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SASP: Efficacy of *Pa* SASPject against *Pseudomonas aeruginosa* ATCC 27853 in a Mouse Lung Model

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ABSTRACT

Background: SASP is a unique antibacterial protein that halts DNA replication and gene expression. Found in bacterial spores, SASP binds to bacterial DNA in a non-sequence specific manner, making resistance extremely unlikely, and rapidly kills vegetative bacterial cells due to inhibition of DNA transcription and replication. SASPject technology delivers SASP genes to target bacteria using nano-delivery vehicles (NDV). The *in vivo* efficacy of *Pseudomonas aeruginosa* (*Pa*) SASPject has been assessed in an immunocompetent mouse lung model of *P. aeruginosa* infection over 24 hours.

Method: Female BALB/C mice were infected with *P. aeruginosa* ATCC 27853 at 5 x 10^7 cfu/mouse, by intra-nasal (IN) administration. *Pa* SASPject was administered once, 2 hours post infection, by intravenous (IV) administration at 5 x 10^{12} units/kg (U/kg). Controls were ceftazidime and vehicle (Tris-buffered saline containing 4 mM calcium chloride, 1 mM magnesium sulphate and 10 % glycerol (w/v)). After 6 or 24 hours, mice were euthanised and the lungs were harvested. Tissue was homogenised and plated for quantitative tissue burden counts onto trypticase soy agar plates supplemented with 5% sheep's blood.

Results: Harvested lungs contained a high level of *P. aeruginosa* infection at 24 hours post-infection (8x10^{10} cfu/g lung tissue in vehicle treated mice). IV-administered *Pa* SASPject significantly reduced *P.aeruginosa* burdens in lung tissue 24 hours post-infection (ANOVA analysis, P=0.002) with a geometric mean of 2.7x10^4 cfu/g, a >6-log reduction compared to the vehicle group. At 6 hours, post infection, SASPject caused a 3-log reduction in bacteria in the lungs (SASPject = 7.0 x 10^6 cfu/g; Vehicle = 1.5 x 10^{10} cfu/g).

Conclusions: *Pa* SASPject shows rapid activity in murine lungs where the animals are exposed to a high infectious dose of *Pa* cells. *Pa* SASPject is the first IV-formulated SASPject with the advantage of rapid cidal activity and thus potential to accelerate the speed of cure.

INTRODUCTION

The emergence of multidrug resistant Gram negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli, Acinetobacter baumannii,* and *Klebsiella spp.* amongst others, has complicated antibiotic therapy against these organisms. Emergence of recent antibiotic resistant bacteria such as NDM-1 expressing *Enterobacteriaceae*, and more recently *P. aeruginosa* and *A. baumanii,* have highlighted the urgent need for new and novel therapies to treat antibiotic-resistant bacterial infections.

SASPject comprises delivery of broad-spectrum antibacterial proteins called SASP, or small acid-soluble spore protein(s), to selected bacterial species using targetable nano-delivery vehicles (NDVs). SASPs are the first molecules in a new class of antibacterial proteins called bDIPs (bacterial DNA inactivator proteins). SASP are non-sequence specific DNA binding proteins which bind to bacterial DNA, disrupting the normal functioning of the DNA processing enzymes - DNA and RNA polymerases - leading to a rapid cessation of DNA replication and transcription, and therefore causing rapid cell death (Figure 1.)

Pa SASPject is in development for the treatment of serious *P. aeruginosa* infections. Rapid bactericidal action of *Pa* SASPject has been demonstrated *in vitro*, together with broad spectrum of activity against >500 clinical *P. aeruginosa* isolates (2). In this study the activity of PT3 in a murine model of *P. aeruginosa* lung infection was assessed.

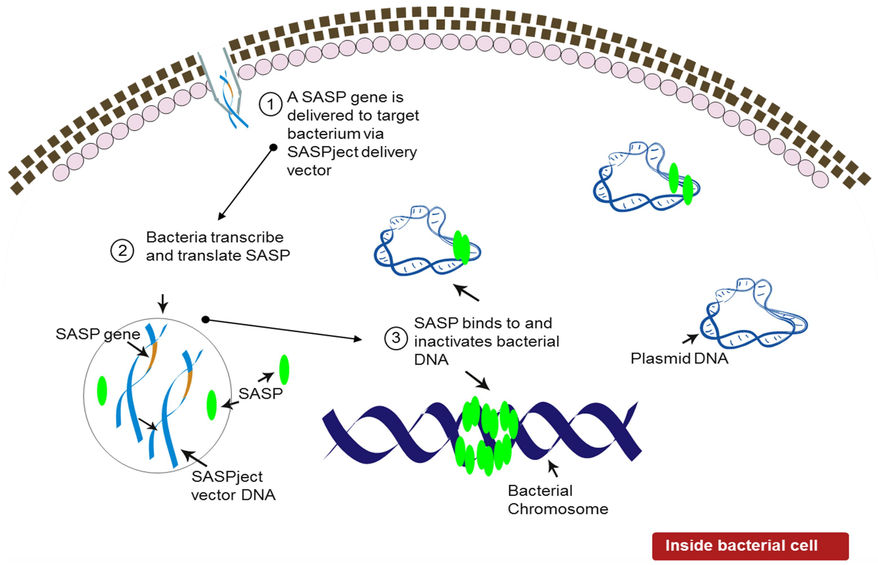

Figure 1 Mechanism of action of *Pa* SASPject

METHODS

Murine Lung model

Mouse Strain Mice used in this study were supplied by Harlan and were pathogen free. The strain of mouse used was BALB/c. Mice were 7-8 weeks old and 18-20 g upon receipt, and were allowed to acclimatise for 3 days.

