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Historical perspective

Formulation, stabilisation and encapsulation of bacteriophage for phage therapy

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ABSTRACT

Against a backdrop of global antibiotic resistance and increasing awareness of the importance of the human microbiota, there has been resurgent interest in the potential use of bacteriophages for therapeutic purposes, known as phage therapy. A number of phage therapy phase I and II clinical trials have concluded, and shown phages don't present significant adverse safety concerns. These clinical trials used simple phage suspensions without any formulation and phage stability was of secondary concern. Phages have a limited stability in solution, and undergo a significant drop in phage titre during processing and storage which is unacceptable if phages are to become regulated pharmaceuticals, where stable dosage and well defined pharmacokinetics and pharmacodynamics are *de rigueur*. Animal studies have shown that the efficacy of phage therapy outcomes depend on the phage concentration (i.e. the dose) delivered at the site of infection, and their ability to target and kill bacteria, arresting bacterial growth and clearing the infection. In addition, in vitro and animal studies have shown the importance of using phage cocktails rather than single phage preparations to achieve better therapy outcomes. The in vivo reduction of phage concentration due to interactions with host antibodies or other clearance mechanisms may necessitate repeated dosing of phages, or sustained release approaches. Modelling of phage-bacterium population dynamics reinforces these points. Surprisingly little attention has been devoted to the effect of formulation on phage therapy outcomes, given the need for phage cocktails, where each phage within a cocktail may require significantly different formulation to retain a high enough infective dose.

This review firstly looks at the clinical needs and challenges (informed through a review of key animal studies evaluating phage therapy) associated with treatment of acute and chronic infections and the drivers for phage encapsulation. An important driver for formulation and encapsulation is shelf life and storage of phage to ensure reproducible dosages. Other drivers include formulation of phage for encapsulation in micro- and nanoparticles for effective delivery, encapsulation in stimuli responsive systems for triggered controlled or sustained release at the targeted site of infection. Encapsulation of phage (e.g. in liposomes) may also be used to increase the circulation time of phage for treating systemic infections, for prophylactic treatment or to treat intracellular infections. We then proceed to document approaches used in the published literature on the formulation and stabilisation of phage for storage and encapsulation of bacteriophage in micro- and nanostructured materials using freeze drying (lyophilization), spray drying, in emulsions e.g. ointments, polymeric microparticles, nanoparticles and liposomes. As phage therapy moves forward towards Phase III clinical trials, the review concludes by looking at promising new approaches for micro- and nanoencapsulation of phages and how these may address gaps in the field.

1. Introduction

The discovery of antibiotics and the subsequent control of bacterial infections may be regarded as a significant achievement of modern medicine. Surgery, transplantation and chemotherapy rely heavily on the control of bacterial infections. Broad spectrum antibiotics are highly

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attractive since they may be used against a wide range of bacteria without the need to identify the infection causing bacterial agent. This advantage has also resulted in significant misuse and overuse of antibiotics contributing to the emergence of antibiotic resistance [1]. Bacterial resistance to antibiotics has become a significant problem in the treatment of a wide range of infections where the bacteria commonly causing the infections have become highly resistant to many classes of antibiotics including third generation cephalosporins, carbapenems and fluoroquinolones [2]. Recent evidence of plasmid mediated colistin resistance in Enterobacteriaceae is particularly troubling [3]. Between 1940 and 1962 > 20 new classes of antibiotics came to market; since then the antibiotic pipeline has produced only two new classes [4]. Coates et al. [4] estimate that to stem the rising tide of antibiotic resistance 20 new classes of antibiotics are urgently needed. It is highly unlikely that this goal will be achievable even if governments provided stronger economic incentives to industry to develop new broad spectrum antibiotics. Czaplewski et al. [5] call for a sustained, concerted and coordinated international effort and discuss the need to invest in the development of alternatives to antibiotics (non-compound approaches) including probiotics, vaccines as well bacteriophage and phage derived lysins.

