Bacteriophage Therapy : An Alternative to Conventional Antibiotics

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Abstract

Bacteriophage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. We reviewed the studies that dealt with the therapeutic use of phages from 1966-1996 and few latest ongoing phage therapy projects via internet. Phages were used topically, orally or systemically in Polish and Soviet studies. The success rate found in these studies was 80-95% with few gastrointeslinal or allergic side effects. British studies also demonstrated significant efficacy of phages against *Escherichia coli*, *Acinetobacter spp*., *Pseudomonas spp* and *Staphylococcus aureus*. US studies dealt with improving the bioavailability of phage. Problems faced in these studies have also been discussed. In conclusion, phage therapy may prove as an important alternative to antibiotics for treating multidrug resistant pathogens.

Introduction

Multidrug resistant bacteria are a serious health problem these days. Persistant use of broad spectrum antibiotics leads to further drug resistance in these organism. Rigorous research activities are ongoing to develop alternate methods of treatment of infections caused by these microorganisms. Phage therapy seems to be a good option for this problem.

Bacteriophages are bacterial viruses that invade bacterial cells and are amongst the most abundant living entities on earth playing important roles in maintaining the natural abundance and distribution of microorganisms. The antibacterial power of phages has been harnessed with increasing effectiveness for nearly 70 years.

Bacteriophage Genetics

Bacteriophages are the viruses with either DNA or RNA as the genetic material and both single and double stranded forms of each are known. The structure is same as that found universally among living organisms: a polynucleotide chain consisting of a deoxyribose (or ribose) phosphate backbone to which are attached specific sequences of the four nucleotides - adenine, thymine (or uracil), guanine and cytosine; in all, except the single stranded phages, two such complementary chain are paired together in a double helix.[1]

Phages have a developmental cycle within the host bacteria which can be lytic or lysogenic. The lytic cycle comprises a series of events that occur between attachment of phage particle to a bacterial cell and its subsequent release of daughter phage particles. It consist of four phages: adsorption of phage to host cell. Penetration of phage nucleic acid, intracellular development and final release of daughter phage particles.

The lysogenic cycle on the other hand comprises replication of phage nucleic acid together with the host genes for several generations without major metabolic consequences for the cell. This is a latent mode of infection and it occurs at a very low frequency. The phage genes in this state may occasionally revert to lytic cycle, leading to release of phages particles, this property is known as lysogeny and phage that can develop both lytically and lysogenically are said to be temperate phages.[1]

History

Bacteriophages were discovered in 1915 by Towart.[2] He described degenerative changes present in staphylococcal colonies isolated from calf lymph, which could be transmitted serially by application of culture filtrates from the original growth. Felix Herelle in 1917[2] observed that filtrates of faeces culture from dysentery patients induced transmissible lysis of a broth culture of dysentery bacillus. He suggested that the lytic agent was a virus and gave it the name "Bactriophage". Intensive studies on the therapeutic use of phages for treating infectious diseases were taken up in 1920.[3,4] *Vibrio cholerae* was the first bacteria against which phage therapy was tried but the activity of phage was found to be much higher in vitro than in vivo.[3] These studies were later abandoned due to the introduction of broad spectrum antibiotics which were cheap and an easy alternative. Now, in the present era of multidrug resistant pathogens, we need

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to aggressively explore the possibility of phage therapy, taking full advantage of the power of modern biological tools to enhance their effectiveness.

Source of Phages

Phage for a given bacterium can be isolated wherever that bacterium grows, such as in faeces, sewage, soil, hot springs oceans. Water from the Ganges (India) has been found to be a rich source of vibrio phages. The problem is not in isolating phage against particular bacteria but in selecting the ones most likely to be useful for clinical purposes. This includes lytic phages that have high efficacy and broad spectrum activity on clinically important strains. These should not be temperate phages that carry toxigenic genes. Phages should be such that they can be readily produced in large quantity and should be stable during storage.