Bacterial Strain *Pseudomonas aeruginosa* strain ATCC27853 from American Type Culture Collection was used throughout the study.

Infection Bacteria were grown overnight on trypticase soy agar (TSA) plates supplemented with 5 % sheep's blood. Bacteria were removed from the plate using a swab and resuspended in trypticase soy broth (TSB), and the optical density assessed (OD_{600}). Bacteria were diluted to 8.5 $\log_{10}$/ml. Mice were anaesthetised using isoflurane, brought to a surgical plane and infected with a total infectious dose of 7.5 $\log_{10}$ per mouse by slowly introducing 0.05 ml of bacteria into the nares of each mouse. Mice were held in a vertical position until each dose was inhaled.

Antibacterial Therapy Antibacterial treatment was initiated 2 hours post infection by intravenous (IV) injection into the tail vein. PT3 was used at 1.5 x 10^{11} U/ml, and was administered once at 5 ml/kg; ceftazidime was used at 128 µg/ml and administered at 5 ml/kg. The vehicle control group was treated with vehicle buffer (Tris-buffered saline containing 4 mM calcium chloride, 1 mM magnesium sulphate and 10 % glycerol (w/v)) at 5 ml/kg IV. Ceftazidime data not shown as response was poor and dose adjustment required.

Endpoint At 6 and 24 hours post infection, the clinical condition of all animals was assessed prior to them being humanely euthanized. Immediately post euthanasia, lungs were removed and weighed before being homogenised in 2 ml TSB using a mini-bead beater. Homogenate was serially diluted and plated for cfu counts after 24 hours growth. Bacterial load in lung tissue was calculated as cfu/g tissue.

Monitoring NDV levels in mouse lungs

Mice were administered 2 x 10^{10} U of *Pa* NDV by injection of 0.1 ml into the lateral tail vein. 30 minutes later, mice were humanely euthanised and lung tissue was homogenised and and serially diluted for NDV counts using plaque assay.

RESULTS

Distribution of* Pa *NDV to the lungs

• *Pa* NDV is well distributed to the lungs 30 minutes after IV administration (Table 1)

ROA (Time post administration)	Total Administered Amount of *Pa* NDV (U)	Amount of NDV in Lung tissue post administration (U/g)
IV (30 m)	2 x 10^{10}	2.5 x 10^9

Table 1 Distribution of *Pa* NDV to mouse lungs by intravenous administration.

Activity of* Pa *SASPject in a Murine Pneumonia Model

• A model of *P. aeruginosa* infection was established in immunocompetent mice, with bacterial burden levels reaching 10.2 and 10.9 $\log_{10}$ cfu/g tissue at 6 and 24 hours respectively post infection.

• *Pa* SASPject showed rapid significant decreases in lung burden, with 3-log and 6-log lower biodurden levels in *Pa* SASPject treated mouse lung tissue compared with vehicle treated mice after 6 and 24 hours (P=0.002) respectively (4 and 22 hours post-treatment).

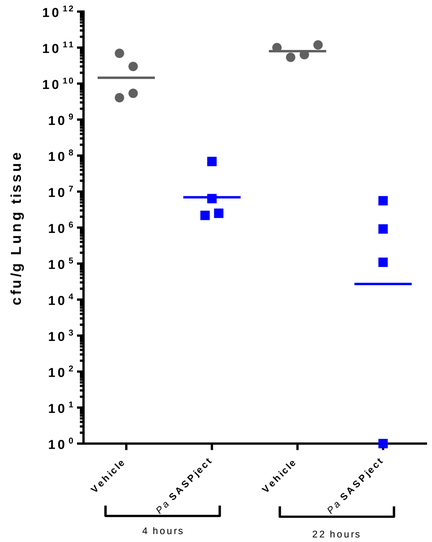

Figure 2 Effect of *Pa* SASPject on mouse lung biodurden over time (time of single dose = 0h; time post treatment shown)

CONCLUSIONS

• PT3-NDV is well distributed to the lungs following IV administration.

• SASPject PT3 shows rapid activity against *P. aeruginosa* in a mouse pneumonia model, when delivered via a single dose intravenously, with further reductions in lung biodurden seen 22 hours post-treatment.

• Supported by *in vitro* spectrum of activity data *Pa* SASPject has the potential to be used to treat MDR *P. aeruginosa* infections

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2. Cass *et al* (2014). **SASP: Microbiological characterisation of a novel therapeutic targeting MDR *Pseudomonas aeruginosa*.** ICAAC Poster F-1548, Washington, 2014.