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Apidaecins: Linear Peptide Antibiotics for the Treatment of Serious Gram-Negative Infections

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Background

Apidaecins are linear peptide antibiotics (LPAs) with a novel mechanism of action that are aimed at the treatment of serious, drug-resistant Gram-negative infections. Apidaecin 1b, isolated from *Apis melifera* (honeybee), is a linear, proline-rich antimicrobial peptide. Unlike most other classes of antimicrobial peptides (AMPs), apidaecins selectively enter bacterial cells without membrane disruption. As a result, they exhibit no mammalian cytotoxicity or hemolytic activity. Apidaecins exert their antibacterial activity through inhibition of protein translation, by binding to the exit tunnel of existing 70S bacterial ribosome which blocks the dissociation of release factors and by preventing the assembly of additional bacterial ribosome [1,2,3].

Material/Methods

Peptides were synthesized by Fmoc/tBu-chemistry and purified by RP-HPLC. Peptides were incubated with human bronchoalveolar lavage (BAL, pooled from five males), serum and plasma, respectively, at a total concentration of 75 µg/mL under gentle shaking at 37°C. After precipitation of the proteins in the serum samples in the presence of trichloroacetic acid (TCA; 3% w/v, final concentration), the remaining intact peptide amount was quantified using reversed-phase chromatography (RPC). Degradation products were identified with mass spectrometry. For plasma and human BAL, solid phase extraction (SPE) cartridges from Waters (HLB prime) were utilized. Minimal inhibitory concentration was determined according to CLSI standard methods using 25% cation-adjusted Muller-Hinton broth (MHB II).

Table 1. Half-life time (hours) in different mammalian serums, EDTA plasma, and human bronchoalveolar lavage (BAL).

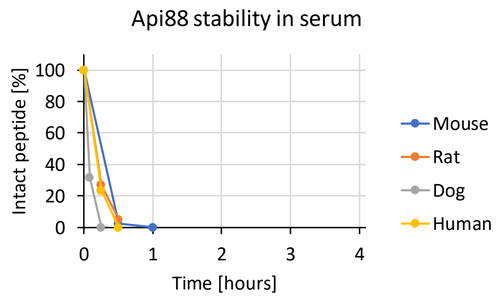
Name	Peptide sequence*	Mouse		Rat		Beagle dog		Human		Human BAL	Ref.
		Serum	Plasma	Serum	Plasma	Serum	Plasma	Serum	Plasma		
Api 1b	GNNRPVYIPRPQPPHPRL-OH	4.1	>4	7.2	>4	n.d.	>4	>4	>4	n.d.	[4]
Api88	gu-ONNRPVYIPRPRPPHPRL-NH_2	0.06	0.5	0.2	2.3	0.06	>4	0.1	>4	>2	[4]
Api137	gu-ONNRPVYIPRPRPPHPRL-OH	5.6	>4	>6	>4	>4	>4	>4	>4	>20	[4]
Api755	gu-OIORPVYOPRPRPPHPRL-OH	4.5	2.4	n.d.#	>4	n.d.	>4	>4	>4	n.d.	[5]
Api795	gu-OIOIORPVYOPRPRPPHPRL-OH	5.5	3.4	>6	>4	n.d.	>4	>4	>4	n.d.	[6]

*gu, O, r denote N,N,N´,N´-tetramethylguanidin, L-ornithine, and D-arginine, respectively. # n.d. = not determined

Table 2. MIC (mg/L) profile[a] of exemplar compounds vs. panel 1 strains.

Name	*E. coli* (n=8)	*K. pneumoniae* (n=6)	*A. baumannii* (n=5)	*P. aeruginosa* (n=.5)
Api88	1	0.5	64	>64
Api137	2	1	64	>64
Api795	1	1	16	8

[a] mean MICs in 25% diluted MHB II across n strains tested



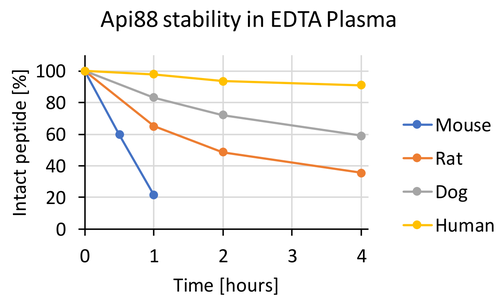


Figure 1. Degradation curves of LPA after incubation in plasma and serum. Peptides were analyzed using RPC and peak areas were normalized to time point 0h. Mouse and rat matrices were obtained from PAA Laboratories and Innovative Research. Dog and human matrices were pooled from five beagle dogs and three human male volunteers, respectively.

Table 3. MIC[a] data (mg/L) vs. panel 2 multi-drug resistant strains of *E. coli* (*Eco*) and *K. pneumoniae* (*Kpn*).

