3 segments/group):
  - Pretreatment control (rinse, homogenize, plate [as below] to determine initial CFUs)
  - Buffer control (i.e., treatment with Lactated Ringer's solution alone)
  - 3 separate CF-301 concentrations: 1µg/mL (clinically relevant), 10 and 100 µg/mL
  - 3 separate DAP concentrations: 1µg/mL (clinically relevant), 10 and 100 µg/mL
  - CF-301 and DAP at concentrations used above
- Segments (buffer control and treatments with CF-301, DAP, and each combination) were incubated at 37°C for 18 hours, then rinsed with PBS
- Catheter segments were homogenized (Precellys 24 tissue homogenizer, Bertin Technologies) according to a standard methodology designed to recover and quantitate biofilm bacteria (**7**)
- Surviving bacteria were enumerated by quantitative plating on:
  - TSA blood agar plate (CFU counts, assess hemolytic phenotype and purity of culture)
  - CHROMagar (selection for *S. aureus,* distinguish from *S. epidermidis*)
  - Mannitol salt agar with 4 µg/mL OXA plate (selection for OXA^R phenotype)
- Coagulase test, species identification by sequencing of 16s rRNA amplicons, determine MICs for CF-301, DAP, VAN, and OXA

Visual Assessment of Biofilm Formation on Catheter

- ❑ Explanted human hemodialysis catheter (Panel **A**)
- ❑ Adherent biofilm in catheter lumen (Panel **B**)
- ❑ Biofilm visualized by differential interference contrast (DIC) microscopy, x2000 mag (Panel **C**)
- ❑ Same field as (**C**), visualization of Biofilm Stain, x2000 mag (Panel **D**)



- Adherent mucoid biofilm observed across the internal lumen of catheter
- Biofilm stain identified clusters of cocci adhering to internal lumen

Quantitative Plating Results

- ❑ Enumeration of surviving bacteria from each of 5 overall treatment groups

Treatment Groups	CF-301 (µg/mL)	DAP (µg/mL)	Log_{10} CFU/g of Catheter
Pretreatment Control[a]	n.a.	n.a.	2.94
Buffer Control[b]	0	0	2.88
CF-301 Alone	1	0	<LOD[c]
	10	0	<LOD
	100	0	<LOD
DAP Alone	0	1	3.76
	0	10	<LOD
	0	100	<LOD
CF-301+DAP (Combos)[d]	1	1	<LOD

[a]The pretreatment group was used to determine baseline CFUs prior to any treatment, thus the indicated treatments are "not applicable" (n.a.)
[b]"0" is used to indicate that no CF-301 or DAP was used in the Buffer Control. The Buffer Control is treated with Lactated Ringer's solution alone.
[c]The limit of detection (LOD) is 0.7 Log_{10} CFU/g
[d]All combinations with CF-301 at 100, 10, and 1 µg/mL yielded no CFUs

- CF-301 eradicated biofilm at the clinically relevant concentration of 1 µg/mL (the MIC_{90} for CF-301 against *S. aureus*) and at higher concentrations.
- DAP did not eradicate biofilm at the clinically relevant concentration of 1 µg/mL. Clearance was observed at 10X and 100X MIC.
- Addition of CF-301 (1 µg/mL) to clinically relevant DAP concentration (1 µg/mL) resulted in eradication of biofilm

Identification and Susceptibilities of Recovered Biofilm Bacteria

- ❑ Random colonies selected from pretreatment and buffer control groups were subjected to a series of phenotypic and genotypic analyses
- ❑ Analyses included MIC determinations, coagulase test, and 16s amplicon sequencing

16S homology (100%)	MIC (µg/mL)				Coagulase
	CF-301	DAP	VAN	OXA	
S. capitis	1	2	2	256	-
S. capitis	1	2	2	256	-
S. epidermidis	0.125	1	2	64	-
S. capitis	1	2	2	256	-
S. capitis	1	2	2	256	-
S. epidermidis	0.125	1	2	32	-
S. capitis	1	2	0.5	256	-
S. epidermidis	0.125	0.5	2	32	-
S. capitis	1	2	0.5	256	-
S. capitis	1	2	2	256	-
S. epidermidis	0.125	0.5	2	64	-
S. aureus	1	2	0.5	256	+
S. capitis	1	2	2	256	-
S. capitis	1	2	2	256	-
S. aureus	1	1	2	256	+
S. capitis	2	2	1	256	-

*Control colonies were also analyzed including *Enterococcus faecalis* strain ATCC 29212, *S. epidermidis* strain ATCC 12228, and *S. aureus* strain MW2

- 3 distinct biofilm-forming pathogens were identified from the catheter: *Staphylococcus capitis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*
- Based on OXA MIC values, the catheter was colonized with Methicillin-Resistant *S. aureus* (MRSA), Methicillin-Resistant *S. epidermidis* and Methicillin-Resistant *S. capitis*
- All staphylococcal isolates identified were susceptible to DAP and VAN

Conclusion

- This is the first pilot study evaluating the ability of CF-301 to eradicate biofilm formed in a human host
- CF-301 alone, at a clinically relevant concentration, eradicated biofilm formed on an explanted hemodialysis permacatheter in a patient with suspected *S. aureus* bacteremia
- DAP did not clear the biofilm at a clinically relevant concentration, however, the addition of CF-301 to DAP resulted in eradication of the biofilm
- Data from this pilot study provide evidence of the efficacy of CF-301 in eradicating biofilms formed by various staphylococcal species (including MRSA) in the setting of a human CRBSI; further investigation is justified

References

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