Linear Peptide Antibiotics (LPAs) as an innovative approach to fight antimicrobial resistance

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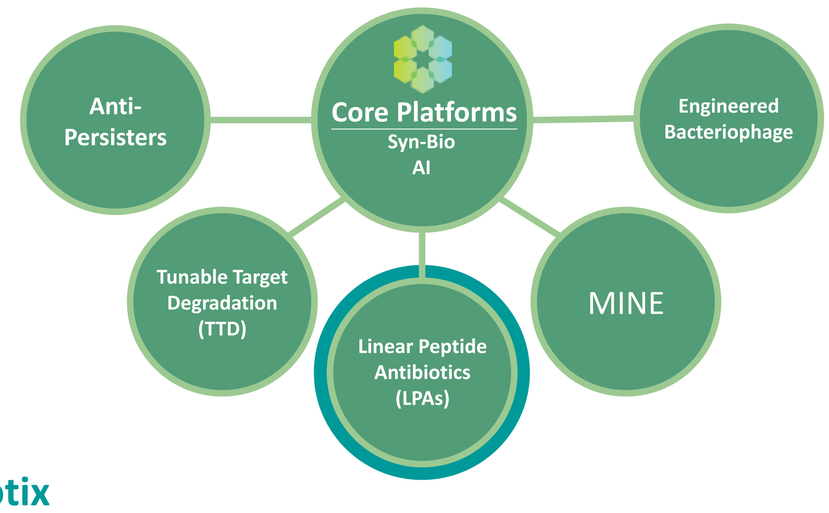

About EnBiotix

EnBiotix is an engineered antibiotics company utilizing novel machine-learning systems and synthetic biology technologies to enable the devel-opment of both new antibiotics and potentiators of existing antibiotics, with the potential to transform antibiotic spectrum of activity and resis-tance profile. With antibiotic-resistant and -tolerant infections rapidly be-coming a global health crisis, EnBiotix's robust product pipeline addresses a wide range of acute and chronic infections to significantly impact the lives of patients.

Background

The extensive spread of antimicrobial resistance continues to be a growing health risk. Proline-rich antimicrobial peptides (PrAMPs) are potentially new drugs to treat AMR. PrAMPs are derived from naturally occurring peptides from a wide range of organisms. They constitute an important component of the innate immune system. Apidaecins and oncocins isolated originally from insects are part of EnBiotix LPA platform. LPAs are predominantly active against *Enterobacteriaceae*, and act, without lysing the cells, by inhibiting protein translation due to interactions with ribosomal protein complexes without lysing the cells. Oncocins bind to the protein exit tunnel of matured 70S ribosomes blocking and destabilizing the initiation complex [1]. Apidaecins appear to use two different mechanisms i) interfering with the assembly of the 50S subunit leading to inconvertible pre50S particles and ii) binding to the exit tunnel trapping release factors RF1 and RF2 [2, 3]. 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).

Objectives

Proteolytic stability of therapeutic peptides is a dominant concern in the development of peptide-based drugs. Here we studied the stability in different blood derived matrices of human, dog, mouse, and rats as well as in human bronchoalveolar lavage (hBAL). Identification of proteolytic labile peptide bonds allows for modification of the adjacent amino acid residues to yield derivatives with potent pharmacokinetics.

Table 1. Peptide sequences and half-life times in murine and rat serum and human BAL.

Name	Peptide sequence	Stability in serum ($t_{½}$/ hours)		Factor Rat/ Mouse	Stability in human BAL ($t_{½}$/ 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	VDKPPYLPRPRPPRrIYNr-NH_2	> 6	>8	~1	>20	[4]
Onc143	VRKPPYLPRPRWPRRIYNR-NH_2	0.5	n.d.	n.a.	0.4	[5]
Onc158	VRKPPYLPRPRWPROIYNO-NH_2	1.7	1.6	1	<0.5	[5]
Onc166	VrKPPYLPRPRWPRrIYNr-NH_2	7.2	2.7	3	15	[5]
Onc223	VRKPPYLPRPRWPRRIYNR-OH	< 0.5	n.d.	n.a.	0.2	[5]
Api88	gu-ONNRPVYIPRPRPPHPRL-NH_2	0.2	0.08	3	>2	[6]
Api137	gu-ONNRPVYIPRPRPPHPRL-OH	> 6	5.8	>1	>20	[6]

