Pharmacodynamics of non-replicating viruses, bacteriocins and lysins

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The pharmacodynamics of antibiotics and many other chemotherapeutic agents is often governed by a ‘multi-hit’ kinetics, which requires the binding of several molecules of the therapeutic agent for the killing of their targets. In contrast, the pharmacodynamics of novel alternative therapeutic agents, such as phages and bacteriocins against bacterial infections or viruses engineered to target tumour cells, is governed by a ‘single-hit’ kinetics according to which the agent will kill once it is bound to its target. In addition to requiring only a single molecule for killing, these agents bind irreversibly to their targets. Here, we explore the pharmacodynamics of such ‘irreversible, single-hit inhibitors’ using mathematical models. We focus on agents that do not replicate, i.e. in the case of phage therapy, we deal only with non-lytic phages and in the case of cancer treatment, we restrict our analysis to replication of incompetent viruses. We study the impact of adsorption on dead cells, heterogeneity in adsorption rates and spatial compartmentalization.

Keywords: phage therapy; non-lytic phage; bacteriocins; lysins; pharmacodynamics; mathematical model

1. INTRODUCTION

Many therapies consist of administering drugs to a patient, with the goal of killing or modifying a target population of cells. Antibiotic treatment of bacterial infections and chemotherapy of tumours are the familiar examples. The drugs in these therapies often obey a multi-hit kinetics, in which multiple molecules of the antibiotic agent are required to bind to a target cell to kill it (Tagg *et al.* 1976). However, not all potential agents operate this way. With some agents, the binding of a single molecule to a target cell will suffice for its killing. The agents falling into this latter category include viruses as well as proteins produced by bacteria to kill other bacteria, the so-called bacteriocins and possibly lysins (Mossie *et al.* 1979; Riley & Mee 1985; Fischetti 2003). Unlike common antibiotic drugs, the binding of these agents to their targets is effectively irreversible. The dynamics and kinetics of these ‘irreversible, single-hit inhibitors’ have been worked out for a few special cases (Hedges 1966; Payne & Jansen 2001), and these models have been explored only narrowly. One reason for this lack of attention is that these types of therapies have not been aggressively pursued by industry. Yet the increasing problem of drug-resistant bacteria motivates a consideration of new approaches to control infection.

This paper explores the mathematical basis of agents that are governed by a single-hit kinetics and bind irreversibly to their targets. We take previous models as a starting point and extend those results to accommodate greater complexity. These models should be useful in assessing the feasibility of treatments using such

irreversible, single-hit inhibitors and should be helpful in deciding which drug properties to modify for improved efficacy. The specific context for our models is non-replicating phages attacking a liquid population of bacteria, but there are many other applications to which the models also apply. Non-replicating phages may seem to offer a somewhat counter-intuitive agent for treating infections, since the major advantage of phages lies in their ability to replicate. Yet many of the advantages of phages for treating infections accrue whether the phages replicate or not (such as their ability to locate and infect specific cell types), and if a phage fails to lyse the host, then deadly endotoxins are not released. Furthermore, a non-replicating phage may be used in combination with other agents, even replicating phages, to help offset drawbacks of non-replication.

2. MODEL STRUCTURE AND PARAMETERS

The models here will assume that infections follow mass-action dynamics of phage and bacterial densities as pioneered by the early phage school (Adams 1959). The phage (P) and bacterial (B) populations will be characterized by their densities per millilitre, and the abundance of collisions between them which result in infections is given by δPB , where δ is the adsorption rate. These models therefore do not apply to the structured populations of cells (e.g. solid masses) because those structures are not mixed and defy the mass-action assumption. In most of our models, neither population is allowed to replicate—the phage by design and the bacteria for analytical convenience. The phages are lost by two processes: infection and clearance by the host. Bacteria are lost only from infection by a phage. A ‘static’ bacterial population may be obtained in some infections, and phage adsorption may be dynamically fast enough to justify ignoring bacterial growth, but this assumption is

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best accepted in the spirit that the results provide a minimum estimate of the dose required (see electronic supplementary material for some extensions to allow bacterial replication). These models may also apply to replication-competent phages when the cell density is too low to maintain phage populations and hence when successful treatment requires addition of sufficient phages to overwhelm the bacteria prior to phage replication.

As noted earlier, the dynamics of phage infecting bacteria are not to be equated with that of classical antibiotics interacting with the bacterial population. First, and most importantly, one phage is enough to kill a bacterial cell ('quantal' or 'single-hit' kinetics), whereas in general, many molecules of classical antibiotics are required for killing ('molar' or 'multi-hit' kinetics). Second, for a phage, there is no equivalent to an 'off rate' or dissociation constant, whereas classical antibiotics can dissociate from their targets. The models we develop obey the 'single-hit' kinetics (Jacob *et al.* 1952) that adsorption of a virus to a cell kills both the virus and the cell (perhaps with a probability of less than 1). The usual method of assessing whether the dynamics of an agent conforms to a single-hit kinetics is to vary agent concentration and plot log(survival) versus concentration, taken at a fixed time point and for a fixed concentration of cells. Assuming a Poisson distribution of phage adsorbing to cells, the survivors (proportion S) are those that adsorb no phage,

$$S = e^{-C},$$

where C is the concentration of the lethal agent. Taking logs, we obtain:

$$\ln(S) = -C.$$

Hence, for a single-hit agent, a linear relation between log of survival with concentration is predicted (Hedges 1966; Reynolds & Reeves 1969). As noted by Hedges (1966), this simple relationship assumes that the agent is present in such abundance that any loss of it during the measurement phase can be ignored. The problem becomes more complicated when one must account for the decay in both cell and agent concentrations. Here, we develop models to account for losses of both cells and agent, deriving the time courses of these declines as well as considering the effects of various realistic extensions of the basic model.

