Stemming the tide of antibiotic resistance by exploiting bacteriophages

The growing prevalence of antibiotic resistance is a global crisis. It is predicted that by 2050, antibiotic resistance-related deaths will exceed by 10 million per year. Thus, there is an urgent need for alternative strategies that can either replace or supplement antibiotic use. Bacteriophages and their encoded lytic proteins, called endolysins, have both shown promise as antibiotic alternatives. Bacteriophages were first investigated as therapeutics nearly a century ago, but the success of antibiotics led to phage therapy being largely abandoned in Western medicine until recently. While sporadic reports of life-saving successes in the ad hoc use of phage therapy have emerged, properly designed, robust clinical trials and clear regulatory guidelines are required before the true potential of phage therapy can be realized. In addition, despite endolysin research still being in its infancy, the early successes of endolysin-based therapeutics already entering clinical trials are an exciting glimpse into the future. No stone can be left unturned in the discovery and development of novel therapeutics if we are to ensure a future supply of effective treatments for bacterial infections.

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Introduction

Since Alexander Fleming serendipitously discovered penicillin in 1929, antibiotics have been used extensively, and often indiscriminately, in human medicine, agriculture and food processing, saving many lives. Unfortunately, the golden age of antibiotics has come to an end, with the rise in antibiotic resistance rendering some drugs almost completely ineffective. Around the globe, bacterial strains showing resistance to almost all available antibiotics are being reported with increasing frequency, essentially heralding a return to the pre-antibiotic era. Compounding the situation is the stagnation of antibiotic discovery and development – only 15 new antibiotics have been approved in the last 20 years compared with 63 in the 20 years before 2000. It is obvious then that alternative approaches are urgently needed.

Bacteriophage

Amid the array of strategies currently being investigated, bacteriophages and their derived lytic proteins, called endolysins, are emerging as promising candidates to supplement or replace antibiotic use.

Bacteriophages, or phages, are viruses that only infect bacteria. Almost as soon as they were discovered in 1915, and long before Fleming discovered penicillin, phages were investigated for their potential use as antibacterial agents. However, these early attempts at phage therapy were limited by a poor understanding of phage biology and variable success in the treatment of bacterial infections. The subsequent discovery of antibiotic drugs and their successful application during World War II led to Western medicine largely abandoning phage-based antimicrobials. Notably though, phage therapy has remained prevalent in some Eastern European countries, particularly Poland and Georgia. Now, in the face of the current antibiotic resistance crisis, phage therapy has once again become a hot topic with researchers worldwide.

Phages are the most abundant organisms on the planet, with an estimated $10^{31}$ phage particles in the biosphere. Yet, like most other viruses, phages lack the machinery for energy generation and protein production, and therefore rely on their hosts for propagation. After penetrating bacterial host cells and transferring their genetic material (Figure 1), phages parasitize the host machinery to produce a slew of new virus particles, killing the host cell in the process. However, this hijacking of the host is not always immediate, as the phage can take either of two pathways following infection of a bacterial cell. While virulent phages immediately initiate reproduction, causing the host cells to burst open and release the viral progeny, temperate phages insert their genetic material into the host genome, where they are reproduced along with the host until they are triggered to begin replicating as in the case of virulent phage.
Virulent phages are, therefore, better candidates for therapeutics, as they immediately begin killing the host bacteria and are less likely to horizontally transfer genes.

**Phages as therapeutics**

Unlike antibiotics which have broad impact on the human microflora, a key advantage of phage therapy is that it offers a targeted approach with no effects on other related, but potentially beneficial, bacteria. With the host ranges of phage described to date restricted to at least a single known isolate, and at the most several bacterial species, this targeted approach is likely to reduce the opportunity for development of acquired resistance.

Phage therapy can be deployed as a type of personalized medicine. If the species and strain of a bacterial pathogen can be identified from a clinical specimen, an appropriate phage can be selected from an arsenal of previously characterized isolates and formulated as a personalized therapeutic. An alternative approach is the preparation of a mixture of phages, known as a phage cocktail, which may be more appropriate if a patient requires urgent therapeutic intervention. Phage cocktails have the advantage of being effective against a wider range of bacteria and, because they can target a bacterial strain in different ways, the risk of resistance is reduced. Although phage cocktails are likely to have a better clinical outcome, selecting an appropriate mix of phages is complex. For example, the pharmacokinetics and efficacy of individual phages may not simply be additive, as demonstrated by the ‘depressor effect’, whereby dissimilar phages have a detrimental impact on each other following coinfection.

