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-specific sequence 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 (NDVs). 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 10^11 units/kg (LgU). Controls were ceftazidime and vehicle (Triis-bufffered 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 (8x10^5 cfu/g lungs tissue in 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 2.7x10^6 cfu/g, a 4-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 10^7 cfu/g; Vehicle = 1.5 x 10^11 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 in- formulated SASPject with the advantage of rapid calial 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. among others, has complicated antibiotic therapy against these organisms. Emergence of recent antibiotic resistant bacteria such as NDM-1 expressing Enterobacteriaceae, and more recently Pa aeruginosa and A. baumannii, have highlighted the urgent need for new and novel therapies to treat antibiotic-resistant bacterial infections.

SASPject comprises delivery of broad-spectrum antibiotic proteins called SASP, or small acid-soluble spore proteins(s), to selected bacterial species using targetable nano-delivery vehicles (NDVs). SASPjects are the first molecules in a new class of antibacterial proteins called IDPs (Bacterial DNA inactivating 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 bacterial activity against Pa aeruginosa has been demonstrated in vitro, together with broad spectrum of activity against >500 clinical Pa aeruginosa isolates (2). In this study the activity of PT3 in a murine model of P. aeruginosa lung infection was assessed.

METHODS

Marine 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 (OD600). Bacteria were diluted to 8.5 log6/mL. Mice were anaesthetised using isoflurane, brought to a surgical plane and infected with a total infectious dose of 7.5 log6 , 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 inhale.

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 units/ml, and was administered once at 5 mM/kg; ceftazidime was used at 128 μg/ml and administered at 5 mM/kg. The vehicle control group was treated with vehicle buffer (Triis-buffered saline containing 4 mM calcium chloride, 1 mM magnesium sulphate and 10 % glycerol (w/v)) at 5 mM/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 euthanised. 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 cfug tissue.

REFERENCES
