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Dawn of antimicrobials: Using phages to synergize with antibiotics to counteract bacteria

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Abstract:

Currently, the misuse of antibiotics has led to a global crisis of antibiotic resistance, and people have to start seeking new strategies to solve this problem. Traditional phage therapy has been valued for its advantages of strong bactericidal efficacy and self-replication. However, the use of phage alone may confront a series of problems, such as a narrow antimicrobial spectrum, phage resistance, and immunogenicity. If phage and antibiotics are used in combination, they can synergistically lyse host bacteria and better control or eradicate bacterial infections. Therefore, their combination has recently begun to become a hotspot for research and application of phage therapy. In this paper, by collating the research literature and clinical reports on the combined application of phage and antibiotics, we explore and summarize the synergistic mechanism of phage and antibiotics, the mechanism of bacterial resistance, and the limitations of this cocktail therapy.

Keywords: phage therapy, phage-antibiotic combination therapy, antibiotic resistance, lysis spectrum

1 Introduction

In recent years, the emergence of drug-resistant pathogenic bacteria has become one of the threats to human life and health. In the decades since penicillin (against Gram-positive bacteria) and streptomycin (against Gram-negative bacteria) were introduced into the clinic, the abuse of various types of antibiotics and bacterial evolution has resulted in more than 700,000 deaths per year globally due to drug-resistant bacterial infections, and it is predicted that by 2050, this value will rise to 10 million people per year [1], exceeding the number of cancer deaths. The mere utilization of antibiotics is clearly no longer effective in the treatment of bacterial diseases, and it may also result in recurrent infections [2], wasting a large amount of human and material resources in the treatment process. In addition, with the discovery of vancomycin-resistant Staphylococcus aureus [3] (a super-resistant bacterium), the problem of antibiotic resistance has gradually become a major pharmaceutical barrier to human health, and we are beginning to become helpless against super-resistant bacteria. Therefore, research on how to inhibit bacterial resistance or effectively get rid of resistant bacteria is crucial. In the course of this research, a combined antimicrobial approach of phages and antibiotics has been discovered, and this approach is of great research significance. Phages have strong bactericidal specificity, low development cost, few side effects, and can kill drug-resistant strains of bacteria. their combined application with antibiotics can reduce the use of antibiotics and antibiotic resistance crisis, which has a wide range of application prospects.

The paper will focus on the mechanism of the synergistic antimicrobial activity of phages and antibiotics, the emergence of bacterial resistance, and the limitations and shortcomings of the therapy

2 Basic mechanics of phage-antibiotic cocktail therapy

2.1 Description of phage

Phage is a general term for a class of viruses that infect microorganisms such as bacteria, fungi, and algae. Phages are composed of nucleic acids and proteins and contain only one type of nucleic acid (DNA or RNA), which are widely found in various environments such as water sources, soil, and even in humans and animals and are closely related to human health [4]. There are many types of phages, and according to relevant literature, the number of phages in the earth's biosphere can be up to 10^{30}~10^{32}, which is ten times the number of bacteria, and it is considered to be the most abundant biological agent on earth. As a kind of virus, phage has some of the characteristics of viruses: it is tiny, does not have a complete cellular structure, contains only a single nucleic acid, and can be regarded as a kind of organism that "preys" on bacteria. Phages are more resistant to physical and chemical factors than ordinary bacterial propagules, generally lose their activity only after continuous heating at 75 °C for more than

30 min, and are sensitive to ultraviolet light.

2.2 Formation of Antibiotic Resistance

By and large, bacterial resistance to antibiotics can be divided into two categories: congenital (e.g., the natural structure of cell walls and membranes inherent in bacteria) and acquired [5] (Namely, tolerant mutations develop in the bacterial genome, such as mutations in antibiotic-targeting genes, which then accumulate in the course of natural selection.). Currently, four main types of mechanisms for bacterial drug tolerance formation have been identified, including Alteration of antibiotic-specific receptors on the bacterial cell wall, alteration of cell membrane permeability, formation of antibiotic efflux pumps (efflux systems), and bio-inactivation of specific enzymes [5].

The antibiotic efflux pump is one of the most common mechanisms of drug tolerance in several pathogenic bacteria [6]. Efflux pumps are transporter proteins in bacterial membranes that participate in the regulation of the internal environment by pumping out toxic substances, community-sensing molecules, biofilm-forming molecules, and bacterial virulence factors. Based on their energy source, efflux pumps have been classified as primary and secondary: efflux pumps that drive substrate translocation across membranes through chemical energy generated by ATP hydrolysis are defined as primary efflux pumps, whereas efflux pumps that derive their energy from the electrochemical potential of protons are defined as secondary efflux pumps. Currently, six major efflux pump families have been identified in bacteria, namely the ATP-binding cassette superfamily (ABC), the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion family (MATE), the resistance nodulation cell division family (RND), the small multidrug-resistant family (SMR) and the proteobacterial antimicrobial compound efflux family (PACE) [6]. The variety and lack of specificity of efflux pumps means that several different efflux pumps can efflux a single antibiotic, and a single efflux pump can efflux different drug substrates.

