Background: SASPs are a class of unique antibacterial proteins that prevent DNA replication and gene transcription through binding to bacterial DNA. The mode of SASP binding is DNA sequence-independent and thus mutations have no impact upon SASP activity. Phico's SASP technology uses a synthetic biology approach to create nano-delivery vehicles (NDV), capable of delivering a SASP gene expression system target to bacteria. SASP production in situ in target cells is rapidly bactericidal. NDV gene delivery activity has been assessed against a large diverse range of recent, geographically diverse clinical isolates – an essential requirement for novel antibacterial technologies. Furthermore, the rapid bactericidal activity of Pseudomonas aeruginosa (Pa) SASP against Pa has been assessed.

Methods: Spectrum activity: 339 clinical Pa strains from multiple sites across Asia, EU, North and South America were grown in Luria Bertani broth supplemented with magnesium, calcium and glucose (LB); “10” cfu was used to inoculate LB soft agar and poured onto LB plates. 10 μl spots of a preparation of 10^9 Pa NDV U were inoculated onto the soft agar overlay. Kill efficiency: Diluted overnight suspensions of MDR Pa 2046 were inoculated (final inoculum 10^5 cfu/mL) in LB broth; “10” Pa LB U/mL were added and incubated statically at 37°C with total Visible Counts every hour (h) for 6 h.

Results: Pa NDV gene delivery activity was assessed in 92% of Pa strains, spanning carbapenem, ampicillin, fluoroquinolone and polymyxin resistance profiles. Pa SASPject was rapidly bactericidal with Pa cells reduced to below the detection limit of 50 cfu/ml within 1 h. Continued monitoring over 6 h showed no bacterial regrowth.

Conclusion: Pa NDV screening demonstrated a broad spectrum against an extensive range of recent, geographically diverse clinical Pa. P. aeruginosa strains enabling utility in Pa-SASPject. Furthermore, SASPject’s unique mode of action, together with its rapid bactericidal activity indicates a potential role in addressing the unmet clinical need for novel antibacterial approaches with activity against Gram negative pathogens.

ABSTRACT

SASPject: Microbiological Characterisation of a Novel Therapeutic Targeting MDR Pseudomonas aeruginosa

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INTRODUCTION

The emergence of multidrug resistant Gram-negative bacteria such as Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii, and Klebsiella spp. amongst others is a global public health issue and has highlighted the urgent need for new therapeutic options, ideally with novel mechanisms of action.

SASPjectTM is a new class of antibacterial currently under development (1). The SASPject platform technology comprises NDV’s designed to deliver a gene encoding an antibacterial protein (SASP) into target bacteria (Figure 1). SASPs are small proteins expressed by sporing bacteria which, when delivered into bacteria, non-specifically, inactivate bacterial DNA and lead to rapid cell death. Due to the specific nature of the NDV-bacterium interaction, single-species or multi-species or genera can be selectively targeted. Consequently, non-selective inhibition of commercial bacteria, which is a significant drawback of some conventional antibiotic therapies, can be avoided.

Previously, a SASP antibacterial (PTL2) against Staphylococcus aureus, including a diverse range of antibiotic-resistant strains including MRSA and VISA/VNSA (2), has been developed. PTL2 successfully passed through a Phase I clinical trial for nasal desensitisation of S. aureus in humans and demonstrated an excellent safety profile (2, 3). Pa NDV is in development for treating P. aeruginosa, including MDR strains. Here we report the first data on the binding and gene transfer efficacy of Pa NDV against a diverse range of clinical Pa: aeruginosa isolates from around the globe and demonstrate the rapid mode of action Pa SASPject.

METHODS

Background: SASPs are a class of unique antibacterial proteins that prevent DNA replication and gene transcription through binding to bacterial DNA. The mode of SASP binding is DNA sequence-independent and thus mutations have no impact upon SASP activity. Phico’s SASP Technology uses a synthetic biology approach to create nano-delivery vehicles (NDV), capable of delivering a SASP gene expression system target to bacteria. SASP production in situ in target cells is rapidly bactericidal. NDV gene delivery activity has been assessed against a large diverse range of recent, geographically diverse clinical isolates – an essential requirement for novel antibacterial technologies. Furthermore, the rapid bactericidal activity of Pseudomonas aeruginosa (Pa) SASP against Pa has been assessed.

Methods: Spectrum activity: 339 clinical Pa strains from multiple sites across Asia, EU, North and South America were grown in Luria Bertani broth supplemented with magnesium, calcium and glucose (LB); “10” cfu was used to inoculate LB soft agar and poured onto LB plates. 10 μl spots of a preparation of 10^9 Pa NDV U were inoculated onto the soft agar overlay. Kill efficiency: Diluted overnight suspensions of MDR Pa 2046 were inoculated (final inoculum 10^5 cfu/mL) in LB broth; “10” Pa LB U/mL were added and incubated statically at 37°C with total Visible Counts every hour (h) for 6 h.

RESULTS

• Pa NDV demonstrated binding and gene delivery against 92% of P. aeruginosa isolates across the EU, Asia and Americas.

• Activity was demonstrated against clinical isolates of P. aeruginosa resistant to a wide array of antimicrobial agents including aminoglycosides and carbapenems (Figure 2).

• Pa SASPject was bactericidal against MDR P. aeruginosa strain 2046 and an adjunctive hyper-expressing mucA22 mutant of PAO1 within 1 h (Figure 3).

• Continued monitoring demonstrated no re-growth of either P. aeruginosa strains within 6 h.

CONCLUSIONS

• The binding and gene delivery spectrum of Pa NDV was impressive against a geographically diverse range of clinically significant P. aeruginosa isolates, including MDR strains.

• Kill of P. aeruginosa PAO1 by Pa SASPject was rapid and bactericidal within 1 h and reflected observations with PTL2 against MRSA.

• As a new class of antibiotics, SASPject represents an attractive and novel therapeutic option against MDR P. aeruginosa, given the rapid bacterial killing and also ability of SASP to limit horizontal transfer of antibiotic resistance genes in vitro (4).

• Furthermore, the low propensity for resistance development to SASPject’s unique mode of action (1, 5) further demonstrates the advantages of this new class of antibiotics.

• Further studies are required to characterise Pa SASP binding and gene delivery in vivo.

REFERENCES


ACKNOWLEDGEMENTS

• Phico Therapeutics Ltd is grateful to Professor Mark Wilkin (University of Leeds & The General Infirmary, Leeds, UK), Professor Vittorio Sambrì (SIRAC, Italy) and Professor Eugenio Giannitrapani (ATTICON, Greece).