ERADICATE MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA BIOFILMS AND PERSISTERS WITH SPLUNC1-DERIVED NOVEL ANTIMICROBIAL PEPTIDES

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ABSTRACT

Background: Pseudomonas aeruginosa is one of the most lethal pathogens that causes chronic respiratory infections in cystic fibrosis (CF) patients. With the current shortage of newly developed antibiotics, respiratory failure induced by bacterial infection is still the main cause of death in CF. Antimicrobial peptides (AMPs) are a new class of promising therapeutics that could potentially target current difficult-to-treat multi-drug resistant (MDR) bacterial infections. The goal of this study is to understand the bacterial killing mechanism, efficacy against MDR P. aeruginosa and in vivo safety of novel AMPs that were derived from respiratory host defense protein SPLUNC1 (collectively named A4-AMPs). Methods: Rationally designed novel AMPs were synthesized based on an antimicrobial motif of SPLUNC1 and screened against a panel of more than 70 MDR clinical P. aeruginosa isolates. Antimicrobial potency was determined by growth inhibition and various biofilm prevention assays. Mode of action through bacterial membrane permeation was confirmed by changes of membrane potential and microscopic images including SEM, TEM, and AFM. Cellular toxicity was evaluated using RBC, WBC, and airway epithelial cells. Safety profile and efficacy were determined using murine pneumonia and bacteremia models.

Results: De novo synthesized A4-AMPs overcome multiple shortcomings that are normally associated with natural AMPs and demonstrated excellent antimicrobial activity that are not achievable by natural AMPs. Multiple A4-AMPs also showed superior bactericidal and anti-biofilm activities against MDR-P. aeruginosa isolates compared to current standard of care antibiotics including Tobramycin, Cefazidime, and Ciprofloxacin. The lead compound A4-112 has demonstrated significantly lower toxicity and better efficacy than colistin, the last resort antibiotics. A4-112 directly perturbs bacterial membrane that results in very fast bacterial lysing and killing. In vitro and in vivo studies also showed negligible RBC and WBC toxicity and high efficacy against P. aeruginosa-induced murine respiratory infection with a therapeutic index >100 in eliminating P. aeruginosa-induced pneumonia.

Conclusions: Our data demonstrated promising therapeutic potential of A4-AMPs as the next generation novel antimicrobials. The low toxicity and high efficacy against MDR-P. aeruginosa warrant further investigation and exploration of A4-112 in eradicating persistent P. aeruginosa-associated biofilm, colonization, and infection in CF sufferers.

METHODS

- Scanning electron microscope (SEM) and Transmission electron microscopy (TEM)
- Growth inhibition assay (GIA) for MIC screening on 96-well microplates in MHB
- Biofilm prevention assays on abiotic and biotic (airway epithelial cells) surfaces
- RBC and WBC toxicity assays
- In vivo pneumonia and bacteremia models in mouse

Table 1: Antimicrobial Activity, Antibiotics tested include: 1 Amikacin, 2 Aminoglycosides, 3 Cefotidine, 4 Cefazidime, 5 Ciprofloxacin, 6 Colistin, 7 Chloramphenicol, 8 Gentamicin, 9 Imipenem, 10 Levofloxacin, 11 Meropenem, 12 Piperacillin/tazobactam, 13 Tobramycin

<table>
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<tr>
<th>CDC Pseudomonas aeruginosa isolates</th>
<th>A4-lead (2 μM)</th>
<th>Colistin (2 μM)</th>
<th>A4-lead (4 μM)</th>
<th>Colistin (4 μM)</th>
<th>A4-lead (8 μM)</th>
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Figure 1 | Structural Origin of A4-AMPs, SPLUNC1 / EPF1/4
Airway-Specific Secretory Protein Host Defense Molecules

Table 2 | Antimicrobial Activity

- **Dispersal**
- **Formation**
- **Disruption**

A4-AMPS Biofilm Prevention & Eradication

- **A4-lead** (2 μM)
- **Colistin** (2 μM)
- **A4-lead** (4 μM)
- **Colistin** (4 μM)
- **A4-lead** (8 μM)
- **Colistin** (8 μM)

Figure 2 | P. aeruginosa PA01 SEM imaging with or without A4-112 treatment (μM, 15 minutes).

Figure 3 | P. aeruginosa PA01 TEM imaging with or without A4-112 treatment (μM, 15 minutes).

**DISCUSSION**

- A4 is a novel broad-spectrum AMP effective against various GPRGNMDR pathogens.
- A4 is superior to Standard of Care antibiotics and other existing AMPs.
- A4 has outstanding efficacy in animal infectious models.
- A4 is of very low toxicity, results in excellent SI (LD50/MIC) and TI (MTD/tI).
- Goal - bring A4 to clinical use in overcoming MDR bacterial infection in CF

Acknowledgements

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References
