European Review for Medical and Pharmacological Sciences 2021; 25 (1 Suppl): 101-107

Naturally-occurring and cultured bacteriophages in human therapy

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Abstract. – OBJECTIVE: The aim of the study was to show the importance of developing techniques that could exploit the potential of bacteriophages as therapeutics or food supplements.

MATERIALS AND METHODS: PubMed database was searched using the following combination of keywords: (bacteriophage) AND (human therapy); (natural bacteriophage) AND (application).

RESULTS: The increasing antibiotic resistance of many bacterial strains is making standard antibiotic treatments less effective. Phage therapy provides a non-antibiotic alternative with greater specificity and without harmful effects on the human microbiota. Phages target their specific bacteria, replicate, and then, destroy the host pathogen. Bacteriophages may be administered by several routes, including topical, oral and intravenous. They not only destroy the host pathogen but, in some cases, increase the sensitivity of host bacteria to antibiotics. Various studies have shown that combining phage therapy and antibiotic treatment can be effective against bacterial infections. Clinical trials of phage therapy have shown promising results for various human diseases and conditions. With advances in genetic engineering and molecular techniques, bacteriophages will be able to target a wide range of bacteria.

CONCLUSIONS: In the future, phage therapy promises to become an effective therapeutic option for bacterial infections. Since many potentially beneficial bacteriophages can be found in food, supplements containing bacteriophages could be designed to remodel gut microbiota and eliminate pathogenic bacteria. Remodeling of gut microbiota could correct gut dysbiosis. The order of phages known to have these promising activities is *Caudovirales*, especially the families *Siphoviridae* and *Myoviridae*.

Key Words:
Phage therapy, Bacteriophage, Antibiotic resistance, Food supplement.

Introduction

The constant increase in multidrug-resistant organisms, such as ESKAPE pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species, has significantly reduced the effectiveness of antimicrobials. The increasingly narrow range of effective antibiotics makes it urgent to find new non-antibiotic options. Bacteriophages or phages are viruses that exploit the reproductive machinery of bacteria for their biological cycle. Antibiotic resistance is leading researchers to examine the prospects of phage therapy[1].

For more than a century, phage therapy was used to treat bacterial infections and this approach has been particularly explored in Georgia and Russia[2]. The universal decrease in antibiotic effectiveness has stimulated renewed interest in phage therapy. Phage therapy typically relies on naturally-occurring bacteriophages of the pathogen and lysis of bacterial cells at the infection site. Advances in biotechnology have extended the phage therapeutic repertoire to new strategies using bioengineered phages or purified lytic phage proteins. Recent research on the use of phages and their lytic proteins, especially against bacterial infections with multidrug resistance, suggests that phage therapy may be used as an alternative to antibiotics or to supplement antibiotic therapy[3].

There are approximately 10^{30}-10^{31} bacteriophages in the biosphere, 10 times more than the total population of bacterial cells. Bacteriophages are also an integral part of the human microbiome. They evidently have great potential as an

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alternative to antibiotics in human medicine to protect cells from bacterial infections[4-6] or as food supplements[7,8].

Phages in Natural Habitats

The oceans are estimated to host a population of 4×10^{30} viruses, with phages being the dominant viral group[9]. The most abundant phages in marine environments belong to the order *Caudovirales*[10,11], tailed phages with double stranded DNA. The order contains three families based on morphological differences: *Siphoviridae, Myoviridae* and *Podoviridae*[12,13]. The most abundant of these is the *Siphoviridae* phage, which can also be found in many other habitats[14,15]. Studies[16] on phages in various soil types from different geographical locations have estimated an average of 10^9 phage particles per gram of dry soil. Phages found naturally in soil and water are ecologically significant. They probably modulate bacterial growth rates and selectively impact the diversity of bacterial communities[17,18]. Interestingly, Ganges River water has been found to have bactericidal properties and to contain lytic phages specifically targeting *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia*[19].

Bacteriophages in thermal springs may explain some of the health benefits of thermal waters. Phages are abundant in hot springs and are important components of thermophilic communities: both bacteria and phages replicate in the water. Phages act as predators of hot spring microbes[20].

Thermal water is currently used for the treatment of certain skin diseases, including psoriasis, acne, atopic dermatitis, contact dermatitis, seborrheic dermatitis, recurrent upper respiratory tract infections, rhinitis and rhinosinusitis[21-23]. The beneficial effects of thermal water may be associated with its microbial component (bacteria and phages).

Phages Isolated and Cultured in the Laboratory for Therapeutic Use

Phage therapy has a promising future because it is an economically viable, environmentally friendly, potential alternative to antibiotics. However, there are certain criteria to consider before a phage can be deployed as a biocontrol agent. These include host specificity, absence of virulence genes or genes encoding toxins, phage life cycle, ability to integrate into the host genome and resistance to host defense mechanisms[24].