(a) Model 1

We begin with a simple model that is easily expanded to encompass greater reality. A population of bacteria is mixed with a population of phage, and when a phage adsorbs to a bacterium, both the bacterium and the phage instantly 'die', such that the phage can no longer infect other bacteria and the bacterium can no longer be infected by other phages. Importantly, in this model, the bacterium also instantly loses the ability to adsorb other phages. As the phage is non-replicating, no phage progeny are released from the infection. Denote

$B(t)$	density of live bacteria at time t (units ml^{-1})
$P(t)$	density of phage at time t (units ml^{-1})
δ	adsorption rate of phage to bacteria (units ml min^{-1})
λ	clearance rate of phage due to factors other than adsorption to bacteria (units min^{-1}).

The adsorption rate, δ , gives the rate at which a single bacterium collides with a single phage particle and results in an infection. Its theoretical upper limit is near $10^{-8} \text{ ml min}^{-1}$ for phage, and the adsorption rates typical for lab media are near $10^{-9} \text{ ml min}^{-1}$ (Adams 1959). For colicins, the theoretical upper limit is near $4 \times 10^{-8} \text{ ml min}^{-1}$, but the observed values tend to be in the range of 10^{-10} – $10^{-11} \text{ ml min}^{-1}$, varying somewhat with media (Reynolds & Reeves 1969). Since the phage is not allowed to replicate, other phage properties, such as burst size and latent period, need not be specified. As time progresses, phage attack and kill some of the bacteria, and the concentrations of both decline equally from these infections. However, the phage concentration also declines from clearance by the host. From these behaviours, a rather simple set of dynamics follows:

$$\frac{dB}{dt} = -\delta PB, \quad (2.1)$$

$$\frac{dP}{dt} = -P[\lambda + \delta B], \quad (2.2)$$

where variables are functions of time unless indicated otherwise. The initial conditions are simply that $B(0)$ live bacteria and $P(0)$ phage are present at the outset.

A standard approach used with these types of models is to set all the derivatives to 0 and solve for relationships among the parameters at equilibrium. This approach does not work here because the equilibrium depends on starting conditions. In particular, the phage concentration decays to 0 no matter what the starting density is and the final bacterial density varies with the number of phages added initially (see figure 1b). Short of obtaining a general solution to these equations, our goal is to describe the relationship between the initial phage density and the ultimate number of surviving bacteria. Towards this end, the system of equations may be solved by separation of variables. Equation (2.1) provides an expression for P that may be substituted in equation (2.2) to obtain

$$P(t) - B(t) - \lambda \delta \ln B(t) = c, \quad (2.3)$$

where c is a constant whose value is set by initial conditions.

Using the fact that this result holds at all times and the phage concentration eventually decays to 0,

$$P(0) = B(0) - B(\infty) + \frac{\lambda}{\delta} \ln \frac{B(0)}{B(\infty)} \quad (2.4)$$

(Payne & Jansen 2001). All the terms have units ml^{-1} . This equation assumes that all hits are lethal. If instead only a fraction of adsorptions, α , are lethal, the right-hand side of equation (2.4) is merely multiplied by α^{-1} .

Figure 1 illustrates various properties of equations (2.3) and (2.4). The time course of bacterial and phage densities is shown in figure 1a and the phase-plane profiles are shown in figure 1b. The phage dose required to reduce the bacterial density by a certain amount is shown in figure 1c, and here, it can be seen that this particular model 1 leads to the most favourable outcome (lowest dose of phage necessary) of all the models considered here. However, for the parameters used in figure 1, the effect is small.

Equation (2.4) has an easy interpretation which is not evident from these figures. It gives the amount of phage, $P(0)$, needed to reduce the initial bacterial count, $B(0)$, to

Figure 1. Comparison of the dynamics of model 1 in which dead bacteria do not adsorb (solid lines), model 2 in which dead bacteria adsorb (dashed lines), model 3 in which two types of bacteria are present (dotted lines), and model 4 in which there are two compartments (dashed-dotted lines). (a) Time course of bacterial (green) and phage (black) densities. In all the models, the bacterial densities decline only initially when phage densities are high, and then attain a stable level. The phage densities decline approximately exponentially (in model 2 exactly exponentially). (b) Phase–plane plots. The arrows denote the direction of time. The higher the initial phage density, the greater the reduction of the bacterial population. The B – P phase–plane profiles can be derived analytically (see equation (2.3)). (c) The phage dose, $P(0)$, required for to reduce the bacterial population to a certain fraction of the initial density, $B(0)$. For these graphs, we set $B(0) = 10^6$ and $P(0) = 3 \times 10^7$ and $\lambda = 5 \times 10^{-3}$. In models 1 and 2, we assumed $\delta = 10^{-9}$, and, in model 3, we assumed that $\delta_1 = 0.7 \times 10^{-9}$, $\delta_2 = 1.7 \times 10^{-9}$, and that the initial frequency of the first bacterial subpopulation is 0.3. (With these parameters the initial mean adsorption rate of the total population is the same as the adsorption rate $\delta = 10^{-9}$ in models 1 and 2.) Lastly, in model 4 we assumed $x = 0.01$ and $V = 2.5$.

