Rapid Viral Expansion and Short Drug Half-Life Explain the Incomplete Effectiveness of Current Herpes Simplex Virus 2-Directed Antiviral Agents

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The nucleoside analogues acyclovir (ACV) and famciclovir (FCV) reduce the frequency and severity of herpes simplex virus 2 (HSV-2) genital shedding, yet despite their high potency in vitro and a lack of induced drug resistance, frequent episodes of breakthrough mucosal shedding occur. We tested a published stochastic, spatial mathematical model of HSV-2 replication and spread, in concert with pharmacokinetic and pharmacodynamic equations, against virologic data from clinical trials of twice-daily acyclovir and famciclovir suppression. The model reproduced the key features of clinical trial data, including genital shedding episode rate, expansion and decay dynamics, and heterogeneous peak viral production and duration. In simulations, these agents shortened episode duration by limiting the extent of viral production by 1 to 2 log units and limiting the formation of secondary ulcers by ~50%. However, drug concentrations were noninhibitory during 42% of the dosing cycle. Even if drug concentrations were high at episode initiation, prolonged episodes were often ensued due to drug decay over ensuing hours and subsequent rebound of rapidly replicating HSV-2. The local CD8+ T-cell density was more predictive of episode viral production (R2 = 0.42) and duration (R2 = 0.21) than the drug concentration at episode onset (R2 = 0.14 and 0.05, respectively), though the model projected that an agent with an equivalent potency but a two times longer half-life would decrease shedding by 80% compared to that of standard twice-daily regimens. Therefore, long half-life is a key characteristic of any agent that might fully suppress HSV-2 reactivations.

The agents currently licensed for treatment of herpes simplex virus 2 (HSV-2) infection, acyclovir (ACV), famciclovir (FCV), and valacyclovir (VCV), decrease genital tract viral shedding and lesion rates (1–3), yet they do not eliminate shedding, even when given three times daily at maximal doses (4). The reason that shedding episodes lasting several days still occur in most treated patients is unknown. Drug resistance does not explain breakthrough shedding and is usually clinically relevant only in immunocompromised hosts (5). Other possibilities include inadequate penetration of drug into tissue or breakthrough replication due to subtherapeutic concentrations. Recent evidence highlights that certain antiretroviral agents bind cooperatively with viral target enzymes (6, 7). A lack of cooperative binding is another possible mechanism for diminished antiviral potency.

We recently published a stochastic spatial model of HSV-2 pathogenesis (8) that provides hypotheses for the shedding patterns observed in persons off therapy. In simulations of this model off treatment, viral spread occurs in several stages: neurons release HSV-2 in a slow drip throughout the genital tract. Mucosal replication is initiated when epithelial cells are infected (9). Rapid cell-to-cell spread of HSV ensues within a single ulcer, and the extent of infection inversely correlates with the local density of resident HSV-2-specific CD8+ T cells (10). While clearance of infected cells within an ulcer is achieved in <24 h, secondary seeding of adjacent microenvironments can prolong shedding. During severe episodes lasting more than a few days, HSV-2 concurrently expands and decays rapidly within numerous genital tract microenvironments (8). This prediction of the model is supported by clinical observations of serpiginous formation of many ulcers across genital skin, wide spatial dispersion of HSV DNA during reactivations (11), and highly localized acquired immune responses at ulcer sites (8, 12, 13). The model precisely recapitulates detailed features of shedding episode dynamics and variability in untreated persons (8).

Here we expand this model of HSV-2 reactivation dynamics by considering the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of ACV and FCV to develop hypotheses for the breakthrough shedding observed during twice-daily therapy. Our model reproduces detailed kinetic characteristics of shedding episodes in patients enrolled in randomized controlled trials of ACV and FCV therapy and predicts that these agents are potent when drug concentrations peak and immunological clearance of infected cells temporarily predominates over viral replication, leading to viral decay. However, drug concentrations exceed the 50% effective concentration (EC50) during less than 60% of the dosing interval, allowing rapid reexpansion of HSV during trough concentrations. Dosing of agents twice daily limits initiation of satellite lesions of HSV replication, thereby decreasing episode duration. This study highlights that PK/PD parameters can explain treatment shortcomings if analyzed in concert with the dynamic temperospatial properties of the virus.

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MATERIALS AND METHODS

Overview. The overall purpose of this study was to develop a comprehensive mathematical model of HSV-2 pathogenesis during antiviral therapy that captures the fundamental impact of viral replication and spread, the cytolytic immune response against infected cells, and the PK/PD principles of the drug. First, we tested models for their ability to reproduce the most relevant dynamic features of HSV-2 shedding from clinical trials. Next, we analyzed emergent properties of model simulations to develop hypotheses for the persistent shedding observed despite the use of high doses of the agents most commonly used against HSV-2, ACV and FCV.

