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ORIGINAL ARTICLE

Phage therapy is an important replacement for the antibiotic resistance

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ABSTRACT

Antibiotic resistance has become a significant and growing threat to public and environmental health. The emergence of multiple drug-resistant bacteria has prompted interest in alternatives to conventional antimicrobial. One of the possible replacement options for antibiotics is the use of bacteriophages as antimicrobial. We were forced to look for a new approach in treatment. Phage therapy is an important alternative antibiotic in the current of drug-resistance pathogens. In this way, poisoning bacteria bacteriophage bacteria infect and replicate in bacteria, in this therapy, identify the type of virus per person and can be targeted manipulation of harmful bacteria and then returned the person and invented phage therapy. We discuss the advantages and disadvantages of bacteriophages as therapeutic agents in this regard. And so describe a brief history of bacteriophages and clinical studies on their use in bacterial disease. Much hope is placed in genetic modifications of bacteriophages prevents the development of phage-resistant bacteria.

Keywords: antibiotic resistance, bacteriophage, phage therapy

INTRODUCTION

Phages are the antibodies protecting diseases from getting infectious and are the only natural hosts derived from the body defense mechanism that can completely prevent infections. The antibodies with the ability to provide both preventive and therapeutic methods were discovered first by Bering in the late 19th century (1893) and indicated the fight of inactive antibodies provided by the infected animal immune system against diphtheria, which led to production of antibodies for the treatment of humans. Subsequently, horses, sheep, chickens, and human sera were collected, mixed together and used as therapeutic agents to treat some infectious diseases like diphtheria, tetanus, pneumococcal pneumonia, meningitis, and other diseases. As they produced toxins, they were used to mediate the production of toxins, including the preparation of polyclonal antibodies from sera, showing disease improvement in patients (11-1). Due this success, it is used for diseases caused by bacteria, viruses and protozoa for cancer, metabolism and hormonal disorders and in the diagnosis of lymphatic malignancy, tissue identification, immunosorbent assay attached to enzymes, radioimmunoassays and serotypes of microorganisms (9). However, since 1980, they have been used as antimicrobial agents given their reducing effects on costs involved in the development and testing new drugs. In parallel, the alarming rise in antibiotic resistance has been used as a leading public health concern in the 20th century in infections like Staphylococcus aureus and Pseudomonas aeruginosa (5). Antibody therapy has emerged as an important alternative method for various diseases to overcome the limitations of antibiotic use. Moreover, antibiotics can only be used for bacterial infections, whereas antibody therapy can be used for a wide range of antibodies uses like bacterial and viral infections. However, antibody competition can be used in certain conditions where it lacks growth, including the treatment of diseases for which no treatment has been effective: for treating snake bites as well as after exposure to rabies, cytomegalovirus, respiratory tract virus, hepatitis A virus, hepatitis B virus, smallpox, and measles where they are preventative (36-34). Monoclonal antibodies have potential applications in diagnostic, therapeutic, and drug delivery systems.

Definition of bacteriophage: Phages are viruses that infect bacteria with a DNA or RNA genome package in a protein coat. They are the duplicate portion of the “foreign” fragments of Escherichia coli DNA and a different representation of the conventional expression systems in the foreign gene that bind to a phage-encoding protein sequence. The genetic fusion of the foreign amino acid sequence of the proteins is fusion-dependent. A compound is incorporated called phage or virion particles that exit the cell, so that the foreign peptide or domain of the protein is shown on the outer surface. Phage libraries are linked to different regions of the gene-made immunoglobulin (like single-stranded, heavy chain (VH)) and variable domain (VL), Fab fragments, or single VH or VL domain. Every library is derived from cDNA and from immune or simple B cells. DNA library will be a phage in the surface protein blocker gene (III) and displayed on their surface as the structure of an antibody fused to the surface protein. The phages needed have readily been isolated and enriched and used to produce antibodies by selecting conjugated antigens and infecting Escherichia coli bacteria. The antibodies can be isolated and cloned to produce monoclonal antibodies to transfer to human cells. As this library is a random combination of V regions of light and heavy chains, it is expressed only by B cells in vivo.