While Western countries remain cautious, others such as Georgia have adopted phage therapy into their therapeutic repertoire, including the availability of over-the-counter phage-based medications for a range of uses. Yet, despite the ongoing use of phage therapy, there is a lack of comprehensive clinical data, largely owing to previous clinical studies being non-randomized and uncontrolled. Importantly though, all evidence to date suggests that the immune response elicited by phage-based therapies does not pose a significant health risk to patients. Regardless, more information on the immunogenicity of phage therapies should be gathered before their widespread introduction.

More robust trials evaluating the safety and efficacy of phage-based therapies are starting to be published (Figure 2). In 2009, independent controlled clinical trials hinted at the enormous potential of phage-based therapeutics. One of these studies, conducted in the USA, confirmed that there were no adverse health effects
associated with a phage cocktail for the treatment of chronic venous leg ulcers, although the efficacy of the treatment required further investigation. In the UK, phage therapy was associated with a positive clinical outcome in a randomized, double-blind, placebo-controlled Phase I/II clinical trial for the treatment of ear infections caused by antibiotic-resistant *Pseudomonas aeruginosa*. Since then, phage research has progressed in leaps and bounds, highlighted by the approval of the first intravenous clinical study in the USA in 2019.

The life-saving potential of targeted phage treatments against antibiotic-resistant bacteria has been emphasized in several high-profile cases. In 2008, a 68-year-old patient in the USA was diagnosed with multidrug-resistant *Acinetobacter baumannii* infection of the pancreas. As a last resort, an intravenous phage treatment was approved as an emergency investigational new drug. Researchers screened a library of phages to identify isolates capable of tackling the infection and prepared a bespoke phage cocktail. This life-saving treatment was then administered to the patient, who made a complete recovery. Experimental therapy was also successfully used in the UK to treat a *Mycobacterium abscessus* lung infection in a teenager with cystic fibrosis.

However, phage-based therapeutics need not be limited to currently known strains. Advances in gene sequencing and our understanding of phage genetics have created an opportunity to engineer phages with more desirable properties. For example, killing ability can be enhanced, host specificity can be modified to achieve a narrower or broader spectrum and immunogenicity can be reduced. The ability of phages to deliver genetic material into the host genome could also be exploited to introduce genes that increase bacterial sensitivity to antibiotics. Thus, tailor-made phages for therapeutic use offer significant clinical potential.
Endolysins

Although phages were identified in 1915, the potential therapeutic properties of their lytic enzymes were not recognized until 2001. Endolysins are enzymes that cleave peptidoglycan in the bacterial cell wall from within, allowing the release of progeny phages following replication (Figure 1). Peptidoglycan is a mesh-like structure composed of amino acids and glycan strands that is essential for the structural integrity of a cell.

Figure 3. Endolysins interact with peptidoglycan. a) A typical peptidoglycan monomer comprising sugar units, $N$-acetylmuramic acid (MurNAc), $N$-acetylglucosamine (GlcNAc) and a chain of amino acids, the composition of which varies among bacterial species. These chains are cross-linked to the amino acid chain of another peptidoglycan unit. b) Different types of endolysin cleave each of the bonds found within peptidoglycan. c) Exogenously applied endolysins cleave the bonds within the peptidoglycan of Gram-positive bacteria, resulting in cell lysis. d) External application of endolysins is generally ineffective against Gram-negative bacteria due to the presence of an outer membrane structure that is not present in Gram-positive bacteria. An endolysin must be modified, so that it can penetrate the outer membrane to gain access to the peptidoglycan, where it can then cause cell lysis.
Staphefekt™ has shown promise as a component of gels only just starting to blossom, a modified endolysin, although endolysin-based therapeutic research is for endolysins to replace or be used synergistically with antibiotics in therapeutics.

