June 2009 *Volume 3 Issue 1*

Trade Science Inc.

Research & Reviews in

BioSciences

Review

RRBS, 3(1), 2009 [93-98]

Bacteriophage therapy: A potential use of phages in medical field

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Received: 7th April, 2009 ; Accepted: 12th April, 2009

ABSTRACT

Phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Phage therapy has many potential applications in human medicine, dentistry, veterinary science, and agriculture. With the increase in antibiotic resistance, the use of bacteriophage as antimicrobial agents is being re-examined to supplement antibiotic treatment. Potential advantages of using phage result from their specificity and ability to multiply.

KEYWORDS

Phage therapy;
Drug resistance;
Bacterial infections.

INTRODUCTION

All over the world, the resistance of bacteria to antibiotics is becoming a grave medical problem[1]. Independent of the resources of the medical system, whenever antibiotics are used the development of resistance is a logical consequence; like all other living organisms, bacteria adapt to changing environmental conditions in a continuous process of evolution. Ironically, resistance is promoted by both the overuse of antibiotics as well as insufficiency of dose. In industrialized countries, bacteria are developing multiple resistance to a range of antibiotics, which threatens to make the achievements of modern medicine futile. In developing countries basic medical care is already endangered by single resistance to inexpensive common generic antibiotics, particularly because of the concomitant increase in immunosuppressed patients. The concern that humankind is reentering the preantibiotic era has become very real, and the development of alternative antiinfection modalities has become one of the highest priorities of modern medicine and biotechnology.

Prior to the discovery and widespread use of antibiotics, it was suggested that bacterial infections could be prevented and/or treated by the administration of bacteriophages. Although the early clinical studies with bacteriophages were not vigorously pursued in the United States[2] and Western Europe, phages continued to be utilized in the former Soviet Union and Eastern Europe. The results of these studies were extensively published in non-English (primarily Russian, Georgian, and Polish) journals and, therefore, were not readily available to the western scientific community. In this minireview, we briefly describe the history of bacteriophage discovery and the early clinical studies with phages and we review the recent literature emphasizing research conducted by the various authors

A bacteriophage (from 'bacteria' and Greek *phagein* "to eat") is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, *phage*. If the target host of a phage therapy

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treatment is not an animal, then the term "biocontrol" (as in phage-mediated biocontrol of bacteria) is sometimes employed rather than "phage therapy".

Phages are estimated to be the most widely distributed and diverse entities in the biosphere[1]. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×10^8 virions per milliliter have been found in microbial mats at the surface[3], and up to 70% of marine bacteria may be infected by phages[4].

They have been used for over 90 years as an alternative to antibiotics in the former Soviet Union and Eastern Europe[5]. They are seen as a possible therapy against multi drug resistant strains of many bacteria[6-9].

Classification of phages

The dsDNA tailed phages, or *Caudovirales*, account for 95% of all the phages reported in the scientific literature, and possibly make up the majority of phages on the planet[1]. However, there are other phages that occur abundantly in the biosphere, phages with different virions, genomes and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid (TABLE 1).

Phage therapy

Since ancient times, it was reported that the waters of the rivers Ganga and Jamuna in India possessed astonishing antibacterial properties. In 1896, Earnest reported that something in the waters of the Ganga and Jamuna rivers in India had marked antibacterial action against cholera and could pass through a very fine porcelain filter[11].

In 1915, British bacteriologist Frederick Twort[10] superintendent of the Brown Institution of London, discovered a small agent that infected and killed bacteria. French-Canadian microbiologist Félix d'Herelle, working at the Pasteur Institute in Paris, announced on September 3, 1917 that he had discovered "An invisible, antagonistic microbe of the dysentery bacillus". D'Hérelle called the virus a bacteriophage or bacteria-eater (from the Greek *phagein* meaning to eat). He also recorded a dramatic account of a man suffering from dysentery who was restored to good health by the bacteriophages.

Early studies of phage therapy

Not long after his discovery, d'Herelle used phages to treat dysentery, in what was probably the first attempt to use bacteriophages therapeutically. The studies were conducted at the Hospital des Enfants-Malades in Paris[11] in 1919 under the clinical supervision of Professor Victor-Henri Hutinel, the hospital's Chief of Pediatrics. The phage preparation was ingested by d'Herelle, Hutinel, and several hospital interns in order to confirm its safety before administering it the next day to a 12-year-old boy with severe dysentery. The patient's symptoms ceased after a single administration of d'Herelle's antidysentery phage, and the boy fully recovered within a few days. The efficacy of the phage preparation was "confirmed" shortly afterwards, when three additional patients having bacterial dysentery and treated with one dose of the preparation started to recover within 24 h of treatment. However, the results of these studies were not immediately published and, therefore, the first reported application of phages to treat infectious diseases of humans came in 1921 from Richard Bruynoghe and Joseph Maisin[13], who used bacteriophages to treat staphylococcal skin disease. The

