HIV

Drug Effectiveness Explained: The Mathematics of Antiviral Agents for HIV

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Antiretroviral therapy has improved the quality and length of life of millions of individuals affected by human immunodeficiency virus type 1 (HIV-1). The capacity of these drugs to indefinitely suppress HIV—which has a well-known capacity for escaping antiviral pressures—is surprising. In this issue of *Science Translational Medicine*, Shen *et al*. use a combination of mathematical modeling and experiment to examine the potency of anti-HIV drugs. They show that non-nucleoside reverse transcriptase inhibitors and protease inhibitors exhibit cooperative dose-response curves—a finding that has implications for the treatment of HIV as well as other viral infections, such as hepatitis C.

INTRODUCTION

Antiretroviral therapy has changed human immunodeficiency virus type 1 (HIV-1) infection from a death sentence to a treatable chronic illness. Since the mid-1990s when combination therapy first became available (*1*, *2*), the death rate from HIV has declined substantially (*3*). Although a cure has been elusive, long-lasting and perhaps lifelong control of HIV replication can be achieved in essentially all patients who have access to these drugs and who are able to adhere to the dosing requirements. The fact that therapy is so effective remains a puzzle. HIV replicates at a high titer and mutates constantly. Resistance to certain drugs given as monotherapy can emerge and dominate in the virus population in 14 days (*4*). The virus is known to replicate in several cellular and tissue sanctuaries; any one of these might serve as a source for low-level replication and eventual escape. Given the robust capacity for the virus to evolve in response to almost any antiviral pressure, many clinicians, researchers, and patients remain dubious about the prediction that antiretroviral drugs will work for everyone and for decades, yet the emerging clinical data suggest that this is indeed happening. In this issue of *Science Translational Medicine*, Shen and colleagues provide novel insights into the effectiveness of these drugs (*5*). This work might provide insights relevant to other antiviral strategies (including emerging work on the management of hepatitis C) and might even inform the future development of safer combination regimens for HIV as well as possibly provide insight into how to cure HIV infection.

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DOSE RESPONSE

It was recognized around the 16th century that a drug's effect depended on its dose. A prescription written in 1562 for a mixture of hyoscyamus and opium stated that giving one dram to a person caused sleep, but added "If you want him to sleep less, give him less" (*6*). The notion that a drug's dose and effect are related is a basic tenet of pharmacology and is generally summarized by an experimentally derived dose-response curve. Determining the dose that gives 50% of the maximum response is one way to quantify the potency of a drug; For example, for an enzyme inhibitor such as a protease or reverse transcriptase inhibitor, one can determine the IC_{50} (the concentration of a drug needed to produce 50% of the maximal inhibitory effect in vitro) or the ED_{50} (the plasma concentration of a drug required to obtain 50% of the maximal effect in vivo). Frequently, the dose-response curve is an S-shaped curve, such as those shown in Fig. 1.

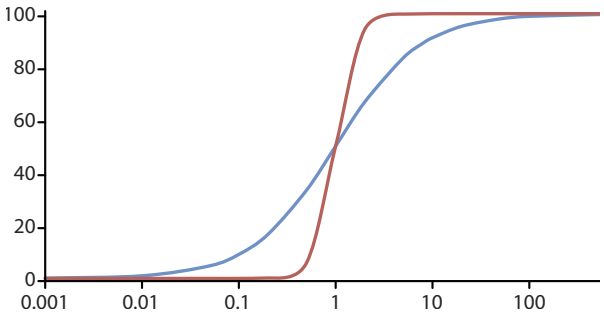

Fig. 1. Dose-response curves. A typical dose-response curve is shown in blue. The IC_{50} is the dose that leads to 50% of the maximal response; for both curves, the IC_{50} is 1. Each curve has a different slope *m*, which is also known as the Hill coefficient. For the blue curve, $m = 1$, whereas the red curve has a steeper slope of $m = 4$. In other words, the higher the Hill coefficient, the steeper the curve, and the more potent the drug at small increases in concentration above the IC_{50}. Curves with $m > 1$ usually arise from cooperative interactions.

Dose-response curves with the same IC_{50} can have varying steepness or slope (Fig. 1). Both the red and blue dose-response curves have an IC_{50} of 1; yet, for the red curve a dose of 5 generates 99.8% of the maximal response, whereas for the blue curve a dose of 5 yields 83% of the maximum response. A steep dose-response curve means that a drug concentration slightly above the IC_{50} can lead to extremely high levels of inhibition. Furthermore, if the maximum drug concentration that one can obtain in a patient is high (for example, 100 in Fig. 1) then the trough concentration can be 95% lower without any noticeable loss in drug effectiveness; this is true for the red dose-response curve, but not for the blue curve. Thus, the steepness of the dose-response curve has important clinical implications.

