COMMENTARY

Phage therapy: The peculiar kinetics of self-replicating pharmaceuticals

The specter of antibiotic-resistant bacteria has provoked renewed interest in the possible use of bacteriophages to control bacterial infections. We argue that clinical application of phage therapy has been held back by a failure to appreciate the extent to which the pharmacokinetics of self-replicating agents differ from those of normal drugs. For self-replicating pharmaceutical agents, treatment outcome depends critically on various density-dependent thresholds, often with apparently paradoxical consequences. An ability to predict these thresholds and associated critical time points is a necessity if phage therapy is to become clinically practicable. (Clin Pharmacol Ther 2000;68:225-30.)

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With the rising prevalence of antibiotic-resistant bacteria,[1,2] alternatives to treatment with antibiotics are receiving increased attention. One such alternative is the possible therapeutic use of bacteriophages—viruses that parasitize and kill bacteria. The suggestion of administering phages as pharmaceutical agents has been mooted for more than 80 years, and every so often is picked up and trumpeted by the media as a possible "magic bullet." But in spite of several recent experiments in the United States and the United Kingdom that have helped rekindle aspirations (reviewed by Alisky et al[3] and Barrow and Soothill[4]), the approach remains unused in a clinical setting in the West.

Studies of bacteriophage therapy have had a history of being rather hit or miss; phages that actively replicate in and lyse bacteria in vitro do not always do so in vivo. This means that large or repeated doses are often required to effect benefits in live hosts, even when results have been readily achieved in cell culture. The poor predictability of outcome has been attributed to a plethora of causes, including contamination, phage-bacteria specificity, horizontal toxin transfer via temperate phages, antiphage host immune response, and bacterial coevolution.

Two recent review articles[3,4] summarize experimental results and argue for a more focused agenda of research on phage therapy. Both reviews acknowledge the apparently wayward nature of many of the results and discuss some of the practical difficulties that will have to be overcome. Yet, although the putative benefits of the use of replicating organisms as pharmaceutical agents have been well rehearsed, the fact that self-replicating agents have quite different kinetics to standard drugs appears to be unappreciated within the phage therapy literature. In this article we explain why this is a critical omission, one that contributes to the current inability to readily project from results obtained in vitro to usage in vivo, and one that must be rectified. The key point is that replication and infection processes of both bacteria and phage are density-dependent in ways that give rise to novel phenomena that do not occur with nonreplicating pharmaceuticals. We argue that many of the supposedly paradoxical features of phage therapy can readily be explained if only one shifts from a viewpoint based in chemical reaction kinetics to one rooted in ecological interactions.

KINETICS OF SELF-REPLICATING ORGANISMS

Bacteriophages may control bacterial infections in two ways. Under "active treatment" most of the bacte-

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Supported by the Natural Environment Research Council (UK) and The Wellcome Trust.
Received for publication April 6, 2000; accepted June 15, 2000.
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0009-9236/2000/$12.00 + 0 **13/1/109520**
doi:10.1067/mcp.2000.109520