Phage history: The phage history is studied for better understanding of the future outlook and the plan of phage in modern medicine. From the old times, there have been documented reports of river water ability in treating infectious diseases like leprosy. However, Hankin (1896) published a report on antibacterial activity against Vibrio cholerae, had observed in the Ganges and Jumna rivers in India, and suggested that an unknown substance was responsible for limiting the spread of cholera. Two years later, the Russian microbiologist Gamalia saw a similar phenomenon while working with Bacillus subtilis. Similar observations of bacteriophages were made from 1898 to 1918. Nonetheless, another British microbiologist, Frederick Tuart, hypothesized the existence of an advanced virus but did not pursue it for different reasons,

including financial problems (20). A French-Canadian microbiologist first observed the presence of bacteriophages in locusts in 1910. In the laboratory, while growing them on agar, he observed growthless halos, which he called Raplach, and claimed that they are caused by viral parasites. Six years later, he proposed the name "bacteriophage" (36). In 1917, phage testing in human patients began under the supervision of Professor Victor Henry at the Mothers and Infants Hospital in Paris. D'Herelle observed immunity by eating phage, and then, he demonstrated its effectiveness by prescribing them to a 12-year-old boy with severe dysentery. The patient's symptoms stopped after treatment, and he recovered completely. Phage anti-diarrheal was then injected into three other patients, all of whom began to recover within 24 hours of treatment (17). In 1923, two physicians from Baylor College of Medicine reported the successful results of a phage therapy experiment in the United States and concluded that the bacteriophages have great potential as a new weapon against infectious diseases (24).

Phage therapy more effective than antibiotic therapy: Very specific bacteriophages minimize the risk of secondary infections, yet antibiotics target both pathogens and the normal flora of the patients that can end in secondary infections or sometimes more severe infections. Moreover, bacteriophages are concentrated at the site of infection, yet antibiotics travel throughout the body and are not concentrated at the site of infection. Bacteriophages have no side effects; however, resistant bacteria, allergies (sometimes even fatal anaphylactic reactions), and secondary infections are common side effects of antibiotic therapy (23). Ultimately, adaptive bacteriophages are selected according to the natural environmental selection. Isolating and identifying existing bacteria are faster than discovering and developing new antibiotics, which can take several years and cost millions of dollars for clinical trials. Moreover, although the bacteria can become resistant to antibiotics, phages can mutate and thus could be suitable for coping with the resistant bacteria (24-27). When phage therapy and antibiotic therapy are combined, they create synergy (37).

Using phages in treatment: Discovering phages has been recommended as the possible therapeutic agents to kill bacteria. Given their nature, bacteriophages have many advantages as therapeutic agents. Firstly, they act very specifically and effectively against their target bacteria, so they are more effective in combating antibiotics and do not even eliminate the natural microflora in host organisms. Phages are not infected human or animal cells, and are available to us through different management methods (35). The action mechanism of bacteriophages differs from that of antibiotics, making them effective against multidrug-resistant bacteria. Moreover, the process of selecting phage-resistant bacteria is ten times slower than that of antibiotic-resistant bacteria (30). It has to be stated that the production of phage is easy, fast and relatively inexpensive. Phage is used in the treatment of infectious diseases in plants and animals to (3). Phage is used in humans in the treatment of diseases like dysentery, skin infections, pulmonary infections, meningitis, and infected wounds or myelitis caused by various organisms, including Staphylococcus aureus, Streptococcus spp., Escherichia coli, P. aeruginosa, Shigella spp. and Salmonella. High levels of phage-treated effect were observed in vancomycin-resistant and Enterococcus faecium (VRE) - infected mice. Injection alone (PFU 3 10 108 (plaque formation unit)) of an active phage 45 minutes after administration (109 CFU (colonies) of VRE was enough to eradicate the pathogen and relieve clinical symptoms in 100% of the mice tested. Even when phage was used in the final stages, about 50% of mice completely recovered (3). Phage may be used in urinary tract infections. Preliminary clinical trial in patients infected with E. coli, Pseudomonas, Klebsiella spp., Enterobacter spp., and Staphylococcus aureus indicate a high level of phage effect (20). The spread of multidrug-resistant P. aeruginosa infection has convinced researchers to conduct clinical studies with phage preparation (malignant bacteriophage clone cocktail). In vitro activity of such cocktails was observed for 99.5% of the 206 species tested and the therapeutic efficacy in studies in white mice ranged from 80-100% and above that of ciprofloxacin (50-80%). A combination of antibiotics and phage guarantees 100% therapeutic effect. Using phages in mice with burn wounds besides subcutaneous injection in damp Pseudomonas aeruginosa-infected veins have proven good therapeutic results, largely dependent on phage production effective route (87%). In general infection, injection was intraperitoneal and less effective compared to intramuscular and subcutaneous injection (13-14). Most cases of phage therapy in humans have been subject to routine close monitoring for clinical trials. However, the results of positive treatment and the absence of side effects have stimulated growing interest in this type of treatment and research in bacteriophage biology. In Poland, at an institute of immunology and experimental treatment methods from the Polish Academy of Sciences in Wroclaw used active phage therapy applications (1957). Using phages in the treating different infections has reduced the use of antibiotics, and thus has reduced the spread of multidrug-resistant bacteria. Phage treatment had some problems, mainly related to the introduction of foreign genetic material into the patient body associated with the possibility of transmitting undesirable gene traits. Thus, the researchers focused on the phage enzyme involved in the destruction of bacterial walls and their shells. These are lytic enzymes where hydrolyze peptidoglycans, i.e. both protein and carbohydrate molecules are hydrolyzed. These enzymes are active in active bacteria both in the environment and inside the host cell (2011). The effect of lysis has been confirmed in vivo on Streptococcus pyogenes and Basil anthracis (Nelson et al., 2001) and in antibiotic-resistant strains of Staphylococcus aureus (O'Flaherty et al., 2005).