A function that describes many dose-response curves and has a long and venerable history in biochemistry was applied empirically by Hill in 1910 to describe the binding of oxygen to hemoglobin (*7*). This Hill equation—which has also been used to describe the effectiveness of a drug, such as interferon for the treatment of hepatitis C (*8*, *9*) or an antiretroviral for the treatment of HIV (*10*)—is written as

$$\varepsilon = \frac{D^m}{IC_{50}^m + D^m} \quad (1)$$

where *D* is the drug dose; *m* is the Hill coefficient; and ε is the response or the effectiveness of the drug, which varies between 0 (no effect of the drug) and 1 (the maximal effect). For the blue curve in Fig. 1, $m = 1$, whereas for the red curve $m = 4$. The higher the Hill coefficient, the steeper the dose-response curve. For oxygen binding to he-

moglobin, the Hill coefficient is $m = 4$ and represents the fact that four oxygen molecules can bind to each hemoglobin molecule and that the binding is cooperative; in other words, the binding of one oxygen molecule makes it easier for the subsequent molecules to bind.

In this issue of *Science Translational Medicine*, Shen *et al.* (*5*) discuss the conceptual basis for dose-response curves for anti-HIV drugs that have slopes or Hill coefficients greater than 1. Rather than using the Hill equation which yields an S-shaped dose-response curve (Fig. 1), they used a logarithmic transformation to generate an equivalent linear plot called a median effects plot (*11*). In an earlier publication, Shen and colleagues measured the Hill coefficient—or, as described by the authors, the "slope parameter"—for a large number of anti-HIV compounds (*10*). They showed that the slope parameter of the dose-response curve was characteristic of a drug class, with $m = 1$ applying to nucleoside reverse transcriptase inhibitors (NRTIs) and integrase inhibitors, whereas $m > 1$ for non-nucleoside reverse transcriptase inhibitors (NNRTIs), fusion inhibitors, and protease inhibitors (PIs), with an m of approximately 1.7 for NNRTIs and fusion inhibitors and m between 1.8 and 4.5 for PIs (*10*). Thus, antiretroviral drugs acting through different mechanisms show distinct slopes of their dose-response curves. In classical ligand–receptor binding theory, slopes of m greater than 1 imply that the binding is cooperative. It is surprising that the binding of a small molecule, such as a PI, to an enzyme that contains a single drug-binding site follows a mathematical law that describes cooperative binding. Typically, small molecules that bind a target molecule with a single drug-binding site generate a dose-response curve that is noncooperative and that has a slope parameter of $m = 1$ (Fig. 1).

THE CRITICAL SUBSET MODEL

The new insight described by Shen *et al.* (*5*), which explains the cooperative dose-response curves of anti-HIV drugs in the PI and NNRTI classes, is that multiple copies of the target molecule within virions or infected cells need to be inactivated by a drug in order to block HIV infection. As an example, consider HIV-1 protease, which is the enzyme that processes the Gag and Gag-Pol polyproteins to yield mature, infectious HIV virions. If protease activity is lacking, then immature, noninfectious viral particles are produced. Rosé *et al.* showed that if wild-type HIV protease was replaced by a mutant form so that the protease activity was 25% of the wild-type activity, then the viral particles produced were morphologically similar to wild-type virions and only a slight reduction in infectivity was observed (*12*). However, if a mutant with only 2% of the wild-type protease activity was used, then noninfectious particles were produced. Thus, there appears to be a threshold level of protease activity required for processing HIV polyproteins and generating infectious particles.

In light of the work by Rosé *et al.* (*12*) and related work by Ambrose *et al.* (*13*), Shen and colleagues put forth the idea that a critical number of HIV enzymes would need to be inhibited by a drug in order to reduce HIV infectivity. Using the law of mass-action, they formulated this idea mathematically and called it the "critical subset model." Applying the model to PIs, the authors considered a virion containing a total of n_T protease enzymes. The binding of a PI to any one enzyme renders it inactive. If a critical subset c, or more of the protease molecules remain active (that is, have no drug bound), then they assume the virus is infectious; conversely, if fewer than c protease molecules remained active, then the virus was assumed to be noninfectious. In the experiment by Rosé *et al.* (*12*), the critical subset would have been between 2 and 25% of the protease molecules. Because the number of protease enzymes in a virion might not be the same in all virions, Shen *et al.* considered the effect of allowing n_T to vary among virions. They also showed that the slope of a dose-response curve is determined by n_T and c. Further, for PIs and NNRTIs they demonstrated that the dose-response curve they had predicted using the critical subset model was able to well-approximate experimental data measuring the infectivity of HIV versus drug dose in single-round infectivity assays (*5*). The model was further tested experimentally by modulating the number of functional drug targets per virus. Dose-response curves generated by the model again fit the data well.