Clinical trial data. We selected data for mathematical model fitting from crossover trials in which participants served as their own controls and obtained daily swabs of genital secretions on and off therapy for quantitative measures of HSV DNA (1, 14). All study subjects signed informed consent prior to enrollment and the human subjects protocols were approved by the University of Washington Institutional Review Board. Because ACV and FCV have similar effects on quantitative shedding frequency and pattern, we combined data from two trials to allow a larger sample size; 92 participants took ACV at 400 mg twice daily, and 64 took FCV at 250 mg twice daily (3). Participants were swabbed for ≥30 days while on and off therapy, with a 1- to 2-week washout period between treatment with drug and placebo. We analyzed 8,142 swab specimens from participants while they were off therapy and 7,059 swab specimens from participants while they were on therapy.

Spatial mathematical model. All model simulations and movies were implemented using the C++ programming language. Statistical analyses were performed with Stata (version 10) and Microsoft Excel software. The baseline model employed in our analysis is a stochastic model of HSV-2 pathogenesis which was validated against a large set of data on shedding dynamics in patients off therapy (8). The natural history of HSV-2 infection in untreated patients consists of unpredictable but frequent (approximately weekly) shedding episodes of highly variable duration (hours to weeks) and peak viral load (100 to 108 HSV DNA copies). We classified the episode rate, as well as the heterogeneity of the episode peak, expansion and decay slopes, duration, and first and last genomic copy number of HSV (Fig. 1A), from 1,020 episodes in 531 study participants (22). The baseline model has successfully reproduced each of these general features of shedding in merged data from all study participants when simulated over prolonged time periods of approximately 10 years (8).

To capture that genital lesions consist of multiple herpetic ulcers, the model’s equations follow viral and CD8 T lymphocyte activity in 300 spatially distinct microregions. In simulations, genital tract shedding is periodically initiated by a slow drip of virus from neuronal endings. Episodes...
initiate in a microregion following infection of a single keratinocyte. Further infected cells are produced by cell-to-cell spread of cell-associated virus and eliminated by expansion of regional CD8^+ T cells, which slowly decay only after no infected cells remain. Adjacent model regions may be seeded with new infection when the local cell-free HSV-2 load exceeds a threshold. The most complex feature of nearly all episodes of more than 3 days’ duration is frequent viral rebound (16): the model explains this phenomenon to be a result of concurrent viral expansion and decay in multiple spatially discrete genital tract regions. A schematic of the model is illustrated in Fig. 1B, while equations and parameters are described in more detail in the supplemental material.

Model fitting to trial participant data while off therapy. We first tested the off-therapy model against 262 HSV-2 episodes that occurred when participants were taking placebo. Shedding episodes have highly variable durations and levels of viral production and extremely rapid early expansion and late decay phases (8). The goal of the model simulations was to reproduce the diversity of episodes captured with the histograms (Fig. 2).

Episode duration was unknown in many episodes that were ongoing at the time of swabbing initiation or did not terminate prior to completion of swabbing. Omitting such episodes would bias the data toward brief episodes, while inclusion allows episodes of unknown duration. Therefore, we performed separate analyses inclusive of episodes with known and unknown duration. The latter episodes were assumed to be 1 day longer than the observed duration.

For model fitting, each of the episode characteristics was weighted to achieve an equal level of importance. Parameter value search and fitting techniques, including those that use residual sum of squares (RSS) and the Akaike information criterion (AIC), are described in the supplemental material.

PK model. We next added antiviral therapy to the model. A single-compartment PK equation was added to the spatial model to capture the exponential decay of antiviral drugs (D) with a serum half-life (t_{1/2}). We assumed linear expansion to the maximal drug concentration after the first dose (C_{max}) over the time to C_{max} after ingestion (T_{max}) rather than first-order drug absorption. This simplification has no impact on simulated shedding, as >90% of the dosing cycle is spent in drug decay rather than expansion. Decay occurred due to dD/dt = -x D, where x represents the drug decay rate. The serum t_{1/2} was equal to ln(0.5)/x.
PD models in genital mucosa and neuronal tissue. The sites of possible drug activity are illustrated in Fig. 1B with red arrows. A PD equation described an instantaneous effect of drug on viral replication. ACV and FCV are DNA polymerase inhibitors whose metabolites chain terminate viral DNA strands at the replication site (17). The 50% effective concentration \( \text{EC}_{50} \) (in \( \mu \text{g/ml} \)) is defined as the drug concentration at which the replication rate per keratinocyte or neuron is decreased by 50%. PK and PD models are linked by \( D/E\text{EC}_{50} \) where \( \text{EC}_{50} \) is invariant and \( D \) fluctuates according to the PKs. We assumed that the rate of HSV DNA production \( (p_p) \) within infected skin cells was \( p_p/(1 + (D/E\text{EC}_{50})) \), where \( p_p \) is the number of HSV DNA copies per cell per day without drug. As it is uncertain whether ACV inhibits HSV replication in neural tissue, we tested competing models with and without drug activity in neurons for fit to the data. If an effect was assumed, then the rate of HSV release from neurons \( (n_{eu}) \) was \( n_{eu}/[1 + (D/E\text{EC}_{50})] \), where \( n_{eu} \) is the number of HSV DNA copies per cell per day released from neuronal endings without drug.