The selected phage strains need to be tested against 200 strains of other pathogenic and conditionally pathogenic enterobactericiae. For therapeutic phages the ability to lyse host mutants resistant to other therapeutic phages in a given mixture is important.

Phage Bioderm

A non-toxic, autodegrading perforated biopolymer complex containing phages and other therapeutic compounds is called a phage bioderm. Wounds and burns, osteomyelitis and periodontal diseases have been treated with artificial skin containing these bioderms and this product has been marketed by Ministry of Health Institute in Tbilisi. In a study conducted on 306 clinical isolates of *Serratia marcescens* in vitro using specific phage strains and phage type, 20-95% lysis was reported. Environmental decontamination has also been tried using salmonella phages. At 48 hours it was seen that bacteria were totally eliminated when phages were introduced.[5]

Potential problems faced in dealing with these bioderms is the induction of toxin genes e.g. lambdoid phages which carry cholera toxin or filamentous phages involved in diphtheria. Another difficulty is the rapid release of bacterial endotoxins due to the lytic effect of phage. This can lead to a Jarish Herxheimer reaction which can be fatal. Development of antibodies against phages may also lead to their decreased effectiveness and cost is another limitation to their use as a routine.

Special Advantages

Phages have specific properties which give them advantages as therapeutic agents. They are self-replicating as well as self-limiting. They continue to multiply and penetrate deeper as long as local infection as present. This is in sharp contrast to antibiotics which decrease in concentration below the site of infection. Phages are lytic against specific bacteria so they can be targeted more specifically than antibiotics which are active against a group of bacteria. Phages do not harm normal intestinal microflora.[6] Antibiotics have side effects which can be serious. But phages have been used in millions of patients without any reported side effects. Phages can be used prophylactically as well as in established infections. The self-perpetuating nature of phages in the presence of susceptible bacteria, makes multiple administrations[7,8] unnecessary. It also allows transfer of administered phages between animals in a farmyard.[9,10]

Clinical Studies

Phages therapy trials have been carried out in USSR, France, Poland and USA for dysentery, wound infections, burns in children hospitals and infectious diseases hospitals.

Polish Studies

In the study by Slopek and Coworkers, 372 cases of staphylococcal infected patients were treated of which 151 cases were of mixed infection including *Staphylococcus*. Positive results were seen in 75% of infected ulcerated varicose vein cases and 100% in gastrointestinal infection, pericarditis and furunculosis due to *Staphylococcus*. Response rates were lower for patients more than 60 years of age and in patients with mixed infections. Importantly, parallel administration of antibiotics diminished the effectiveness of phage therapy from 95.2% to 84.9%.[5]

Cislo *et al* studied the effect of concomittant topical and oral phage therapy on infected skin ulcers in 31 patients. There was marked improvement in 74.2% (23/31) patients while in seven patients the study could not be conducted due to side effects of phage therapy in the form of either eczema or pain or vomiting.[5]

Weber *et al* in 1987 assessed the extent to which orally administered anti-staphylococcal and anti-pseudomonal phage penetrated into the serum or urine of 56 patients with suppurative bacterial infection. By tenth day of therapy, it was found that 84% serum samples and 35% urine samples contained phage indicating a high bio-availability.[5]

Russian Studies

In 1974, studies were conducted in Tbilisi Georgia, by Sakandelidze *et al* who treated patients of antibiotic resistant osteomyelitis, lung abscess, peritonitis and post-surgical wound infections with pyophage which is mixture of phage active against *Staphylococcus*, *Proteus* and *Streptococcus* or diphage (*Staphylococcus* and *Proteus* phages). Phage was applied subcutaneously or via the surgical drain daily for 5-10 days and improvement was seen in 92% of these cases.[5]

In 1989, Kochetkova *et al* conducted a detailed and quantitative study in 131 cancer patients suffering post-operative wound infections. Phages produced positive clinical results in 81.5% of patients. Phage therapy was found to be more effective for cutaneous wound infections by local application than for pleural empyema, mediastinitis or osteomyelitis. Antipseudomonal phages were found to be most effective (86.7%) followed by staphylococcal (74.7%) phages.[5]