2.3 Mechanisms of combined action of phage and antibiotics

2.3.1 Synergistic effects of antibiotics on phages

Comeau et al. [8] found that phage plaques were significantly enlarged, and phage titers were significantly increased after cefotaxime treatment, showing a synergistic effect. Some scholars have found similar synergistic effects using different phage-antibiotic combinations (e.g., phage KS12 and KS14 with meropenem, ciprofloxacin, and tetracycline [9]; phage σ-1 with ceftriaxone [10]). Moreover, the morphology of certain bacteria is altered after antibiotic treatment [11], and antibiotics can exert pressure to induce a delay in bacterial lysis, thereby increasing phage lysis [12]. In addition, antibiotics can enhance the phage lysis of bacteria. It has been found that phages can function to lyse bacteria through lytic enzymes. Phage lytic enzymes are proteins synthesized during the late stage of phage replication in host bacteria [13], which are able to lyse peptidoglycan contained in the cell wall, leading to the rupture of the bacteriophage and the release of the zygotic phage. Related studies have shown that lytic enzymes are able to digest peptidoglycan and form pores in the cell wall, thus exerting their antibacterial activity [14]. Synergistic effects have also been observed when certain phage lytic enzymes are combined with antibiotics [15-19]. Phage lysis of the cell releases the zygotic phage and lytic enzymes into the surrounding environment, and subsequently, antibiotics attack the cell wall and cell membrane to make it defective, so the lytic enzymes in the environment reach the peptidoglycan from the outside without any difficulty and act to lyse the cell.

2.3.2 Synergistic effects of phages on antibiotics

When phages and antibiotics are applied in combination, phages using the drug efflux pump as a receptor force drug-resistant bacteria to mutate their efflux pumps and thus develop phage resistance, leading to increased susceptibility to antibiotics and a reduction in their drug resistance [7]. In addition, some phages are selected for virulence factor-deficient mutants by binding to virulence factors, causing phage resistance along with reduced virulence [7].

3 Case Studies

In 1917, French scientist d' Herelle discovered phage and used it for the first time in the treatment of bacillary dysentery, which opened the era of phage therapy in the treatment of human diseases. With the improvement of phage therapy, especially the phage-antibiotic cocktail therapy, the applicable strains of phage therapy have been gradually expanded, and diseases caused by bacteria such as *Vibrio cholerae*, *Shigella*, *Enterobacteriaceae*, and *Streptococcus*, to name a few, have been gradually conquered.

3.1 Klebsiella pneumoniae

Klebsiella pneumoniae is a common Gram-negative pathogen and has become the most common conditionally pathogenic organism other than *Escherichia coli*. This pathogen can be transmitted through contaminated food or water and has a high level of antibiotic resistance, which has become a major challenge threatening human health.

Currently, phage-antibiotic combination therapies have made some progress in dealing with this pathogen: Xin Shi [19] successfully cured a patient with a urinary tract

infection caused by drug-resistant *Klebsiella pneumoniae* after four courses of treatment using a *Klebsiella pneumoniae* phage-antibiotic cocktail preparation. According to the SIP team, a patient with severe pneumonia suffering from carbapenem-resistant *Klebsiella pneumoniae* was discharged from the ventilator after 5 ml of phage cocktail nebulization treatment at a titer of 10^9 PFU/mL, with the disappearance of *Klebsiella pneumoniae* from the sputum culture of his subject.

3.2 Acinetobacter baumannii

A. baumannii is a gram-negative, conditionally pathogenic bacterium. It can induce respiratory tract infections, urinary tract infections, bacteremia, secondary meningitis, and ventilator-associated pneumonia. The misuse of antibiotics, especially broad-spectrum antibiotics, has led to a gradual increase in the number of multidrug-resistant *Acinetobacter baumannii*, carbapenem-resistant *Acinetobacter baumannii*, and pan-drug-resistant *Acinetobacter baumannii*. On 1 March 2017, WHO released a list of 12 categories of superbugs, and the bacterium is at the top of the list, which is a major hygiene problem for mankind.

According to Chao Ma [22] et al., the best elimination of this pathogen was achieved by the simultaneous addition of phage and antibiotics. Nir-Paz [20] reported a reduction in the total number of colonies and tissue healing observed in a patient with left tibia infection induced by drug-resistant *Acinetobacter baumannii* after receiving a combination of phage-antibiotic therapy. Clinical studies such as those by Schooley [21] reported that a 68-year-old diabetic patient suffering from necrotizing pancreatitis and infected by multidrug-resistant *Acinetobacter baumannii* was cured by a combination of phage specific for the organism and minocycline.