PD models: possible cooperative binding of drug to its target. We also tested a competing model, \( p_p/[1 + (D/E\text{EC}_{50})^m] \), which includes the Hill coefficient \( (m) \). The Hill coefficient is included to capture the fact that antiviral therapies have dramatically different potencies according to the mode of action: HIV-1 strains targeting protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (nNRTIs) bind targets cooperatively, such that inhibition of enzyme activity is enhanced as drug concentrations increase. These agents have values of \( m \) ranging from 2 to 5 and are therefore far more potent than nucleoside reverse transcriptase inhibitors (NRTIs), for which \( m \) is equal to 1 (6, 7).

While the Hill coefficient has never been formally measured for ACV or FCV, a similar phenomenon could theoretically occur with ACV and FCV: ACV triphosphate (ACV-TP) and penciclovir triphosphate (PCV-TP) competitively bind the 3’ end on both elongating viral DNA strands, outcompeting the abundant supply of guanine nucleotides which are used by viral DNA polymerase for DNA synthesis. As a result, the chain of replicating DNA is in close proximity to the DNA replication fork, as well as to each other. It is therefore theoretically possible that ACV-TP binding to the leading chain and lagging chain could occur synergistically. We include models in which \( p_p \) is equal to \( p_p/[1 + (D/E\text{EC}_{50})^m] \), \( n_{eu} \) is equal to \( n_{eu}/[1 + (D/E\text{EC}_{50})^m] \), and \( m \) is an unknown fitting parameter. If these models demonstrate an improved fit to the data (with an estimated value for \( m \) of >1) compared with that of the model without binding synergy \( [1 + (D/E\text{EC}_{50})] \), then this would imply the possibility of cooperative binding of a drug to its multivalent target in vivo.

PK/PD model parameterization. On the basis of similar PK/PD characteristics, ACV and FCV were modeled under a single-parameter regime. ACV at 400 mg and FCV at 250 mg have similar \( \text{EC}_{50} \) (1.2 and 1.6 \( \mu \text{g/ml} \), respectively), \( T_{\text{max}} \) (2 and 1 h, respectively), and \( t_{1/2} \) (3 and 2.3 h, respectively) values (15, 19). The intracellular half-life of active phosphorylated FCV (penciclovir-TP) exceeds that of phosphorylated ACV (acyclovir-TP) in HSV-2-infected cells (20 h versus <1 h). However, there is no apparent benefit of a prolonged intracellular FCV half-life, perhaps because most infected cells produce virus for 1 to 2 h in vivo due to rapid CEB lymphocyte-mediated lysis (9). As such, the plasma FCV half-life is likely to exceed the half-life of FCV in infected cells. In simulations, \( T_{\text{max}} \) and \( t_{1/2} \) were fixed at 2 and 3 h, respectively, with a \( C_{\text{max}} \) of 1.4 \( \mu \text{g/ml} \) assumed for both agents.

Model fitting to trial data while on therapy. We tested the models inclusive of PKs/PDs against 115 episodes that occurred when participants were taking therapy (Fig. 2). We carried forward viral and immune parameter values for the spatial model from the placebo simulations. Simulations lasted 30 to 60 days with daily sampling. \( \text{EC}_{50} \) was an unknown fitting parameter in all simulations. While \( \text{EC}_{50} \) values are roughly similar for ACV and FCV, estimates based on the results of plaque reduction assays are reliable only for resistant isolates with \( \text{EC}_{50} \) of >2 \( \mu \text{g/ml} \) (8.9 \( \mu \text{M ACV and 6.2} \mu \text{M FCV}) estimates vary by 1 to 2 log units between cell lines for sensitive isolates (20). Moreover, the conditions used for these cell culture-based assays may or may not represent in vivo infection. The Hill coefficient was included as a second unknown fitting parameter in the model inclusive of cooperative binding.