Recently (1993), phage study conducted by Miliutina and coworkers compared phage plus antibiotics against antibiotics alone to treat generalized bacterial dysentery and

salmonella in paediatric patients and found that phage plus antibiotic could treat certain infections resistant to antibiotics alone.[5]

British Studies

In Britain, studies were conducted by Smith and Huggin's in 1982-83 using mice, calves, piglets and lamb models.[11] In 1992, Soothill tested the efficacy of phages in experimental *Acinetobacter*, *Pseudomonas* and staphylococcal infection in mice and their study reported limited use of phage therapy in staphylococcal infections. However, in 1994, Soothill turned attention to the problem of cutaneous infection in burns patients in whom skin grafts fail due to bacterial infection. The study was conduced in guinea pigs and split skin grafts were taken and full thickness defects were created. Prior to reimplantation of graft, wound bed was infected with *Pseudomonas* strain with its respective phage. After five days, grafts were accepted and they vascularized.[12]

US Studies

In 1973, Geier *et al* found that phage lambda (λ) which had previously been instilled in the blood or peritoneum of mice, was totally cleared from these locations within 48 hours. However, a relatively high titre of λ persisted in the spleen upto seven days after administration.[13] In 1996, Merril *et al* found a way to overcome this sequestration of phage in the reticuloendothelial system.[14] He injected λ phage into mice and isolated the phage. This phage was regrown in a mutant *E. coli* strain repeatedly ten times. This serial cycling of phage proved effective in selecting λ with an anino acid mutation in major phage head protein E that enabled it to evade the reticuloendothelial system.

Recent Studies

Recently a study conducted in Japan has highlighted the protective effects of phages against experimentally induced bacterial infections of cultured fish[6] (aquaculture).

Problems

Problems faced in phage studies were poor understanding of heterogenity and ecology of both the phages and the bacteria involved. There was also difficulty in selecting appropriate mixture of phages of high virulence against the target bacteria. Failure to correctly and appropriately characterize the phage proportions was another reason for hampering the progress of these studies. Phages with high activity in vitro are more active in vivo.[8] Maintenance of phages by regular propagation needs expertise and an established set up.

Resistance of bacteria to lysis by phages was another important challenge to these workers since phage resistant mutants are fairly common in *Pseudomonas plecoglossicida* cultures.[6] Often bacteria harbouring plasmids confer resistance. *E. coli* harbouring the mutant RP4 plasmid become resistant to pili specific bacteriaphage PRDI and GU5.[8]

In phage therapy, it is essential to optimise gastric pH if oral formulations are being tried. Lastly phages mediate genetic exchange among bacteria i.e. transudation or phage conversion. It is well known that some temperate phages contribute to bacterial virulence. Temperate phages with broad infectivity amongst species strongly supports antiphage activity.[15]

Conclusion

Use of antibiotics along with pahges have been tried with limited benefit because antibiotics are more likely to inhibit phage effectiveness than to enhance it and combination may result in problem/resistant genes. Hence it is concluded that phage therapy alone is more effective.

In medicine today phages find many applications. They are used for typing of clinical bacterial strains for in situ bacterial detection through labelled phages, (TB, Listeria), phage display system for vaccines, control of food pathogens and for drug and gene delivery using defective phage with targeted receptor. Phage therapy for eliminating multidrug resistant bacteria is gaining importance. However, there is a need to carry out further studies on phages as therapeutic agents using specific phage strains against the corresponding bacterial hosts. Phages should be essentially free of contaminating bacterial toxin and also capable of evading the reticulendothelial system.

(Phage related programmed which are going on in different countries are : "Gangagen" in India; "Guelph" in Canada (beef industry development fund); "Biophage" in Montreal, phage Tech Inc; 'Phagen AB' in Sweden and 3-M Corp in Russia. Hexal pharmaceuticals are also supporting phage therapy work at Medical University of *S. Carolina)*.