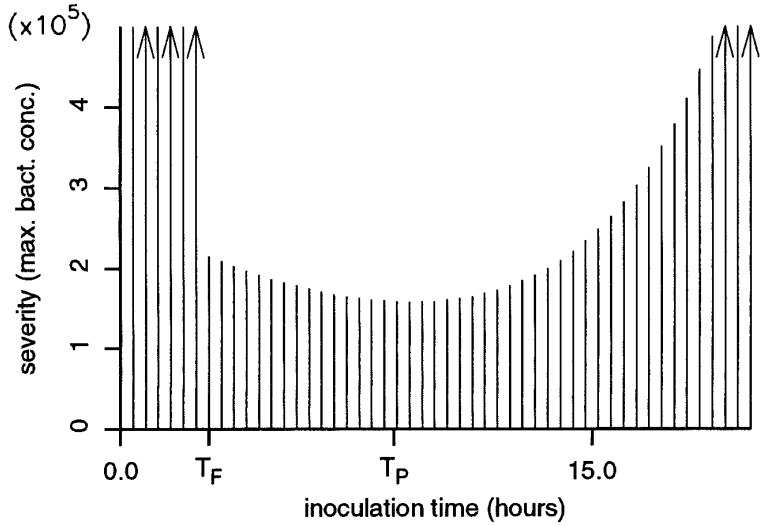

Fig 1. Importance of timing in phage therapy. This figure is based on numerical (computer) simulations of the kinetics of a generalized phage-bacteria system, as described in Appendix B. Each data point marks the disease severity (y-axis) for the same hypothetical patient, but under different treatment timings (x-axis). Notice that earlier inoculation does not necessarily lead to reduced disease severity. In this illustrative example the optimal inoculation time is around 9 hours after initial infection, and treatment fails completely if administered within 3 hours after infection. The points marked T_P and T_F are the predicted proliferation onset time and failure threshold time, respectively, as estimated using the formulae in Appendix A. Determining actual values of the proliferation onset time and the failure threshold time for real phage-bacteria systems will be an important objective of future studies.

ria (the target parasites) are killed by secondary infections after extensive reproduction and transmission of the phage (the antagonist). If the phages do not increase in number, there can still be "passive treatment" in which the initial phage dosage is large enough to inundate the bacteria by primary infection alone. In kinetic terms passive treatment is little different from traditional antibiotic and chemotherapies, missing the distinctive advantages provided by antagonist self-replication. This active/passive dichotomy is true also for agricultural biocontrol,[5] although the parallel appears to have passed unnoticed so far .

Whether a population of antagonistic phage increases in number depends on a measure termed its "basic reproductive number." The basic reproductive number is a key concept in epidemiology,[6] but it is equally applicable to any infective system, including bacteria infected by bacteriophage. The basic reproductive number, usually referred to as R_0, represents the average number of new infections arising per infected cell. The reason the basic reproductive number is useful is because the value of R_0 can be predicted by use of a formula on the basis of the basic life-history parameters involved (that is, a formula described by properties such as the mean replication rate of bacteria, the mortality rate of healthy bacteria, the lysis rate of phage-infected bacteria, and so forth). Details of the formula are given in Appendix A. Only if the basic reproductive number is greater than unity do phage numbers increase, as required for active therapy. The basic reproductive number of the phage depends on the density of bacteria present, and thus active therapy is only possible if the density of bacteria exceeds a critical threshold, which we term the *proliferation threshold* and represent by X_P. The proliferation threshold is the bacterial density at which the basic reproductive number of the phage equals unity. Understanding the role of the proliferation threshold is critical in interpreting the outcome of phage therapy. As with the basic reproductive number, the proliferation threshold may be predicted by use of a formula on the basis of basic life-history parameters (Appendix A).

An important feature that distinguishes bacteriophage therapy from most other forms of biocontrol is that the bacteria are themselves undergoing explosive growth during the time scale of the treatment. It is therefore help-

ful to conceptualize the problem in terms of time. The increasing density of bacteria means that there is a specific point in time before which active phage replication cannot occur, simply because the density of bacteria is too low to sustain a growing phage population. Active phage replication is possible only after the time when the density of bacteria exceeds the proliferation threshold. We term this time point the *proliferation onset time,* represented by T_P. The proliferation onset time is the time at which the basic reproductive number becomes greater than one: it is another key value for which one can derive a formula on the basis of basic life-history parameters (Appendix A). The proliferation onset time is a critical concept for which there is no equivalent in the world of standard (nonreplicating) pharmaceutical agents. The importance of the proliferation onset time lies in the fact that active phage therapy is only possible if the proliferation onset time occurs earlier than the time when any natural host responses (ie, immune or toxic response of the host of the bacteria) would become significant. A system with too late a proliferation onset time will not display active replication in vivo, even if it does so in vitro. It is significant that numbers of bacteria used in cell culture rarely reflect cell densities in live hosts; one should not be surprised that experiment trials predicated on cultured experiments are often thwarted.

