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Engineering the Antibiotics of Tomorrow

Evaluation of Proline-rich antimicrobial Peptides for Treatment of Ventilator-Associated Pneumoniae

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BACKGROUND

Proline-rich antimicrobial peptides from insects are predominantly active against Gram-negative Enterobacteriaceae by inhibiting protein translation due to interactions with ribosomal protein complexes without lysing the cells [1]. Recently, we also expanded the activity spectrum to non-fermenters like *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (apidaecin peptides) and Gram-positive *Staphylococcus aureus* (oncocin peptides) [2,3]. Oncocins bind to the protein exit tunnel of matured 70S ribosomes blocking and destabilizing the initiation complex. Apidaecins appear to use two different mechanisms i) interfering with the assembly of the 50S subunit leading to inconvertible 50S particles and ii) binding to the exit tunnel trapping release factors RF1 and RF2.

Promising derivatives of both families showed high in vivo efficacy against *E. coli* and *K. pneumoniae* (including antibiotic resistant strains) in different murine infection models (intraperitoneal sepsis and thigh abscess). In the current project we evaluate the potential of PrAMPs for treatment of serious drug-resistant lung infections starting with assessing the stability of the PrAMPs against proteases present in the lung, serum, and plasma. Potential cleavage site will be stabilized by amino acid exchange and the antimicrobial activity of optimized derivatives will be tested.

MATERIAL/METHODS

Peptides were synthesized by Fmoc/^{t}Bu-chemistry and purified by RP-HPLC to >90% purity and the product was confirmed by mass spectrometry. Lyophilized peptides were reconstituted in water at 3 g/L. Peptides were incubated with human bronchoalveolar lavage (hBAL) and murine and rat serum, respectively, at a total concentration of 75 µg/mL under gently shaking at 37°C. After precipitation of the proteins in the presence of trichloroacetic acid (3% w/v, final concentration), the remaining intact peptide amount was quantified using reversed-phase chromatography. Degradation products were identified with mass spectrometry. For hBAL, solid phase extraction cartridges from Waters (HLB prime) were utilized. Samples of hBAL (45 µL) were diluted with aqueous phosphoric acid (4% v/v; 955 µL) and loaded on solid phase extraction cartridges (HLB prime; Waters). After washing with 0.1% aqueous trifluoroacetic acid (TFA, 1 mL), peptides were eluted with 60% aqueous acetonitrile containing 0.1% TFA (1 mL) and analyzed using HPLC

Table 1. Peptide stability in murine and rat serum.

Name	Peptide sequence	Stability in serum (half life time/hours)		Factor Rat/ Mouse	Stability in human BAL (half life time/ hours)	Ref.
		Rat	Mouse			
Onc18	VDKPPYLPRPRPPRRIYNR-NH_2	1.6	0.4	4	>2	[4]
Onc72	VDKPPYLPRPRPPROIYNO-NH_2	6.3	2.9	2	>20	[4]
Onc112	VDKPPYLPRPRPPRrIYNrNH$_2$	> 6	>8	~1	>20	[4]
Onc143	VRKPPYLPRPRWPRRIYNR-NH_2	0.5	n.d.	n.a.	0.4	[3]
Onc158	VRKPPYLPRPRWPROIYNO-NH_2	1.7	1.6	1	<0.5	[3]
Onc166	VrKPPYLPRPRWPRrIYNr-NH_2	7.2	2.7	3	15	[3]
Onc223	VRKPPYLPRPRWPRRIYNR-OH	< 0.5	n.d.	n.a.	0.2	[3]
Api88	gu-ONNRPVYIPRPRPPHPRL-NH_2	0.2	0.08	3	>2	[5]
Api137	gu-ONNRPVYIPRPRPPHPRL-OH	> 6	5.8	>1	>20	[5]

*gu, O, r denote N,N,N´,N´-tetramethylguanidin, L-ornithine, and D-arginine, respectively.

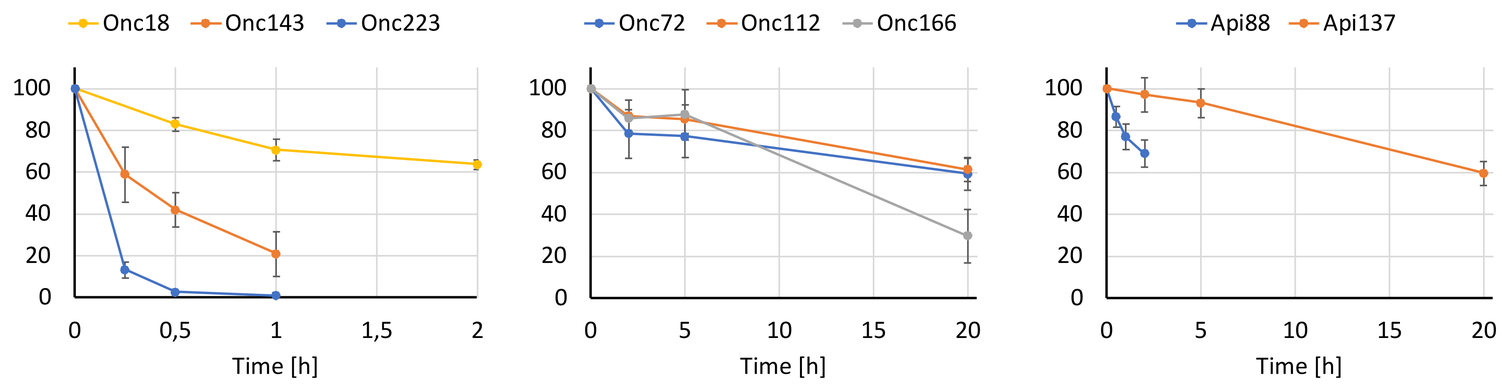

Figure 1. Degradation curves of PrAMPs after incubation in pooled human BAL (n=5). Peptides were analyzed after solid phase extraction using UV-HPLC and peak areas were normalized to time point 0h.

RESULTS

Stability of PrAMPs against mammalian proteases have usually been in murine serum and published along with optimized sequences (see References). The stability in rat serum was evaluated here for the first time and showed around three-fold higher half life times than in mouse serum (Table 1). Sampling of BAL from mouse yields only around 0.5 mL murine BAL. Thus, for this study human BAL available at higher amounts was pooled from five individuals with healthy lungs. When incubated in this matrix, three peptides were remarkably stable and more than 50% Onc72, Onc112, and Api137 remaining after 20 hours (Figure 1). Similarly to serum, L-ornithine and D-arginine on position 15 and 19 stabilized the oncocins against proteolytic degradation whereas tryptophane on position 12 installed an additional cleavage site leading to lower Stabilities for Onc143 and Onc223.

CONCLUSION

Apidaecin peptides show remarkable stability against proteases in human lungs and from this point of view seem to be applicable as an inhalation therapy without further modification. Oncocin peptides containing Trp-12, which improves activity against *S. aureus*, showed additional degradation sites N-terminal to this residue. Higher stabilities in rat serum indicates that in vivo efficacy in lung infection models should be studied in rats.

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