Applications

The use of phages to treat bacterial infections dates back to the 1920s, following the discoveries of Félix d'Herelle[25] and subsequently Bruynoghe and Maisin, who treated staphylococcal infections with phage therapy[26]. Around that time, several successful experiments investigated the possibility for treating various bacterial infections in humans by phages, but were not further developed due to the advent of antibiotics and lack of knowledge of phage biology and phage specificity[26,27]. Although countries like Georgia, Russia and Poland have continued practicing phage therapy since the 1930s, only in the last 30 years this approach has regained attention in other countries as a result of the threat of multidrug-resistant pathogenic strains[28,29].

Administration

Unlike antibiotics, a single dose of phages may be sufficient to eradicate 100% of the bacterial population. However, the route of administration has a direct effect on the efficiency of phage therapy. In order to achieve the maximum effect, high bacteriophage concentrations need to be delivered in proximity of the target pathogen by appropriate dosing and administration[30,31]. Phage kinetics describes the amount and variations in phage particles available in a living system. Phage dynamics describe and measure the physiological effects of phages on a living system. Phage kinetics and dynamics are of utmost importance in therapeutic applications because the interactions between phages, bacteria and eukaryotic cells are complex and also involve the immune system of the animal or human[32,33]. *In vivo* phage kinetics and dynamics depend on many critical factors, such as adsorption to the host, latency period, initial phage dose, rate of clearance from body fluids, ability to replicate *in situ*, the anatomy of the animal or human being treated, host resistance mechanisms, phage distribution in the body, and immune system effects on the bacteriophage[32,33].

Phages Against Clinically Significant Pathogens

Recent studies have used animal models to explore phage therapy against a wide range of clinically significant pathogens. In gut-derived sepsis caused by *P. aeruginosa*, oral administration of phages saved almost 66.7% of the experimental mice from death, against 0% in the control group[34]. A single dose of bacteriophage with parallel administration of *Clostridium difficile* in

a *C. difficile*-induced ileocecitis hamster model was sufficient to prevent infection. Likewise, a single strain of intraperitoneally administered phage along with vancomycin-resistant *Enterococcus faecium*[35] in bacteremia mouse models was sufficient to save 100% of the mice; the same was found with imipenem-resistant *P. aeruginosa* and extended spectrum β-lactamase producing *E. coli*[36,37]. Further animal studies showed similar promising results against multidrug-resistant *E. coli* clone O25:H4-ST131, *A. baumannii*, *Vibrio parahaemolyticus* and *S. aureus*. Some studies even showed that bacteriophages could restore antibiotic sensitivity of antibiotic-resistant bacteria, such as multidrug-resistant *P. aeruginosa*. Since its first therapeutic uses, phage therapy, alone or in combination with antibiotics (phage cocktail) has been successful in treating surgical wounds[2], skin infections and burns[38,39], infected ulcers[40], nosocomial infections[41,42], respiratory tract infections[43,44], urinary tract infections[45,46], orthopedic implant-associated infections[47-49], ear infections[40], gastrointestinal diseases[50,51] and septicemia with acute kidney damage[31]. For instance, a cosmetic formulation containing phages against *S. aureus* has been developed for the treatment of skin diseases[52].

Naturally-Occurring Bacteriophage Applications

Bacteriophage-Containing Food and Prophage Inducers

Most phages in the human gut are integrated in the genomes of their bacterial hosts as prophages[53]. Prophages are induced by certain foods and chemicals, including soy sauce[54], nicotine[55], sunscreen[56] and antibiotics[57]. Prophage induction in *B. thetaiotaomicron*, *E. faecalis* and *S. aureus* can alter the relative abundances of these bacteria. Strong prophage inducers are stevia, clove, propolis, uva-ursi, aspartame and grapefruit seed extract. In mice, an extract containing propolis can mitigate the gut community composition and inflammatory markers resulting from high fat diets[58]. Common foods, herbs and dietary supplements can manipulate the relative abundances of gut bacteria through prophage induction, making diet-induced prophage activation a regular occurrence in the gut ecosystem[58]. In addition, fermented foods like sauerkraut may contain phage particles. Phages isolated from sauerkraut fermentation belong to three virus families (*Myoviridae*, *Siphoviridae* and *Podoviridae)* of the order *Caudovirales*[59]. These foods can be employed as natural sources of phages for human therapy or food supplementation to restore the human microbiota[6]. Many phages related to the Bacillus bacterial group can be isolated from the soybean fermentation process. Although phages are usually considered detrimental for food fermentation, phages found naturally in food may also limit foodborne pathogenic bacteria[60]. Most cucumber fermentations are driven by lactic acid bacteria, naturally present on fresh cucumbers[61]. Although lactic acid bacteria are naturally associated with fresh cucumbers, Gram-negative bacteria are initially predominant[62]. Successful fermentation can be achieved by favoring the growth of lactic acid bacteria over other microorganisms. Some Gram-negative bacteria, such as *Salmonella* and *Escherichia coli* O157:H7 can cause foodborne illness, and phage infection to control the growth of Gram-negative bacteria is often overlooked[61]. According to a study, a phage isolated from cucumber fermentation is able to infect the Gram-negative *Escherichia coli* O157:H7[63]. At least eight phages able to infect Gram-negative bacteria can be isolated from the fermentation. All belong to the order *Caudovirales* (*Myoviridae*, *Siphoviridae* and *Podoviridae*)[61]. Lactococcus phages BM13 and Q33 are the most common in fermented milk and have a genome closely related to phages infecting Enterococci and Clostridia[64]. Enterococci are important nosocomial pathogens[65]. Alterations in the gut microbiota associated with antibiotic treatment may stimulate the growth of resistant strains and the onset of *Clostridium difficile* infection[66]. Phages that can infect and eliminate Enterococci and Clostridia could therefore be useful in the management of conditions associated with these pathogens, especially antibiotic-resistant strains[67].

