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Stronger together? Perspectives on phage-antibiotic synergy in clinical applications of phage therapy

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Abstract

Increasingly, clinical infections are becoming recalcitrant or completely resistant to antibiotics treatment and multidrug resistance is rising alarmingly. Patients suffering from infections that used to be treated successfully by antibiotic regimens are running out of the treatment options. Bacteriophage (phage) therapy, long practiced in parts of Eastern Europe and the states of the former Soviet Union, is now being reevaluated as a treatment option complementary to and synergistic with antibiotic treatments. We discuss some current studies that have addressed synergistic killing activity between phages and antibiotics, the issues of treatment order and antibiotic class, and point to considerations that will have to be addressed by future studies. Overall, co-treatments with phages and antibiotics promise to extend the utility of antibiotics in current use. Nevertheless, a lot of work, both basic and clinical, remains to be done before such co-treatments become routine options in the hospital setting.

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Introduction

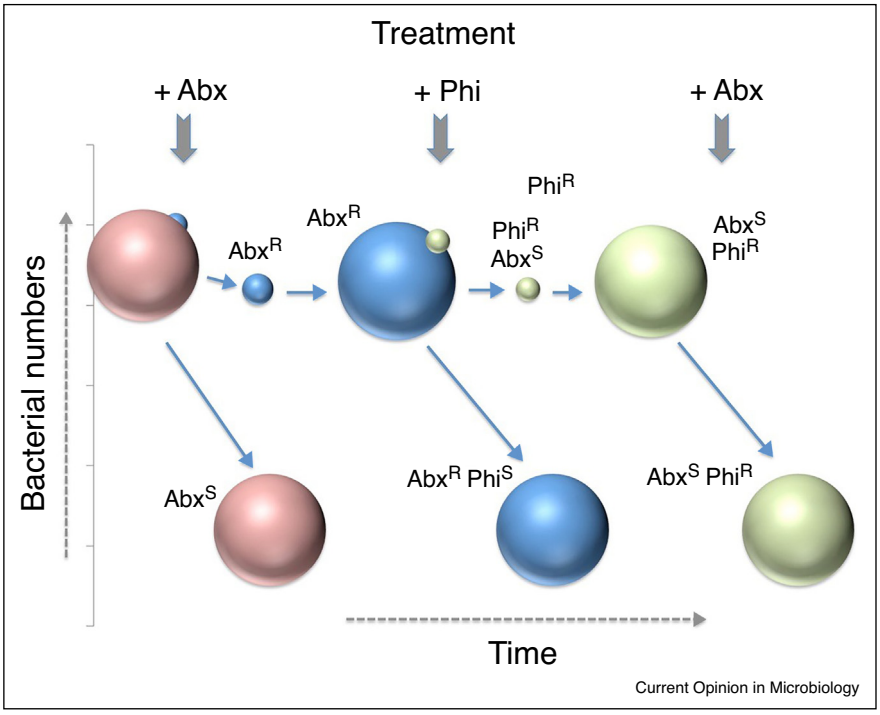
Bacteriophage (phage) therapy has been used to treat bacterial infections in some parts of Eastern Europe since Felix d'Herelle first treated pediatric cases of dysentery in 1919. Because of the development of antibiotics, Western countries have largely ignored phage therapy, until the growing global crisis of antimicrobial resistance has renewed interest in phage therapy to treat multi-drug resistant (MDR) bacterial infections [1]. In one such case, therapeutic phage cocktails were administered intravenously and into the catheters to successfully treat a San Diego resident suffering from a systemic *Acinetobacter baumannii* infection [2••]. Synergy was observed between two of the phages used, a myophage and a podophage that targeted different receptors, and minocycline. This result suggested that phage predation imposed selective pressure on *A. baumannii* and favored the outgrowth of bacterial isolates with reduced capsule production, and consequently lower virulence. The general rationale for synergy between antibiotics and phages is diagrammed in Figure 1. This mini-review discusses examples of phage-antibiotic synergy and the potential of harnessing this synergy as a therapeutic strategy in considerations for future studies.

Phage-antibiotic synergy: what we know

While phage-antibiotic synergy had previously been observed *in vitro* and in animal models [3,4••], its potential in treating complex MDR bacterial infections in humans is gaining greater attention [1,2,5,6••,7]. For example, an *in vitro* study by Lin *et al.* [8] with *Pseudomonas aeruginosa* investigated the interaction between nebulized phages and antibiotics and found that synergy was both strain-dependent and antibiotic-dependent. In one clinical strain, FADD1-PA001, synergy occurred when the podophage PEV20 and one of three antibiotics (ciprofloxacin, amikacin, or colistin) were used in combination. In the case of strain JIP865, synergy was also observed between phage PEV20 and ciprofloxacin, amikacin or tobramycin. However, complete inhibition of bacterial growth only occurred when phage PEV20 was combined with ciprofloxacin. By contrast, no synergy was observed when phage PEV20 was combined with amikacin, aztreonam, ciprofloxacin, colistin, or tobramycin in the case of strain 20844n [8]. In another study, Jansen *et al.* [9•] identified a T4-like phage KARL-1, which infects eight MDR strains of *A. baumannii*. The KARL-1 phage showed pronounced synergy with meropenem, but more modest synergy with ciprofloxacin or colistin.

