

Bacteriophage Therapy: A War Against Antibiotic Resistant Bacteria

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Abstract

Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. Recent examples of the use of bacteriophages in controlling bacterial infections are presented, some of which show therapeutic promise. The therapeutic use of bacteriophages, possibly in combination with antibiotics, may be a valuable approach. However, it is also quite clear that the safe and controlled use of phage therapy will require detailed information on the properties and behavior of specific phage–bacterium systems, both in vitro and especially in vivo. In vivo susceptibility of bacterial pathogens to bacteriophages is still largely poorly understood and future research on more phage–bacterium systems has to be undertaken to define the requirements for successful phage treatments.

INTRODUCTION

The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immunosuppressed patients. The concern that humankind is reentering the “preantibiotics” era has become very real, and the development of alternative ant infection modalities has become one of the highest priorities of modern medicine and biotechnology.

BACTERIOPHAGES

Viruses that attack bacteria were observed by Twort and d’Herelle in 1915 and 1917. They observed that broth cultures of certain intestinal bacteria could be dissolved by addition of a bacteria-free filtrate obtained from sewage. The lysis of the bacterial cells was said to be brought about by a virus which meant a “filterable poison”

Probably every known bacterium is subject to infection by one or more viruses or “bacteriophages” as they are known (“phage” for short, from Gr. “phagein” meaning “to eat” or “to nibble”). Most research has been done on the phages that attack *E. coli*, especially the T-phages and phage lambda. Like most viruses, bacteriophages typically carry only the genetic information needed for replication of their nucleic acid and synthesis of their protein coats. When phages infect

their host cell, the order of business is to replicate their nucleic acid and to produce the protective protein coat. But they cannot do this alone. They require precursors, energy generation and ribosomes supplied by their bacterial host cell.

Bacterial cells can undergo one of two types of infections by viruses termed lytic infections and lysogenic (temperate) infections. In *E. coli*, lytic infections are caused by a group seven phages known as the T-phages, while lysogenic infections are caused by the phage lambda.

LYTIC INFECTIONS

The T-phages, T1 through T7, are referred to as lytic phages because they always bring about the lysis and death of their host cell, the bacterium *E. coli*. T-phages contain double-stranded DNA as their genetic material. In addition to their protein coat or capsid (also referred to as the “head”), T-phages also possess a tail and some related structures. A diagram and electron micrograph of bacteriophage T4 is shown below. The tail includes a core, a tail sheath, base plate, tail pins, and tail fibers, all of which are composed of different proteins. The tail and related structures of bacteriophages are generally involved in attachment of the phage and securing the entry of the viral nucleic acid into the host cell.

Before viral infection, the cell is involved in replication of its own DNA and transcription and translation of its own

genetic information to carry out biosynthesis, growth and cell division. After infection, the viral DNA takes over the machinery of the host cell and uses it to produce the nucleic acids and proteins needed for production of new virus particles. Viral DNA replaces the host cell DNA as a template for both replication (to produce more viral DNA) and transcription (to produce viral mRNA). Viral mRNAs are then translated, using host cell ribosomes, tRNAs and amino acids, into viral proteins such as the coat or tail proteins. The process of DNA replication, synthesis of proteins, and viral assembly is a carefully coordinated and timed event.

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PHAGE THERAPY

Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. Phage therapy is an alternative to antibiotics being developed for clinical use by research groups in Eastern Europe and the U.S. After having been extensively used and developed mainly in former Soviet Union countries for about 90 years, phage therapies for a variety of bacterial and poly microbial infections are now becoming available on an experimental basis in other countries, including the U.S. The principles of phage therapy have potential applications not only in human medicine, but also in dentistry, veterinary science, food science and agriculture.

An important benefit of phage therapy is derived from the observation that bacteriophages are much more specific than most antibiotics that are in clinical use. Theoretically, phage therapy is harmless to the eukaryotic host undergoing therapy, and it should not affect the beneficial normal flora of the host. Phage therapy also has few, if any, side effects, as opposed to drugs, and does not stress the liver. Since

phages are self-replicating in their target bacterial cell, a single, small dose is theoretically efficacious. On the other hand, this specificity may also be disadvantageous because a specific phage will only kill a bacterium if it is a match to the specific subspecies. Thus, phage mixtures may be applied to improve the chances of success, or clinical 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. They are reported to be especially successful where bacteria have constructed a biofilm composed of a polysaccharide matrix that antibiotics cannot penetrate.