Sensitivity analyses. Upon identifying the best-fitting models and parameter values, we performed several sets of sensitivity analyses. First, we individually adjusted individual PK/PD parameter values within predefined ranges to demonstrate the relative potencies of existing and theoretical antiviral agents at different doses. Second, we performed global sensitivity analyses of PK/PD parameters to explore which PK/PD values are most likely to impact clinical outcomes. All model parameters \( (C_{\text{max}}, T_{\text{max}}, \text{and } \text{EC}_{50}) \) were randomly drawn from probability distribution functions (pdf’s), which were derived according to a literature review (15, 19, 20). The Hill coefficient was randomly selected for values between 1 and 2. Third, we performed global sensitivity analyses of PK/PD parameters, as well as viral and immunological parameters that we previously demonstrated to have the greatest impact on shedding rate (21), to establish whether the outcome of antiviral therapy is most likely to be impacted by underlying characteristics of the host immune system, host drug metabolism, or viral strain \( (\text{EC}_{50}) \).

For most of the sensitivity analyses, the outcome of interest was the shedding rate. We first generated a unique parameter set and then simulated 200 episodes. Sixty-day simulations were performed with each parameter set to establish a shedding rate. We also used global sensitivity analyses to assess for model fit using least squares and Akaike information criteria as outcomes (see the supplemental material). For these simulations, 60-day model realizations were continued, with episode results tabulated cumulatively until 200 episodes were generated for each set.

RESULTS

Model fit to HSV-2 shedding off therapy. The off-therapy model accurately reproduced characteristics of 262 shedding episodes in 156 untreated persons (Fig. 2A to G) with a low residual sum of squares (RSS) of 1.16 and an Akaike information criterion (AIC) of -138. Optimized viral and immune parameter values approximated those from fitting to a larger published data set (see Table S1 in the supplemental material) with several fitting techniques (8, 21).

Close model fit to HSV-2 shedding on therapy assuming drug activity in neurons. The actual episode rate observed on twice-daily antiviral therapy was 8.0/year. The model assuming drug activity in neurons accurately reproduced this rate (8.9/year), while the model assuming no activity in neurons substantially overestimated the episode rate (15.9/year). The model assuming no activity in neurons also had considerably poorer overall fitting scores (higher values) compared with that of the model inclusive of neuronal antiviral activity: RSS, 3.0 versus 1.5, and AIC, -107 versus -133. Therefore, the relative likelihood of the model without antiviral activity in neurons was \( 2.3 \times 10^{-6} \).

The addition of the PK/PD activity of ACV or FCV in neurons as well as skin resulted in a close fit to 115 episodes on therapy, including quantitative shedding frequency, episode duration, peak, first, and last HSV DNA copy number, episode rate, and episode expansion and decay slopes (Fig. 2A to G). In both the empirical and modeled data, antiviral therapy decreased the shedding rate (Fig. 2A) and duration (Fig. 2B), peak HSV DNA copy number (Fig. 2C), first HSV DNA copy number (Fig. 2D), and rate (Fig. 2E) of episodes, while it increased the peak-to-termination clearance slopes (Fig. 2G). In both empirical and modeled data, antiviral therapy had no effect on the last positive copy number (Fig. 2E) or the initiation-to-peak expansion slope (Fig. 2G) of episodes, reflecting equivalent immune clearance of free virions both in the presence and in the absence of therapy.
Close model fit irrespective of inclusion of the Hill coefficient. A model which allowed variability in the Hill coefficient allowed a marginally better fit by RSS (1.5) and AIC (−133) than the model without inclusion of the Hill coefficient (RSS = 1.9 and AIC = −128). The relative likelihood of the model without the Hill coefficient was 0.08. However, our multiparameter global sensitivity analysis demonstrated that the value for \( m \) had no demonstrable effect on the shedding rate (\( R^2 = 0.0003 \)) or the RSS value (\( R^2 = 0.005 \)), which is in direct contrast to the findings for other PK/PD parameters, such as \( C_{\text{max}} \) and \( EC_{50} \) (described below). Therefore, we conclude that \( m \) could not be precisely estimated from our data and does not appear to be of high relevance in determining shedding outcomes or model fit.