Endolysins as therapeutics

Although endolysin-based therapeutic research is only just starting to blossom, a modified endolysin, Staphefekt™, has shown promise as a component of gels and creams for the treatment of Staphylococcus aureus-associated skin conditions such as eczema. Several clinical trials investigating the efficacy of endolysin-based antimicrobials against Gram-positive bacteria have been conducted, including Phase II and Ia trials of therapeutics aimed at treating S. aureus bacteraemia (Figure 2).

Exogenous application of endolysins is generally more effective against Gram-positive bacteria such as S. aureus than Gram-negative bacteria such as Escherichia coli, as Gram-negative bacterial cells have an additional protective outer membrane (Figure 3a and d). This membrane acts as a barrier that must be overcome before the endolysin can gain access to the peptidoglycan. While there are some examples of endolysins that can naturally pass through this barrier, in general, the enzymes must be modified to facilitate outer membrane penetration. Fortunately, there are a growing number of strategies to accomplish this goal, including the addition of amphipathic helixes or peptides with polycationic properties, as exemplified by Artilysins®, or by exploiting outer membrane transport mechanisms. As new and more effective strategies are developed to overcome this technical hurdle, research can focus on the efficacy of these proteins as therapeutic agents.

An exciting feature of endolysins is the seeming lack of resistance to endolysin activity, probably resulting from the co-evolution of phage (and their endolysins) with bacteria. While there has been a concerning rise in resistance to other peptidoglycan-targeting enzymes such as lysozymes, no equivalent observations have been made for endolysins. Peptidoglycan is essential for the structural integrity of a bacterial cell. Even small modifications, such as those seen in the development of resistance, can deleteriously impact the integrity of the cell, selecting against the emergence of resistance-causing modifications.

Without a doubt, protein engineering strategies will play an important role in the future of endolysin-based therapeutics. As with phages themselves, endolysins are amenable to modification. As such, there are already a growing number of engineered endolysins with improved lytic activity, increased stability or modified specificity, with engineering almost a prerequisite for endolysins to be effective against Gram-negative bacteria. This burgeoning field of endolysin research is, therefore, set to help realize the potential of endolysin-based therapeutics against antibiotic-resistant bacteria sooner rather than later.

Regulatory barriers to development and implementation

While, out of necessity, interest in phage therapy is growing, there are a number of barriers that deter researchers and biotech companies from investing in research and development in this area.

A major obstacle is the lack of clear regulatory pathways for phage-based therapies. For example, phages are classified as ‘drugs’ in the USA, but ‘medicinal products’ in the European Union. However, given the unique properties of phages, including being self-replicating while also self-limiting to the site of infection as they will only multiply in the presence of the targeted, infection-causing bacteria, a dedicated framework may be more appropriate. In addition, the average time for therapeutic products to be approved after the completion of clinical trials is 12 years and comes at a cost of millions of dollars. Therefore, because phage cocktails can be formulated on a case-by-case basis, and even stock phage cocktails would require constant updating in response to the evolution of pathogens, the current processes of drug approval are not a good fit for phage therapy. Belgium has made progress in this area, developing the Magistral Phage Medicine Strategy in 2018, which allows custom phage-based prescriptions to be legally formulated by a pharmacist.

Intellectual property protection has also been perceived as a limitation to development. This is because unmodified phages cannot be patented and phage therapy generally relies on phages naturally occurring in the environment. This has seen the focus of intellectual protection resting on novel processes and formulations, bespoke collections and maintenance of trade secrets.

Conclusions

While antibiotics have been an incredibly effective tool for improving public health, saving countless lives, we now face a crisis of antibiotic resistance. The ensuing urgent need to explore alternative strategies has revived interest in phage therapy, a strategy first investigated as early as 1915. Aimed by modern genome sequencing techniques, rapid advances in phage therapy have been
Drug Discovery

made in recent years, with several life-saving examples highlighting the potential of phage therapy for treating antibiotic-resistant bacterial infections. While a reboot of current regulatory pathways is needed to facilitate the development and widespread application of phage-based therapeutics, all current indications are that these miniature killing machines may just be the answer we have been looking for.

Further reading


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