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Hurdles in bacteriophage therapy: Deconstructing the parameters

Jessica Tsonos [a,b,c,d], Dieter Vandenheuvel [d], Yves Briers [d], Henri De Greve [b,c], Jean-Pierre Hernalsteens [a], Rob Lavigne [d,*]

[a] Viral Genetics Research Group, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[b] Structural and Molecular Microbiology, VIB, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[c] Department Structural Biology, VIB, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[d] Laboratory of Gene Technology, KU Leuven, Kasteelpark Arenberg 21, 3001 Leuven, Belgium

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ABSTRACT

Bacterial infections in animals impact our food production, leading to economic losses due to food rejection and the need for preventive and curative measures. Since the onset of the antibiotic era, the rise of antibiotic-resistant pathogens is causing scares in health care and food producing facilities worldwide. In the search of new therapeutics, re-evaluation of bacteriophage (phage) therapy, using naturally occurring bacterial viruses to tackle infections, is gaining interest. Many studies report about phage therapy success, showing the value and power of these natural viruses.

Although phages carry some interesting traits and their basic biology is now well understood, this review argues that phage therapy has not revealed all of its secrets and many parameters remain understudied, making the outcome of phage therapy highly variable depending on the disease incidence. These difficulties include poorly understood mechanisms of phage penetration and distribution throughout the body, the variable expression and accessibility of phage receptors on the bacterial host in *in vivo* conditions and the unusual (non-linear) phage pharmacokinetics. These parameters are not easily measured in realistic *in vivo* settings, but are nevertheless important hurdles to overcome the high variability of phage therapy trials. This critical approach is in accordance with Goethe's statement; "*Difficulties increase the nearer we get to the goal*". However, since the importance of the goal itself also rises, both difficulties and goal justify the need for additional in depth research in this domain.

1. Introduction

Bacterial infections are the cause of many human and animal health problems. The latter have a large impact on our food production, including meat, eggs and dairy. Product rejection, necessary to avoid human food-borne diseases, leads to huge economic losses for the farmers. The need to reduce bacterial infections in the food animal industry becomes clear in the Food and Agricultural Organization (FAO) statistical yearbook 2013, reporting an average annual meat consumption per capita of 80 kg in developed countries. In 2010, the worldwide meat production totaled to 3×10^8 tons (FAO, 2013). In 2011, the New York Times reported one of the largest meat recalls, being 36 million pounds of ground turkey, linked to a *Salmonella* outbreak where a strain resistant to many commonly prescribed antibiotics was involved (Neuman, August 4, 2011).

* Corresponding author. Tel.: +32 16 37 95 24; fax: +32 16 3 21965.
E-mail addresses: jessica.tsonos@gmail.com (J. Tsonos), dieter.vandenheuvel@biw.kuleuven.be (D. Vandenheuvel), yves.briers@biw.kuleuven.be (Y. Briers), hdegreve@vub.ac.be (H. De Greve), jphernal@vub.ac.be (J.-P. Hernalsteens), rob.lavigne@kuleuven.be (R. Lavigne).

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The use of lytic bacterial viruses (bacteriophages), being the natural predators of bacteria, to treat bacterial infections and its efficient development in the veterinary sector may therefore have a significant economic impact, especially since antibiotic resistance becomes a major public health crisis. Antibiotic residues in food intended for human consumption and the compulsory withholding period to protect human from exposure to antibiotics would no longer be a concern if phage therapy is implemented at large scale. Indeed, phage therapy holds many intrinsic advantages. Bacteriophages are naturally occurring viruses that infect and lyse bacteria. They self-replicate exponentially at the infection site and leave the commensal flora unaffected because of their narrow infection spectrum. Their possible autonomous transfer between animals could be useful in agricultural applications (Loc-Carrillo and Abedon, 2011). Because of their ubiquitous presence in nature, the isolation of phages active against specific bacterial strains should be simple.

The concept of phage therapy emerged quickly after the discovery of bacteriophages in the beginning of the 20th century by Twort and d'Herelle (Inal, 2003). After d'Herelle determined the safety of his phage preparation by oral and subcutaneous self-administration, he opened the door for the development of this new kind of therapy. With his commercial laboratory in Paris, d'Herelle was the first to introduce bacteriophages as therapeutic agents (Summers, 2001; Kutateladze and Adamia, 2008).

