Use of Rational Design and Artificial Intelligence to Improve the Therapeutic Potential of Antimicrobial Peptides.

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INTRODUCTION & AIM

The objective of our work is to modify the residues of Lynronne family of AMPs, originally isolated from cow rumen [1], to further improve their antimicrobial activity while reducing their toxicity against human cells. For this and keeping in mind that the nature and number of cationic residues can impact activity and/or toxicity, we designed, synthesized, and purified Lynronnes analogues containing the same number of cationic charges but changing the type of cationic residues.

Peptides were tested in terms of i) antimicrobial activity (Minimum Inhibitory Concentration) on ESKAPE bacteria and ii) toxicity against human cells.

In addition, new sequences were predicted using MRL (Molecular Reinforcement Learning) based on Lynronne properties (cationic charges and hydrophobicity).

METHOD

Lynronne	Sequence
L1 (L/D)	LPRRNRWSKIWKKVVTVFS-NH_2
L1R	LPRRNRWSRIWRRVVTVFS-NH_2
L1K	LPKKNKWSKIWKKVVTVFS-NH_2
L1I	LPKKNKWSRIWRRVVTVFS-NH_2
L1H	LPHHNHWSHIWHHVVTVFS-NH_2
L2 (L/D)	HLRRINKLLTRIGLYRHAFG-NH_2
L2R	RLRRINRLLTRIGLYRRAFG-NH_2
L2K	KLKKINKLLTKIGLYKKAFG-NH_2
L2I	HLKKINRLLTKIGLYKHAFG-NH_2
L2H	HLHHINHLLTHIGLYHHAFG-NH_2
L3 (L/D)	NRFTARFRRTPWRLCLQFRQ-NH_2
L3K	NKFTAKFKKTPWKLCLQFKQ-NH_2
L3H	NHFTAHFHHTPWHLCLQFHQ-NH_2

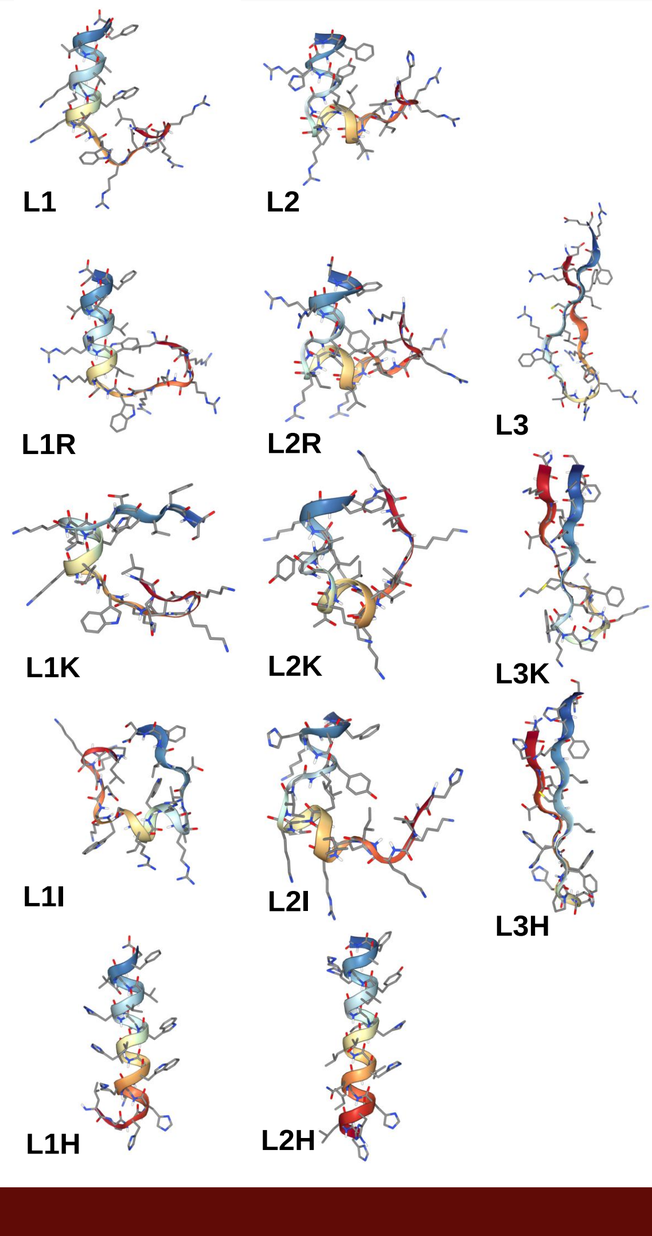

Figure 1 : Sequences and 3D structures predicted in aqueous solution of Lynronne-1,2,3 and analogues. Structures were obtained using PEP-FOLD4, a *de novo* approach aimed at predicting peptide structures from amino acid sequences.