Species	Strain	Comment	Api88	Api137	Api795
Eco	ATCC 25922	Wild type	1	1	1
Serum effect on MICs					
Eco	ATCC 25922	50% Serum	8	2	0.5
Effect of SbmA mutant					
Eco	EN453	ΔSbmA	4	8	1
Colistin-R clinical isolates					
Eco	EN482	MCR-1 (>4)	0.5	0.5	1
Eco	EN485	MCR-1 (>4)	0.5	0.5	2
Kpn	EN498	ColR (>16)	1	2	4
Kpn	EN249	ColR (>16)	0.5	1	2
Kpn	EN499	ColR (>16)	0.25	1	2
Strains resistant to ribosome-targeting antibiotics					
Eco	EN135	MDR	8	16	4
Eco	EN790	rmtC/floR	0.25	0.5	1
Eco	EN1107	rmtB/erm(B)	0.25	1	1
Eco	CH6504	tet(M)	0.25	0.5	2
Eco	EN531	erm(C)	1	4	2
Eco	EN1077	armA/rmtB	≤0.125	≤0.125	0.5
Eco	EN1111	armA	1	0.5	1
Kpn	EN1115	rmtf	0.25	0.25	0.5

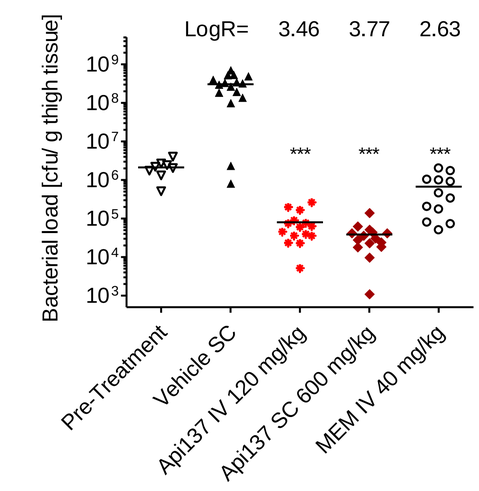


Figure 2. Api137 reduces cfu burden by ~3.8 log units in E. coli ATCC 25922 thigh infection model. Dosing IV = 6 x 20 mg/kg (52 µmol/kg total); SC = 6 x 100 mg/kg (262 mmol/kg total); MEM = meropenem (104 µmol/kg total)

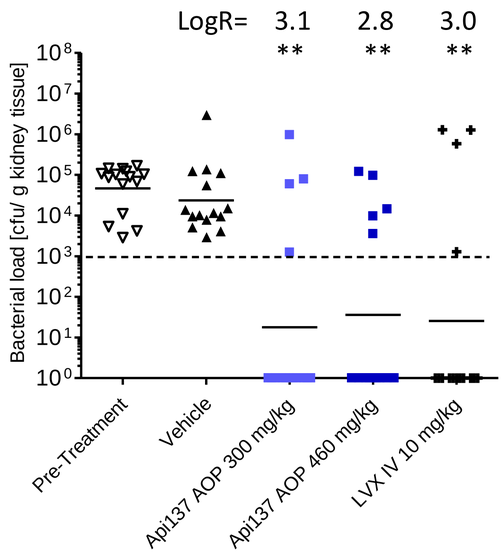


Figure 3. Api137 significantly reduces kidney burden of *E. coli* 1527 in ascending UTI model. Bacteria (7 x 10^7 cfu/mice) were administered via intra-urethral injection. ALZET® osmotic pumps were implanted at 24 and 48 h post infection delivering Api137 at 19.2 and 12.8 mg/kg/h. Levofloxacin (LVX) was administered BID for a total daily dose of 10 mg/kg. Viable cells were analyzed after 76 h.

References

[1] Krizsan A. 2014 *Angewandte Chemie Int. Ed.*; [2] Krizsan A. 2015 *ChemBioChem*; [3] Florin T. 2017 *Nat. Struct. Mol. Biol.*; [4] Berthold N. 2013 *AAC*; [5] Bluhm M.E.C. 2015 *EJMC;* [6] Bluhm M.E.C. et al 2017 *Frontiers*

Results and Conclusion

MICs across a set of Gram- strains including MDR, efflux-defective, and colistin-resistant strains are quite promising. In the limited studies undertaken we have seen correlation between MICs in 25% diluted medium and in vivo efficacy. In addition, the effect of serum on the MICs is minimal, there is only a small MIC shift in a SbmA knockout *E. coli* strain, and there is no evidence of cross-resistance with strains resistant to ribosome-targeting antibiotics. Finally, the proteolytic stability was higher overall in non-rodent matrices and greatest in human. Taken together with previously reported encouraging in vivo results (in sepsis, thigh infection, and urinary tract infection (UTI) models), this further in vitro characterization of apidaecins supports their continued development as therapeutics, initially for complicated UTIs with a view toward expansion into complicated intra-abdominal infections and ventilated-pneumonias.

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