Bacteriophage therapy for the treatment of *P. aeruginosa* infections in cystic fibrosis patients

Susan Lehman[1], Steven Branston[1], Frenk Smrekar[1], Rishi Pabary[2,3], Eric Alton[2,3], Jane Davies[2,3], Sandra Morales[1]*
AmpliPhi Biosciences[1], Department of Paediatric Respiratory medicine, Royal Brompton Hospital, London[2], Imperial College London[3]

Introduction

Chronic lung infections caused by *Pseudomonas aeruginosa* (PA) are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. In some cases effective antibiotic therapy is no longer available, with multi-drug resistant (MDR) forms of these bacteria becoming increasingly challenging to treat.

New means of controlling MDR PA infections are urgently needed. Bacteriophage (phage) therapy is a potential therapeutic tool for the treatment of bacterial infections. However, due to the specific nature of phages, questions have been raised about the clinical practicality of bacteriophage based products and their ability to be effective against a range of clinical isolates.

We previously reported the development of three prototype phage mixes and shown that phages are efficacious in reducing both bacterial load and inflammation in a murine lung infection model [1]. In this study, we have expanded the in vitro testing and developed a phage mix active against relevant clinical PA isolates collected from around the world. In addition, we demonstrated the efficacy of the phage mix in vivo in a murine lung infection model.

Methods

In vitro activty: Lytic phages were isolated from environmental sources in Australia and England and screened against a collection of 67 *P. aeruginosa* from CF patients. A prototype combination of four phages was tested against 429 global CF and non-CF clinical isolates collected between 2007 to 2015. Isolates included both antibiotic susceptible/resistant and mucoid/non-mucoid.

Nebulizer suitability: Phages were added to 6 nebulizers and nebulized according to manufacturer's recommended use. Viable titers of collected, nebulized material was compared to that of the starting material.

In vivo activity: Immunocompetent CD-1 female mice were inoculated intranasally (IN) with 6.26 $\log_{10}$CFU in 50 µL of TSB. At 2 hrs post-infection (hpi), 50 µL of 4-phage mix was administered IN to three dosage groups (n=5) consisting of $7.5x10^9$, $7.5x10^8$, and $7.5x10^7$ PFU/mL per phage (for a total of $1.5x10^9$, $1.5x10^8$, or $1.5x10^7$ PFU administered). A second identical dose was given IN at 6 hpi. Meropenem (25 mg/kg) was administered subcutaneously at 2 and 6 hpi to a fourth group. A fifth group was infected, but treated with the phage diluent. All mice were euthanized at 24 h and CFU/lung pair determined. Statistics were performed using Tukey's multiple comparisons test.

In vitro Results

Broad Spectrum of Activity: Serial dilutions of phage mix (10^8 PFU/ml) was spotted onto lawns of CF and non-CF *P. aeruginosa* strains. Each strain was considered sensitive if ≥ 20 plaques were observed.

Table 1. Activity of phage mix against global *P. aeruginosa* isolates.

Number of Isolates	Area (Year of Isolation)	Type	% Activity of phage mix	% of sensitive isolates hit by ≥2 phages in mix
67*	UK/AU/USA (2012-13)	CF	95.5%	87.5%
120	UK (2012-13)	CF	85.0%	95.0%
60	AU (2007-2013)	CF	93.3%	98.2%
40	USA (2014)	CF	87.5%	91.4%
82	UK (2015)	CF	81.7%	92.5%
60	USA/EU/AU (2013)	Non-CF	83.3%	80.0%
369	Total CF Isolates		87.8%	93.2%
429	Total isolates (CF + Non-CF)		87.2%	91.4%

*Reference panel used for phage selection includes 67 distinct CF strains that included well characterized CF epidemic clones. UK: United Kingdom; AU: Australia; USA: The United States of America. % of activity=[no. sensitive isolates/total no. of isolates tested] x 100.

Nebulizer Suitability: Of seven nebulizers tested, the 5 vibrating mesh devices yielded acceptable results in terms of phage viability.

