Amurin Peptide App2-M1 Eradicates *Stenotrophomonas maltophilia* Biofilms Formed on Hemodialysis Catheters in the Setting of Human Infection

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Poster #51

Abstract

Background. *S. maltophilia* is a multidrug-resistant Gram-negative (GN) pathogen, associated with high morbidity and mortality particularly in immunocompromised patients, and is a recognized pathogen for cystic fibrosis patients in the United States. A new class of direct lytic agents (DLAs) called amurin peptides are now under development to address serious, life-threatening infections caused by GN pathogens. Amurins exhibit broad-spectrum antimicrobial activity against GN pathogens, as well as a range of other hallmark features including the eradication of biofilms formed in vitro. To extend the analysis of amurin activity, peptide App2-M1 was tested on infected explanted hemodialysis catheters from patients with suspected catheter-related bloodstream infections. This is the first study to assess amurin activity on biofilms formed in the setting of human disease.

Materials/methods: Three infected hemodialysis catheter were removed and discarded from two patients as part of clinical care (patient 1 = catheter A; patient 2 = catheters B and C). Catheter segments were bisected and allotted into different treatment groups (n=8 segments/group) with App2-M1 and buffer control. Clinically relevant concentrations of App2-M1 were used (i.e., 1 and 10 µg/mL). A meropenem control treatment (1 µg/mL) was included for one catheter from patient 2. After 4 h treatments with buffer, App2-M1 or meropenem, samples were homogenized for quantitative plating on Tryptic Soy Agar and a subset of resulting isolates (n=16) were examined by comparative DNA sequencing of the 16S rRNA gene and used to determine MIC values for App2-M1 and meropenem.

Results: App2-M1 eradicated biofilms at 1 and 10 µg/mL. These findings are consistent with *in vitro* observations of minimal biofilm eradication concentrations of ≤2µg/mL for various GN pathogens using App2-M1. Meropenem alone did not eliminate biofilm at 1 µg/mL. Sequence analysis of catheter biofilm bacteria revealed the uniform presence of organisms from the genus *Stenotrophomonas* with App2-M1 MIC values of ≤2 µg/mL.

Conclusions: Amurin App2-M1 eliminates *Stenotrophomonas* biofilms inside explanted hemodialysis catheters at clinically relevant concentrations. These data provide the first evidence of translation of the previously reported, potent *in vitro* antibiofilm activity of App2-MI to an *ex-vivo* eradication of biofilms formed in the setting of human infection.

Introduction

In the setting of catheter-related bloodstream infections (CRBSIs), intraluminal biofilms commonly encase bacteria in matrices of proteoglycans and polysaccharide that facilitate adherence to and persistence on catheter surfaces (**1,2**). Conventional antibiotics are generally unable to eradicate or penetrate biofilms and thus are ineffective at clearing bacteria contained within them. (**3,4**). For this reason, novel antibiofilm strategies are urgently needed.

We previously reported on the potent antibiofilm activity of a family of direct lytic agents (DLAs) called lysins (peptidoglycan hydrolases) (**5**). Exebacase, an antistaphylococcal lysin was shown to eradicate staphylococcal biofilm formed on a hemodialysis catheter in the setting of a human CRBSI and removed as part of clinical care (**6**). In a recently completed Phase 2 clinical trial, exebacase demonstrated 42.8% higher clinical responder rates when used in addition to standard of care antibiotics (SOC) compared to SOC alone for the treatment of biofilm-associated methicillin-resistant *S. aureus* (MRSA) bacteremia including endocarditis (**7**).

In the current study, we report for the first time, the activity of a DLA targeting Gram negative (GN) pathogens against biofilms formed in the setting of human CRBSIs using the explanted human dialysis catheter as a translational model.