The relationship between bacteriophages and bacterial biofilms: Various phages have been used to control biofilms. However, biofilm phage interactions are relatively complex and diverse. Bacteriophages may contaminate bacterial biofilms. Phages may specifically trap bacterial biofilms in EPS, as well as producing enzymes that impair its stability outside the cell. Biofilms may show resistance to phage infection (35). Phage faster contamination relative to plankton cells may increase the proliferation rate of phages (30). On the other hand, the structure and composition of

biofilm, as well as the physiology of biofilm cells, may impose some limitations here. Different imaging techniques like confocal fluorescent microscopy with fluorescence in situ hybridization (FISH) and heterogeneity microscopy of the biofilm structure showed a diverse distribution of cells, matrices, and channels filled with water and pores. The radial motion of the T4 phage molecule showed that many biofilms have open structures with water-filled channels that facilitate phage access to the biofilm (35). Moreover, Lactococcus phage c2 can penetrate into the biofilm by creating water-like channels. A similar phenomenon has been observed with the biofilm of Stenotrophomonas maltophilia with no cells sensitive to this phage (18). Moreover, the fact is that phages can reduce the number of bacterial cells in the biofilm. There are different elements causing a decrease in the lytic effect of phages (temperature, medium composition, EPS matrix type, and so on), and thus phage action is less effective on target cells. Moreover, the metabolic status of bacterial cells in the biofilm may end in problems with phage treatment. As fast-growing cells, they invade the dormant phase cells. However, in some cases, the diversity of biofilm structure, nutrient deficiencies, and bacterial metabolism do not impede any barrier to the lytic phage cycle. Phage size T4, meaning that the number of phages amplified is in the range of 12-200 PFU, depends on the physiological conditions of the host cell and Escherichia coli. Additionally, bacteriophage T4 can infect bacterial cells even though the availability of nutrients remains limited. The increases in the nutrients increase bacterial cell growth. Further studies have shown that phage is a barrier to many phage infections for many biofilm matrices. Doolittle et al. (1996) reported that a phage of Pseudomonas aeruginosa has the ability to reach host cells in the deeper layers of a biofilm that phage cannot penetrate through the biofilm matrix. Furthermore, the biofilm matrix is a reservoir of proteolytic enzymes that can lead to bacteriophage inactivation. The first and foremost step in bacterial infection is the uptake of phage particles into specific receptors on the surface of the bacterial cell. In biofilm, where the TPS bacterial microclones are surrounded, the matrix poses a problematic factor for the phages to reach their receptors on the surface of the target cell. However, it has been observed that some phages can overcome this barrier and penetrate the extracellular matrix due to the "associated" enzyme. This enzyme hydrolyzes EPS so that bacteria can be effective on lipopolysaccharides, outer membrane proteins or other receptors to initiate phage infection reaching their target cells. Hughes et al. (1998a) stated that multiple phages that enable enzymes to invade EPS in many gram-negative bacteria, including enabling bacteria to induce biofilm formation. In culture media, phages are identified by halos of different sizes, obtained around the plaque after infection by a bacterial single cell. The halo is created by bacteria from which EPS is released by the enzyme during the lysis of infected cells (Sutherland et al., 2004). Depolymerization activity was seen in phage SF153b in an action against Enterobacter biofilm (Hughes et al., 1998b). Moreover, concerning anti-P release, Hanlon has shown that phage aeruginosa throughout the structure of the alginate gel, a mixture of properties, reduces phage depolymerization and viscosity of alginate and EPS in Pseudomonas aeruginosa (Hanlon et al., 2001). Another example of an associated enzyme contaminated with bacteriophages is the Pseudomonas aeruginosa cystic fibrosis strain, which can reduce extracellular alginic acids. Microscopic analysis of the halo regions on Pseudomonas putita with a pure recombinant tail φ15 bacteriophage, having EPS activity, clearly shows that many bacteria in the halo regions were completely separated from each other, their EPS material reduce or remove. A small dose of endosylase E, which reduces N-Acetylmuramic acid, is shown as a treatment tool for systemic infections due to E. coliK1 strains in neonatal rats (Moshtaq et al., 2005). However, these enzymes are rarely effective in more than several polysaccharides. The synthesis of depolymerase enzyme in some phages is very useful for biofilm destruction. However, it is not a common trait. Thus, for instance, genetically engineered T7 phages are genes that bring about the production of EPS bacteriophages. Unfortunately, this genetic change leads to a decrease in anti-biofilm activity relative to native phage (Lu and Collins, 2007).

