

SASP: Kill Kinetics against Diverse Antibiotic Resistant *Staphylococcus aureus*

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17 - 20 September 2007

ABSTRACT

Background: SASP comprise a small group of proteins which bind and inactivate bacterial DNA. In this study, a SASP gene has been delivered to *S. aureus* via an *S. aureus*-specific delivery vector, PTSA1.2/A, causing production of SASP in the targeted cells. The aim of the study was to assess the effect of SASP on a range of clinical *S. aureus* strains with diverse susceptibility patterns including MRSA and hetero and non-hetero vancomycin intermediate *S. aureus* (hVISA/VISA).

Methods: The bactericidal activity of SASP was determined using time-kill studies against 18 diverse *S. aureus* strains - 7 geographically separated MRSA, 5 hVISA/VISA, and 6 strains resistant to various antibacterials. Diluted overnight suspensions of the bacteria were inoculated (final inoculum 10⁵ cfu/ml) into Luria-Bertani (LB) broth supplemented with calcium and PTSA1.2/A. For growth controls PTSA1.2/A was replaced with buffer. Tubes were incubated at 37 °C and viable counts performed at 0, 1, 2, 3, 6 and 24 hours. Log reductions in viable count and the area-under-the bacterial kill curve 0-24 hour (AUBKC 24) were calculated.

Results: SASP was bactericidal causing a >2 log drop in bacterial count at 2 hours with 13/18 strains and a >3 log drop at 6 hours with 16/18 strains, including all MRSAs. Existing resistance to methicillin, fluoroquinolones, fusidic acid, rifampicin, tetracycline or macrolides had no impact on the delivery of the SASP gene or action of SASP. The degree and speed of kill of the hVISA/VISA strains was less than other MRSAs (AUBKC 24 h hVISA/VISA 46 ± 9, MRSA 24 ± 2, p<0.05) in two strains regrowth occurred at 24 hours.

Conclusions: SASP displayed bactericidal activity against a diverse range of antibiotic resistant *S. aureus* isolates. A >3 log kill was seen at 6 hours with all *S. aureus* except 3/5 hVISA/VISA strains which showed a >2 log kill at 6 hours. This technology can target specific bacterial species and demonstrate antibacterial activity.

INTRODUCTION

α/β-type small acid-soluble spore proteins (SASP) comprise a group of unique proteins that bind to bacterial DNA and cause a halt in DNA replication and, where bound, inhibition of gene transcription. SASP are produced by Gram positive spore-forming bacteria during sporulation and act to protect the spore DNA from damage due to UV, heat, desiccation and many genotoxic chemicals^{1,2}. SASP range in size from 7-11 kDa and act by binding to and saturating bacterial spore DNA and changing its conformation from B-like to A-like (P. Setlow, pers. commun.). The SASP themselves change from a random coil in their free state to ~60 % α-helix upon DNA binding³. SASP sequences are highly conserved for aerobic and anaerobic spore forming bacteria but show minimal homology to sequences in current databases, and contain no known sequence motifs, including motifs found in DNA-binding proteins⁴. Conservation is greatest across two regions which are involved directly in DNA binding and which become almost entirely α-helical upon binding.

Because SASP block both DNA replication and transcription, these proteins must be removed in order for spores to return to vegetative growth, and ultimately SASP are removed through their cleavage by a spore-specific protease known as germination protease (Gpr). SASP cleavage by Gpr does not take place in the dormant spore because of the spore's low water content. However, the hydration that takes place upon spore germination together with the other unique conditions found in a germinating spore, i.e. low pH and the presence of dipicolinic acid chelated to Ca²⁺ (Ca-DPA), allow rapid SASP cleavage by Gpr, thus freeing up the DNA for transcription and eventual replication.