Stenotrophomonas maltophilia (S. maltophilia) is a multidrug resistant GN pathogen and is associated with substantial morbidity and mortality, particularly in debilitated, hospitalized patients (**8,9**). Furthermore, the incidence of biofilm-associated *S. maltophilia* infections in patients with implanted foreign devices, including catheters is increasing (**10,11,12**). We sought to evaluate the activity of another class of DLAs, amurin peptides, against *S. maltophilia* biofilms formed in the setting of human disease, using the translational model of hemodialysis catheters removed as part of clinical care for CRBSI. Amurins are notable for both their potent bactericidal activities against a range of antibiotic resistant GN pathogens, and in vitro antibiofilm effects which are defined by minimal biofilm eliminating concentrations of ≤4 ug/mL. Here, we used clinically relevant concentrations of the amurin peptide App2-M1 to eradicate biofilm formed inside hemodialysis catheters from 2 patients in the setting of clinical care for suspected GN CRBSI.

References

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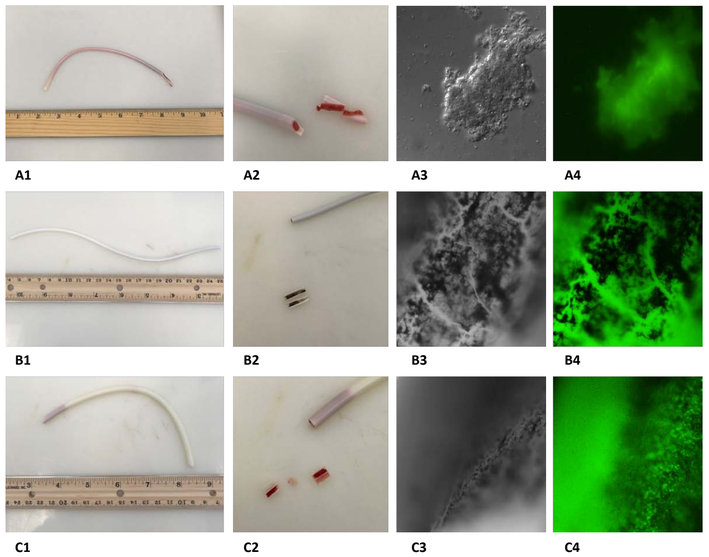
Methods

- Three catheters were removed and discarded from two patients with suspected CRBSI as part of clinical care (patient 1 = catheter A; patient 2 = catheters B and C)
- Catheters were cut into equal length segments and bisected to expose the lumen
- Samples were stained with FilmTracer Calcein Green Biofilm Stain for subsequent microscopy
- Segments were randomized into the following groups (N = 8 segments/group):
  - Lactated Ringer's (LR) pre-treatment control
  - LR treatment control
  - App2-M1 at 1 or 10 µg/mL in LR
  - Meropenem at 1 µg/mL in LR for catheter C
- With the exception of the pre-treatment control, all segments were incubated at 37°C for 4 hours
- All samples (pre-treatment and post-treatment) were homogenized (Precellys 24 tissue homogenizer, Bertin Technologies) according to a standard methodology designed to recover and quantitate biofilm bacteria (**13**)
- Surviving bacteria were enumerated by quantitative plating on TSA blood agar plates
- 16 isolates arising from each pre-treatment and post-treatment LR control were subcultured on TSA agar and used to determine MICs for App2-M1 (and meropenem for catheter C)
- Speciation was performed by MALDI-TOF and sequencing of 16s rRNA amplicons through AccuGENX-ID at Charles River Laboratories

Visual Assessment of Biofilm Formation

❑Explanted hemodialysis catheters (**Panels A1, B1, and C1**)
❑Exposure of intraluminal material (**Panels A2, B2, and C2**)
❑Analysis of intraluminal surface by differential interference contrast (DIC) microscopy, 2000x magnification (**Panels A3, B3, and C3**)
❑Same field as DIC, visualization with Biofilm Stain, x2000 mag (**Panel A4, B4, and C4**)



- Adherent mucoid biofilm observed within the lumen of all three catheters
- Bacterial clusters in biofilm-like structures (stained with calcein green) were observed adhering to internal lumen

Treatment of Catheter A (Patient 1)

❑Enumeration of surviving bacteria from Catheter A

Study groups[a]	App2-M1[a] (µg/mL)	Log_{10} CFU/g[b]
Treatment control	0	3.37
App2-M1 alone	10	<0.7

[A]The concentration of 10 µg/mL was chosen to facilitate bactericidal and antibiofilm activity against as wide a range of GN organisms as possible (based on *in vitro* MIC and MBEC testing)

