Lysins Exhibit Potent Antimicrobial Activity, Synergy and Antibiofilm Effects against *Pseudomonas aeruginosa* in Human Serum and Pulmonary Surfactant

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SUNDAY AAR-695

Abstract

Background: Antibiotic-resistant Gram-negative (GN) pathogens pose a public health threat which necessitates new antimicrobials with novel mechanisms of action. Lysins (cell wall hydrolases), a new class of direct lytic agents (DLAs), represent a novel approach. Exebacase, a novel anti-staphylococcal lysin, recently demonstrated Proof of Concept in Phase 2 with higher clinical responder rates when used in addition to antibiotics vs antibiotics alone for the treatment of *S. aureus* bacteremia/endocarditis. Whereas the therapeutic use of lysins against GN pathogens has previously been precluded by the GN outer membrane (OM) barrier, we recently described lysins designed to penetrate the OM and kill *P. aeruginosa*. We report further characterization of the potent bacteriolytic activities of 4 lead lysins, GN121, GN351, GN370, and GN428, using *in vitro* susceptibility testing formats incorporating human serum or pulmonary surfactant. We furthermore examined killing by fluorescence and TEM.

Material/methods: MICs were determined by broth microdilution in media supplemented with human serum (HuS) or pulmonary surfactant (Survanta). Checkerboard assays and minimal biofilm eradicating concentrations (MBECs) were determined using standard methods. Fluorescence microscopy was performed after LIVE/DEAD staining (ThermoFisher) and TEM was performed using standard methods.

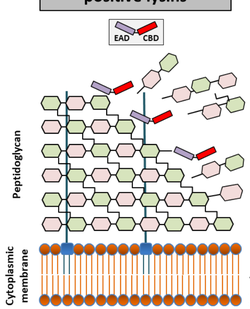
Results: Each anti-pseudomonal lysin demonstrated MIC values of ≤2 µg/mL against clinical isolates in media supplemented with serum or Survanta. The GN lysins also demonstrated synergy with a range of antibiotics (based on fractional inhibitory concentration index (FICI) values of ≤0.5) and potent anti-biofilm activity was observed with MBEC values of ≤2 µg/mL. Rapid bactericidal activity and dissolution of the cell envelope was observed initiating within 5 minutes of treatment using both fluorescence microscopy (LIVE/DEAD staining) and TEM.

Conclusions: These studies demonstrate potent bactericidal activity, synergy with antibiotics, and antibiofilm effects in media containing human biological fluids. This spectrum of activity supports the further development of anti-pseudomonal lysins as potential treatments for serious and life threatening respiratory infections including hospital acquired pneumonia (HAP) and cystic fibrosis exacerbations caused by antibiotic -resistant *P. aeruginosa*.

Objectives

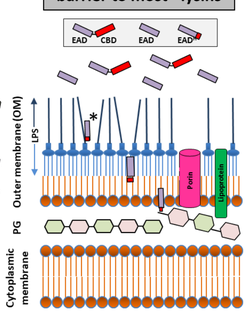
The main technical challenge with developing GN lysins, unlike with lysins targeting Gram-positive pathogens, is the need to penetrate the OM and access subjacent PG substrate in physiological conditions. Four GN lysins were previously identified, in the manner described below, with bacteriolytic activity against *P. aeruginosa* in human blood matrices. Here, we report a more detailed characterization of lysin activity in both human serum and in pulmonary surfactant.

Effective lysis by Gram-positive lysins



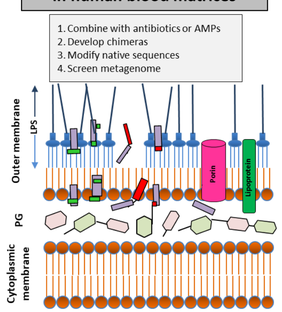
- Gram-positive lysins have an N-terminal enzymatically active domain (EAD) fused to a C-terminal cell wall binding domain (CBD)

Gram-negative OM is a barrier to most* lysins



- Most purified Gram-negative lysins have no antimicrobial activity
- A subset (*) have "intrinsic" activity and are highly active in low ionic strength environments

Challenge: Activate GN lysins in human blood matrices



Identify and characterize native or engineered lysins active in human blood matrices and suitable for study in vitro activity and in vivo efficacy

Potent Activity in Human Serum and Pulmonary Surfactant (Survanta)

MICs (µg/mL) were determined by both microdilution in CAA and CAA supplemented with human serum (12.5%, final concentration) or Survanta (pulmonary surfactant; indicated concentrations). A range of clinical *P. aeruginosa* isolates were used and corresponding meropenem MIC values are shown:

P. aeruginosa strain	Meropenem MIC (µg/mL)	CAA + 12.5% Human Serum MIC (µg/mL)			
		GN121	GN351	GN370	GN428
CFS 1292	32	1	1	2	2
CFS 1557 (PA19)	32	2	4	4	4
CFS 1558 (PA20)	16	0.5	1	0.5	2
CFS 1559 (PA21)	>32	1	2	2	2
CFS 1560 (PA22)	16	1	2	2	2
CFS 1561 (PA23)	16	1	2	2	2
CFS 1562 (PA24)	>32	1	2	2	2
CFS 1766 (ATCC 27853)	1	2	2	4	4
CFS 1539 (PA1)	16	0.5	0.5	1	1
CFS 1540 (PA2)	16	0.5	0.5	1	1
CFS 1541 (PA3)	8	0.5	0.5	1	1
CFS 1596 (PA26)	0.5	0.5	1	1	1
CFS 1597 (PA27)	1	0.5	0.5	0.5	0.5
CFS 1669 (PA41)	<0.25	1	1	2	2
CFS 1674 (PA46)	4	0.5	1	2	2
CFS 1675 (PA47)	4	0.5	0.5	1	1
CFS 1109 (ATCC 17646)	0.5	0.5	1	1	1