3.3 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common Gram-negative bacillus and a conditionally pathogenic organism. It is one of the common pathogens of current hospital-acquired infections and is prone to induce pulmonary infections. In recent years, the bacterium has been included in the list of 12 superbugs by WHO due to the prevalence of antibiotic resistance in the clinic.

Since Soothill [23-24] constructed a model of skin infection in mice using P. aeruginosa phage, the use of P. aeruginosa phage-antibiotic combination for the treatment of various infections induced by its pathogens has gradually matured into clinical use: in 2009, a study used phage cocktail therapy for the treatment of chronic otitis media induced by P. aeruginosa infection, effectively reducing the patient's bacterial load. Nouraldin [25] et al. found that phages act synergistically with amikacin and meropenem to reduce the MIC of antibiotics against *Pseudomonas aeruginosa* from 64 μg/mL to 16 μg/mL and from 64 μg/mL to 32 μg/mL, respectively. In addition, Clara Torres-Barceló et al. applied, Clara Torres-Barceló et al. conducted in vitro susceptibility experiments on Pseudomonas aeruginosa by combining phage with different types and concentrations of antibiotics, and the results showed that the combination therapy had a significant synergistic effect [26]. Frank Oechslin et al. applied phage in combination with ciprofloxacin to treat the mouse model of endocarditis of *P. aeruginosa*, and the same synergistic effect was also achieved [27].

4 Defects and Limitations

At present, in co-therapy, screening to obtain better quality phage has become an important factor in improving the effectiveness of the therapy. phage therapy is still immature in clinical use, and the main problems are as follows:

4.1 Narrow lysis spectrum

The high specificity of phage recognition of host bacteria determines the narrow host spectrum of phage. Through the mutual recognition of phage tail filaments and receptors on the bacterial surface, usually, phage of Gram-negative bacteria act on lipopolysaccharides or lipoproteins on the bacterial surface. In contrast, phage of Gram-positive bacteria acts on peptidoglycans, phosphoglycosanoids, lipophosphoglycosanoids, and related proteins. The specificity of phage is shown in different types of bacteria. In contrast, the pathogenic bacterial types are many, and the bactericidal effect is improved by phage mixture preparation with a broad spectrum of bactericidal or by screening phage particles with a broad spectrum of cleavage. At present, the lack of sufficient numbers and types of potent phages limits the promotion of phage therapy technology to a certain extent [29].

4.2 Highly affected by changes in mutagens

Mutations in either of the host bacteria and phage will affect the lysis of bacteria by phage. Structural changes in the receptor on the host surface produce competitive inhibitory substances in the extracellular surface matrix and the host surface receptor, preventing phage adsorption as well as inhibiting phage DNA injection.

4.3 Problems with therapeutic dosage and duration

Phage can be cleared by immunity in about three days in the body, and only when the bacteria reach a certain number of phages will proliferate. Inoculation too early or inappropriate dosage may result in phage being cleared by the organism before it can play a role. Therefore, grasping the optimal inoculation time, the inoculation method, and

the dose of phage will be a difficult point in implementing phage therapy technology [30].

4.4 Safety issues of phage preparations

Phages carry toxin genes that may cause adverse reactions in the organism, and an important step in the isolation and purification process is the removal of endotoxin. Bacterial endotoxin is an important part of the cell wall of Gram-negative bacteria, and bacterial toxin is released when phage lyses the bacteria, which is toxic to the human body and poses a safety hazard. Currently, phage preparation purification can remove endotoxin from phage particles by affinity chromatography techniques and commercial kits [31].

4.5 Problems with using conditions

At present, there is no fixed standard for phage administration protocols required for phage therapy (e.g., phage-to-antibiotic ratio, oral or intravenous injection, etc.), and the treatment period needs to continue to be explored.

5. Summary

Due to the development of bacterial resistance as a result of the misuse of antibiotics, humans appear to be at their wits' end in the face of disease-causing bacteria. Phage, due to its biological characteristic of specifically recognizing host cells and lysing them, makes it an effective weapon for humans against pathogenic bacteria.

Due to the discovery by James et al. [7], (the mutual synergistic bactericidal effect between phage and antibiotics, and their joint use can reduce bacterial resistance to antibiotics, simultaneously reducing the bacteria's virulence), and the advantages of the cocktail therapy of combining phage and antibiotics for antibacterial treatment with low cost and weak side-effects on the organism, this therapy will become the most powerful strategy for humans to fight against the problem of drug-resistant bacteria.

However, due to technical constraints, such as its narrow cleavage spectrum and the lack of uniform standards for its use, this therapy cannot be industrialized and widely used in the clinic at present. We still need to make efforts to solve these problems.

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