a specific level, $B(\infty)$. The right side of the equation partitions the loss of phage to bacterial infection and clearance. The difference $B(0) - B(\infty)$ is the loss of both phage and bacteria from infection; this equivalence stems from our assumption that infected bacteria instantly lose the ability to adsorb other phages. The term $(\lambda/\delta)\ln(B(0)/B(\infty))$ is the loss due to clearance. The ratio $B(0)/B(\infty)$ is the total number of cells at the outset divided by the number of cells at the end. If 1% of cells survive, the ratio is 100 and goes to infinity as the fraction killed goes up. In addition, the loss due to clearance depends on the clearance rate relative to the adsorption rate. The clearance rates have been measured for some phages in mice, and they range between 10^{-2} min^{-1} and 10^{-3} min^{-1} (see §3; clearance rates for colicins do not seem to have been measured *in vivo*). Adsorption rates in lab media tend to be near $10^{-9} \text{ ml min}^{-1}$, so that the ratio of these two is of the order of 10^6 – 10^7 ml^{-1} . This means that reductions of bacteria of 90–99.9% may require phage concentrations of over 10^7 ml^{-1} just to offset clearance.

The loss of phage to clearance depends on the proportion of bacteria killed, regardless of the absolute abundance of bacteria. In contrast, the loss of phage to infection depends on both the density of bacteria and the fraction killed. For the same proportional killing, the loss to clearance becomes relatively less important as the initial bacterial density is increased. For example, suppose that one determined the amount of phage to kill 99% of $B(0)$ bacteria. The loss to clearance would be $(\lambda/\delta)\ln(100)$ and that to infection would be $0.99B(0)$. Compare this to killing 99% of $10B(0)$ bacteria. The loss of phage to clearance would still be $(\lambda/\delta)\ln(100)$, but the loss to infection would increase 10-fold to $9.9B(0)$. Viewing this from the other direction, the phage lost to clearance explodes to ∞ as higher and higher fractions of bacteria are killed. This effect arises because loss to clearance occurs at a constant rate ($P\lambda$), but loss to bacterial killing occurs in proportion to bacterial density (δPB). As bacterial density gets lower and lower, progressively more phages are lost to clearance as to bacteria killed (the ratio is $\lambda/(\delta B)$). Thus, killing bacteria to extremely low levels entails a huge loss to clearance. As a consequence, the two effects—clearance and infection—are not completely separable, even though this equation partitions them. Note that it is merely as a consequence of allowing $B(t)$ to be continuous with a lower bound of 0 that we obtain the result that complete bacterial eradication is impossible, although the result does legitimately illustrate that clearance becomes progressively more important as live bacterial density wanes, and that complete bacterial clearance by non-replicating phage is not practical.

How could this model be used or tested empirically? An especially simple test would be to monitor an infection for live cells at different time points after phage inoculation (as a test of equation (2.3)). However, the results will be easily interpretable only if the bacteria were not replicating and there were no other sources of bacterial mortality. A less precise but more practical approach would be to use an infection in which a particular phenotypic effect was achieved by a threshold density of inoculated bacteria (e.g. a minimum infectious dose). The phage dose required to counteract the phenotypic effect could then be determined for inocula of bacteria that exceeded the threshold, and those data could be fit to equation (2.4). Here, the time course of infection might be such that bacterial replication

could be ignored. Perhaps the most important use of this model is that it provides a qualitative indication of the dose of phage needed to control different levels of infection [$B(0)$] when some information is already available about controlling one level of infection. Thus, if success has been attained with an infection of 10^7 bacteria per millilitre, how many more phages must be added if the infection is 10^9 bacteria per millilitre? In addition, this model provides a best-case scenario for comparison with several violations of these assumptions, as offered later.

(i) *Multiple versus single inocula*

An interesting question is whether different numbers of bacteria are killed if a quantity of phage is administered in one large dose or sequentially, in several smaller doses that add to the same total. The answer is satisfactorily simple: the bacterial kill depends only on the total number of phages added. In this case, our assumption that the bacteria do not divide is critical, as the addition of phage over time would allow a longer period of bacterial growth than if all the phages were given at once. This result is derived by sequential application of equation (2.4) until a fixed total of phage has been used, noting that the final density of bacteria is independent of the partitioning of phage inocula.

These models depict treatment with an irreversible, single-hit inhibitor under the simplest conditions. The resulting pharmacodynamics is qualitatively different from that of classical antibiotics, which are governed by a multi-hit kinetics (see electronic supplementary material). To explore more realistic infections, we introduce a variety of separate modifications to model 1 in the following sections.