BACTERIAL HOST SPECIFICITY

The bacterial host range of phage is generally narrower than that found in the antibiotics that have been selected for clinical applications. Most phage are specific for one species of bacteria and many are only able to lyse specific strains within a species. This limited host range can be advantageous, in principle, as phage therapy results in less harm to the normal body flora and ecology than commonly used antibiotics, which often disrupt the normal gastrointestinal flora and result in opportunistic secondary infections by organisms such as *Clostridium difficile*. The potential clinical disadvantages associated with the narrow host range of most phage strains is addressed through the development of a large collection of well-characterized phage for a broad range of pathogens, and methods to rapidly determine which of the phage strains in the collection will be effective for any given infection.

Figure 1

Table 1: Comparison of the Prophylactic and/or 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 (15).	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 (59).	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 (76).	A few minor side effects reported (17, 58) 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 (42).
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 (47).
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 (16, 51).	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.

ADVANTAGES OVER ANTIBIOTICS

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.

ADVANTAGES OF BACTERIOPHAGE THERAPY

1. For every type of bacteria known in nature, there is at least one complementary bacteriophage that specifically

infects a single bacterial species. So bacteriophage therapy is possible in all bacterial infections.

2. If a suitable bacteriophage is introduced onto an infected wound, it will continue to increase in numbers as long as there are bacteria to infect and destroy. However, as soon as all the bacteria have been destroyed, the action of the phage will cease and the dormant phage particles will disperse harmlessly.

3. Because phages are so specific to the bacteria they infect, they will not harm other beneficial bacteria present in the intestine and other parts of the body and will not affect the microbial ecosystem in the body.

There is no chance of super infection with other bacteria. The bacterial imbalance caused by treatment with many antibiotics can lead to serious secondary infections involving relatively resistant bacteria, often extending hospitalization time, expense and mortality. This will not occur with specific bacteriophage therapy.

4. Some people are allergic to antibiotics so phage therapy could be a useful alternative for these patients. No patient has ever been known to suffer an allergic reaction to bacteriophages. That may be because phages are omnipresent living organisms on earth, found in soil, water, plants and humans.

5. Phage therapies can be administered to patients in different ways which include pills, injections, enemas, nasal sprays, ointments, etc.

6. Each phage infects a specific bacteria or range of bacteria. A person in hospital, where bacterial infections abound, can be treated with a range of phages targeted at several types of bacteria. They can be given a cocktail of phage types to attack one type of bacteria or they can be given a combination of phage and antibiotic treatment.

7. Phages are considered safe for therapeutic use. No major side effects have been described so far. Only a very few side effects have been reported in the patients undergoing phage therapy. This might be related to extensive liberation of endotoxins from dead bacteria as the phages were destroying the bacteria most effectively. This type of reaction can also happen when antibiotics are used.

11. Since selection of active phages is a natural process, evolutionary arguments support the idea that active phage can be selected against every resistant bacterium, by an ever

ongoing process of natural selection.

12. Production is simple and relatively inexpensive. So the treatment costs of bacterial infections will be reduced. This facilitates their potential applications to underserved populations.

PROBLEMS ASSOCIATED WITH BACTERIOPHAGE THERAPY

1. Because of the high specificity of phages, the disease-causing bacterium has to be identified before the administration of phage therapy. One phage kills only aspecific subgroup of bacteria. One species of bacteria may contain many subgroups. But one antibiotic may kill many different species and subgroups of bacteria simultaneously. So a physician would need to make a specific diagnosis before prescribing a phage treatment.
2. Absences of bacteriophage action efficacy in certain cases were reported. It may be due to insufficient diagnostics and incorrect choice of the method for implementation of a specific phage.
3. The gastric acidity should be neutralized prior to oral phage administration.
4. Bacteriophage with a lytic lifecycle within a well-defined in vitro environment does not ensure that the bacteriophage will always remain lytic under normal physiological conditions found in a body. It may change to adapt lysogenic cycle in some circumstances.
5. Bacteriophages are viruses and, in general, viruses tend to swap genes with each other and other organisms with which they come into contact. So there is a chance of spread of antibiotic resistance in bacteria.
6. Many doctors are scared to give live bacteriophage to the patients.

CONCLUSION

The use of bacteriophages to control bacterial infections

shows therapeutic promise. The worldwide increase of pathogenic bacteria resistant to antibiotics makes it an imperative to exploit alternative strategies to combat this threat. The therapeutic use of bacteriophages –perhaps in combination with antibiotics – may turn out to a valuable approach. However, it is also quite clear that the safe and controlled use of phage therapy will require detailed information on the properties and behavior of the specific phage–bacterium system, both in vitro and especially in vivo. The in vivo susceptibility of bacterial pathogens to bacteriophages is still largely poorly understood.

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