An additional requirement for effective active therapy to occur is that inoculated phages remain in the system until such time as the proliferation onset time is reached. If inoculation is too early then the phage will be purged even before active replication can commence. To avoid this eventuality, inoculation has to be made at least after a special time point (for a given inoculum size). We call this second time point the *failure threshold time,* represented by T_F. As for the other thresholds, the failure threshold time can in principal be estimated from a formula on the basis of the basic life-history parameters (Appendix A).

One of the main paradoxes of phage therapy should now be apparent. For most treatments of most diseases, timely administration is of the essence: the earlier the better. Yet here we find that administration before the proliferation onset time can be disadvantageous, and administration earlier than the failure threshold time will fail completely. Fig 1 shows these phenomena as they appear in computer simulations of a generic phage-bacteria system.

Elsewhere we have predicted an additional paradoxical property of phage therapy, consequent to use of an antibiotic adjuvant to the phage.[7] When phages and antibiotics are administered together, the net result may actually be less efficacious than when phages are used alone. This occurs when the joint inoculation is made before the proliferation onset time and arises because the antibiotics act to delay the times T_F and T_P. This phenomenon, for which a full explanation is given in Payne and Jansen,[7] is unique to the kinetics of self-replicating pharmaceutical agents.

REINTERPRETING THE DATA

The dichotomy between active and passive therapy is reflected in attempts to treat dysentery in patients with leukemia who have undergone immunosuppression. Tolkachera et al[8] found patients given anti-pseudomonad phages recovered after only one course, whereas patients given coli-Proteus needed two to three courses to effect recovery (*Proteus* bacteria concentrations declined only during each course, showing renewed multiplication between courses). In terms of our conceptual framework, these different outcomes are interpreted as the types of phages having different values of the proliferation onset time. The proliferation onset time was early enough for invasion and continued secondary phage replication to occur for antipseudomonad phages (active therapy), but not for coli-Proteus (passive therapy). Berchieri et al[9] used *Salmonella* phages to treat chicks orally infected with *Salmonella typhimurium*, reducing the mortality rate from 60% to 3%. But there was no evidence of in vivo phage multiplication, and large numbers of phage were needed, implying the proliferation onset time to be too late for active replication to play a role. Anti-K1 phage used against *Escherichia coli* infection in mice[10] and cattle[11] readily showed in vivo active replication, implying an early and easily attainable time for the proliferation onset time.

We predict that actively effective therapy should be dependent on the concentration of bacteria, whereas passively effective therapy should be dependent on the concentration of the phage. Soothill[12] examined the ability of phage to control *Pseudomonas aeruginosa* 3719, and *Staphylococcus aureus* 6409. Although both pseudomonas phage and staphylococcal phage showed activity in vitro, neither exhibited active replication in vivo. But whereas treatment of *P. aeruginosa* was effective for doses greater than 1.2×10^7, attempts to treat *S. aureus* with staphylococcal phage failed at all dosages. As with antibiotics, for passive therapy there will be a minimum inundative dose (formula in Appendix A), which appears to be exceeded in the experiments with pseudomonas phage but not in those with staphylococcal phage. This illustrates the point that measurements of dosages required for clearance of in vitro broth culture cannot be used as a direct guide to the qualitative nature of in vivo outcome.

We mentioned above that use of an antibiotic adjuvant may under certain circumstances be disadvantageous. At least one experiment has shown this result.[13] This sounds an important warning to design of combination therapies, which may be compromised unless planned with a full appreciation of the critically time-dependent nature of the phage kinetics.