Both theory and experiment also showed that as the number of functional enzymes was decreased, it was easier to inhibit viral replication at the same drug dose. Thus, the IC_{50} of a drug was not only a property of the drug but also the number of drug targets available in the cell or virion. If there were fewer reverse transcriptase (RT) enzyme molecules in a cell, then fewer need to be bound by a drug to reduce the number of active (unbound) molecules below the critical threshold c. A related argument goes as follows: Assume at drug concentration D of a reverse transcriptase inhibitor (RTI) there is a 99% probability of an RT enzyme being inhibited. Thus, if there is a single RT enzyme in a cell the chance of it not being inhibited (remaining unbound) is 1%; however, if there are 10 RT enzymes in the cell and each has a 99% chance of being inhibited, then the chance that at least one fails to be inhibited is $1 - (0.99)^{10} = 9.6\%$. In other words, the more enzymes there are, the higher the chance that some remain functional. Importantly, for drugs whose targets are abundant biomolecules—such as proteases and RT enzymes in virions and cells, but not integrase (for which only the single molecule bound to the viral genome in cells is the relevant target)—a cooperative dose-response curve exists (Fig. 1, red line).

FAR-REACHING CLINICAL IMPLICATIONS

The observation that drug-mediated inhibition of an enzyme depends on the number of copies of that enzyme has clinical implications beyond those discussed in the article by Shen *et al.* For example, HIV can spread by cell-to-cell transmission, potentially making it harder to inhibit viral replication. During such transmission events, several viral particles can be transferred (*14*, *15*). The presumably higher efficiency of cell-to-cell transfer of HIV might explain recent observations suggesting that, if virus replication persists during therapy, it is happening in areas that may support this type of replication, including the densely packed T cell zones of inductive lymphoid structures (*16*). Inhibiting all replication will probably be a necessary (albeit not sufficient) first step in curing HIV infection, and efforts aimed at enhancing the potency of current regimens will likely be needed. The work by Shen and colleagues (*5*) can and should inform the design of novel curative strategies.

Another, perhaps more practical implication for this work is the design of novel regimens for HIV management. Industry and clinical investigators have long pursued a strategy that avoids the use of nucleoside analogs (which are thought to be toxic). To date, no such strategy has emerged that is both well-tolerated and effective (*17*), and most such strategies have failed owing to efficacy concerns. There are too many pos-

sible drug combinations to test them all in HIV clinical trials; as such, regimens that are selected in part on the basis of having a cooperative dose-response curve ($m > 1$) may play a role in optimizing regimens, both for treatment of naïve patients and for patients who have failed the standard well-characterized, first-line regimens.

The Hill coefficient (slope parameter) is only one component that explains the relative effectiveness of antiretroviral drugs and hence is not able to fully predict what happens in vivo. For example, the drug raltegravir—which has a Hill coefficient of approximately 1—is very effective in treating HIV when administered twice per day. Also, protease inhibitors have the highest Hill coefficient, yet there are some data suggesting that they are less effective than NNRTIs in completely suppressing HIV replication (*17*, *18*). These inconsistencies suggest that other factors, such as tolerability, favorable pharmacologic properties, and where the drugs act during the viral life cycle (*1*), might dominate in defining which regimens are the most effective options for patients.

The critical subset model (*5*) provides an appealing explanation of how steep dose-response curves arise. The steep slope of such dose-response curves implies that small increases of drug concentration can produce a large increase in antiviral activity and, further, that extremely high potencies can be obtained at physiologically relevant concentrations. More importantly, it implies that when drugs with a steep dose-response curve ($m > 1$) are delivered at concentrations greater than their IC_{50}, the drug potency will persist as long as the minimum concentration stays a few fold above the IC_{50}. The work by Shen *et al.* (*5*) suggests why some anti-HIV drugs, specifically the PIs and NNRTIs, have more in vivo potency than others and why some drugs, such as the PI class, might even work as monotherapy (*19*). More broadly, this study might have implications for other antiviral drugs and explain the potency of agents such as the PIs boceprevir and telaprevir for hepatitis C virus (*20, 21*) and explain why the NS5A molecule of HCV appears to be a good drug target (*22*). Drug developers should take heed of this model in choosing future drug targets.

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23. **Funding:** Portions of this work were done under the auspices of the U.S. Department of Energy under contract DE-AC52-06NA25396 and supported by NIH grants R37 AI028433, P20 RR018754, and R01 RR006555 (A.S.P.). S.G.D. was supported in part by the National Institute of Allergy and Infectious Diseases (RO1 AI087145, K24AI069994). **Competing Interests:** The authors declare no competing interests.

10.1126/scitranslmed.3002656

Citation: A. S. Perelson, S. G. Deeks, Drug effectiveness explained: The mathematics of antiviral agents for HIV. *Sci. Transl. Med.* **3**, 91ps30 (2011).