For the model with inclusion of the Hill coefficient as a fitting parameter, the estimated Hill coefficient was 2 for the best-fit model and the parameter value for \( EC_{50} \) using our parameter search algorithm was 0.33 \( \mu g/ml \) (1.48 \( \mu M \) ACV and 1.04 \( \mu M \) FCV). Therefore, at peak concentrations, viral replication in genital skin was reduced by (1.4/0.33)\(^2\), or 18.3, such that viral replication in genital skin was reduced by (1.4/0.21)\(^2\), or 6.7, to 803 HSV DNA copies per cell per hour, when \( C_{\text{max}} \) was equal to \( C_{\text{max}} \) (Figure 2 demonstrates results from this model.) For the model with no inclusion of the Hill coefficient as a fitting parameter, the estimated \( EC_{50} \) was 0.21 \( \mu g/ml \) (0.94 \( \mu M \) ACV and 0.66 \( \mu M \) FCV). For this model, viral replication in genital skin was reduced by 1.4/0.21, or 6.7, to 803 HSV DNA copies per cell per hour, when \( D \) was equal to \( C_{\text{max}} \). Both estimates for the \( EC_{50} \) fall within established ranges for sensitive isolates.

Modeling with continuous versus daily sampling. With the optimized parameter set and model, we analyzed the model output data using continuous sampling, as most shedding episodes last <1 day and are missed with daily sampling. As was found with daily sampling data, simulations with continuous sampling showed that twice-daily ACV/FCV reduced quantitative shedding (see Fig. S1A in the supplemental material). Moreover, these agents decreased the episode rate by approximately 50% (Fig. S1B in the supplemental material) and episode initiation by 57% (see Fig. S1C in the supplemental material). Episodes on therapy were shorter (see Fig. S1D in the supplemental material) and peaked at a lower copy number (see Fig. S1E and F in the supplemental material).

Low correlation between antiviral drug concentration and episode peak viral production and duration. Having established the model fit, we next explored emergent properties of the model to explain persistent HSV-2 shedding on treatment. Given that episodes on therapy had less viral production and a shorter duration, we explored how drug concentrations impacted episode dynamics. We simulated 413 episodes on twice-daily therapy and identified weak predictive relationships between time since drug dosing (a surrogate for the drug concentration at initiation) and episode peak HSV production (Fig. 3A; \( R^2 = 0.14 \)) and duration (Fig. 3B; \( R^2 = 0.05 \)). Mean measures of peak viral load and duration increased gradually according to time since dosing, as measured over 2-h intervals, yet many high-copy-number, prolonged episodes occurred even if drug concentrations were high (Fig. 3A and B).

We next simulated daily dosing and generated 540 episodes. There was no predictive effect of time since drug dosing at episode initiation on peak HSV production (Fig. 3C; \( R^2 = 0.01 \)) or duration (Fig. 3D; \( R^2 = 0.01 \)), mainly because the patterns of episodes that occurred at between 7 and 24 h postdosing (when \( D \) was less than the \( EC_{50} \)) were similar. Mean peak viral load (Fig. 3C) and duration (Fig. 3D) did increase as a function of time since dosing between hours 2 and 8 since episode initiation. Moreover, episodes initiated during the 17-h window with subtherapeutic drug concentrations during daily dosing were of longer mean duration.

**FIG 3** Time since dosing has a minor effect on episode duration and peak viral load. The intensity of red shading indicates the drug level, and white spaces indicate drug concentrations less that the EC \(_{50} \). \( C_{\text{max}} \) occurs 2 h after dosing. Light blue bars are mean values within 2-h intervals. (A and B) Data from 413 simulated episodes on twice-daily drug treatment (63 to 75 episodes per interval). (A) Peak viral production in the initial episode ulcer; (B) episode duration as a function of time since dosing. (C and D) Data from 540 simulated episodes on once-daily drug treatment (39 to 51 episodes per interval). (C) Peak viral load in the initial episode ulcer; (D) episode duration versus time since dosing.
viral load frequently surpassed 10^4 HSV DNA copies per cell; (C and D) CD8^+ T-cell density at episode onset versus peak viral production in initial episode region. (A and B) CD8^+ T-cell density at episode onset versus peak viral production in initial episode region; (C and D) CD8^+ T-cell density at episode onset versus duration per episode.

FIG 4 The local CD8^+ T-cell density at episode onset has a profound effect on local viral production and episode duration in model simulations. Data are from 413 episodes on twice-daily drug treatment, 540 episodes on once-daily drug treatment, and 777 episodes off drug. (A and B) CD8^+ T-cell density at episode onset versus peak viral production in initial episode region; (C and D) CD8^+ T-cell density at episode onset versus duration per episode.

(2.9 versus 2.4 days, P = 0.05) and had higher peak viral loads (4.5 versus 4.2 log_{10} HSV DNA, P = 0.04) than episodes initiated during the 5-h window of subtherapeutic drug concentrations with twice-daily dosing. This result highlights the partial rescue effect of every 12-h rather than daily dosing.