(b) *Model 2: infected/dead cells continue to adsorb virus/phage*

In this section, we turn to yet another difference between phage and antibiotics, which concerns the issue of whether infected bacteria continue to adsorb the drug/phage. In the case of antibiotics they do not, and so dead bacteria do not act as a sink for the drug. For most of the phage, adsorption continues after infection, at least until cell death but possibly beyond, greatly limiting the fraction of bacteria that can be killed. We investigate the impact of adsorption of infected or dead cells in §2(c).

This model is the same as model 1 except that dead bacteria are allowed to continue adsorbing phages. Hence, there is no real distinction between live and dead bacteria. Thus, this model not only represents the case when dead bacteria continue to adsorb phage and take them out of circulation, it also represents cases in which the agent does not kill the cell, but produces some other effect that does not modify the cell's ability to adsorb other agent molecules (as when a virus delivers a gene for transformation). It is nonetheless important to keep track of infected bacterial densities to determine how successful the agent has been in infecting the population.

We use the same variable and parameter definitions as earlier, except that we denote $D(t)$ as the density of dead bacteria at time t .

A total of $B(0)$ live bacteria are present at the outset. As time progresses, phages attack and kill some of the bacteria, but at all the times the combined density of live and dead bacteria remains constant: $B(0) = B(t) + D(t)$. Dead bacteria are merely bacteria that have been infected at

some time in the past; adsorption rates are the same between live and dead bacteria, and infection of dead bacteria is merely a sink for phage. With these assumptions,

$$\frac{dB}{dt} = -\delta PB, \quad (2.5)$$

$$\frac{dD}{dt} = \delta PB, \quad (2.6)$$

$$\frac{dP}{dt} = -P[\lambda + \delta B(0)]. \quad (2.7)$$

Equation (2.7) is an equation of one variable and can be solved directly:

$$P(t) = P(0)e^{-[\lambda + \delta B(0)]t}. \quad (2.8)$$

The phage half-life is $0.69/(\lambda + \delta B(0))$ min. If clearance dominates the loss of phage, then the half-life can be of the order of an hour (given the values of clearance cited earlier). For densities of bacteria of 10^8 ml⁻¹ or more, the phage half-life is less than 10 min and less than what would be the bacterial doubling time if bacteria were replicating. The phage half-life in this model is necessarily shorter than in model 1, because dead cells continue to act as a sink for phage here, whereas many of those phages would remain in the $P(t)$ pool in model 1. The continued adsorption of dead bacteria to phage means that progressively fewer live bacteria are killed with each subsequent phage half-life, because as time progresses, there are increasingly more dead bacteria instead of live bacteria adsorbing to phage. The dashed lines in figure 1a show the time course of bacterial and phage densities predicted by this model.

This system of equations may again be solved by the separation of variables. Equation (2.5) provides an expression for P that may be substituted in equation (2.7) to obtain

$$P(0) = \frac{\delta B(0) + \lambda}{\delta} \ln \frac{B(0)}{B(\infty)}. \quad (2.9)$$

As mentioned earlier, phage loss is partitioned between infection and clearance. It is thus easy to contrast models 1 and 2, and the comparison is interesting. Intuition suggests that more phages will be required to achieve a particular bacterial reduction if dead bacteria continue to adsorb than if they do not. Therefore it is surprising that the loss to clearance ($\lambda/\delta \ln(B(0)/B(\infty))$) is identical in both the models, and it is only the loss of phage due to infections that is substantially different for the two cases. Recall that if the infected cells immediately lose the ability to adsorb (equation (2.4)), then the loss of phage due to infection is $B(0) - B(\infty)$. Indeed, if clearance did not occur, one would need only as many phages as bacteria killed. The upper limit for the loss due to infection in model 1 is thus $B(0)$.

In contrast, when dead cells continue to adsorb, the loss of phage to infection is $B(0) \ln(B(0)/B(\infty))$. The lower bound for this amount is $B(0)$, but it is a potentially much larger amount than $B(0) - B(\infty)$. The two quantities converge to 0 as $B(\infty) \rightarrow B(0)$, but the quantity $\ln(B(0)/B(\infty))$ grows without bound as $B(\infty)$ goes to 0. The difference between the two models also depends on the relative loss to clearance—if clearance dominates phage loss, then the required phage doses are similar. Thus, far more phages are required to infect nearly all cells if dead cells adsorb than if they do not, provided that most phages are lost to infection, as might be expected. Even so, for kills up to

0.01% of the initial amount, the loss to infection is less than $10B(0)$ when dead cells adsorb (versus approx. $B(0)$ if cells do not), so the effect may be manageable. In addition, for initial cell densities of 10^9 ml^{-1} or more, the loss to clearance should be a small fraction of the amount lost to infection. Thus, while the two models differ, the phage inoculum needed for large kills should typically be within an order of magnitude of each other.

Assessing whether infected cells continue to adsorb phage is easily undertaken in a laboratory setting, and for many lytic phages, infection and even cell death do not prevent further adsorption (Adams 1959). However, this rule is not universal (see §3). Data on infection dynamics *in vivo* could be used to infer indirectly whether infected cells continue to adsorb phage, as models 1 and 2 show substantially different dynamics when most phage loss is to infection. Thus, the models may be used comparatively to gain insight to infection details.