The mechanisms behind phage-antibiotic synergy are many. A study of synergy between phage treatment and antibiotics against either planktonic (vegetative) or biofilm-growing methicillin-resistant *Staphylococcus aureus* (MRSA) found that antibiotics were effective at killing the former and ineffective at killing the latter [10•]. In contrast, combinations of phages with certain antibiotics showed marked differences at reducing bacterial growth within biofilms as measured by microcalorimetry. In particular, simultaneous use of phage SB-1 with rifampin

Figure 1



The bubbles represent the infectious agent populations. Successive antibiotic (Abx) and phage (Phi) treatments, shown at the top, both reduce the number of bacteria and impose selective pressure on the populations for resistance. Mutations that confer resistance may affect the overall fitness of the pathogen and select for compensatory mutations to counteract the fitness loss (not shown). R and S refer to resistance or sensitivity.

or with daptomycin resulted in synergistic killing of cells within biofilms. Pre-treatment of either planktonic or biofilm-growing cells with phage SB-1 and subsequent treatment with any of the five antibiotics assayed (rifampin, daptomycin, fosfomycin, ciprofloxacin, or vancomycin) was more effective than co-treatment at preventing bacterial growth synergistically. Phage SB-1 degraded the exopolysaccharide component of a MRSA strain biofilm, as well as reduced the titer of metabolically-inactive cells, such as stationary phase cells or persisters induced either by ciprofloxacin or the protonophore CCCP. These results are particularly intriguing, as some phages only lyse actively growing bacterial cells.

The potential of phages to penetrate and disperse biofilms more effectively than antibiotics was tested in another *in vitro* scenario, in which combinations of phage and antibiotics were used to treat *S. aureus* growing as biofilms [11••]. This study also tested whether the order of treatment affected efficacy. Pre-treating biofilms with antibiotics showed antagonism between the phage SATA-8505 and all the antibiotics used (vancomycin, dicloxacillin, cefazolin, tetracycline, or linezolid). In the case of co-treatment with phages, additive growth inhibition was observed at many or all drug doses tested for linezolid or tetracycline, whereas antagonism was observed between the phage and dicloxacillin or cefazolin. Co-treatment with vancomycin showed additive inhibition at some doses and antagonism at others. Additive reduction in biofilms was observed when phage treatment was administered before dicloxacillin or linezolid, and synergistic reduction was observed with cefazolin or tetracycline, as well as before some lower doses of vancomycin. This study demonstrated that while phages may act synergistically with some antibiotics, at least in part because phages are successful at dispersing biofilms, the order of treatment is critical and may, if reversed, result in antagonistic rather than the desired synergistic effects.

Ho *et al.* [12•] showed that phage NPV-resistant mutants of *Enterococcus faecalis* developed loss-of-function mutations in part of a gene cluster that is involved in synthesis of intermediates in lipoteichoic acids, *epaR*. Phage resistance was attributed to the inability of phage NPV to adsorb to the bacterial capsule. Interestingly, these mutations rendered the NPV-resistant cells to have increased susceptibility to daptomycin, which is a cyclic lipopeptide antibiotic that permeabilizes and depolarizes the cell membrane. Phage-resistant *epaR* mutants also exhibited a decrease in the osmotic stress response, which has a role in the integrity and hydration of the cell [12•]. For

instance, a decrease in external osmotic pressure can cause cells to swell and even burst, whereas an increase in external osmotic pressure can cause cell dehydration.

The development of phage resistance is a common concern with phage therapy. Resistance can often occur by a single mutation of the phage receptor protein on the bacterial cell surface. This may not always be clinically problematic and could even be exploited, for example, when the phage receptor contributes to bacterial pathogenesis. Chan *et al.* [13••] showed that in the case of the P. aeruginosa phage OMKO1, which binds to an outer membrane protein OmpM component of two drug efflux complexes (mexAB and mexXY), only the phage treatment in combination with ciprofloxacin or with ceftazidime reduced bacterial cell density significantly. Importantly, *mexAB* and *mexXY* contribute to the virulence of the biofilm-generating strain associated with infections of prosthetic vascular grafts (PVGI). Phage OMKO1 and ceftazidime were administered together to a patient suffering severe and recurring prosthetic vascular graft infections caused by this *P. aeruginosa* strain, resulting in complete clearing of the infection even after ceftazidime treatment was stopped. Because the treatment was so effective, the status of the phage receptor protein and whether any cells developed phage resistance could not be determined.

