Identification and engineering of a human cathelicidin peptide LL-37mini as a novel antibiotic

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Introduction

Antimicrobial peptides (AMPs) emerge as important candidates for developing new antibiotics against multidrug-resistant (MDR) pathogens. LL-37 is the most widely investigated form of human cathelicidin peptides (*https://aps.unmc.edu*) . Over the past two decades, our lab has demonstrated the antimicrobial activities of various fragments of LL-37 namely SK-24[1], FK-16[2], and KR-12[3]. Although widely utilized, antimicrobial screening in Mueller Hinton broth (MHB) is not ideal as it could mask activity of some candidates such as human cathelicidin LL-37. This poster demonstrated the excellent antibacterial activity of LL-37 against methicillin-resistant Staphylococcus aureus (MRSA) using diluted MHB. Also, based on the screening findings of a small library of ultrashort peptides (≤ 10 amino acids) covering the entire LL-37 sequence, we designed a potent ultra-short peptide (LL-37mini) demonstrating remarkable activity against MDR bacteria including MRSA biofilms without developing resistance.

Methods

- **Antimicrobial assays** were conducted using the broth microdilution method and MIC was determined via a ChroMate Microplate Plate Reader after incubating for 24 hours at 37 ℃.
- **Toxicity assay**[4]**:** Hemolytic assay was done using human red blood cells (2%) after 1 h incubation at 37 ℃. HaCaT cell toxicity was performed using the MTT cell proliferation assay kit.

Results

I. Search a medium for anti-MRSA activity of LL-37

Table 1: Antimicrobial activity (μM) of LL-37 and 17BIPHE2 in different media

		S. aureus USA300		*E. coli* E423-17	
Media		LL-37	17BIPHE2	LL-37	17BIPHE2
MHB	100%	> 32	4	8	2-4
	50%	32	4	4-8	2
	25%	8	4	4	4
	12.5%	4	≤2	2-4	2-4
TSB	100%	>32	2-4	8	4-8
	50%	>32	≤2	4-8	4
	25%	>32	≤2	4	2
	12.5%	>32	≤2	≤2	≤2
LB	100%	>32	≤2	4	2-4
	50%	>32	≤2	4-8	2-4
	25%	>32	4	4	2
	12.5%	>32	4	≤2	≤2

✓ LL-37 inhibited the growth of MRSA with the dilution of MHB, but not the dilution of Tryptic Soy Broth (TSB) nor Luria-Bertani (LB).

II. Screening ultrashort active LL-37 fragments

Figure 1. Antibacterial screening of ultrashort LL-37 fragments in 12.5% MHB led to the identification of KR-8 and RIK-10

Name	Amino acid sequence	EC12.5%	EC100%	SA12.5%	SA100%
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	4	8	2	>64
KR-12	KRIVQRIKDFLR	2	32	2	>64
LL-10	LLGDFFRKSK	32-64	>64	>64	>64
KE-10	KEKIGKEFKR	>64	>64	>64	>64
LR-10	LRNLVPRTES	>64	>64	>64	>64
RK-9	RKSKEKIGK	64	>64	>64	>64
RIK-10	RIKDFLRNLV	2	16	16	>64
KR-8	KRIVQRIK	8	>64	>64	>64
FK-16	FKRIVQRIKDFLRNLV	4	4-8	4	4

EC12.5%: anti-*E. coli* assay in 12.5% MHB, SA12.5% : anti-*S. aureus* assay in 12.5% MHB. Likewise, 100% means rich MHB without dilution. Peptide activity in μM

✓ In 100% MHB, all the peptides (except FK-16) did not display any activity against *S. aureus* USA300 but FK-16 and RIK-10 were active against *E. coli.*

✓ In 12.5% MHB, KR-12, RIK-10, and FK-16 became active against *S. aureus* USA300, while FK-16, KR-12, RIK-10 and, **KR-8** (**eight residue peptide**) displayed activity against *E. coli.*

III. KR-8 based peptide design

Table 2. Peptide designing based on the shortest active template KR-8 and antibacterial activity against ESKAPE pathogens