(c) Model 3: one compartment, two types of bacteria

Bacteria within an infection are not likely to be uniform in physiology. Their locations within tissues will affect resource availability and affect exposure to antimicrobial compounds directed to them. The bacteria in our model are characterized by one parameter, i.e. adsorption rate. The adsorption rates depend on many factors, including the physiological state of the cells (Hadas *et al.* 1997; Abedon *et al.* 2001), metal ion concentration and receptor densities (Hedges 1966), so it is biologically reasonable to consider variation in this parameter. We can thus begin to understand the effect of bacterial heterogeneity by allowing two types of bacteria, differing in adsorption rates, δ_1 and δ_2 . A difference in adsorption rates means that the two types of bacteria are killed at different rates. The bacterium with the highest adsorption rate is killed faster.

Assuming that once-infected cells cannot be re-infected (model 1) and distinguishing bacteria by the adsorption rates (δ_i for B_i), equations are

$$\frac{dB_1}{dt} = -\delta_1 PB_1, \quad (2.10)$$

$$\frac{dB_2}{dt} = -\delta_2 PB_2, \quad (2.11)$$

$$\frac{dP}{dt} = -P[\lambda + \delta_1 B_1 + \delta_2 B_2], \quad (2.12)$$

with solution

$$P(0) = B_1(0) - B_1(\infty) + \frac{\lambda}{\delta_1} \ln \frac{B_1(0)}{B_1(\infty)} + B_2(0) - B_2(\infty) \quad (2.13)$$

$$= B_1(0) - B_1(\infty) + \frac{\lambda}{\delta_1} \ln \frac{B_1(0)}{B_1(\infty)} + B_2(0) \left\{ 1 - \left[\frac{B_1(\infty)}{B_1(0)} \right]^{(\delta_1/\delta_2)} \right\}. \quad (2.14)$$

The solution may take many forms due to the equality $(1/\delta_1) \ln(B_1(0)/B_1(\infty)) = (1/\delta_2) \ln(B_2(0)/B_2(\infty))$. Equation (2.14) represents a solution in which the desired bacterial reduction is set for type 1 (set as $B_1(\infty)$), and all other quantities are known from the initial state of the infection. Equation (2.13) is the same as equation (2.4) except for the last two terms, which represents the loss of phage infecting cell type B_2 . Relatively more phage will be lost to

infection of the host with the higher adsorption rate. However, in comparison to model 1, the maximum addition of phage to accommodate the loss to B_2 is simply $B_2(0)$ and much less if adsorption to B_2 is much slower than to B_1 . In the electronic supplementary material, we show that the variance in adsorption rates generally decreases the rate of bacterial killing when the total bacterial population is counted. The dotted lines in figure 1 also show that heterogeneity in adsorption rates reduces the efficacy of treatment.

Under some parameter values and initial conditions (in particular, when there is a large excess of phage and when the bacterium with higher adsorption rate is at least 90% of the population), treatment of the mixed bacterial population shows a 'biphasic' kill curve: the plot of survivors over time shows a line of sharp exponential decay intersecting with a line of shallower exponential decay (see electronic supplementary material). The shallower exponential decay does not extend indefinitely, of course, because the surviving bacteria eventually reach a constant value as the drug concentration wanes. Biphasic kill curves have been reported for colicins (Mossie *et al.* 1979), and there is an awareness that variance in numbers of receptors per cell can lead to these types of plots (Hedges 1966).

(d) Model 4: compartments

Most infections are localized. The mammalian host presents an environment with membrane boundaries and surfaces that violate the concept of mass action, except perhaps in blood, urine and other fluid environments. In some cases, as with surface wounds, treatment can be applied directly to the site of the infecting bacteria, and treatment of tumours with eukaryotic viruses often involves injection of the virus directly into the tumour (Ganly *et al.* 2000; Heise & Kirn 2000; Nemunaitis *et al.* 2001). In other cases, phage would need to be administered at a site remote to the infection, and the phage be carried by blood or lymph system to the site.

A two-compartment model provides the simplest case of this effect. Again, we assume that bacteria lose the ability to adsorb after a single infection. In the first model, bacteria will be confined to compartment 2, but phage can occur in either compartment 1 or 2 (denoted with a subscript):

$$\frac{dP_1}{dt} = -P_1(\lambda + x) + P_2x, \quad (2.15)$$

$$\frac{dP_2}{dt} = -P_2(\delta B + \lambda + xV) + P_1xV, \quad (2.16)$$

$$\frac{dB}{dt} = -\delta P_2B. \quad (2.17)$$

Exchange of phages between the two compartments is accommodated by the parameter x (the rate of phage exchange between compartments 1 and 2 per minute) and by xV , where V is the dimensionless ratio of the volume of compartment 1 to that of 2. Assuming that the same absolute volume is exchanged between the two compartments, V adjusts for the different proportional effects on the volumes of the two compartments.