Oechslin *et al.* [4••] performed a careful study of synergy between phages and ciprofloxacin in a rat model of endocarditis. The study found that, compared to two bolus treatments, continuous delivery of phages over 24 hours was much more effective, and necessary to achieve high and much more stable phage titers in plasma and in organs (spleen, kidneys, lungs, brain) after 24 hours. The phage-antibiotic synergy was very marked. Both phage treatment alone and the combination phage and antibiotic treatment increased bacterial killing after 6 hours compared to antibiotic treatment alone. However, the combination treatment succeeded in suppressing the growth of bacteria, some of which are presumably phage-resistant mutants, after 24 hours. Interestingly no phage-resistant *P. aeruginosa* mutants were isolated from the implanted valves, suggesting that the resistant mutants may be at a critical disadvantage *in vivo*. To test this possibility, two phage-resistant mutants isolated *in vitro* were tested for their infectivity *in vivo* and found to have significantly lower infectivity and established lower bacterial densities in the infected valve vegetations than the phage-sensitive parent strain. The two phage-resistant mutants were unable to establish infections when inoculated intravenously into rats. One of the phage-resistant mutants affected the *pilT* and abrogated twitching motility, while the other, a deletion of over 350 kb and 342 genes, included the loss of *galU*. The latter mutant had lost the O antigen, as expected, and had shortened LPS chains. Therefore, mutations arising in response to phage infection led the bacterial populations down a path of attenuated virulence.

Considerations for future studies

Synergy between antibiotics and phage treatment may be extremely beneficial clinically. However, the actual mechanistic basis for these synergies is often unknown and mostly speculative. So far, few conclusions can be drawn, in part because antibiotics with similar modes of action, for example inhibition of cell wall synthesis, may result in different outcomes when combined with specific phages. Future experiments need to dissect these conflicting observations.

A critical factor missing from *in vitro* studies and rarely studied in animal models is the contribution of the immune system during phage therapy. An infectious agent entering a host is immediately exposed, in healthy individuals, to components of the innate immune system, including granulocytes (neutrophils, basophils, and eosinophils). Their associated effector functions, such as reactive oxygen species (ROS) and antimicrobial peptides, are most likely synergistic with antibiotics. In dissecting the contributions of innate immunity during phage therapy, Roach *et al.* [14••] showed that in the absence of innate immune activation in MyD88$^{-/-}$ mice, phage PAK_P1 was unable to cure an acute respiratory infection with *P. aeruginosa*. Indeed, phages were able to reduce the number of phage-sensitive bacteria in the lungs, but in the absence of immune intervention, phage-resistant mutants arose and perpetuated the infection to fatality [13••]. The study further showed that phages and neutrophils worked synergistically to control both the phage-sensitive and phage-resistant infection counterparts and was required for efficacious treatment. Cytokine analyses of lung tissues indicated that synergy was largely dependent on the phages and antimicrobial activities of neutrophils rather than direct phage-neutrophil interaction. Moreover, although adaptive immunity did not play a role during phage therapy of an acute infection, B-cell and T-cell responses are likely to play a synergistic role during longer lasting and chronic infections. However, antibody responses toward phage particles may counteract their therapeutic action. The contribution of the adaptive immune system to phage therapy, whether positive and/or negative, remains to be studied in depth. In short, work in the area of interactions between both innate and adaptive immunity and phage therapy is critically important but still in the earliest stages.

Proponents of phage therapy should keep in mind lessons from the adaptation of bacterial pathogens to antibiotic treatment [15]. The fitness costs of antibiotic resistance mutations are balanced with compensatory mutations that reduce fitness deficits and may or may not counter the original antibiotic resistance phenotype. Phage resistance mutations may lead to the rise of compensatory mutations, and the consequences—pro or con—of such compensatory mutations to the benefits of phage therapy will need to be determined.

In some cases, the activity of the most effective antibiotics may be augmented by triggering the lytic growth of temperate (lysogenic) prophages harbored inside pathogenic strains. However, none of the studies we examined reported whether antibiotic therapy resulted in release of temperate phage particles, either from the infectious agent or from the host's own resident microbiota. In most cases, we view DNA damage-inducing antibiotics like ciprofloxacin and other fluoroquinolones as the classic inducers of prophages, but other antibiotics treatments (e.g. those that induce envelope stress or oxidative stress), may also contribute to prophages entering their lytic growth phase through indirect induction of DNA damage and the damage repair response [16–18]. Further studies to address the contributions of prophage induction to phage-antibiotic synergy are needed.

Final comments

Current studies continue to show the great promise of phage therapy. They also highlight the need for translational studies examining how to best exploit potential phage-antibiotic synergy. In the absence of randomized clinical trials that establish efficacy, each application of phage therapy to a life-threatening illness remains experimental, and combined treatment with antibiotics needs to be performed with care so as to prevent antagonism and optimize health outcomes. Rapid diagnostic tests that determine bacterial susceptibility to antibiotics and phage-antibiotic interactions need to be available.

Assuming that future studies that uncover mechanisms of antibiotic re-sensitization are successful, these may underscore two potential uses for phage therapy to treat MDR bacterial infections: 1) using phages to directly lyse bacterial host cells; and 2) using phages to indirectly attack bacteria by the selective pressure they exert to re-sensitize bacteria to antibiotic(s). Having either of these approaches as a viable treatment regimen to treat MDR bacterial infections would be a significant advance against the growing global crisis of antimicrobial resistance; having both would be a game-changer [19].

Conflict of interest statement

Nothing declare.

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