TABLE 1 : ICTV classification of phages[1]

Order	Family	Morphology	Nucleic acid
Caudovirales	*Myoviridae*	Non-enveloped, contractile tail	Linear dsDNA
	Siphoviridae	Non-enveloped, long non-contractile tail	Linear dsDNA
	Podoviridae	Non-enveloped, short noncontractile tail	Linear dsDNA
	Tectiviridae	Non-enveloped, isometric	Linear dsDNA
	Corticoviridae	Non-enveloped, isometric	Circular dsDNA
	Lipothrixviridae	Enveloped, rod-shaped	Linear dsDNA
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA
	Rudiviridae	Non-enveloped, rod-shaped	Linear dsDNA
	Fuselloviridae	Non-enveloped, lemon-shaped	Circular dsDNA
	Inoviridae	Non-enveloped, filamentous	Circular ssDNA
	Microviridae	Non-enveloped, isometric	Circular ssDNA
	Leviviridae	Non-enveloped, isometric	Linear ssRNA

bacteriophages were injected into and around surgically opened lesions, and the authors reported regression of the infections within 24 to 48 h. Several similarly promising studies followed[13,14], and encouraged by these early results, d'Herelle and others continued studies of the therapeutic use of phages (e.g., d'Herelle used various phage preparations to treat thousands people having cholera and/or bubonic plague in India[11] In addition, several companies began active commercial production of phages against various bacterial pathogens.

Problems of early phage therapy research

Despite all the properties of lytic phages that would seem to favor their clinical use, they are not commonly used prophylactically or therapeutically throughout the world and their efficacy is still a matter of controversy. Many factors have contributed to this situation.

Failure to establish rigorous proof of efficacy

One of the most important factors that have interfered with documenting the value of phage therapy has been the paucity of appropriately conducted, placebo-controlled studies. Ironically, d'Herelle caused substantial long-term harm to his idea of phages as therapeutic agents because, in his eagerness to transfer his laboratory studies to hospital and community settings, he performed clinical studies with phages without including placebo groups of patients. Starting with the first known use of phages in humans (the Enfants-Malades trials) and in all subsequent trials, d'Herelle administered phages to all sick patients. This failure to include placebo groups may be explained by the possibility that d'Herelle might have been reluctant to deprive anyone of therapy he believed could save his or her life. It could also have been due to the personal scientific style of d'Herelle, as he also failed to include placebo groups during his studies with chickens, when ethical considerations were not an issue[16] Similar failures were very common during the early history of phage therapy, and therefore the results frequently were controversial. To address this controversy, the Council on Pharmacy and Chemistry of the American Medical Association requested that a full review of the available literature on phage therapy be prepared for the Council's consideration. Consequently, Monroe Eaton and Stanhope Bayne-Jones reviewed more than 100 papers on bacteriophage therapy and in 1934 they published a detailed review of phage therapy[17]. This report is one of the most detailed reviews of phage therapy ever published, and its conclusions were clearly not in favor of phage therapy. Among other conclusions, the authors stated that "d'Herelle's theory that the material is a living virus parasite of bacteria has not been proved. On the contrary, the facts appear to indicate that the material is inanimate, possibly an enzyme". The authors further stated that "since it has not been shown conclusively that bacteriophage is a living organism, it is unwarranted to attribute its effect on cultures of bacteria or its possible therapeutic action to a vital property of the substance". At the present time it is clear that the above conclusions of the report were incorrect. However, the report delivered a severe blow to the interest of Western scientists in evaluating the utility of phages for therapeutic purposes and it undoubtedly had a strong negative impact on the enthusiasm of funding agencies to support therapeutic phage research. In addition, 7 years after the Eaton-Bayne-Jones report, a second unfavourable report was published by Albert Krueger and Jane Scribner[18] as a sequel to the Eaton-Bayne-Jones report. The authors justified the need to write the second review because "much more information about both phage itself and its clinical utility has accumulated". However, the authors' conclusions about the nature of phages also was incorrect since they stated "It is a protein of high molecular weight and appears to be formed from a precursor originating within the bacterium". The authors further concluded that "it is equally evident that phage solutions possess no measurable degree of superiority over well known and accepted preparations". Although the authors suggested that further evaluation of the therapeutic potential of phages might be warranted under thoroughly controlled conditions, their assessment (together with that of Eaton and Bayne-Jones) effectively stopped all major studies of phage therapy in the United States. In addition, a few years after the review was published, antibiotics became widely available, which further contributed to the decline of interest in phage therapy in the West. This was not affected by the continuing studies in the former Soviet Union and Eastern Europe since-as discussed above-many of these studies were not available to the international scientific community and/or were conducted in a manner which did not allow rigorous analysis of the author's conclusions.