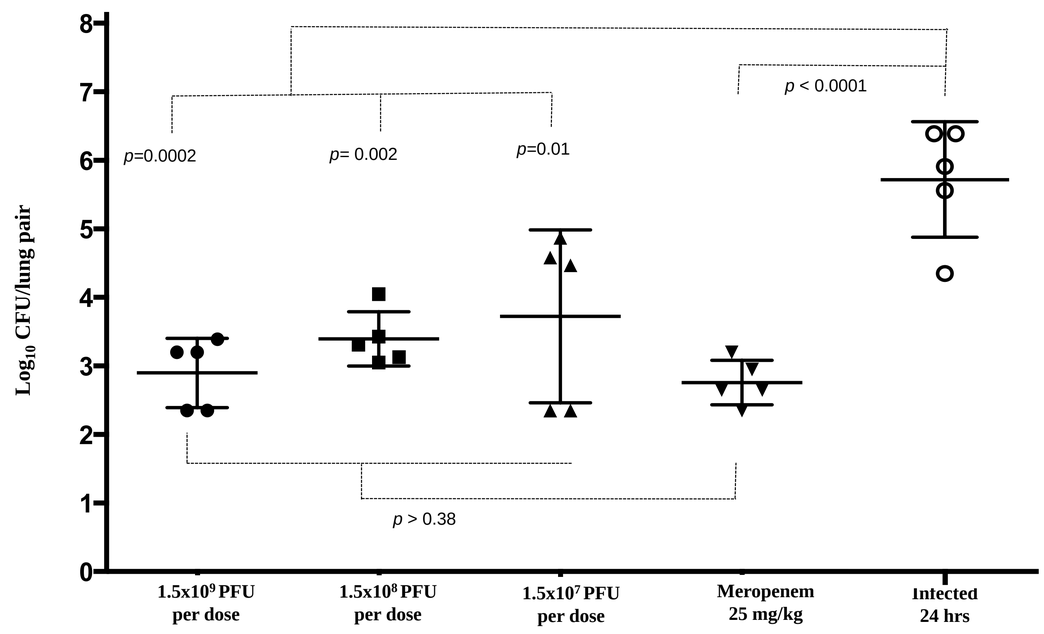
Table 2. Effect of nebulization on phage viable titer.

Nebulizer	Type	Mean Change in Titer ($\log_{10}$ PFU/mL) For Four Individual Phages	Conclusion
1	vibrating mesh	0.83 to -0.63	✓
2	breath-actuated jet	1.08 to -2.09	X
3	jet	-1.33 to -2.01	X
4	vibrating mesh	0.23 to -0.43	✓
5	vibrating mesh	0.49 to -0.38	✓
6	vibrating mesh	-0.07 to -0.15	✓
7	vibrating mesh	-0.05 to -0.33	✓

In vivo Results

Efficacy in Murine Lung Infection Model: Phage mix administered at the three doses had efficacy similar to meropenem in a *P. aeruginosa* murine lung model of infection. However, there seems to be a non-significant trend suggesting a possible dose-dependent effect. The model used virulent strains PA14.

Figure 1. Bacterial load reduction by phage mix and meropenem in 24 h acute murine lung infection



Discussion

- Four-phage mix is capable of infecting *P. aeruginosa* clinical isolates collected around the world
  - includes both antibiotic susceptible & resistant strains,
  - Includes both mucoid & non-mucoid CF strains.
- The broad range of activity addresses concerns that the specificity of phages could make this therapy impractical in the clinical environment. However, it is likely that, like the flu vaccines, these broad spectrum preparations will need to be reformulated over time as the bacterial populations evolve.
- The phage mix administered at the three doses demonstrated efficacy similar to meropenem in a *P. aeruginosa* murine lung model of infection.
- In addition, we have confirmed the usability of the phage mix for:
  - ✓ Clinical use (exclusively lytic, efficacious in vivo)
  - ✓ Nebulisation (no significant decreases in titre were observed)
  - ✓ GMP Manufacturing (long-term stability, current process optimisation)

The use of phages as therapeutic tools continues to be a viable option for the treatment of PA infections in CF patients. AmpliPhi Biosciences, in collaboration with the Brompton Hospital, plan to evaluate the safety and efficacy of AB-PA01 in CF patients.

References

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2. Kutter, E. and Sulakvelidze, A. (2005). Bacteriophages: Biology and Applications. Boca Raton, FL: CRC Press.

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Author's Contact details: Sandra Morales, spm@ampliphibio.com, +61 (2) 9905 7634. AmpliPhi Biosciences is a public US company focused on the development of bacteriophage-based treatments