2. Bacteriophage as novel antimicrobials

Bacteriophages (viruses that infect bacteria) are highly abundant in the environment and may be the source of low cost antimicrobials. The focus of recent phage therapy approaches is on the use of lytic tailed phages all of which belong to the *Caudovirales* and include the *Myoviridae*, *Siphoviridae* and *Podoviridae* families [6]. Members of the *Caudovirales* have an icosahedral capsid head that contains double-stranded DNA (15–500 kbp), and a tail with surface receptor proteins that interact with surface features on the host bacterium [7]. Successful phage therapy requires interaction between the phage and the bacterium resulting in adsorption of the phage to the host bacterium followed by injection of the phage DNA. The pharmacodynamics of this process has been modelled using mathematical models based on colloidal interactions [8–10]. Upon infection, the phage replication cycle ensues culminating (after a short latency period) in cell lysis and the liberation of multiple phage virions (with a burst size that is typically between 10 and 100 [11]). Gill and Hyman [12] and Weber-Dabrowska et al. [13] provide an overview of key considerations related to phage choice, isolation and purification for phage therapy. Phage therapy practice relies on the isolation of naturally occurring phage abundantly found in the environment [14]. Typically, phage are isolated from the environment and screened against commonly occurring pathogenic bacterial strains (to identify host ranges) and then evaluated using in vitro and in vivo animal models. The limitation of host ranges is overcome through the use of phage cocktails to ensure sufficient coverage of commonly occurring strains and the use of phage mixtures targeting different receptors reduces the probability of encountering phage resistant bacterial mutants. Phages are generally manufactured using standard fermentation process technology. In brief, host bacteria are grown in liquid culture in a bioreactor. During the log growth phase, phages are added to the bioreactor to infect the bacteria. Incubation of phage with bacteria results in phage adsorption to the bacteria, infection and following a short lag period, release of bacteriophage virions. The resulting lysate contains the product (the amplified phage) along with bacterial debris and residual fermentation media. Removal of cellular debris is typically done using centrifugation and/or filtration. Ion exchange, gel filtration etc. can be used to further purify the bacteriophage (e.g. for the removal of host cell proteins, host cell DNA or endotoxin for Gram negative bacteria). Typically phage may then be re-suspended in simple saline or buffer and stored under refrigerated conditions or processed further e.g. spray dried to improve storage shelf life or encapsulated in micro- or nanoparticle formulations.

Phage are unique antimicrobials in that in the presence of host bacteria, they are able to increase their numbers by infecting the bacteria and producing virion progeny whilst minimally affecting the overall microbiota and body tissues. Phage carrying polysaccharide depolymerases (polysaccharide degrading enzymes) in their structure may be able to disrupt biofilms [15–17]. For example *Enterococcus* and *Staphylococcus* phage capable of destroying biofilms have been reported [18,19]. In addition to their potential as human biotherapeutics, phage are being developed for agricultural use to rid the environment and domestic animals of pathogens that could contaminate the food supply chain [20], in aquaculture for the treatment of fish pathogens [21] and for the control of infections in intensively farmed poultry [22,23]. Recent advances in molecular biology and sequencing technology have improved our basic understanding of how bacteriophage interact with bacteria and have opened new possibilities for utilising phage, including genetically engineered phage, for potential therapeutic and diagnostic applications [7,24].

3. Phage therapy for acute and chronic infections

Most of the recent phage therapy studies (using small vertebrate animals) have investigated treatments focusing on acute infections (Table 1). In acute infections the specific infection causing bacterium may be identified using suitable rapid diagnostic methods (e.g. lateral flow assays, PCR, MALDI-TOF Mass Spectrometry). In such instances, narrow spectrum antimicrobials such as bacteriophages may provide a suitable therapeutic alternative where organisms are resistant to frontline antibiotics or to reduce the use of broad spectrum antibiotics as part of a global effort towards antibiotic stewardship. An example of this is in cases of urinary tract infections where a significant proportion of the cases are caused by a particular pathotype *Escherichia coli* with specific virulence factors [25]. Animal studies have shown that phage may be effective in certain instances e.g. in treating acute respiratory infections caused by *Pseudomonas aeruginosa* [26–28] and in the treatment of systemic infections caused by *S. aureus* [29]. A significant focus of phage therapy studies in animals has been around respiratory infections, gastrointestinal infections and infections of the skin and wounds (Table 1). Phage therapy studies with animals has shown that in certain instances, it may help in reducing the densities of the infecting bacterial populations to levels that may allow the host immune response to mount a successful defence and clear the infection [26,27,30].

A number of in vivo phage studies (with animals and humans) have suggested that phage therapy may be beneficial in the treatment of difficult to treat antibiotic resistant pulmonary infections (e.g. cystic fibrosis [26] and pneumonia [31,32]), topical and wound infections [33,34] and gastrointestinal infections [35].

3.1. Challenges of antibiotic resistance for respiratory infections

Cystic fibrosis (CF) is a genetic disease of the lung resulting in reduced hydration and thickening of secretions covering the respiratory epithelium. Highly viscous mucus is not cleared by the epithelial cells and eventually leads to chronic inflammation and bacterial infections.