Phages as vaccines or treatment systems: In spite of the large number of publications on phage therapy, there are few reports on the pharmacokinetics of phage therapy. Several reports on this subject (5-32) show that phages enter the bloodstream of laboratory animals (after a single oral dose) in two to four hours, and are found in internal organs (liver, spleen, kidney and so on). This process takes about ten hours. Moreover, information on phage persistence shows that phages can remain in the human body for a relatively long time up to several days (32). Nonetheless, more studies are needed to obtain accurate information on the pharmacological properties of lytic phages including full-scale toxicology studies is required before lytic phages can be used as a therapeutic agent in Western countries (23). Phage trait for targeting host cells is converting itself as a delivery system for bacterial molecules. Likewise, non-lytic phages producing microbial protein in mouse models were effective for E. coli (37). Phages alone have been reported to impair the biofilm synthesis of target organisms like growing Staphylococcus epidermidis. Phages are modified by bioengineering methods to attack the biofilm Phage to E. coli, T7, an enzyme that reduces B-1, 6-N-acetyld-glucosamine (an important component of the biofilm). During bacterial cell lysis, where the enzymes are released into the extracellular environment, results in the deletion of the target phage gene to bind to its target receptor and inject its own DNA (25). This is potentially more effective in inhibiting toxin production and bactericide (39). Exposure to methicillin-resistant S. aureus increases the killing rate to ≥ 99.9% in five minutes with inoculation of 105 × 1 organisms and the killing rate of ≥ 99.9% to 10 minutes by inoculation of 1 × 107.

The advantages of using antibodies production by phages: Recombinant antibodies are more flexible than monoclonal antibodies in basic capabilities and can have more flexibility during their production process using in vitro production processes and have more opportunities for post-generation optimization than conventional monoclonal antibodies. This technology can be used to ease the production, screening and maturation of the selected binder

too. The easy access of gene sequences makes it possible to better synthesis methods understand.

CONCLUSION

Bacteriophages are a diverse group of viruses that can be easily manipulated, and thus could be used in sciences like biotechnology, research and therapy. The purpose of this review paper was to study a wide range of studies by researchers, scientists, and biotechnologists accelerating the development of biotechnology by inserting phages and using them. The fact is that currently we must have reached a critical point in the treatment of infectious diseases: new drugs have a relatively lower natural ability than bacteria to withstand antibiotics. The result is that some of our most potent drugs are becoming ineffective. According to the previous studies and examples, this review paper find considered the use of bacteriophage to treat or prevent bacterial infections promising. Therapeutic use of bacteriophages, possibly combined with antibiotics, may be a valuable method. Undoubtedly, bacteriophage is going to be useful in the biological control of pathogens for food safety and public health. Some critical concerns over using phages are their safety and effectiveness in immune responses. Growth optimization strategies and phage purification are the issues in need of being tackled. The pharmacokinetics of various phages may vary. Given the rapid progress in biotechnology and molecular biology, it is hoped that the phages that can answer many questions can also be used as biocontrol agents not only in agriculture, but also used in the oil industry. Moreover, phages are used as vehicles for vaccines (both DNA and proteins), and as systems for many proteins and antibodies used to detect pathogenic bacteria. The details given in this paper provide an overview of the range of applications that phages can help in the fields of biotechnology and medical science: applications of phages range from diagnosis of the disease, through phage typing and its prevention (phage vaccine), to treatment (phage therapy). Phages can be useful for humans in many cases. By making a combination of phages, it has become an easy treatment for a wide range of drug-resistant bacterial infections that are even resistant to the latest generation of antibiotics. A phage alone can be useful in treating a bacterial infection by lysing a bacterial cell - it has lytic potential. At the same time, phage versatility allows us to use antibodies against bacteria present on the phage surface. Likewise, a protective antigen can be used as phage DNA or a vaccine. A combination of genetically modified phages could be more helpful in solving all of these issues. Phages could prove useful in preventing food spoilage, and treating bacterial infections of plants too.

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