Synergy between antimicrobial peptides derived from aurein 2.2 and IDR- 1018 and commonly used antibiotics

Lauren A. Walgren[1], Evan F. Haney[2], Robert E.W. Hancock[2], Suzana K. Straus[1]

[1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T 1Z1, lwalgren@chem.ubc.ca, sstraus@chem.ubc.ca; [2] Centre for Microbial Diseases and Immunity Research, Department of Microbiology and Immunology, University of British Columbia, 2259 Lower Mall Research Station, Vancouver, BC, Canada, V6T 1Z4

Abstract: Antibiotic resistance has become a large public health problem due to the frequent and unrestricted use of antibiotics. Thus, there is an urgent need to find novel antibacterial therapeutics or a combination of antibacterial agents to treat antibiotic-resistant bacteria.[1] This study investigated the synergy between two antimicrobial peptides (AMPs) and broad-spectrum antibiotics used against a number of ESKAPE pathogens, namely *P. aeruginosa*, *S. aureus*, *A. baumannii* and *E. faecium*. One of the AMPs, denoted peptide 73, was derived from the natural host defense peptide aurein 2.2 [1,2]. The other AMP (IDR-3002) was derived through in silico quantitative structure-activity relationship (QSAR) models [3]. The minimum inhibitory concentration (MIC) of each AMP was evaluated against each of the 4 strains listed above. Based on the MIC found for each AMP, a checkerboard assay was performed to investigate the synergy between the peptides and antibiotics, as expressed by the fractional inhibitory concentration (FIC). Neither peptide showed synergistic effects with antibiotics when tested against the Gram-positive bacteria (*S. aureus* and *E. faecium*). However, each AMP combined with polymyxin B showed synergistic activity against antibiotic sensitive strains of *P. aeruginosa* and *A. baumannii*. The results will be presented in light of using AMP/antibiotic combinations to combat antibiotic resistance.

Introduction

- Antibiotic-resistance is emerging rapidly worldwide due to the overuse and misuse of antibiotics.
- Antimicrobial peptides (AMPs) are considered to be promising alternatives, because they result in little to no resistance.
- One AMP of interest to our group is aurein 2.2, secreted by the amphibian *Litoria aurea*. In previous work, two potent analogs of aurein 2.2, peptides 73 and 77, were discovered and found to be more active than aurein 2.2 [1,2].
- Peptide IDR-3002 was generated by quantitative structure-activity relationship (QSAR) models and found to have an 8-fold increased antibiofilm potency *in vitro* compared to its parent peptide, IDR-1018 [3].
- More recently, we have developed a version of 73, named peptide L73[4], which incorporates a linker sequence that can be cleaved by enzymes such as matrix metalloproteinases, secreted at sites of bacterial infection.

Peptide	Sequence (all peptides have an amidated C-terminus)
73	R L W D I V R R W V G W L
IDR-3002	I L V R W I R W R I Q W
L73	G P L G V R G K R L W D I V R R W V G W L
73*	V R G K R L W D I V R R W V G W L
3002*	V R G K I L V R W I R W R I Q W

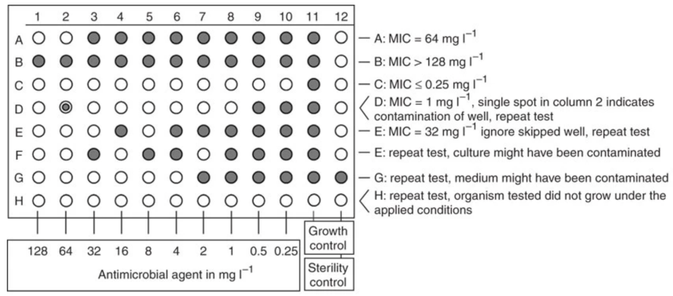
Objectives

- Synthesize peptides 73* and 3002*
- Test the antimicrobial activity of 73* and 3002* against several ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter cloacae)* pathogens
- Test the effect of combining either peptide 73* or 3002* in combination with several broad-spectrum antibiotics against ESKAPE pathogens