References

1. Benett PM, Howe TGB. Bacterial and bacteriophage genetics. Topley and Wilson's Microbiology and Microbial Infections. 9th ed. 1998;2:231-86.
2. Topley WWC, Wilson GS. The Principles of Bacteriology and Immunity. New York William Wood and Company 1929;1:224-33.
3. Adams MH. Bacteriophages, New York: Interscience Publishers Inc. 1959:1-2.
4. Brock TD. Milestones in microbiology. Washington DC: American Society of Microbiology 1961;1:157-9.
5. Alisky J, Iczkowski K, Rapoport A, Troitsky N. Bacteriophages show promise as antimicrobial agents. *J Infect* 1998;36:5-15.
6. Nakai T, Park SC. Bacteriophage therapy of infectious disease in aquaculture. *Res Microbiol* 2002;153:13-8.
7. Barrow PA, Soothill JS. Bacteriophage therapy and prophylaxis : rediscovery and renewed assessment of potential. *Trends Microbiol* 1997;5:268-71.
8. Smith HW, Huggins MB. Successful treatment of experimental Escherichia coli infections in mice using phage: its general superiority over antibiotic. *J Gen Microbiol* 1982;128:307-18.
9. Berchieri Jr A, Lovell MA, Barrow PA. The activity in the chicken alimentary tract of bacteriophages lytic for Salmonella typhimurium. Res Microbiol 1991;142:541-9.
10. Smith HW, Huggins MB, Shaw KM. Factors influencing the survival and multiplication of bacteriophage in calves and in

their environment. *J Gen Microbiol* 1987;133:1127-35.

11. Smith HW, Huggins MB. Effectiveness of phages in treating experimental Escherichia coli diarrhea in calves, piglets and lambs. *J Gen Microbiol* 1983;129:2659-75.
12. Soothill JS. Bacteriophage prevents destruction of skin grafts by Pseudomonas aeruginosa. *Burns* 1994;20:209-11.
13. Geier MR, Triggg ME, Merril CR. Fate of bacteriophage lambda in non-immune germ-free mice. *Nature* 1973;246:221-2.
14. Merril CR, Biswas B, Carlton R, Jensen NC, Creed GJ, Zullo S, *et al*. Long circulating bacteriophage as antibacterial agents. *Proc Natl Acad Sci USA* 1996;93:3188-92.
15. Jensea EC, Schrader HS, Rieland B, Thompson TL, Lee KW, Nickerson KW, *et al*. Prevalence of broad-host-range lytic bacteriophages of Sphaero tilus natans, Escherichia coli and Pseudomonas aeruginosa. *Appl Environ Microbiol* 1998;64:575-80.

Book Review

Action Potential to Arrhythmias

by KP Misra

In the recent years, the globe has witnessed tremendous growth in the field of Cardiac Medicine with equal focus on advanced technologies in diagnostic and therapeutic strategies including the understanding of genetic abnormalities. The advancements in Electrophysiology and its application in the understanding of simple and complex cardiac arrhythmias paved the way for evolving current management strategies for arrhythmias, Radio Frequency Ablation and implantation of Cardiac Defibrillator devices.

I know Dr. K. P. Misra for the last few decades as an outstanding teacher, eloquent speaker and above all as an exemplary Physician and Cardiologist. It is not an easy job to conduct excellent Electrocardiogram and Arrhythmia sessions across the length and breadth of this country which he has undertaken. His already published books and video teaching cassettes on arrhythmia serve as a desktop reference manual in physician's chambers.

It is highly befitting that Dr. K. P. Misra, has put in enormous efforts in bringing out a book on 'ACTION POTENTIAL TO ARRHYTHMIAS', at this juncture as a jewel on the crown for Senior Consultants. In the rapidly changing scenario of Cardiology wherein any new discovery tends to undermine classic Electrophysiological study, Dr. Misra has in his usual intuitive and explicit style breathed new life into this important subject.

The structuring and the style of presentation of the subject undoubtedly make medical men to posses a copy as a reference book. I congratulate him for his stupendous work and appreciate him for the time that he has devoted to create this valuable book.

S Thanikachalam

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