School of Biomedical Sciences – Department of Biochemistry and Microbiology

Role of Outer Membrane Impermeability in *Psuedomonas aeruginosa* Resistance to Liptin D7

Jake Romoser, Dr. Franklin Champlin

CENTER FOR HEALTH SCIENCES
OKLAHOMA STATE UNIVERSITY

ABSTRACT

BACKGROUND: Pseudomonas aeruginosa is an opportunistic pathogen that is intrinsically resistant to hydrophobic molecules, yet is susceptive to chemically sensitization to low concentrations of the biocide triclosan using the outer membrane permeabilizer compound 48/80. In order to obtain a better understanding of these properties in P. aeruginosa, it was decided to examine disparate hydrophobic substances thought to also possess antibacterial potential. Researchers working with Dr. Dennis Burns at Wichita State University have recently synthesized and characterized a novel class of picket porphyrins (liptins) and showed them to have antibacterial properties in previous studies.
METHODS: The minimal inhibitory concentrations (MICs) were determined for the model liptin molecule (d7) using a conventional macro-broth dilution bioassay. Pasteurella multocida was included as a reference organism because of its permeable outer membrane. The next steps would have been to repeat the analysis in the presence of outer membrane permeabilizer compound 48/80, as well as to perform disc agar diffusion assays to eliminate the possible inhibitory effects of ethanol.
RESULTS: The liptin d7 did not readily dissolve in absolute ethanol, thereby resulting in a turbid suspension. P. aeruginosa and P. multocida were both found to be resistant to liptin d7 with MICs of 32 and 64 µg/mL, respectively.
CONCLUSION: Because the MICs were so high, both organisms were deemed liptin d7 resistant. We were subsequently informed by our collaborators that the liptin derivative sent to us may have been incorrectly labeled and was in fact a less soluble and less inhibitory derivative than the intended liptin d7. This halted further study of the compound due to an inability to obtain the active form to perform the analyses.

INTRODUCTION

P. aeruginosa is an opportunistic pathogen that is intrinsically resistant to hydrophobic molecules, yet can be chemically sensitized to low triclosan concentrations using the outer membrane permeabilizer compound 48/80. In order to reach a better understanding of these properties in P. aeruginosa, it is helpful to investigate other hydrophobic antibacterial substances that can be used as informational probes. Researchers working with Dr. Dennis Burns at Wichita State University have recently synthesized and characterized a novel class of picket porphyrins (liptins) and showed them to have antibacterial properties in previous studies.

METHODS

- The minimal inhibitory concentrations (MICs) were determined for the model liptin molecule (d7) using a conventional macro-broth dilution bioassays (see dilution scheme).
- The liptin d7 did not readily dissolve in the absolute ethanol, which required the use of an increased amount of ethanol.
- *Pasteurella multocida* was included as a reference organism because of its permeable outer membrane.
- The next steps would have been to repeat the analysis in the presence of outer membrane permeabilizer compound 48/80, as well as to perform disc agar diffusion assays to eliminate the possible inhibitory effects of ethanol.

RESULTS

Pseudomonas aeruginosa

Run No. 1		Run No. 2		Run No. 3		Run No. 4	
Concentration (µg/mL)	Visual Turbidity	Concentration (µg/mL)	Visual Turbidity	Concentration (µg/mL)	Visual Turbidity	Concentration (µg/mL)	Visual Turbidity
64	-	64	-	64	-	64	-
32	-	32	++	32	-	32	-
16	+++	16	+++	16	+++	16	+++
4	+++	4	+++	4	+++	4	+++
2	+++	2	+++	2	+++	2	+++
(+) Control	+++	(+) Control	+++	(+) Control	+++	(+) Control	+++
(-) Control	-	(-) Control	-	(-) Control	-	(-) Control	-
4% ETOH	-	4% ETOH	-	4% ETOH	-	4% ETOH	-

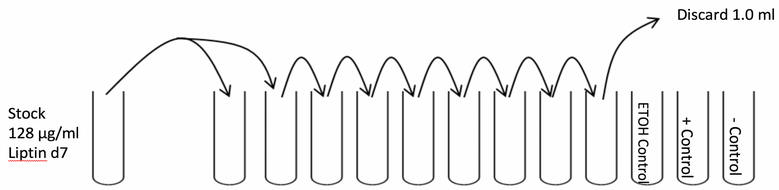
Pasteurella multocida

Run No. 1		Run No. 2		Run No. 3		Run No. 4	
Concentration (µg/mL)	Visual Turbidity	Concentration (µg/mL)	Visual Turbidity	Concentration (µg/mL)	Visual Turbidity	Concentration (µg/mL)	Visual Turbidity
64	-	64	-	64	-	64	-
32	++	32	+++	32	+++	32	+++
16	++	16	+++	16	+++	16	+++
4	++	4	+++	4	+++	4	+++
2	++	2	+++	2	+++	2	+++
(+) Control	+++	(+) Control	+++	(+) Control	+++	(+) Control	+++
(-) Control	-	(-) Control	-	(-) Control	-	(-) Control	-
4% ETOH	-	4% ETOH	-	4% ETOH	-	4% ETOH	-

Susceptibility to liptin d7 results summary

P. aeruginosa		*P. multocida*	
MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
32	>32	64	>64

Dilution scheme for macro-broth dilution bioassay



Tube:	1	2	3	4	5	6	7	8	9	10	11	12
Diluent (ml):	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.92	1.0	2.0
Antibiotic (µg/ml):	64	32	16	8	4	2	1	0.5	0.25	0	0	0
Inoculum (ml):	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0
4% ETOH (µl):	0	0	0	0	0	0	0	0	0	100	0	0

SPECIFIC AIMS

- Determine minimal inhibitory and bactericidal concentrations for the model liptin molecule (d7) using conventional macro-broth dilution bioassays
- Repeat the same bioassays in the presence of outer membrane permeabilizer compound 48/80

CONCLUSION

- Both *P. aeruginosa* and *P. multocida* appeared to be resistant, which could be due to the compound losing potency over time.
- Because there was no visible growth in the ethanol control tubes, it cannot be determined whether the ethanol, liptin, or a combination of both was causing the inhibition.
- Because the MICs were so high, both organisms were deemed liptin d7 resistant.
- We were subsequently informed by our collaborators that the liptin derivative sent to us may have been incorrectly labeled and was in fact a less soluble and less inhibitory derivative than the intended liptin d7.

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