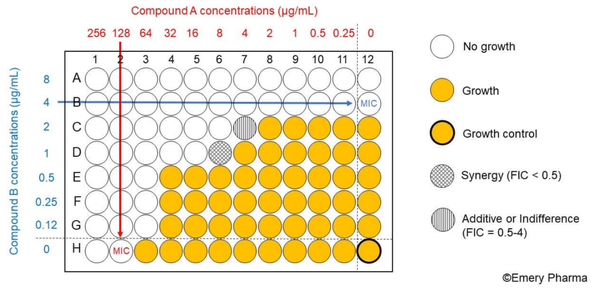
Methods

Peptides 73* and 3002* were synthesized using solid-phase peptide synthesis (CS Bio Co., Menlo Park, CA, USA) using Fmoc chemistry. The crude product was purified using reversed-phase high-performance liquid chromatography (RP-HPLC), using a Phenomenex (Torrance, CA, USA) C4 semi-preparative column (20.0 µm, 2.1 cm × 25.0 cm) on a Waters 600 system (Mississauga, Ontario, Canada). The minimum inhibitory concentration (MIC) of each AMP was then evaluated against *P. aeruginosa*, *S. aureus*, *A. baumannii* and *E. faecium.* The MIC was defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Based on the MIC found for each AMP, a checkerboard assay was performed to investigate the synergy between the peptides and 7 antibiotics, as expressed by the fractional inhibitory concentration (FIC). FIC was calculated using the formula below, where FIC A is the concentration of drug A in a well divided by the MIC of drug A alone, and FIC B is the concentration of drug B in the well divided by the MIC of drug B alone. The combination is considered synergistic when the FIC is less than 0.5, additive or indifferent when the FIC is between or equal to 0.5 and 4, and antagonistic when the FIC is greater than 4.

MIC Assays[5]



Checkerboard Synergy assays[6]



$$FIC = FIC_A + FIC_B$$

$$FIC = \frac{Concentration\ of\ A}{MIC\ A} + \frac{Concentration\ of\ B}{MIC\ B}$$

Peptides 73* and 3002* display antimicrobial activity against Gram-positive and Gram-negative bacteria

	MIC (µg/mL)			
	Gram-positive		Gram-negative	
	S. aureus	*E. faecium*	*P. aeruginosa*	*A. baumanii*
73*	2	16	8	4
3002*	4	32	16	8
Vancomycin	0.125	-	> 64	-
Ciprofloxacin	4.0	-	2	-
Gentamicin	0.25	16	0.125	-
Rifampicin	0.125	-	16	-
Erythromycin	0.5	-	> 64	-
Polymyxin B	0.25	> 128	1	0.5
Meropenem	0.25	-	0.25	-

Polymyxin B displays synergistic activity in combination with 73* and 3002* in *P. aeruginosa and A. baumannii*

	FIC							
	Gram-positive				Gram-negative			
Antibiotic	*S. aureus*		*E. faecium*		*P. aeruginosa*		*A. baumanii*	
	73*	3002*	73*	3002*	73*	3002*	73*	3002*
Ciprofloxacin	2.0	2.0	-	-	2.5	1.5	-	-
Vancomycin	1.5	1.5	-	-	-	-	-	-
Gentamicin	1.5	-	1.5	1.5	1.5	-	-	-
Rifampicin	2.5	-	-	-	1	-	-	-
Erythromycin	2.5	-	-	-	-	-	-	-
Meropenem	1.5	-	-	-	2.25	-	-	-
Polymyxin B	0.75	0.75	-	-	0.375	0.375	0.375	0.3125

Conclusions

- Peptides 73* and 3002* display antimicrobial activity against both Gram-negative and Gram-positive bacteria.
- The combination of either peptide 73* or peptide 3002* with polymyxin B works synergistically against two Gram-negative bacteria *P. aeruginosa* and *A. baumannii.*
- Peptides 73* and 3002* when combined with polymyxin B and other antibiotics in Gram-positive bacteria, *S. aureus* and *E. faecium*, only produced an additive effect. However, the FIC for polymyxin B combined with both peptides was slightly higher than the synergy cut-off (FIC $\leq$ 0.5) in *S. aureus*.
- Other combinations of peptide 3002* and peptide 73* with broad spectrum antibiotics showed no synergistic effect, but none were considered to be antagonistic.
- Further studies will explore the conjugation of polymyxin B and these peptides and will further determine the mechanism of synergy between AMPs and antibiotics.

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References

1.Kumar, P. et al. Antimicrobial Peptide-Polymer Conjugates with High Activity: Influence of Polymer Molecular Weight and Peptide Sequence on Antimicrobial Activity, Proteolysis, and Biocompatibility. ACS Appl. Mater. Interfaces 9, (2017).

2. Raheem, N. et al. Insights into the mechanism of action of two analogues of aurein 2.2. Biochim. Biophys. Acta - Biomembr. 1862, (2020).

3. Haney, E. F. et al. Computer-aided Discovery of Peptides that Specifically Attack Bacterial Biofilms. Sci. Rep. 8, 1–12 (2018).

4. Drayton. M.; Alford M. A.; Pletzer D.; Haney E.F.; Machado Y.; Luo H.D.; Overall C.M.; Kizhakkedath J.N.; Hancock R.E.W.; Straus S.K. Enzymatically Releasable Polyethylene Glycol – Host Defense Peptide Conjugates with Improved Activity and Biocompatibility. *In review at JControlled Release*.**2021.**

5. Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *Nat. Protoc.* **2008**, *3* (2), 163–175. https://doi.org/10.1038/nprot.2007.521.

6. Antimicrobial Synergy Study - Checkerboard Assay. *Microbiology and Cell Biology, Medicinal Chemistry - Emery Pharma*.