Bactericidal Activity of P128 on Coagulase Negative Staphylococci: Alone and in Combination with Antibiotics

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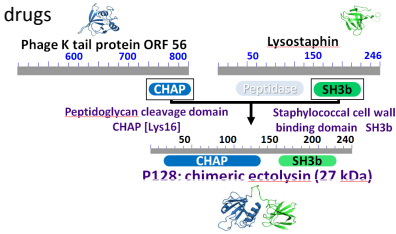
Aim: To demonstrate *in vitro* killing potential of P128 alone and in combination with antibiotics on three clinically significant CoNS species, *S. epidermidis, S. haemolyticus* and *S. lugdunensis*.

Introduction

P128: an engineered Ectolysin with unique properties

- P128 is a chimeric recombinant ectolysin* that is being developed to treat systemic and topical *Staphylococcus* infections.
- P128 demonstrates potent bactericidal activity against >120 clinical isolates of *S. aureus* including MRSA.
- P128 shows potent bactericidal activity in disrupting MRSA biofilms and also killing biofilm embedded bacteria.
- P128 shows high degree of synergy in combination with standard-of-care (SoC) drugs



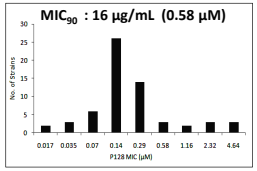
(*Ectolysins - phage lysin involved in cleaving the peptidoglycan from outside during DNA injection*)

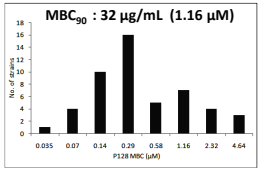
- **Coagulase-negative staphylococci (CoNS)** are increasingly being recongnised as causing serious disease.
  - CoNS account for large number of hospital acquired blood stream infections.
  - *S. epidermidis* is the most commonly isolated causative agent in catheter related bloodstream infections (CRBSI)
  - *S. haemolyticus* is the second most common species isolated from human blood cultures.
  - Pathogenesis of *S. lugdunensis* endocarditis, although less frequent, is associated with high rates of mortality, similar to *S. aureus.*
- CoNS strains showed a trend towards increasing drug resistance.
  - More than 90% of the *S. epidermidis* strains are resistant to methicillin.
  - Emergence of CoNS strains showing resistance to drugs such as linezolid has made the situation even more alarming.

Methods and Results

MIC and MBC Assays: The MIC of P128 was determined using a modified clinical and laboratory standards institute (CLSI) broth micro dilution procedure.

MIC & MBC distribution of P128 on 62 CoNS strains





CoNS species	MIC in µg/ml (µM)		
	MIC range	MIC_{50}	MIC_{90}
S. epidermidis (37)	0.5-128 (0.017-4.64)	4 (0.14)	8 (0.29)
S. haemolyticus (16)	2-128 (0.07-4.64)	8 (0.29)	64 (2.32)
S. lugdunensis (9)	4-8 (0.14-0.29)	4 (0.14)	8 (0.29)

CoNS species	MBC in µg/ml (µM)		
	MBC range	MBC_{50}	MBC_{90}
S. epidermidis (37)	0.5 - 128 (0.017 - 4.64)	8 (0.29)	32 (1.16)
S. haemolyticus (16)	4 - 128 (0.14 - 4.64)	16 (0.58)	64 (2.32)
S. lugdunensis (9)	8 - 16 (0.29 - 0.58)	8 (0.29)	16 (0.58)

All three species of CoNS tested showed equal susceptibility to P128 in terms of MIC_{90} and MBC_{90} values observed.

Combinations of P128 and antibiotics – Checkerboard assay

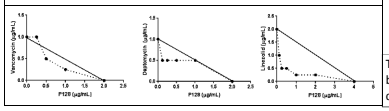
- Microtiter plate wells precoated with 0.5% bovine serum albumin
- 50 µl of a CoNS culture at a final density of 5 x 10^5 CFU/ml
- Two fold dilutions of P128 (25 µl) and / or an antibiotic (25 µl) in CAMHB
- The plates incubated under static conditions at 35°C for 18 h.