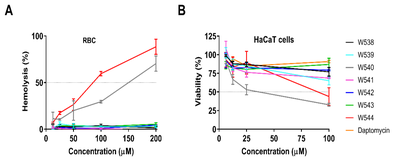
Peptide	Amino acid sequence	Q	Pho	*S. aureus* USA300	*E. coli* E423-17	*P. aeruginosa* E416-17	*K. pneumoniae* E406-17	*A. baumannii* B28-16
W538	KRIVQRIK	+5	38%	>32	>32	>32	>32	>32
W539	KRIWQRIK	+5	38%	>32	>32	>32	>32	>32
W540	KRIWQRWK	+5	38%	>32	>32	>32	>32	>32
W541	KRWWQRWK	+5	38%	>32	8	>32	>32	>32
W542	KRWWQWWK	+4	50%	8	4	4-8	>32	32
W543	RRWWRWWR	+5	50%	4-8	8	8	>32	>32
W544	RRWWRWWL	+4	63%	4	4	4	8-16	8-16

. Q: net charge; Pho: hydrophobic content; W538 = KR-8; MIC values are in μM.

✓ The W542, W543 and W544 peptides were active against *S. aureus* USA300, *E. coli* and *P. aeruginosa*, while W541 was active only against *E. coli.*

IV. Toxicity and antimicrobial robustness assay of the designed peptides

Figure 2. Cytotoxicity of designed peptides in (A) human red blood cells (RBCs) and (B) HaCaT cells



✓ W543 (named **LL-37mini)** was least hemolytic and toxic to HaCaT cells and hence, it was selected as a candidate for additional studies.

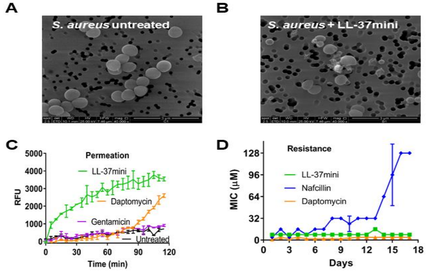
Table 3. Antimicrobial robustness of LL-37mini and antibiotics against *S. aureus* USA300 in MHB (MIC values are in μM, ND: Not determined).

Peptide/ Antibiotic	pH 7.2	pH 6.3	NaCl (150 mM)	10% Serum	20% Serum
LL-37mini	8	16	8	64	ND
17BIPHE2	4	16	4	>64	ND
Daptomycin	1	1	1	1	1
Amikacin	8	32	32	32	32-64

✓ Both acidic pH and physiological salts had a minimal impact on the activity of LL-37mini. However, 10% human serum reduced the activity of by 8-fold.

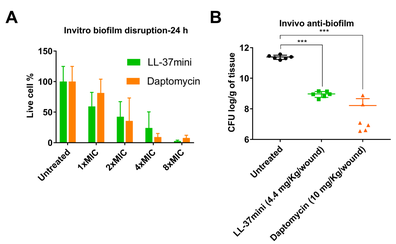
V. Antibacterial and antibiofilm activity of LL-37mini

Figure 3. Mechanism of killing and resistance development of LL-37mini in *S. aureus* USA300 .



✓ LL-37mini caused MRSA membrane damage and cell lysis.
✓ Multiple passage experiment showed no change in the MIC values of LL-37mini after 16 passages.

Figure 4. Antibiofilm activity of LL-37mini against *S. aureus* USA300 in vitro and in vivo.



✓ Dose-dependent effect of LL-37mini was observed against the 24 h preformed biofilms in vitro (A) .
✓ LL-37mini caused a 2 log decrease in bacterial CFU compared to the untreated control (B).

Conclusions

1. This poster has demonstrated that a diluted MHB medium enabled the observation of antibacterial and antibiofilm activity of human cathelicidin LL-37.
2. The ultra-short Peptide LL-37mini engineered based on the KR-8 template showed in vitro and in vivo efficacy and was selective.
3. LL-37mini constitutes a novel lead for developing new antimicrobial and antibiofilm agents.
4. The diluted medium obtained here may be useful for initial antimicrobial screen to identify antimicrobials from natural sources or artificial libraries.

References

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