Effectiveness of bacteriophages in the sputum of cystic fibrosis patients

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Abstract

Bacteriophages have been shown to be effective for treating acute infections of the respiratory tract caused by antibiotic-resistant bacteria in animal models, but no evidence has yet been presented of their activity against pathogens in complex biological samples from chronically infected patients. We assessed the efficacy of a cocktail of ten bacteriophages infecting *Pseudomonas aeruginosa* following its addition to 58 sputum samples from cystic fibrosis (CF) patients collected at three different hospitals. Ten samples that did not contain *P. aeruginosa* were not analysed further. In the remaining 48 samples, the addition of bacteriophages led to a significant decrease in the levels of *P. aeruginosa* strains, as shown by comparison with controls, taking two variables (time and bacteriophages) into account ($p = 0.024$). In 45.8% of these samples, this decrease was accompanied by an increase in the number of bacteriophages. We also tested each of the ten bacteriophages individually against 20 colonies from each of these 48 samples and detected bacteriophage-susceptible bacteria in 64.6% of the samples. An analysis of the clinical data revealed no correlation between patient age, sex, duration of *P. aeruginosa* colonization, antibiotic treatment, FEV1 (forced expiratory volume in the first second) and the efficacy of bacteriophages. The demonstration that bacteriophages infect their bacterial hosts in the sputum environment, regardless of the clinical characteristics of the patients, represents a major step towards the development of bacteriophage therapy to treat chronic lung infections.

Keywords: Chronic infection, phage therapy, *Pseudomonas aeruginosa*, pulmonary infection
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Introduction

Despite improvements in patient management, infection control policies, early detection and eradication therapies that have increased the mean life expectancy of cystic fibrosis (CF) patients to about 37 years, most of these patients eventually succumb to chronic pulmonary bacterial infections [1–4]. The most prominent pathogen in CF patients, the gram-negative bacterium *Pseudomonas aeruginosa*, is becoming increasingly resistant to antibiotics [5], leading to a gradual decrease in the clinical benefits of antibiotic treatment over time.

In the environment, microbial communities are controlled by various mechanisms, including the antagonistic action of their specific viruses, through the combined activity of temperate and virulent bacteriophages [6–10]. Bacteriophages were used in medicine (phage therapy), back in the early 20th century, before the discovery of the first antibiotics [11,12]. With the current alarming increase in the frequency of infections caused by antibiotic-resistant pathogens and the lack

of new antibiotics, phage therapy is returning to the spotlight. There is recent support from experimental data and experience accumulated over 80 years in some European countries (Georgia, Russia and Poland) for the use of virulent bacteriophages for treating lung infections [13–20]. As a further step towards applications of bacteriophages in human medicine, we evaluated their potential to infect bacteria in the challenging environment of the lungs, by performing an *ex vivo* study on sputum samples from 58 chronically infected CF patients.

Methods

Study design

We carried out a multicentre cross-sectional study on sputum samples from 58 CF patients recruited from CF centres in Montpellier (n = 23), Nancy (n = 20) and the Necker Hospital in Paris (n = 15). This study was approved by the regional ethics committee (Nîmes, registered under number 2011-A01197-34) and declared to ClinicalTrial.gov (no. NCT01818206).

Bacteria and bacteriophage strains

We used the *P. aeruginosa* PAK strain to amplify bacteriophages PAK_P1, PAK_P2, PAK_P3, PAK_P4 and PAK_P5; the CHA strain to amplify P3_CHA[20] and CHA_P1[19]; the PAO1 strain for PhiKZ and LUZ19; and the Aa245 strain for LBL3. Bacteriophages PhiKZ, LUZ19 and LBL3 were kindly provided by R. Lavigne, KU Leuven, Belgium. The four indicative strains (PAK, CHA, PAO1 and Aa245) were cultured at 37°C in LB medium, with shaking, and bacteriophage lysates were prepared and purified as described elsewhere [20]. The cocktail of these 10 bacteriophages was freshly prepared from bacteriophage solutions, each of which had been titrated on the corresponding host the day before sample processing and on the day of processing. Bacteriophage titration was performed by serial dilutions spotted in triplicate on bacterial lawns. This cocktail was assembled from bacteriophages available in our laboratory without any prior knowledge of their efficacy on a large collection of CF *P. aeruginosa* strains.

New bacteriophages infecting colony #4 from sputum sample 04 were isolated as described elsewhere [18].

Sputum sample processing

Four aliquots of sputum samples were used to evaluate the count of bacteria and bacteriophages before and after addition of the bacteriophage cocktail over 6 h (see Fig. 1 and Data S1). Bacteria were selected on cetrimide agar and bacteriophage counts were obtained using the four indicative strains.

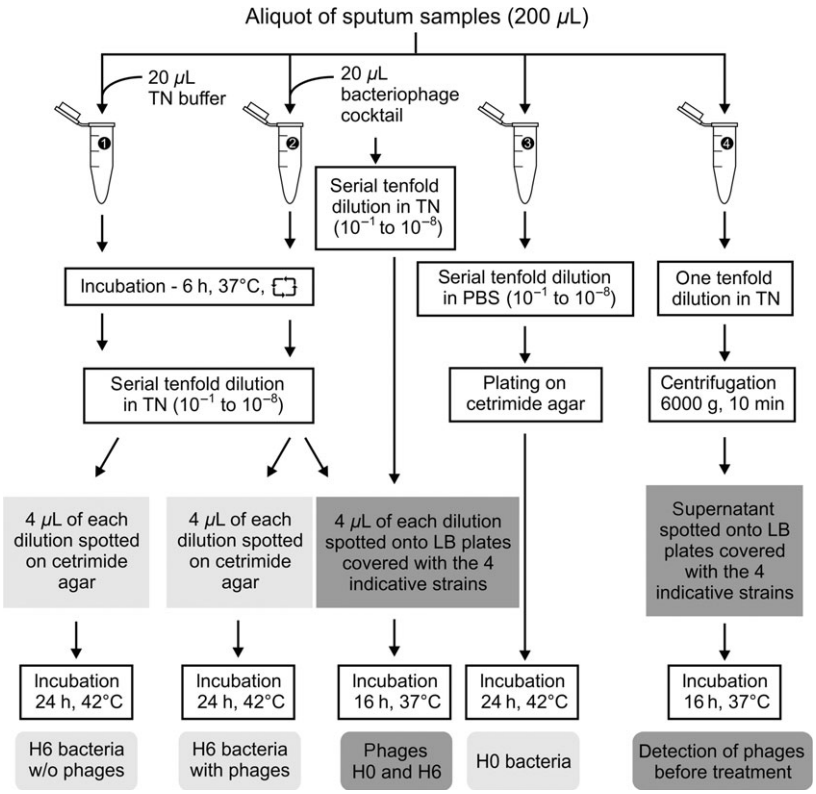

FIG. 1. Schematic diagram of the processing of sputum samples.