RESULTS & DISCUSSION

MIC (µg/mL)	Gram +			Gram -				
	E. faecalis	*E. faecium*	*S. aureus*	*A. baumannii*	*E. cloacae*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
L1	62,5 (125)	31,25 (62,5)	7,8 (7,8)	3,9 (3,9)	62,5 (62,5)	3,9 (3,9)	3,9 (3,9)	7,8 (7,8)
L1D	7,8 (7,8)	3,9 (7,8)	7,8 (7,8)	3,9 (3,9)	31,25 (31,25)	3,9 (3,9)	7,8 (7,8)	7,8 (15,6)
L1R	31,25 (62,5)	31,25 (31,25)	15,6 (15,6)	7,8 (7,8)	31,25 (31,25)	3,9 (3,9)	3,9 (3,9)	15,6 (15,6)
L1K	125 (250)	125 (250)	7,8 (7,8)	3,9 (3,9)	62,5 (62,5	1,9 (1,9)	3,9 (3,9)	7,8 (7,8)
L1I	62,5 (125)	31,25 (62,5)	7,8 (7,8)	3,9 (3,9)	31,25 (31,25)	1,9 (1,9)	1,9 (1,9)	7,8 (7,8)
L1H	>500	>500	>500	>500	>500	>500	>500	>500
L2	>500	>500	125 (125)	3,9 (7,8)	250 (500)	7,8 (7,8)	3,9 (3,9)	7,8 (15,6)
L2D	3,9 (7,8)	3,9 (7,8)	31,25 (31,25)	3,9 (3,9)	31,25 (62,5)	1,9 (1,9)	7,8 (7,8)	7,8 (15,6)
L2R	250 (250)	250 (250)	31,25 (31,25)	3,9 (3,9)	250 (250)	3,9 (3,9)	1,9 (3,9)	7,8 (15,6)
L2K	>500	>500	500 (500)	7,8 (31,25)	>500	0,95 (0,95)	31,25 (31,25)	3,9 (3,9)
L2I	>500	>500	500 (500)	3,9 (125)	>500	1,9 (3,9)	3,9 (3,9)	15,6 (15,6)
L2H	>500	>500	>500	>500	>500	250 (250)	>500	>500
L3	62,5 (125)	31,25 (62,5)	62,5 (62,5)	31,25 (125)	125 (500)	31,25 (62,5)	31,25 (125)	62,5 (250)
L3D	31,25 (62,5)	31,25 (31,25)	31,25 (31,25)	31,25 (31,25)	31,25 (125)	15,6 (15,6)	31,25 (62,5)	62,5 (125)
L3K	62,5 (62,5)	62,5 (62,5)	62,5 (125)	15,6 (31,25)	250 (>500)	31,25 (31,25)	31,25 (62,5)	62,5 (125)
L3H	>500	>500	>500	>500	>500	>500	>500	>500

Table 1 : Antimicrobial activities of Lynronne-1,2,3 and analogues. Results are expressed as Minimum Inhibitory Concentration (MIC) in µg/mL. Minimum Bactericidal Concentration (MBC) were determined using resazurin. The color gradient indicates changes in the antimicrobial activity of analogues with green and red indicating an improved or decreased activity compared to parent peptide.

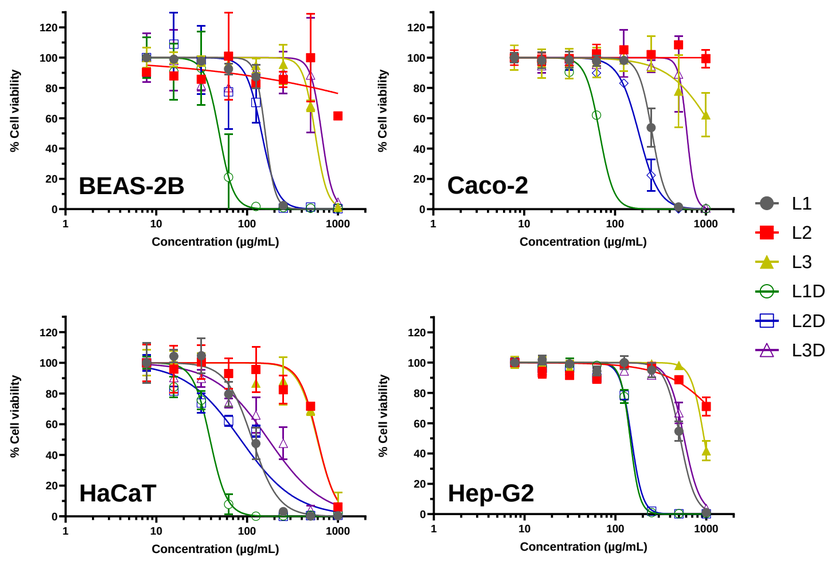

Figure 2 : Innocuity testing of Lynronne-1,2,3 and analogues. Human cells corresponding to the lung (BEAS-2B), the intestine (Caco-2), the skin (HaCaT), and the liver (Hep-G2) were exposed for 48h to increasing concentrations of peptides before measurement of the cell viability using resazurin. Results are expressed as means +/- SD (n=3).