GN lysin	CAA MIC (µg/mL)	Fold Change in MIC for CAA + Survanta (%)*						
		25	12.5	6.25	3.125	1.5625	0.7813	0.3906
121	2	4	2	2	2	1	1	1
351	2	2	2	2	1	1	1	1
428	4	4	2	1	1	1	1	1
370	4	4	2	2	1	1	1	1

*determined using *P. aeruginosa* strain CFS 1292

P. aeruginosa strain	Fold Change in MIC for CAA + 6.25% Survanta*			
	GN121	GN351	GN370	GN428
CFS 1292	1	2	1	1
CFS 1557 (PA19)	2	1	0.5	0.5
CFS 1558 (PA20)	2	2	1	1
CFS 1559 (PA21)	2	2	1	1
CFS 1560 (PA22)	2	2	1	1
CFS 1561 (PA23)	1	1	1	1
CFS 1562 (PA24)	2	1	0.5	1
CFS 1766 (ATCC 27853)	1	1	1	2
CFS 1539 (PA1)	1	1	0.5	0.5
CFS 1540 (PA2)	1	1	1	1
CFS 1541 (PA3)	2	2	1	1
CFS 1596 (PA26)	2	2	1	1
CFS 1597 (PA27)	2	1	0.5	0.5
CFS 1669 (PA41)	2	0.5	0.5	0.5
CFS 1674 (PA46)	2	2	0.5	1
CFS 1675 (PA47)	1	0.5	0.5	0.5
CFS 1109 (ATCC 17646)	2	1	1	1

*6.25% corresponds to 1.5 mg/mL phospholipids

- GN lysins are active in the presence of 12.5% human serum against a range of clinical *P. aeruginosa* isolates, including meropenem-resistant forms
- GN lysin activity is not inhibited in the presence of pulmonary surfactant

Synergy with Meropenem in Human Serum

Checkerboard assays were performed in CAA with 12.5% human serum. Mean FICI values were determined (≤0.5 = synergy; >0.5-4 = additive; and >4 = antagonism). A range of clinical *P. aeruginosa* isolates were used.

P. aeruginosa strain	Mean FICI values			
	GN121	GN351	GN370	GN428
CFS 1292	0.292	0.219	0.219	0.219
CFS 1557 (PA19)	0.427	0.292	0.240	0.198
CFS 1558 (PA20)	0.156	0.177	0.109	0.135
CFS 1559 (PA21)	0.229	0.177	0.438	0.396
CFS 1560 (PA22)	0.313	0.323	0.198	0.229
CFS 1561 (PA23)	0.198	0.240	0.240	0.323
CFS 1562 (PA24)	0.214	0.177	0.240	0.198
CFS 1766 (ATCC 27853)	0.229	0.109	0.156	0.156

- GN lysins synergize with meropenem against all *P. aeruginosa* isolates tested in serum
- Each synergistic combination demonstrated reductions of meropenem MICs to below the breakpoint value for meropenem and is consistent with resensitization

Antibiofilm Activity in Human Serum

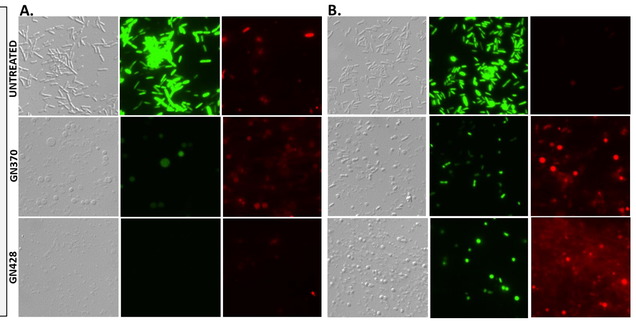
Minimum biofilm eliminating concentrations (MBECs) were determined using CAA supplemented with 12.5% human serum.

- The GN lysins exert a potent antibiofilm effect in the MBEC assay
- MBEC values were similar to those observed in the MIC assay

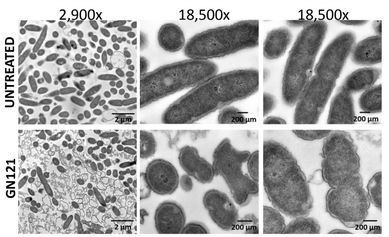
GN lysin	MBEC (µg/mL)
121	0.25
351	0.5
428	1
370	1

Visualization of Rapid Killing in Human Serum and Pulmonary Surfactant

Strain CFS 1292 in (**A**) 6.25% Survanta or (**B**) 100% HuS for 30 min and treated with GN370 or GN428 (10 µg/mL) for 30 min with LIVE/DEAD stain (Sytox Green, live cells; Propidium iodide (red), damaged or dead cells). Bright field and corresponding fluoresence images (10 ms exp.) are shown.



Strain CFS 1292 in 100% HuS for 30 min and treated with GN121 (10 µg/mL) for 15 min before fixation and analysis by SEM



Summary and Conclusions

- The lead GN lysins at ContraFect, including GN121, GN351, GN370 and GN429, are highly active in human serum and pulmonary surfactant, demonstrating antibiofilm effects and synergy with antibiotics in both.
- These findings support the further development of GN lysins as potential therapeutic agents for the treatment of serious, potentially life-threatening respiratory infections caused by antibiotic-resistant *P. aeruginosa*