The concepts we have set down make testable predictions and point toward those aspects that must be investigated further if more refined models are to be developed. The implications for therapeutic protocols are notable. For most diseases early administration is desirable, and yet this is not true for active bacteriophage therapy (Fig 1). The optimal policy is to administer the phage as close as possible to the proliferation onset time itself. To inoculate later is to waste useful time during which phage could actively multiply with positive feedback. To inoculate earlier is to place the system in a phase during which phage are lost while waiting for the active phase to start. Moreover, if inoculation is made before the failure threshold time, then treatment will fail completely. All of these predictions should be easily testable, at least qualitatively. Some data describing the time-course of infection are available,[10,14] but there are as yet no explicit studies of the dependency of outcome on inoculation time. It is worth noting that the pivotal role of the proliferation onset time is likely to be more readily observable for low initial bacterial doses. The formulas listed in Appendix A are given in terms of biologically meaningful parameters, most of which will be measurable in vitro, and thus quantitative tests should also be possible. Refinement of the modeling to suit specific systems should become practicable once such measurements become available.

IMPLICATIONS: A ROLE FOR "PHARMACO-ECOLOGY"?

Recent optimistic reviews have argued for bacteriophages as promising antimicrobial agents, warranting further investigation and development.[3,4] But many challenges remain. Various suggestions have been made regarding why the in vivo activity of bacteriophages is typically unpredictable: contamination, horizontal toxin transfer, host immune responses, phage-bacteria specificity, and mutation. Against this background, we suggest that many of the enigmatic aspects of phage therapy most likely are not of genetic or molecular origin but are a natural consequence of the intrinsically nonlinear and density-dependent world of self-replicating agents. Although it is clear that the future clinical viability of bacteriophage therapy will depend on gaining detailed information on specific infections via molecular biologic and genetic manipulation techniques, our message is that it will also depend on interpreting that information as part of a kinetic process of "ecological interactions." Extrapolation from in vitro measurements to in vivo expectations requires appreciation that kinetic behavior is realized in a context of density-dependent thresholds and associated critical times.

Our predictions are based on a simple generic model of the "pharmaco-ecology" of phage-bacteria interactions. This is not to deny the importance of the details of the pathophysiology, but rather it is a means of emphasizing those qualitative consequences of phage therapy that arise uniquely as a result of the self-replication of the phage. It is these that may appear most unfamiliar and counterintuitive from the standpoint of traditional pharmacology. The concepts explicating the phage-bacteria system have many parallels in theories within ecology and epidemiology that deal with the population dynamics of predator-prey and host-pathogen interactions. It is likely that useful ideas and methodology may be drawn from these areas and perhaps also from experience gained in other forms of biological control. We argue for the incorporation of explicit models of density-dependent replication, to stand alongside knowledge of the relevant physiology and molecular biology if a complete and predictive understanding of phage therapy is to be achieved. We would equally urge theoreticians working on agricultural biocontrol to turn their skills toward medical applications—in a recent edited volume on the theoretical underpinnings of biocontrol no mention was made of possible parallels in phage therapy.[15]

But could awareness of the critical time points help guide timing of phage administration in clinical reality? That is, will it be possible to predict the proliferation onset time and the failure threshold time in particular individuals in a therapeutic setting? These are difficult problems that ideally need techniques for the rapid assay of actual in vivo bacterial densities and growth rates to be developed. We suspect the theory most likely will play a role in helping to shape laboratory studies. A quantitative understanding of the way that the two critical time points are determined by the life-history of the phage-bacteria system is of great benefit, because it points directly to which aspects of phage biology might best be manipulated to enhance the prospects of phage therapy. For example, the important role of phage loss has been demonstrated in experiments by Merril et al,[16] in which long-circulating strains of phages were developed and shown to be more effective antibacterial agents. The formulas in Appendix A expose the mechanisms underlying this effect by

making explicit the way that the proliferation onset time and the failure threshold time depend on the parameter describing rate of phage loss.

We have described how theoretical modeling of the "pharmacoecology" can reveal which biologic parameters are most important; but it will require the ingenuity of experimentalists and clinicians to engineer these parameters in ways most advantageous to the practicability of phage therapy. We look forward to seeing the results of that ingenuity over the coming years. Bacteriophage therapy has many potential strengths, but surely future studies must be performed with an awareness of the peculiar kinetics intrinsic and unique to the interactions of self-replicating pharmaceutical agents.

We thank Bob May and Hester Korthals Altes for advice.