Equations 2.15–2.17 lead to expressions for the required phage dose similar to those for the previous models. If all the phages are added to the bacteria-free

Figure 2. Bacterial survival, $B(\infty)/B(0)$, in the multi-compartment model. (a) Bacterial survival as a function of the adsorption rate δ . In a multi-compartment model with five compartments (dashed-dotted line), the efficacy decreases much less with increasing adsorption rate, δ , than in the single-compartment model 1 (solid line). (b) Bacterial survival in each of the five compartments (assuming $\delta = 10^{-9}$). (c) Bacterial survival as a function of the number of compartments. The parameters in this plot are the same as in figure 1 except that we set $P(0) = 2 \times 10^7$. The migration rate between compartments was assumed to be $x = 0.01$ (black), and $x = 0.1$ (red).

compartment 1, then

$$P_1(0) = G \left[B(0) - B(\infty) + \frac{H}{\delta} \ln \frac{B(0)}{B(\infty)} \right]. \quad (2.18)$$

If, instead, all the phages are added to compartment 2 containing bacteria, then

$$P_2(0) = B(0) - B(\infty) + \frac{H}{\delta} \ln \frac{B(0)}{B(\infty)}. \quad (2.19)$$

Here, $G = (\lambda/x + 1)/V$ and $H = \lambda(1 + 1/G)$. At the boundaries of no exchange and of free exchange between compartments, both equations reduce to their

corresponding single-compartment counterparts, although when both compartments are fully mixed, the phage occupy a larger volume $(1 + V)$ than the bacteria. The most interesting result concerns the addition of phage to the empty compartment (2.18), representing the addition of phage to a site remote from the infection. Here, intuition agrees with the results; low exchange rates (relative to the clearance rate) require substantial increases in the number of phages needed, because clearance removes many of the phages before they enter the compartment with bacteria. This result is seen quantitatively by the fact that $G \rightarrow \infty$ as $x \rightarrow 0$.

An extended version of model 4 assumes a series of n compartments, subscripted with i ($1 \leq i \leq n$). Although a more general case can be solved, we will consider that phage move unidirectionally at the same rate from all compartments, but bacteria remain within their compartment. The equations are

$$\frac{dB_i}{dt} = -\delta P_i B_i, \quad (2.20)$$

$$\frac{dP_i}{dt} = -\delta P_i B_i + x P_{i-1} - (x + \lambda) P_i, \quad (2.21)$$

subject to the constraint that $P_0(t) = 0$ for all t (because there is no compartment 0), and phages diffuse out of the final compartment at the usual rate, but do not go into a compartment with bacteria. The solution takes a similar form as before:

$$P_i(0) = B_i(0) - B_i(\infty) + \frac{x + \lambda}{\delta} \ln \left[\frac{B_i(0)}{B_i(\infty)} \right] - \frac{x}{\delta} \ln \left[\frac{B_{i-1}(0)}{B_{i-1}(\infty)} \right], \quad (2.22)$$

subject to the appropriate boundary conditions. If phages are added only to the first compartment, then all other compartments must satisfy

$$B_i(\infty) + \frac{x + \lambda}{\delta} \ln B_i(\infty) = B_i(0) + \frac{x + \lambda}{\delta} \ln B_i(0) - \frac{x}{\delta} \ln \left[\frac{B_{i-1}(0)}{B_{i-1}(\infty)} \right]. \quad (2.23)$$

The initial conditions specify the number of phages added to compartment 1 as well as the starting numbers of bacteria in all the compartments. Except for compartment 1, $P_i(0) = P_i(\infty) = 0$. The unknowns are thus the $B_i(\infty)$, and all except the first equation have two such unknowns. Starting with compartment 1 (the B_{i-1} terms are absent from this first equation because there is no compartment 0), the equation may be solved for its single unknown, $B_1(\infty)$. This solution then enables the equation for the second compartment to be solved, and so on. The killing of bacteria in compartments other than the first is measured entirely by the term $-(x/\delta) \ln[B_{i-1}(0)/B_{i-1}(\infty)] = (x/\delta) \ln[B_{i-1}(\infty)/B_{i-1}(0)]$. The first fraction in each product is a measure of the rate at which phages move between the compartments: high transfer rates (x) and low adsorption rates (δ) enhance transfer and the effect of adsorption rate is the paradoxical one that more phages are available for transfer when fewer have adsorbed to bacteria. The extent of killing in compartment i also increases with the extent of killing in $i-1$, but it increases logarithmically with the survival fraction. Here, the effect is that a high kill in compartment $i-1$ means that there were lots of phages in that compartment to be transferred to i .

Figure 2a shows the dependence of the survival of the total bacterial population on the adsorption rate, δ , and the transfer rate, x , for a model with five compartments. The efficacy of treatment is best for high adsorption rates and low transfer rates. High transfer rates are disadvantageous in this model because phages transferred from the last compartment are lost. In this respect, our n -compartment model differs from our two-compartment model, in which phage could not leave both the compartments. Figure 2b shows the reduction in each of the five compartments. With a low transfer rate, $x=0.01$, killing is quite good in at least some compartments. In contrast, with a high transfer rate, $x=0.1$, killing is generally low owing to this effect. Figure 2c shows the bacterial reduction for differing numbers of compartments. Interestingly, for a given transfer rate, maximum killing is achieved at an intermediate number of compartments. Overall, we can conclude that the existence of compartments reduces the efficacy of treatment.