[b]Surviving bacteria were enumerated after 24 hrs of incubation at 37 °C. The limit of detection is 0.7 Log_{10} CFU/g of catheter

- Bacterial colonies recovered from each control group exhibited a uniform phenotype on blood agar plates suggesting a mono-microbial biofilm
- App2-M1 completely removed the biofilm at a concentration of 10 µg/mL
- First evidence of the ability of amurin peptides to clear biofilms formed in human host

Treatment of Catheters B & C (Patient 2)

❑Enumeration of surviving bacteria from Catheters B and C

Study groups[a]	Catheter B	Catheter C
	Log_{10} CFU/g[c]	Log_{10} CFU/g[d]
Treatment control	4.24	4.22
App2-M1 (1 µg/mL)	<0.7	<0.7
Meropenem (1,10,100 µg/mL)	n.d.	3.16

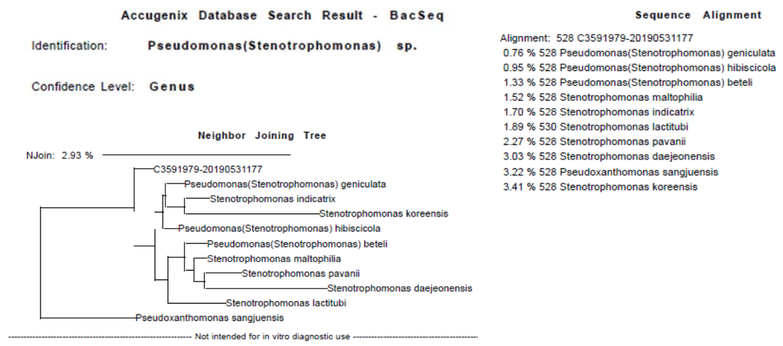
[a]The 1 µg/mL concentration was chosen for App2-M1 to determine the minimal amount needed to clear biofilm; for catheter A, the concentration of 10 µg/mL was sufficient

[c,d]Surviving bacteria were enumerated after 24 hrs of incubation at 37 °C

- App2-M1 removed the biofilm at a concentration of 1 µg/mL
- The meropenem treatments did not eradicate the biofilm
- Recovered bacterial colonies exhibited a uniform phenotype on blood agar plates suggesting a mono-microbial biofilm for each catheter
- Similar colony morphologies were observed for all bacteria from catheters B and C (and A as well), suggesting the same or a similar causative agent

Speciation of Biofilm Bacteria

❑16S rRNA amplicon sequencing we as used to identify the unknown organism/s colonizing each catheter



- For all organisms recovered for each of the 3 catheters, 16S rRNA amplicon sequencing identified the presence of *Stenotrophomonas* spp.
- MALDI-TOF confirmed the presence of *S. maltophilia* in each catheter

Minimal Inhibitory Concentration Assays

❑Multiple colonies recovered from each of the three catheters were used to determine MICS for both App2-M1 and meropenem

		MIC (µg/mL)[a]	
Catheter	Organism	App2-M1	Meropenem
A	*S. maltophilia*	2	>32
B	*S. maltophilia*	1	>32
C	*S. maltophilia*	1	>32

[A]For each catheter, the recovered colonies yielded the identical App2-M1 and meropenem MIC values, which are shown

- Organisms from each of the 3 catheters yielded similar App2-M1 and meropenem MICs
- The *S. maltophilia* strain/s colonizing each catheter were meropenem-resistant

Findings and conclusions

- **This is the first study evaluating the ability of amurin peptide (App2-M1), to eradicate biofilm formed during human infection with a resistant Gram-negative pathogen**
- **App2-M1 alone, at clinically relevant concentrations of 1-10 µg/mL, eradicated biofilms formed by *S. maltophilia* on explanted hemodialysis permacatheter from patients with suspected GN CRBSI**
- **Meropenem treatments had no effect on biofilm viability**
- **Data from this translational study provide evidence of the efficacy of App2-M1 in eradicating biofilms formed by GN pathogens in the setting of a human infection**
- **These promising findings support the potential therapeutic potential of amurin peptides to treat antibiotic resistant GN infections**