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APPENDIX A. FORMULAE FOR CRITICAL THRESHOLDS AND TIME POINTS

In the formulae, a represents the replication coefficient of the bacteria, b the transmission coefficient, k the lysis rate, L the burst size, and m the decay rate of free phages. The bacterial dose is size x_0 at time zero, and the phage dose is of size v_ϕ at time t_ϕ. A simulation model on the basis of these life-history parameters is described in Appendix B. Here we analyze the critical thresholds. The basic reproductive number, R_0, is defined as the number of secondary infections per infected cell. Each infected bacterial cell can divide and will thus give rise to a cell line that, on average, will exist for a time $1/(k - a)$, during which this lineage will produce $L\,k/(k - a)$ virus particles. Each of these will cause on average $bx/(bx + m)$ new infections. The total number of secondary infections per infection is therefore

$$R_0 = \frac{Lk}{(k-a)} \frac{bx(t)}{(bx(t)+m)} \tag{1}$$

The proliferation threshold X_P (analogous to the eradication threshold in epidemiology[6]) is calculated from the condition $R_0 = 1$. The phage can increase in number only when $x(t) > X_P$, where

$$X_P = \frac{m(k-a)}{b(k(L-1)+a)} \approx \frac{m(k-a)}{bkL} \tag{2}$$

Assuming that initially the bacteria increase exponentially with $x(t) = x_0 e^{at}$, then the proliferation onset time T_P is found from the condition $x(T_P) = X_P$, giving

$$T_P \approx \frac{1}{a} \ln\left(\frac{m(k-a)}{bkLx_0}\right) \tag{3}$$

For active therapy to be successful also requires phages to still be present at the proliferation onset time. No exact formula is possible for this condition, but we derived[7] an approximation for the condition $t_\phi > T_F$, where

$$T_F \approx T_P - \frac{1}{m} \ln v_\phi - \frac{1}{a} \tag{4}$$

If active therapy is not possible, then a minimum inundatory dose must be exceeded to effect passive therapy, where the minimum dose is given by

$$V_I = \frac{a}{b} \tag{5}$$

With these critical thresholds and time points, the qualitative outcome of therapy can be categorized depending on the dosage and timing of inoculation.

APPENDIX B. NUMERICAL SIMULATIONS

The formulas given in Appendix A predict various density and timing thresholds. We tested the existence of these thresholds by use of numerical simulations of a mathematical model describing the kinetics of a generalized phage-bacteria system of the form:

$$dx/dt = ax - bvx \tag{6}$$

$$dy/dt = ay + bvx - ky \tag{7}$$

$$dv/dt = kLy - bvx - mv \tag{8}$$

where x(t) represents the number of uninfected bacteria, y(t) the infected bacteria, and v(t) the free phage. For parameter definitions see Appendix A. Exact values of the parameters for specific systems are generally not reported, but estimates are available from modeling studies,[17,18] and by inference from time series data in experimental studies.[10,16] No direct estimates of the transmission parameter b are available, but values were derived by use of estimates of V_I and a substituted into equation of Appendix A. For simulation of our "generic" kinetic system we explored a range of different combinations of inferred parameter values. For all biologically reasonable values the same qualitative properties were observed in the simulations.

Fig 1 was produced from numerical simulations run with the following typical parameter values (time units: hours): $a = 0.3$, $b = 10^{-6}$, $k = 1.2$, $L = 100$, $m = 1.8$; phage inoculation size $v_\phi = 100$. Thus for this example the bacterial replicative cycle is around 3 hours, the phage-infected bacteria decay on a time scale of about 1 hour, each lytic burst releases an average of 100 new phage particles, and the free phage decay on a timescale of around half an hour. To produce Fig 1 the only quantity varied on different runs of the simulations was the inoculation time; for each run the maximum concentration of bacteria (used as a surrogate measure of disease severity) was recorded. For the illustrative example in Fig 1, it was assumed that the infection is controlled only be active phage therapy, with no host immune response. The predicted estimates of T_P and T_F are marked. Only inoculation after T_F has any effect; the optimal inoculation time is at T_P.