Advantages over antibiotics

An important theoretical benefit of phage therapy

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TABLE 2 : Therapeutic use of phages and antibiotics

Bacteriophages	Antibiotics	Comments
Very specific (i.e., usually affect only the targeted bacterial species); therefore, dysbiosis and chances of developing secondary infections are avoided[19].	Antibiotics target both pathogenic microorganisms and normal microflora. This affects the microbial balance in the patient, which may lead to serious secondary infections.	High specificity may be considered to be a disadvantage of phages because the disease-causing bacterium must be identified before phage therapy can be successfully initiated. Antibiotics have a higher probability of being effective than phages when the identity of the etiologic agent has not been determined.
Replicate at the site of infection and are thus available where they are most needed[20].	They are metabolized and eliminated from the body and do not necessarily concentrate at the site of infection.	The "exponential growth" of phages at the site of infection may require less frequent phage administration in order to achieve the optimal therapeutic effect.
No serious side effects have been described.	Multiple side effects, including intestinal disorders, allergies, and secondary infections (e.g., yeast infections) have been reported[23].	A few minor side effects reported[21, 22] for therapeutic phages may have been due to the liberation of endotoxins from bacteria lysed in vivo by the phages. Such effects also may be observed when antibiotics are used[24].
Phage-resistant bacteria remain susceptible to other phages having a similar target range.	Resistance to antibiotics is not limited to targeted bacteria.	Because of their more broad-spectrum activity, antibiotics select for many resistant bacterial species, not just for resistant mutants of the targeted bacteria[25].
Selecting new phages (e.g., against phage-resistant bacteria) is a relatively rapid process that can frequently be accomplished in days or weeks.	Developing a new antibiotic (e.g., against antibiotic-resistant bacteria) is a time-consuming process and may take several years[26-28].	Evolutionary arguments support the idea that active phages can be selected against every antibiotic-resistant or phage-resistant bacterium by the ever-ongoing process of natural selection.

is that bacteriophages can be much more specific than more common drugs, so can be chosen to be harmless to not only the host organism (human, animal, or plant), but also other beneficial bacteria, such as gut flora, reducing the chances of opportunistic infections. They also have a high therapeutic index, that is, phage therapy gives rise to few if any side effects, as opposed to drugs, and does not stress the liver. Because phages replicate *in vivo*, a smaller effective dose can be used. On the other hand, this specificity is also a disadvantage: A phage will only kill a bacterium if it is a match to the specific strain. Thus, phage mixtures are often applied to improve the chances of success, or samples can be taken and an appropriate phage identified and grown.

Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antibiotics, particularly in the country of Georgia[7,14]. They tend to be more successful than antibiotics where there is a biofilm covered by a polysaccharide layer, which antibiotics typically cannot penetrate. In the West, no therapies are currently authorized for use on humans, although phages for killing food poisoning bacteria (*Listeria*) are now in use[16].

Phage therapy can be very effective in certain conditions and has some unique advantages over antibiotics. Bacteria also develop resistance to phages, but it is incomparably easier to develop new phage than new antibiotic. A few weeks versus years are needed to obtain new phage for new strain of resistant bacteria. As bacteria evolve resistance, the relevant phages naturally evolve alongside. When super bacterium appears, the super phage already attacks it. We just need to derive it from the same environment. Phages have special advantage for localized use, because they penetrate deeper as long as the infection is present, rather than decrease rapidly in concentration below the surface like antibiotics. The phages stop reproducing once as the specific bacteria they target are destroyed. Phages do not develop secondary resistance, which is quite often in antibiotics. With the increasing incidence of antibiotic resistant bacteria and a deficit in the development of new classes of antibiotics to counteract them, there is a need to apply phages in a range of infections.

Lytic phages are similar to antibiotics in that they have remarkable antibacterial activity. However, therapeutic phages have some advantages over antibiotics, and phages have been reported to be more effective than antibiotics in treating certain infections in humans and experimentally infected animals. TABLE 2 signifies therapeutic use of bacteriophages against antibiotic resistant bacteria.

Potential benefits

A potential benefit of phage therapy is freedom from the severe adverse effects of antibiotics. Also it would possibly be fast-acting, once the exact bacteria are identified and the phages administered. Another benefit of phage therapy is that although bacteria are able to develop resistance to phages the resistance might be easier to overcome.