High correlation between CD8^+ T-cell density and episode peak viral load and duration both off and on therapy. We next investigated whether the simulated episode severity was sensitive to the local CD8^+ T-cell density at the time of initiation, as in prior studies (8, 10). We simulated 777 episodes off therapy, 413 episodes on twice-daily therapy, and 540 episodes on daily therapy. CD8^+ T-cell density predicted peak viral production in the first infected model region, though this effect was stronger off therapy (R^2 = 0.42) than on daily therapy (R^2 = 0.32) or twice-daily ACV or FCV therapy (R^2 = 0.21). For episodes with >10^4 HSV DNA copies at the peak, the predictive effect of CD8^+ T-cell density was extremely high; twice-daily antiviral therapy had the added effect of lowering episode peaks by 1 to 2 log units (Fig. 4A) at a given CD8^+ density, while this effect occurred only partially and only during certain episodes with daily dosing (Fig. 4B). The local CD8^+ lymphocyte density also correlated inversely with episode duration off therapy and on daily or twice-daily therapy (R^2 = 0.21, 0.21, and 0.19, respectively) (Fig. 4C and D).

The basic reproductive number (R_0) denotes the number of cells infected by virions produced from a single cell at episode onset. In the absence of therapy, increasing CD8^+ T-cell density correlated with a decrease in the regional value of R_0 (see Fig. S2A in the supplemental material; R^2 = 0.92); in turn, an R_0 value of <1 (log R_0 < 0) predicted rapid local control of replication before the HSV DNA copy number exceeded 10^4 when R_0 was >1, the viral load frequently surpassed 10^4 HSV DNA copies and R_0 predicted the peak viral load (see Fig. S2B in the supplemental material; R^2 = 0.93) (9, 10). On therapy, R_0 was less dependent on CD8^+ T-cell density at episode onset (R^2 = 0.56) and varied according to drug concentration (see Fig. S2A in the supplemental material); even when R_0 was >1 at episode onset, peak viral production could vary by ~2 log units for a given R_0 (see Fig. S2B in the supplemental material). Because rapid drug concentration fluctuations correlated with rapid shifts in R_0 over several hours, R_0 was less predictive of viral production for episodes with >10^4 HSV DNA copies (R^2 = 0.48).

Effects of fluctuating drug concentrations on HSV-2 shedding. Despite potent inhibition of viral replication when the drug concentration reached C_{max} in simulations, drug decayed rapidly and concentrations were below the EC_{50} for 5 h (42%) of the 12-h dosing interval, allowing full levels of replication at 5,380 HSV DNA copies per cell per hour. Therefore, to explain the paradox that twice-daily therapy predictably limited viral production while initial drug concentrations had only a subtle effect on episode peak viral production and duration, we plotted drug concentrations along with viral load for two simulated episodes. Shortly before drug concentrations peaked during viral expansion, there was usually conversion to viral decay (Fig. 5A and B) due to unsupported immune activity against infected cells, with a decrease in viral slope (Fig. 5C). Occasionally, early during episodes (Fig. 5A and B) or during rapid reexpansion (Fig. 5B, day 5), high concentrations of antiviral therapy decreased the expansion rate, but it was not enough to induce a transition to viral decay. Nevertheless, the drug exerted its most potent effect when concentrations peaked during a time point with a high expansion slope (Fig. 5D). In fact, when drug concentrations peaked during decay, there was no impact on the HSV clearance rate (Fig. 5C and D).

Antiviral concentrations decayed rapidly and virus reexpanded during 5-h troughs, explaining the narrow window of protection during a dosing interval. However, twice-daily dosing ensured that drug concentrations peaked multiple times during prolonged episodes: the net effect was an ~1- to 2-log-unit blunting of peaks (Fig. 4A). This blunting effect occurred inconsistently with daily dosing (Fig. 4B). This suggests that maintaining high drug con-
centrations over an entire episode would have an enduring effect on viral replication and spread.

The effect of various drug concentrations can be conceptualized as a twice-daily resetting of the reproductive number by a factor of \( \frac{1}{H_{100}} \) in all 300 model regions; in many regions, this resulted in an \( R_0 \) value of 1 for some of the dosing cycle. If episodes initiated within a region with a low CD8 density and, therefore, an \( R_0 \) value of >1, then peak drug concentrations only briefly drove \( R_0 \) below the viral containment threshold (\( R_0 < 1 \)); the rapid cycling of drug concentrations explains why the initial drug concentration had only a limited effect on peak HSV DNA copy number with twice-daily dosing. Spatial visualization of shedding during therapy revealed that many regions remained vulnerable to high-copy-number shedding with \( R_0 \) values of >1 throughout the dosing cycle (see Movie S1 in the supplemental material).