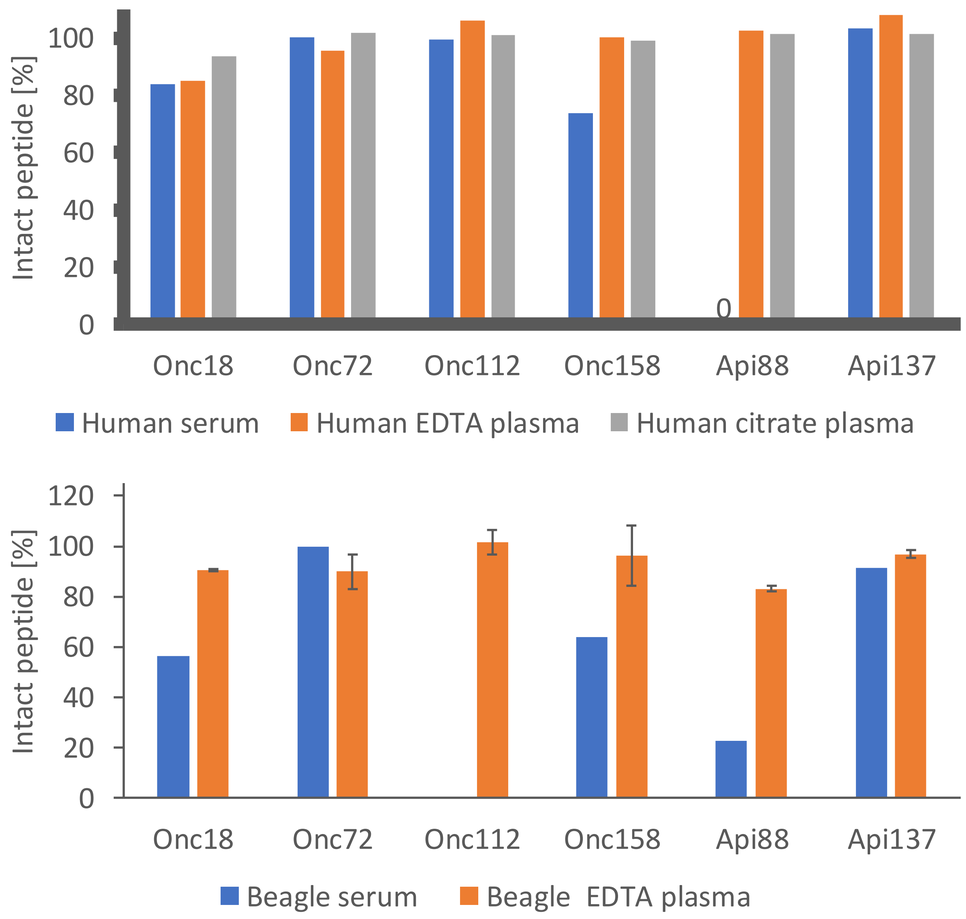

Figure 1. Degradation of LPAs in human (TOP) and beagle (BOTTOM) plasma and serum. After one hour incubation, LPAs were analyzed after serum protein precipitation and plasma SPE, respectively, using UV-HPLC.

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 hBAL, serum and plasma, 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 and beagle dog EDTA plasma, solid phase extraction (SPE) was utilized. For protein denaturation and depletion, samples (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.

Results and Conclusion

The stability in rat, beagle, and human serum was evaluated here for the first time and showed around three-fold higher half-life times than in mouse serum (Table 1). Importantly, the degradation of LPAs in human and beagle plasma after an hour incubation is very low (≤20%) and more than 80% peptide was recovered (Figure 1 and 2). The most stable derivatives in all matrices were Onc72, Onc112, and Api137. Additionally, the first study of LPA stability in human BAL revealed three remarkably stable peptides with more than 50% of Onc72, Onc112, and Api137 remaining after 20 hours (Table 1). Previously shown for other therapeutic peptides, lower peptide stability in mouse serum that translates in rat and non-rodent species to higher stabilities will most likely lead to more promising pharmacokinetics.

References

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