FICI: Fractional Inhibitory Concentration Index

≤ 0.5 = Synergy
0.5-1.0 = Additive
1.0-4.0 = Indifferent
≥4.0 = Antagonist

CoNS strain	FIC index of P128 and antibiotics combinations			
	Vancomycin	Daptomycin	Linezolid	Oxacillin
S. epidermidis				
NRS 873	0.70	0.38	0.45	0.25
NRS 867	1.06	0.29	0.50	0.09
NRS 859	0.95	0.60	0.40	0.28

Synergy of P128 with SoC antibiotics, vancomycin, daptomycin and linezolid, on planktonic cells of *S. epidermidis* NRS 859 represented in the form of isobolograms.



CoNS strain	FIC index of P128 and antibiotics combinations			
	Vancomycin	Daptomycin	Linezolid	Oxacillin
S. haemolyticus				
NRS 50	0.56	0.33	0.38	0.24
HM 1164	0.29	0.72	0.37	0.30
S. lugdunensis				
NRS 881	0.47	0.29	0.35	0.50
NR 46406	0.28	0.18	0.40	0.40

The individual MIC values of P128 and the drugs have been joined by a solid line, while the MIC values obtained in P128 and drug combinations have been joined by a dotted line.

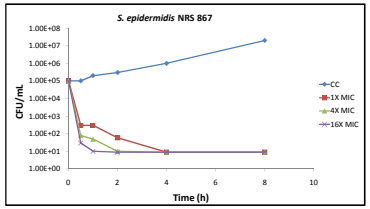
Combinations of P128 and SoC drugs resulted in FIC indices ranging from 0.18 to 1.0 suggesting a synergistic inhibition or an additive effect.
No antagonism was observed with P128 and antibiotic combinations.

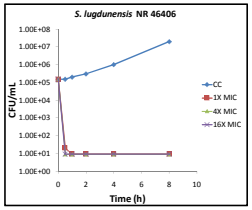
MIC of P128 in Fetal calf serum

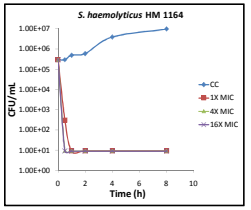
Strains	MIC of P128 in µg/mL (µM)		Fold reduction in serum MIC
	CAMHB	Fetal calf serum	
S. epidermidis			
NRS 859	4 (0.214	0.12 (0.004)	32
NRS 848	16 (0.58)	4 (0.14)	4
S. lugdunensis			
NR 46406	16 (0.58)	0.5 (0.018)	32
NRS 881	4 (0.14)	0.5 (0.018)	8
S. haemolyticus			
HM 1164	8 (0.29)	0.25 (0.009)	32
NRS 50	125 (4.5)	8 (0.29)	16

Serum had a potentiating effect on P128 MIC.
Serum MIC values on CoNS strains were reduced ~4 – 32 fold when compared to MIC in broth.

Time-Kill Kinetics: To evaluate concentration-dependent bactericidal activity of P128 on CoNS cultures, time-kill assays were performed in accordance with the CLSI guidelines.
(The detection limit in time-kill assays was 10 CFU/ml).







P128 caused rapid loss of viability in all six CoNS strains tested.
P128 at 1x MIC caused more than 4 log reduction in CFU in 30 minutes.
At 8 hours no viable cells were found in P128 treated cultures.

Conclusions

- **P128 inhibits growth of clinically important species of CoNS *viz. S. epidermidis, S. haemolyticus* and *S. lugdunensis* at low concentrations.**
- **Lower MIC values seen in serum compared to the broth predicts that P128 might show a therapeutic effect in humans at low concentrations.**
- **Low MBC values and rapid TKK of P128 demonstrated its strong bactericidal effect on diverse clinical isolates of CoNS.**
- **P128 shows strong synergy with SoC antibiotics on all the three species of CoNS tested.**

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