**Mechanism by which antiviral therapy limits episode duration.** Antiviral agents decreased the frequency of viral loads of >10^6 HSV DNA copies, thereby decreasing the probability of regional viral seeding and shortening episode duration (8). We plotted peak episode copy number versus duration for 262 and 155 empirically derived shedding episodes off and on twice-daily therapy, respectively (Fig. 6B). For most episodes with <10^6 HSV DNA copies produced in the initial ulcer-forming region, we noted a linear relationship between peak copy number and duration. These episodes generally were limited to one spatial region in simulations. This linear relationship disappeared above 10^6 HSV DNA copies due to the presence of longer episodes arising from more secondary ulcer formation (Fig. 6C).

**Predicting optimal characteristics of antiviral agents.** In clinical studies, breakthrough viral shedding episodes occur with maximal doses of ACV or VCV (4). (VCV at 1,000 mg achieves a \( C_{max} \) 7.5 times greater than that of ACV at 800 mg.) Using our model, we performed a single-parameter sensitivity analysis to test the effects of various dosing scenarios on the shedding rate. Higher and more frequent dosing led to a decrease in shedding rates. However, even ACV at 800 mg five times daily and VCV at 2,000 mg three times daily allowed rare breakthrough shedding at high levels (>10^6 HSV DNA copies) (Table 1); these optimized ACV and VCV regimens were predicted by our model to result in 70- and 2,400-fold reductions in the replication rate at the drug peak, respectively, and 8- and 64-fold reductions at the drug trough, respectively, yet the potential for very rare high-copy-
number shedding episodes existed even with these potent regimens, due to episode initiation in regions with low CD8$^+$ lymphocyte density when drug concentrations were in decline.

We expanded the sensitivity analysis to assess which antiviral characteristics would most limit shedding. A version of ACV with double the half-life would decrease the shedding rate by 80% compared to that achieved with the standard ACV regimen (Table 1).

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Sheding rate (%) of $&gt;10^7$ HSV DNA copies/mL</th>
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<td></td>
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<tr>
<td>No therapy</td>
<td>23.7/8.2</td>
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<tr>
<td>ACV at 400 mg every 12 h/FCV at 500 mg every 12 h ($m = 2$, half-life = 3 h)</td>
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<tr>
<td>ACV at 400 mg daily</td>
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<tr>
<td>ACV at 400 mg every 8 h</td>
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<td>ACV at 800 mg every 4.8 h</td>
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</tbody>
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We next assumed that $m$ was equal to 5 to account for the potent cooperative binding that virtually eliminates replication when high drug concentrations are present. If the drug half-life remained short, cooperative binding had a dose-dependent impact on the frequency of shedding of $>10^6$ HSV DNA copies/ml but a limited effect on the overall shedding frequency. A drug with a high Hill coefficient and long half-life would allow very rare breakthrough shedding at levels comparable to those achieved with high-dose ACV and VCV (Table 1).

**FIG 6** Antiviral therapy decreases average episode duration by limiting regional peak viral production and decreasing the probability of secondary ulcer formation. (A) Empirical data from 115 episodes on drug and 262 episodes off drug; (B and C) simulated data from 413 episodes on drug and 777 episodes off drug; (A and B) episode duration as a function of peak HSV DNA copy number of the initiating ulcer during an episode: the linear relationship between these two outcomes disappears at peaks of $>10^6$ HSV DNA copies due to formation of secondary ulcers, which prolong episodes and lead to nonmonotonic viral clearance; high-copy-number shedding and prolonged episodes are more common without drug. (C) Percentage of simulated episodes with secondary ulcers: 19% of episodes on drug versus 44% of episodes off drug have secondary ulcers.

DISCUSSION

Our study identifies short half-life as the likely reason that ACV and FCV only partially suppress shedding and long half-life as an
important goal of novel antiviral drug development. ACV and FCV limit HSV replication when the drug concentration peaks, but even with twice-daily dosing, concentrations are inadequate nearly 50% of the time. A drug with comparable potency but a prolonged half-life could prevent the majority of high-level shedding and possibly transmission. This might explain why continuous ACV infusion is effective for severe HSV infections, even when the virus is resistant (23, 24). This concept is relevant for other infections, such as influenza, which are characterized by quick replication and spread and intense, immunological containment. Oseltamivir, the most commonly used influenza medication, is only partially effective in limiting flu symptoms, perhaps also due to its short half-life (6 to 10 h) (25).