Bacteriophages are often very specific, targeting only one or a few strains of bacteria. Traditional antibiotics usually have more wide-ranging effect, killing both harmful bacteria and useful bacteria such as those facilitating food digestion. The specificity of bacteriophages might reduce the chance that useful bacteria are killed when fighting an infection.

Increasing evidence shows the ability of phages to travel to a required site including the brain, where the blood brain barrier can be crossed and multiply in the presence of an appropriate bacterial host, to combat infections such as meningitis. However the patient's immune system can, in some cases mount an immune response to the phage (2 out of 44 patients in a Polish trial[12]. This might possibly be therapeutically significant.

Development and production is faster than antibiotics, on condition that the required recognition molecules are known. Research groups in the West are engineering a broader spectrum phage and also target MRSA treatments in a variety of forms - including impregnated wound dressings, preventative treatment for burn victims, phage-impregnated sutures. Enzobiotics are a new development at Rockefeller University that create enzymes from phage. These show potential for preventing secondary bacterial infections e.g. pneumonia developing with patients suffering from flu, otitis etc. Some bacteria such as multiple resistant *Klebsiella pneumoniae, Pseudomonas sps*[29] have no non toxic antibiotics available, and yet killing of the bacteria via intraperitoneal, intravenous or intranasal of phages in vivo has been shown to work in laboratory tests[9].

Phage therapy is generally considered safe. As with antibiotic therapy and other methods of countering bacterial infections, endotoxins are released by the bacteria as they are destroyed within the patient (Herxheimer reaction)[30,31]. This can cause symptoms of fever, or in extreme cases toxic shock (a problem also seen with antibiotics) is possible[28]. This complication can be avoided by using genetically engineered bacteriophages; which have had their gene responsible for producing endolysin removed. Without this gene the host bacterium still dies but remains intact because the lysis is disabled.

The development of phage-neutralizing antibodies is another possible problem which may hamper phage effectiveness in lysing targeted bacteria in vivo. Indeed, the development of neutralizing antibodies after parenteral administration of phages has been well documented[32]. However it is unclear how significant a problem this may be during phage therapy, especially when phages are administered orally and/or locally. In theory, the development of neutralizing antibodies should not be a significant obstacle during the initial treatment of acute infections, because the kinetics of phage action is much faster than is the host's production of neutralizing antibodies. Also, it is not clear how long the antibodies will remain in circulation. Thus, careful studies must be conducted to address the validity of this concern. For example, if administration of phages elicits only a brief, mild antibody response in the patient, phages given at a later time (e.g., to treat a recurring, acute infection) should not be affected. However, if phageneutralizing antibodies are still present at the time the second course of treatment is necessary or if a rapid anamnestic immune response occurs before the phages exert their action, the value of repeated administration of increased doses of phages or of the administration of different phages having the same spectrum of activity but a different antigenic profile must be determined. Another concern regarding the therapeutic use of lytic phages is that the development of phage resistance may hamper their effectiveness. Bacterial resistance to phages will unquestionably develop, although according to some authors[33] the rate of developing resistance to phages is approximately 10-fold lower than that to antibiotics. The rate of developing resistance against phages can be partially circumvented by using several phages in one preparation[9] (much like using two or more antibiotics simultaneously). Most importantly, when resistance against a given phage occurs, it should be possible to select rapidly (in a few days or weeks) a new phage active against the phage-resistant bacteria. It is also unclear how effective phages would be in treating diseases caused by intracellular pathogens[27] (e.g., *Salmonella* species), where bacteria multiply primarily inside human cells and are inaccessible to phages. It is

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possible that phages will have only limited utility in treating infections caused by intracellular pathogens; however, phages have been reported[34] to be effective in preventing salmonellosis in children.

In summary, bacteriophages have several characteristics that make them potentially attractive therapeutic agents. They are (i) highly specific and very effective in lysing targeted pathogenic bacteria, (ii) safe, as underscored by their extensive clinical use in Eastern Europe and the former Soviet Union and the commercial sale of phages in the 1940s in the United States, and (iii) rapidly modifiable to combat the emergence of newly arising bacterial threats. In addition, a large number of publications, suggest that phages may be effective therapeutic agents in clinical settings. Granted, many of these studies do not meet the current rigorous standards for clinical trials and there still remain many important questions that must be addressed before lytic phages can be widely endorsed for therapeutic use. However, we think that there is a sufficient body of data and a desperate enough need to find alternative treatment modalities against rapidly emerging, antibiotic-resistant bacteria to warrant further studies in the field of phage therapy.

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