3. DISCUSSION

The infective powers of viruses can be harnessed for many beneficial purposes, such as gene delivery, vaccination, killing cancer cells and killing bacteria (Smith & Huggins 1982; Fisher *et al.* 1996; Ganly *et al.* 2000; Heise & Kirn 2000; Heise *et al.* 2000; Nemunaitis *et al.* 2001; Payne & Jansen 2001; Bull *et al.* 2002). In some of these applications, it is desirable or necessary to use viruses incapable of replicating, whether to avoid viral amplification and spread to non-intended cell populations or to avoid killing the cells they infect (as with gene therapy and vaccines). Even when using phages to kill bacteria, where it seems obvious that phage replication is desirable, there are good reasons to avoid phage replication because replication of most phages entails lysis, which releases bacterial toxins (Westwater *et al.* 2003). Indeed, recent empirical work with an experimental infection in mice showed that high doses of a non-lytic phage (incapable of replication) conferred better mouse survival than a replicating/lytic form of the same phage; the non-lytic phage even performed better than a β -lactam antibiotic, which also lyses cells (Matsuda *et al.* 2005). Thus, while the benefit of replication to a phage used to treat an infection seems obvious, endotoxin release is a serious by-product of phage replication. Nonetheless, it is not our intent in this paper to advocate non-replicating phages or other agents for therapy. Instead, we accept that there may be valid reasons to pursue such methods, and we provided models that should facilitate developing the technologies. Furthermore, a non-replicating virus may constitute only a part of a successful treatment strategy, and it will be necessary to understand how to maximize its efficiency in combination with other therapies.

Viruses obey a special type of kinetics in which attachment of a virion to a target cell is close to irreversible and the attachment of a single virion is enough to produce its effect on that cell ('single-hit' kinetics). Bacteriocins, which are proteins made by bacteria to kill other bacteria, operate with a similar kinetics (Jacob *et al.* 1952; Shannon & Hedges 1967; Reynolds & Reeves 1969; Mossie *et al.* 1979; Riley & Mee 1985), and it seems likely that phage lysins applied

externally to bacteria behave in a similar manner (Fischetti 2003). In principle, the combination of the irreversible attachment and the single-hit kinetics should lead to a high efficacy of such agents as compared to classical antibiotics. The problem is that various properties of the host and the virus contribute to a substantial inefficiency, whereby a much lower proportion of cells become infected than is predicted with perfect non-interfering infections.

Our models quantify this viral inefficiency and indicate the inoculum size needed to infect a predetermined fraction of the target cell population, identifying how different properties of the infection environment affect the outcome. This problem seems to have been given only slight attention for non-replicating viruses. Levin *et al.* (1977) developed some of the first comprehensive models of phages replicating in a sustained population (a chemostat). Their model focused on the maintenance of phage populations, which thus do not admit non-replicating phages. Payne & Jansen (2001) generated the result in our model 1 as a contrast to the effect produced by a replicating phage and thus did not explore it. Wodarz developed a model of viral treatment of tumour cells (Wodarz 2001, 2003); one version of that model considered a non-replicating virus (Wodarz 2003), but that model did not address how much virus was needed, and there was no viral loss when infecting cells.

Our results often match intuition qualitatively, but the magnitudes of effect would be difficult to anticipate without the models, and some complexities are difficult to predict with intuition alone. Although it would be tedious to estimate the parameters comprehensively in an *in vitro* system and to fit the model rigorously, the models may be used in a less precise manner to anticipate qualitative effects of changing phage and bacterial doses and to determine which types of infections are most suitable for the use of a non-replicating phage treatment. Four factors considered to influence viral loss were: (i) clearance rates of the virus; (ii) whether once-infected cells continue to be infected; (iii) populations of target cells that vary in adsorption rates; and (iv) compartments of the target population that defy mass action. In all the cases, the amount of virus needed to achieve a specific level of target cell infection was the sum of two effects: clearance of virus by the host and loss due to virus infecting the target cells. Clearance is capable by itself of eliminating all the viruses, but viruses can infect an appreciable fraction of cells before clearance removes them all, and it is the number of cells infected that interests us.

One important factor whose effect is easily calculated is (ii) stated above: whether or not infected cells continue to adsorb virus. If the infected cells do not adsorb additional virus, then there is a 1-for-1 loss of virus for each cell infected, ignoring clearance. If instead infected/dead cells continue to adsorb virus, the amount of virus lost to infection grows logarithmically with the inverse of bacterial survival rate. Under the most ideal conditions, infecting 99% of the target cells requires virus at 4.6 times the concentration of target cells (ignoring loss due to clearance); each additional 99% reduction of the remaining live cells again requires that much additional viruses. In contrast, if the infected cells do not continue to adsorb, the maximum amount of virus needed to kill all the cells is just equal to the concentration of target cells. There are

many applications in which infected cells would be expected to remain infectible, when the virus did not kill the cell and also when the virus killed the cell slowly or even when the dead cell remained intact. For bacteriophages, heat-killed cells are sometimes used for adsorption rate assays, indicating that dead cells continue to adsorb (Adams 1959). Thus, by knowing merely whether infected bacteria continue to adsorb phage, which can be assessed *in vitro*, it becomes possible to anticipate the relative difficulty of using non-replicating phages for treatment. We note in passing that some phage behaviours are intermediate between the two types of adsorption processes we considered: phage T1 requires a living membrane (energized) for irreversible adsorption, so it presumably can super-infect a cell that is already infected only until the cell dies (Hancock & Braun 1976). How long a cell lives beyond infection with a non-replicating phage would depend on the nature of the replication defect.