An antibacterial platform technology has been developed, named SASPject™, which utilises the ability of SASP to bind to bacterial DNA in a non sequence-specific manner and inhibit DNA activity by preventing replication and transcription. The SASPject™ technology comprises bacterial viruses modified to deliver SASP genes to targeted species of pathogen. The viruses are rendered unable to complete their full life cycle by preventing virus-mediated target cell lysis. The SASPject™ technology represents a new class of antibacterial agent that, due to its unique mode of action, is active against bacteria exhibiting a wide range of antibiotic resistances.

Initial data, using a modified *S. aureus*-specific vector, PTSA1.2/A to deliver a SASP gene, has shown SASP to be rapidly bactericidal against laboratory strains of *Staphylococcus aureus*. The aim of this study was to assess the efficacy of SASP against a range of clinical *S. aureus* strains with a wide range of susceptibility patterns, including methicillin-resistance (MRSA) and hetero and non-hetero vancomycin intermediate *S. aureus* (hVISA/VISA).

MATERIALS & METHODS

Bacterial isolates

18 strains of *S. aureus* were used – 7 geographically different MRSA, 5 hVISA/VISA and 6 strains with resistance to one or more of penicillin (PEN), oxacillin (OXA), tetracycline (TET), erythromycin (ERY), ciprofloxacin (CIP), fusidic acid (FUS).

Time kill studies

Time kill studies were performed in Luria-Bertani broth supplemented with 10 mM CaCl₂ (LBC broth) using a bacterial inoculum of 10⁵ cfu/ml from an overnight culture. Total volume was 3 ml. At T0, PTSA1.2/A (2 x 10⁸ pfu/ml in Tris-buffered saline containing 10 mM CaCl₂ and 1 mM MgSO₄ (ΦTBS)) and the test isolate were added to the LBC broth. For growth controls PTSA1.2/A was substituted with ΦTBS. Aliquots were taken for viable counts at times T0, 1, 2, 3, 4, 6 and 24 hours and spiral plated onto nutrient agar plates. The plates were incubated in air at 37 °C for 18 hours. Antibacterial effect was assessed by drop in viable count at 24 hours.

TABLE 1: Bactericidal effect of PTSA1.2/A on clinical MRSA isolates

Strain	Number of viable cells (cfu/ml) at time (hours)						
	T0	T1	T2	T3	T4	T6	T24
MRSA 31410	120000	<100	NS	NS	NS	NS	NS
MRSA 32985	130000	<100	<100	<100	NS	NS	NS
MRSA 33922	140000	<100	<100	<100	NS	NS	NS
MRSA 33829	160000	220	<100	NS	NS	NS	NS
MRSA 33815	110000	860	<100	NS	NS	NS	NS
MRSA 33024	130000	5600	720	NS	<100	NS	NS
MRSA 33827	190000	3800	1000	<100	<100	<100	NS
MRSA 33820	110000	3200	1100	540	<100	NS	NS
MRSA 31543	130000	3500	660	220	NS	NS	NS
MRSA 31475	150000	4200	1800	520	<100	<100	NS

TABLE 2: Bactericidal effect of PTSA1.2/A on clinical MSSA isolates

Strain	Number of viable cells (cfu/ml) at time (hours)						
	T0	T1	T2	T3	T4	T6	T24
MSSA 30963	100000	220	NS	NS	NS	NS	NS
MSSA 31411	130000	2500	1600	260	<100	<100	NS
MSSA 31465	170000	85000	29000	8500	3000	NS	NS

TABLE 3: Bactericidal effect of PTSA1.2/A on hVISA / VISA

Strain	Number of viable cells (cfu/ml) at time (hours)						
	T0	T1	T2	T3	T4	T6	T24
hVISA 14545	110000	4500	11000	1600	1100	260	NS
hVISA 15818	160000	21000	20000	17000	7300	200	4000
VISA 14547	170000	560	220	<100	<100	620	19000
VISA 20200	120000	1800	1400	340	<100	<100	21000
VISA 24764	100000	27000	28000	6800	2400	260	NS

Key to Tables 1, 2 and 3:

	≤ 1 log reduction in viable count
	1 - 2 logs reduction in viable count
	2 - 3 logs reduction in viable count
	≥ 3 logs reduction in viable count

NS - No surviving cells detected from spiral plating 50 µl aliquot

RESULTS

The bactericidal effect of SASP against the various strains of *S. aureus* is shown in Tables 1, 2 and 3 and Figures 1 and 2.

