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

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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) samples were homogenized for quantitative plating on TSA blood agar plates and a subset of resistant 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-M1 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 cause bloodstream infections in patients with underlying conditions and are an important cause of catheter failure. Infections are associated with substantial morbidity and mortality, particularly in debilitated, hospitalized patients. Stenotrophomonas maltophilia is a multidrug-resistant GN pathogen that 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). We previously reported on the potent antibiofilm activity of a family of direct lytic agents (DLAs) called amurin peptides and polyoxycarboxylate that facilitate adherence to and persistently adhere to catheter surfaces (12). We have now used a family of direct lytic agents (DLAs) called amurin peptides and polyoxycarboxylate that facilitate adherence to and persistently adhere to catheter surfaces (12). Conventional antibiotics are generally unable to eradicate or penetrate biofilms and thus are ineffective at clearing bacteria contained within them. For this reason, novel antibiotic strategies are urgently needed.

We previously reported on the potent antibiofilm activity of a family of direct lytic agents (DLAs) called amurin peptides and polyoxycarboxylate that facilitate adherence to and persistently adhere to catheter surfaces (12). We have now used a family of direct lytic agents (DLAs) called amurin peptides and polyoxycarboxylate that facilitate adherence to and persistently adhere to catheter surfaces (12). Conventional antibiotics are generally unable to eradicate or penetrate biofilms and thus are ineffective at clearing bacteria contained within them. For this reason, novel antibiotic strategies are urgently needed.

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 is a multidrug-resistant GN pathogen and is associated with sustained 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). 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 CRBSIs. Amurins are notable for both their potent bactericidal activity against a range of antibiotic-resistant GN pathogens, and in vitro antibiofilm effects which are defined by minimal biofilm eliminating concentrations of ≤4 µg/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 CRBSIs.

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
• 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 onto 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

Treatment of Catheter A (Patient 1)

- 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
- Bacterial colonies recovered from each control group exhibited a uniform phenotype on blood agar plates

Study groups
- Treatment control
- App2-M1 alone

<table>
<thead>
<tr>
<th>Study groups</th>
<th>App2-M1</th>
<th>Log10 CFU/g</th>
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</thead>
<tbody>
<tr>
<td>Treatment control</td>
<td>0</td>
<td>3.37</td>
</tr>
<tr>
<td>App2-M1 alone</td>
<td>10</td>
<td>&lt;0.7</td>
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The concentration of 10 µg/mL was chosen to facilitate bactericidal and antibiofilm activity at clinically relevant concentrations of 1-10 µg/mL. The 1 µg/mL concentration was chosen for App2-M1 to determine the minimal eradication concentration. Surviving bacteria were enumerated after 24 hrs of incubation at 37°C.

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 catheter 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.

References

6. Oh et al. 2018. ECMCID Poster P1435
7. Fewler et al. 2019. ECMCID Oral Presentation # L0012