	CC_{50} (µg/mL)				MIC range	SI range
	BEAS-2B	Caco-2	HaCaT	Hep-G2		
L1	159±7	256±5	114±6	516±9	3,9 - 62,5	1,8 - 132
L1D	49±4	68±1	39±2	145±6	3,9 - 31,25	1,6 - 37
L1R	228±5	296±11	113±7	508±13	3,9 - 31,25	3,6 - 130
L1K	231±6	297±10	146±5	398±26	1,9 - 125	1,2 - 209
L1I	216±7	272±25	149±8	445±31	1,9 - 62,5	2,4 - 234
L1H	>1000	>1000	>1000	>1000	>500	n.a
L2	>1000	>1000	599±41	>1000	1,9 - >500	1,2 - 526
L2D	145±14	183±6	85±10	150±6	1,9 - 31,25	2,72 - 96
L2R	590	546	354±13	625±31	1,9 - 250	1,4 - 329
L2K	>1000	>1000	>1000	>1000	0,95 - >500	2 - 1052
L2I	>1000	>1000	877±62	>1000	1,9 - >500	1,7 - 526
L2H	>1000	>1000	>1000	>1000	>500	n.a
L3	560±26	>1000	592±33	946±20	31,25 - 125	4,48 - 32
L3D	662±101	619±131	171±16	570±18	15,6 - 62,5	2,7 - 42
L3K	432±41	568	475±71	583±30	15,6 - 250	1,7 - 37
L3H	>1000	>1000	>1000	>1000	>500	n.a

Table 2 : Cytotoxic concentrations (CC_{50}) and Therapeutic Indexes (TI) of peptides determined from Figure 2. with green and red indicating an improved or decreased activity/safety compared to parent peptide.

CONCLUSION

- 16 peptides were synthesized, purified, and characterized in term of biological activities.
- Differences were observed in the antibacterial activity of the different analogs, with all-histidine analogs showing no activity. A decrease in cytotoxicity was observed in most cases with the analogues.
- A more in-depth study including the investigation of the mechanism of action of Lynronnes derivatives will allow to better understand the role of cationic residues in biological activities.
- New sequences were generated and will be soon tested in terms of antimicrobial activities and toxicity.

FUTURE WORK

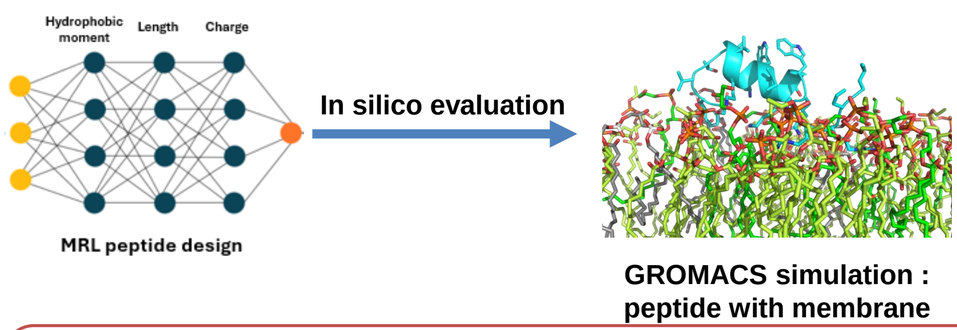

Figure 3 : Use of MRL (Molecular Reinforcement Learning) to generate new sequences based on the properties of Lynronne-1. The sequences were first evaluated by GROMACS before being synthesized and evaluated *in vitro*

[1] Oyama, L. B.; Girdwood, S. E.; Cookson, A. R.; Fernandez-Fuentes, N.; Privé, F.; Vallin, H. E.; Wilkinson, T. J.; Golyshin, P. N.; Golyshina, O. V.; Mikut, R.; Hilpert, K.; Richards, J.; Wootton, M.; Edwards, J. E.; Maresca, M.; Perrier, J.; Lundy, F. T.; Luo, Y.; Zhou, M.; Hess, M.; Mantovani, H. C.; Creevey, C. J.; Huws, S. A. The Rumen Microbiome: An Underexplored Resource for Novel Antimicrobial Discovery. npj Biofilms Microbiomes 2017, 3, 1–9. doi : 10.1038/s41522-017-0042-1.