Phage therapy for chronic *Pseudomonas aeruginosa* infections in Cystic Fibrosis patients

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Abstract

Background: Chronic respiratory infections with *Pseudomonas aeruginosa* (P. aeruginosa) are a major cause of morbidity and mortality in Cystic Fibrosis (CF) patients1. Due to extensive antibiotic use, there are increasing rates of colonization with multi-drug resistant (MDR) *P. aeruginosa* isolates2. In addition, biofilm-forming propensity of many *P. aeruginosa* isolates contributes to treatment failure with antibiotics.

Phages are naturally occurring viruses that infet specific bacteria. Unlike antibiotics, phages are specific to the strain level and therefore have unique advantages in terms of minimization of perturbation of the microbe. They have no capability to infect mammalian cells and so are considered safe.

Results

1 MDR *P. aeruginosa* isolates are highly variable

*P. aeruginosa* isolates were cultured from sputum samples of Cystic Fibrosis patients (n=122 US, n=24 EU). Bioinformatic analysis revealed that 97% (141/146) are MDR isolates, including large number of genes that confer beta lactam and chloramphenicol and streptomycin resistance.

To evaluate the similarity of the *P. aeruginosa* isolates, a phylogenetic tree based on the sequences of these, and other public *P. aeruginosa* isolates was generated. This revealed that the *P. aeruginosa* isolates are highly variable and dispersed across the entire *P. aeruginosa* phylogenetic tree, guaranteeing the genomic diversity of *P. aeruginosa* isolates that are used for isolation and characterization of novel phages.

Phylogenetic tree of *P. aeruginosa* isolates

- *P. aeruginosa* Public Sequence (Ref-Seq) & Biomet
- Biomet *P. aeruginosa* isolates

2 Identification of novel phages for *P. aeruginosa*

Screening of a large library of environmental samples resulted in the isolation of phages recognizing drug resistant *P. aeruginosa* isolates. Sequence analysis of the phages revealed that they belong to different genera in the Caudovirales order, contain linear dsDNA genomes between ~40-180 kbp in length and are devoid of undesirable genes. Scanning electron micrographs of one type of phage virion (*Pseudovirus genus, in the family Myoviridae*), demonstrated its isometric heads and long non-contractile tail.

Example of phage morphology

Phages are self-amplifying therapeutic agents

- Lytic
- Recombinant
- Adsorbed
- Assemble
- Multiply
- Inject

3 Phages infect a broad range of *P. aeruginosa* isolates

High throughput efficiency of plating (EOP) infection assays showed that 3 phages were able to target ~80% of the 146 tested *P. aeruginosa* clinical isolates with most of these isolates being targeted at an efficiency of plating (EOP) of at least 0.01.

Phages + Azt 10X MIC
Phages + Imipenem 100X MIC

4 In vitro activity of phage cocktail against *P. aeruginosa* in biofilm

CF lungs are colonized with *P. aeruginosa* in a biofilm layer. We tested the phages capability to reduce bacterial burden in biofilm. *P. aeruginosa* were grown in conditions allowing biofilm formation, and 24h later the 3-phase cocktail was added for 6 hours. The results revealed that the phages were able to penetrate biofilm and reduce bacterial burden of embedded *P. aeruginosa* (~3 logs). The significant reduction by phage is also corroborated as a visible decrease in biofilm by staining with crystal violet, which stains DNA of dead bacterial cells and the extracellular matrix.

Treatment with the phage cocktail was efficacious in significantly reducing target bacteria encased in biofilm relative to treatment with imipenem (MIC X1000) or vehicle (p-value < 0.0001). Although phage therapy alone achieved a statistically significant reduction in *P. aeruginosa* levels, phages & aztreonam treatment had the highest bacterial burden reduction (~5 logs), suggesting that a synergistic interaction may exist between the phages and aztreonam.

Scanning Transmission Electron Micrographs (STEM) of stained phages infecting *P. aeruginosa* isolates. The head has a diameter of about ~48 nm and the tail length is around ~80 nm

Discussion and Conclusions

The 3-phase cocktail comprised of natural phages demonstrated broad host range, potent antimicrobial activity even in the presence of biofilms, and synergistic activity with antibiotics against clinical *P. aeruginosa* isolates and should be further explored for the treatment of *P. aeruginosa* pulmonary infections in patients with CF.

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