The history of phage therapy passed through four periods: enthusiasm, skepticism, abandonment and reappraisal. Early enthusiasm was generated by a set of encouraging results with the first ones being obtained by d'Herelle against "avian typhosis", a gastrointestinal disease, and bovine hemorrhagic septicemia (Summers, 2001). Several pharmaceutical firms started to sell phage preparations *e.g.*, Eli Lilly, E.R Squibb and Sons, Antipol and The German Bacteriophage Society (Fischetti et al., 2006; Courchesne et al., 2009). The German and Soviet armies treated their soldiers during WWII against dysentery with phages (Hermoso et al., 2007). However, this excitement was short-lived and skepticism against phages as therapeutic agents took the lead. A lack of understanding of basic phage biology, quality control, absence of properly controlled studies and the associated unsuccessful therapeutic results contributed to this skepticism (Hanlon, 2007; Petty et al., 2007). Also, the discovery of penicillin saved thousands of Allied soldiers during WWII and its mass production was further developed in the post-war era to cure civilians and contributed to the downfall of phage therapy in Western medicine (Neushul, 1993). The Council of Pharmacy and Chemistry evaluated phage therapy in the late 1930s and acknowledged positive and negative results. Concern was expressed about the lack of standardization of phage preparations and the criteria for purity (Summers, 2001). In 1959, the World Health Organization came to the conclusion that there were no reasons to continue investigations with phages after the success of tetracycline against cholera (Barrow and Soothill, 1997; Barrow et al., 1998). Unfortunately, because of the widespread use (and misuse) of antibiotics, bacteria developed antibiotic resistance, in some cases to all clinically approved antibiotics. The potential of older methods had to be reexamined, explaining the renewed interest and reappraisal of phage therapy (Sulakvelidze, 2005).

Basic concepts we nowadays understand were completely unknown during the early period shortly after the discovery of phages. The two different replication cycles of phages (lytic & lysogenic) have now been unraveled and the importance of the strictly lytic character of phages (limiting horizontal gene transfer between bacteria) in therapy is well established. In addition, the high-throughput sequencing technologies allow inexpensive sequencing, enabling us to assess the presence of genes coding for factors that increase or generate pathogenicity of the bacteria or for toxins (*e.g.*, Shiga toxin, cholera toxin, botulinum neurotoxin) in the phage genome (Wagner and Waldor, 2002). Phage diversity and specificity was often underestimated and led to wrong phage choices for treatment in the past. Therapists were also not aware of the highly immunogenic character of the bacterial cell wall and outer membrane components found in crude phage lysates, which can lead to severe adverse reactions.

Even if our knowledge on bacteriophages is more extensive than ever before, the clinical outcome of phage therapy trials is still variable, indicating that there are still many parameters which are not yet understood or controlled. In the past, many attempts to improve the success of phage therapy have been based on the isolation of various phages from the environment and the identification of those that are the most effective in preventing mortality from a lethal infection (Levin and Bull, 2004). However, as will be shown in this review article, many more aspects are implicated than only the lytic capacity of phages and their simple administration to an infected organism.

In this review we outline the parameters which should be considered when analyzing the efficacy of whole phage therapy, to prevent the uncertainty and accidental outcome when going from an *in vitro* to an *in vivo* environment. We discuss the difficulties of phage penetration and its influence on therapeutic success. In addition, the importance of a detailed knowledge of the life style of the studied pathogen is highlighted, together with the too often neglected effect of the unusual phage pharmacokinetics. Where possible, available information concerning human phage therapy is mentioned, though human application remains subject to various (non-scientific) limitations (Verbeken et al., 2012). An outline of the review with the different topics is shown in Table 1.

2. Bacteriophage administration and translocation in the body

Depending on the site of infection and the method of administration, phages will need a high or low translocation capacity to reach the site of infection. At the industrial scale of intensive animal husbandry, phages must be administered to the food animals in a non-time-consuming and economically feasible manner. The most straightforward administration methods are either orally, by supplementing the feed or drinking water, or spraying