Our model also displays the complex impact that ACV and FCV therapy have on HSV pathogenesis. Peak drug concentrations provide periods where expansion of virus ceases or severely decelerates in mucosa due to ongoing immunological clearance of infected cells, while the replication rate is limited >15-fold, yet drug concentrations rapidly decay, and several hours later, virus reaccumulates and new cells are infected. Overall, viral peaks are blunted 10- to 100-fold with twice-daily dosing. Therefore, HSV DNA copy number is maintained below 10^6 for a greater proportion of time. As this is a rough threshold for viral seeding of adjacent regions, leading to secondary ulcers, episodes spread to multiple regions less frequently while the patient is on treatment. Indeed, suppressive therapy abrogates new ulcer formation and shortens recurrences (4).

These model predictions illuminate the fact that the optimization of antiviral drug regimens cannot rely on PKs and PDs alone and must consider pathogenesis and the dynamics of the virus and host immunological response. In the case of HSV, antiviral agents exert pressure in concert with a potent and dynamic mucosal immunological response. While antiviral agents lead to peak episode levels that are generally 1 to 2 log units lower than those during comparable episodes off therapy, we predict that the CD8^+ T-cell expansion rate and concentration remains above the EC_{50}.

The most important source of variability may be the short sampling period within the trials: any single parameter value in our model is only slightly predictive of model outcomes, in part because of the stochastic nature of episode initiation in trials and in model simulations. The EC_{50} of the virus is a predictive parameter, highlighting the fact that a binary assignment of each virus as either sensitive or resistant is likely to be inadequate. Peak drug concentration also has a predictive effect, highlighting the importance of host drug metabolism on therapeutic outcome. The Hill coefficient value has no impact on the shedding rate. The relative importance of C_{max} versus that of m likely highlights the former parameter’s impact on increasing the proportion of time that the concentration remains above the EC_{50}.

Our model has several limitations. First, it does not include a detailed characterization of intracellular decay characteristics or the activity of ACV-TP and penciclovir-TP (PCV-TP; the active component of FCV). We believe that this omission is acceptable because our less complex model adequately reproduces a rigorously defined data set with 42 outcome measures (histogram bins). Second, ACV and FCV were modeled together; potentially important drug differences are not captured in this study. Third, it is difficult to validate the prediction that antiviral medications block viral replication in neurons, as animal models do not adequately describe viral latency (32), though a potent antiviral effect against replicating HSV in neuronal culture is easily demonstrated.

A concern for multiparameter models is the potential for multiple model solutions. This is highly unlikely for our PK/PD estimates, as we solve these models for only 1 to 2 unknown parameters and fit to 42 data bins. The model fit to data off therapy does have 6 unknown parameters. While a semimanual approach was used to arrive at parameter values in this paper, in the past we have used algorithms that examine the entire multidimensional parameter space at once (21) and arrived at parameter values very similar to those arrived at in the current study. We therefore feel that viral and immune parameter values are unlikely to represent local minima.

**TABLE 2 Model parameter effect on shedding rate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variability in PK/PD parameters only</th>
<th>Variability in PK/PD, viral, and immune parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max}</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>T_{max}</td>
<td>0.0007</td>
<td>0.002</td>
</tr>
<tr>
<td>EC_{50}</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>m</td>
<td>0.0003</td>
<td>0.0008</td>
</tr>
<tr>
<td>Drug t_{1/2}</td>
<td>0.013</td>
<td>0.04</td>
</tr>
<tr>
<td>C_{max}/EC_{50}</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>(C_{max}/EC_{50})^m</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Viral release rate from neurons</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Viral infectivity</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Free viral clearance rate</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>CD8^+ T-cell expansion rate</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

^2 R indicates the predictive effect of each parameter. Values greater than 0.05 are in bold. Values in italics indicate a negative correlation.
Finally, the predictions of drug efficacy in Table 1 assume a cohort with shedding rates off therapy of 23%, as in the trials used for this study. In fact, shedding rates vary substantially between individuals off treatment, and differences between various drug regimens differ accordingly (4). The model is intended not as a prediction tool for individual infected persons but rather as a means to explain the general phenomenon of breakthrough shedding in the majority of treated persons, even on maximal doses of treatment.

In summary, sustained high levels of antiviral pressure are needed to prevent extensive periods of replication and tissue damage caused by HSV. Once an expansion phase is active beyond several hours, local immune pressure is responsible for viral clearance, though antiviral medicines are useful to lower the extent of viral spread. HSV-directed agents which maintain concentrations greater than the EC_{50} throughout the dosing cycle might eliminate viral shedding more effectively.

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REFERENCES