Loss of virus to the host environment (clearance) represents a potential problem. The effect of viral clearance is measured relative to the adsorption rate in all the single-compartment models (in the two-compartment model, the clearance rate is measured relative to adsorption rate in some terms and is measured relative to compartment exchange rate in other terms). The clearance rates of bacteriophages in mice have been measured at roughly 6×10^{-3} , 3×10^{-3} and just under 2×10^{-3} per minute (Merril *et al.* 1996; Westwater *et al.* 2003). These rates have been estimated from graphs or statements in these papers and hence are approximate. Merrill *et al.* selected two phages for reduced clearance rates and achieved an impressive drop of 3 in the order of magnitude in the 24 h clearance rates, but the per-minute rate change is within a factor of 5. Applying the adsorption rate value of 10^{-9} ml min⁻¹ (obtained for various phages in lab media (Adams 1959)), the loss of phage due to clearance may reach 10^7 ml⁻¹, which could require substantial doses of virus in a large animal. Again, the models allow us to quantify the effects of this process (clearance) without a large amount of empirical work. The clearance rates have not been determined for bacteriocins, but would be of obvious value in anticipating the utility of those toxins for treatment.

The compartments were analysed as a way of violating mass action while retaining the basic model structure. A treatment complication of compartments was identified for the case in which phages are added to a compartment without bacteria and there is also a slow rate of exchange to reach the compartment with bacteria. Clearance can then begin to proceed before many bacteria are infected. A second compartment model considered serial transfer of phage across a connected series of compartments, each containing bacteria. With low levels of exchange, phage efficacy decays rapidly from the compartment of inoculation. This problem is likely to be important in solid tissues and other environments in which cells and viruses are not well mixed.

Although bacteriocins have apparently not previously been addressed with the types of models used here, they have long been appreciated to follow single-hit kinetics, and there are several old observations that can be considered in light of our models. In general, it was commonly assumed that bacteriocins would be administered at much higher

concentrations than the bacterial concentrations, so that the loss of bacteriocins due to adsorption could be ignored (Hedges 1966). One interesting observation was that bacteriocins often followed single-hit kinetics, but calculations based on molecular weight suggested that 100 or more molecules were required to produce a kill (Timmis & Hedges 1972). The biological basis of this incongruity was not resolved, but could possibly have been degradation of the protein preparation. As noted earlier, biphasic survival curves had been observed in cells treated with bacteriocins; our models could accommodate that with multiple adsorption rates, which could stem from variation in bacterial receptors for the bacteriocins (Hedges 1966).

How does our analysis of the pharmacodynamics of irreversible, single-hit inhibitors relate to the mathematical analysis of the pharmacodynamics of classical antibiotics (Garrett 1971; Zhi *et al.* 1986, 1988; Leggett *et al.* 1989; Staneva *et al.* 1994; Hyatt *et al.* 1995; Nolting & Derendorf 1995; Bouvier d'Yvoire & Maire 1996; Nolting *et al.* 1996; Dalla Costa *et al.* 1997; Nix *et al.* 1997; Mouton *et al.* 1997, 2002; Corvaisier *et al.* 1998; Yano *et al.* 1998; Delacher *et al.* 2000; Louie *et al.* 2001; Bonapace *et al.* 2002; Vinks 2002; Boylan *et al.* 2003; Mueller *et al.* 2004; Regoes *et al.* 2004)? There are two main differences. First, most mathematical studies of pharmacodynamics of classical antibiotics after the 1960s focus on the *rate* of killing of the bacterial population induced by the antibiotic agent, rather than on the reduction of the bacterial *population size*. The reason for this is that the data that are analysed in most of the studies are generated *in vitro*, and do not permit ignoring bacterial replication. Second, the approach adopted in most of these studies is phenomenological, i.e. less emphasis is put on the molecular mechanisms of killing (e.g. whether the kinetics is single- or multi-hit). Instead, the pharmacodynamics is described by a sigmoidal relationship between the net bacterial growth rate and the antibiotic concentration—the so-called Zhi model, or $E_{\max}$ model. Although the $E_{\max}$ models are valuable in the rational design of treatment strategies, they are less useful for disentangling the differences between single- and multi-hit kinetics. Thus, our analysis continues along the lines of mathematical work on pharmacodynamics that pre-dates $E_{\max}$ models (e.g. Hedges 1966).

The models we present in this paper are merely a guide to the feasibility of infecting large fractions of a target population of cells. Various complexities may be added to these models, such as including more than two types, combining factors and allowing the target population to grow (see electronic supplementary material). Nonetheless, these models should foreshadow the difficulties and feasibility of using non-replicating viruses. In some cases, knowledge of the proportion of infected cells may be unnecessary, as when delivering a vaccine whose efficacy can be measured in antibody levels. Yet, when a viral delivery method fails to achieve the desired effect, it may be necessary to explore the basis of that failure by ascertaining how many cells are infected. This type of approach will be necessary for that exploration.

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