Bacteriophages From Water

The antimicrobial properties of the waters of Ganges River have long been known but the reasons for these properties were not understood. Ganges water sampled at different points along the river contained different pathogenic species of the *Enterobacteriaceae* group (*Escherichia, Enterobacter, Salmonella, Shigella* and *Klebsiella*) and species of the *Vibrio* genus. Interestingly, bacteriophages were also isolated from the water samples. When these bacteriophages were plated on plates containing all these bacteria, plaques developed after a certain period of time[18,68]. In an-

other study, bacteriophages specific for coliform bacteria were found in samples of Spanish and Israeli drinking water obtained in 1997. These phages could be concentrated and used against their bacterial targets[69].

Bacteriophages From Soil and Clay

Bacteriophages can also be found in soil and clay. In particular, MS2, ΦX174 and T2 phages may be adsorbed on clay (hectorite, saponite, kaolinite, montmorillonite)[70,71]. Since these phages specifically target *E. coli*, clay materials can be used to concentrate bacteriophages that target pathogenic bacteria, like pathogenic strains of *E. coli*.

Advantages and Disadvantages of Naturally-Occurring and Cultured Phages

Phage therapy has two major advantages: phages have high specificity towards their hosts and do not usually cause dysbacteriosis or secondary infections. Phages have not shown any major side effects or toxicity to mammalian cells. The selection and isolation of new phages is not as expensive as the development of antibiotics[72]. Bacterial resistance to phage therapy develops slower than bacterial resistance does to antibiotics, because phage cocktails can be modified by substituting phages, by evolutionary pressure *in vitro* and by genetic engineering. Phages can successfully eliminate multidrug-resistant bacteria and their ability to spread throughout the body after systemic administration and to replicate in their specific host are qualities that cannot be found in antibiotics[73].

The greatest disadvantage of phage therapy is the lack of detailed information about how phages can be used in clinical practice to control bacterial infections. Most experimental and clinical data has been published in Polish and Russian journals, difficult to access due to language and security barriers. Regulatory approval for phage-based therapies is more difficult to obtain than approval for conventional therapies[74]. There is also a lack of well-established and validated protocols for phage therapy posology. The stability and the purity of phage preparations used in clinical trials vary greatly and published data lacks adequate quality controls. Additionally, the assessment of bacteriophage genetic biosafety is complex: they must not contain any toxin, antibiotic resistance or other virulent genes and they must not be able to transfer genes horizontally to human microflora. Another limitation of phage therapy is that the function of all the genes encoded by bacteriophages is not fully understood, therefore genetic engineering and manipulation of these bacteriophages will require greater scrutiny for safety issues[4,75]. Future randomized controlled clinical trials will have to compare the standard of care of antibiotic treatment with the standard of care of antibiotic plus phage therapy. If the combination of phages and antibiotics shows better results than antibiotics alone, then production of these phage-antibiotic combinations would be of great interest to drug companies. These products would open the way for a new antimicrobial pipeline and may well end the "era of antibiotic resistance"[1].

The phages used so far with positive results in the treatment of human diseases are those cultured *in vitro* in laboratories. However, various studies mentioned above show that naturally-occurring phages obtained from water, soil or fermented food can have important biological properties for human health. Phages could therefore be promoted not only as therapeutic agents, but also as food supplements. However, before we can promote their use in humans, greater awareness of the importance of phages must be fostered.

Conclusions

Most *in vitro* studies have established that combined phage-antibiotic therapy is more effective than phage therapy or antibiotic treatment alone. However, the studies performed so far have not fully evaluated or reported the significance of phage-antibiotic combinations. Since many bacteriophages with potential beneficial activity can be found in food, it will be necessary to concentrate bacteriophages in the food that already contains them, or to design food supplements containing phages that occur naturally in common food. These supplements may contain a single bacteriophage species or a cocktail and could be used to remodel gut microbiota and to eliminate pathogenic or potentially pathogenic bacteria, thus improving health and correcting gut dysbiosis. The order of phages with these promising activities is *Caudovirales*, especially the families *Siphoviridae* and *Myoviridae*. Most studies concentrated on isolation of phages *in vitro*, though now more effort is being made to identify and concentrate phages occurring naturally in specific foods. Further research into the unique properties and sta-

bility of phages in different preparations is likely to lead to interesting applications in therapeutics and food supplements.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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