- In rate of kill studies, SASP achieved rapid bactericidal activity against both MSSA and MRSA, with a >3 log reduction in viable count for 12/13 strains within 4 hours and all strains within 6 hours (Tables 1 and 2 and Figure 1).
- SASP achieved a >3 log reduction in viable count at 4 hours for 2 VISA strains, and a >2.5 log reduction in viable count at 6 hours for remaining VISA and 2 hVISA strains (Table 3 and Figure 2). Some regrowth occurred in some strains by 24 hours. The AUBKC 24 for hVISA/VISA strains was 45.7 ± 9.1.
- Against *S. aureus* strains with resistance to one or more antibiotics (excluding vancomycin), SASP achieved a minimum 3 log reduction in viable count by 6 hours. This was maintained over the 24 hour time interval (Table 4).

FIGURE 1: Mean activity of PTSA1.2/A against MRSA and MSSA isolates

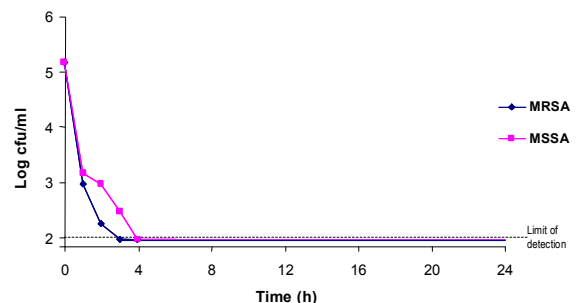


FIGURE 2: Mean activity of PTSA1.2/A against VISA and hVISA isolates

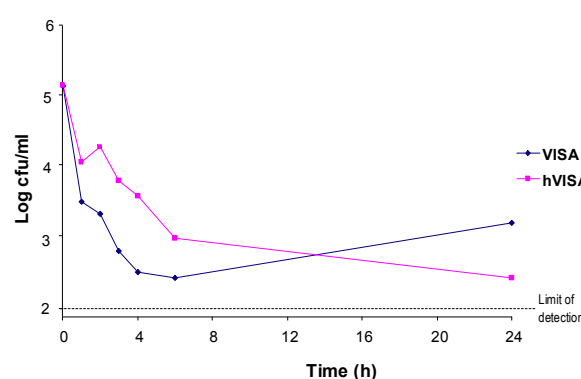


TABLE 4: The antibacterial effect of SASP against *S. aureus* with different antibiotic resistances.

Strain	Resistance	Log reduction in viable count (log cfu/ml) at	
		6 h	24 h
MSSA 31411	PEN	>3.1	>3.1
MSSA 30963	TET	>3.1	>3.1
MSSA 31465	PEN, CIP, RIF	>3.2	>3.2
MRSA 32985	OXA, PEN, ERY, CIP	>3.2	>3.2
MRSA 31410	OXA, PEN, FUS	>3.1	>3.1
MRSA 33024	OXA, PEN, ERY, CIP, GEN	>3.1	>3.1
MRSA 31543	OXA, PEN, ERY, CIP	>3.2	>3.1
MRSA 31475	OXA, PEN, ERY, CIP	>3.2	>3.2

CONCLUSIONS

- The SASPject technology represents a new class of antibacterial agent that can target clinical isolates of *S. aureus*
- SASP is rapidly bactericidal against clinical strains of both MSSA and MRSA.
- Activity of SASP is not reduced by resistance to existing agents except for vancomycin.

REFERENCES

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