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Pharmacokinetic Determinants of Virological Response to Raltegravir in the *In Vitro* Pharmacodynamic Hollow-Fiber Infection Model System

Ashley N. Brown, Jonathan R. Adams, Dodge L. Baluya, George L. Drusano

Institute for Therapeutic Innovation, Department of Medicine, University of Florida, Orlando, Florida, USA

Daily administration (q24h) of raltegravir has been shown to be as efficacious as twice-daily administration (q12h) in the hollow-fiber infection model (HFIM) system. However, q24h regimens were not noninferior to q12h dosing in a clinical trial. We hypothesized that between-patient variability in raltegravir pharmacokinetics (PK) was responsible for the discordance in conclusions between the *in vitro* and *in vivo* studies. Hollow-fiber cartridges were inoculated with HIV-infected H9 cells and uninfected CEM-SS cells. Four cartridges received the total daily exposure (800 mg) q24h and four received half the daily exposure (400 mg) q12h. PK profiles with half-lives of 8, 4, 3, and 2 h were simulated for each dosing interval. Cell-to-cell viral spread was assessed by flow cytometry. Viral inhibition was similar between q24h and q12h dosing at the 8- and 4-h half-lives. The q24h dosing was not as efficacious as the q12h dosing when faster half-lives were simulated; a lack of viral suppression was observed at days 3 and 4 for the 2- and 3-h half-lives, respectively. The discrepancy in conclusions between the *in vitro* HFIM system studies and clinical trials is likely due to the large interindividual variation in raltegravir PK.

Raltegravir is an HIV-1 integrase inhibitor that is used in combination with other antiretroviral agents to treat patients with HIV-1 infections. Raltegravir-based combination chemotherapeutic regimens have been shown to be well tolerated and highly efficacious in both treatment-naive (1–5) and treatment-experienced (6–8) patient populations compared to other combination regimens. Raltegravir is currently administered as a 400-mg twice-daily (BID) dose. A once-daily (QD) raltegravir regimen would be preferable in a clinical setting, since the convenience of a QD regimen would likely improve patient adherence.

Previous work done by others has shown that, once bound, raltegravir remains in the active site of the HIV-1 integrase protein for a long time, demonstrating dissociative half-lives ranging from 7.3 to 8.8 h (9, 10). The long occupancy time of raltegravir in the binding pocket of viral integrase suggests that once-daily dosing (800 mg QD) may be as efficacious as the current regimen of 400 mg BID. Moreover, data generated using an *in vitro* pharmacodynamic (PD) hollow-fiber infection model (HFIM) system supported this speculation (11). Given the strong *in vitro* support of once-daily dosing, Merck conducted a phase 3 noninferiority trial to compare the efficacy of raltegravir administered as 800 mg QD to the standard dosage regimen of 400 mg BID. The findings of this clinical study showed that both regimens yielded high response rates *in vivo*, but once-daily dosing did not achieve the −10% noninferiority margin and was deemed not noninferior to the twice-daily regimen (12). Our objectives for this study were to explain the discordance in conclusions between the *in vitro* experiments and human clinical studies for raltegravir and to identify the pharmacokinetic-pharmacodynamic (PK-PD) determinants for raltegravir that allow for once-daily dosing.

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MATERIALS AND METHODS

Cells and viruses. Human T lymphoblast CEM-SS and H9 cell lines were acquired from the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Cell lines were maintained in cell growth medium (RPMI 1640 medium; HyClone, Logan, UT) supplemented with 10% fetal bovine serum (HyClone), 2.0 mM L-glutamine, and 1% penicillin-streptomycin solution (HyClone).

Proviral DNA for the HIV-1 IIIB strain was provided by Merck, Inc. (West Point, PA). Viral stocks were prepared as previously described (13). Briefly, human kidney 293T cells (ATCC, Manassas, VA) were transfected with the IIIB proviral DNA and incubated for 3 days at 37°C in 5% CO_2. Cell supernatants were collected, centrifuged at 4,000 × *g* for 10 min to remove cell debris, aliquoted, and frozen at −80°C.

Compound. Raltegravir was provided by Merck, Inc. Drug stocks of 10 mg/ml were prepared in sterile double-distilled water, sterilized through a 0.2-μm filter, aliquoted, and stored at −80°C.

Flow cytometry assay of HIV-infected cells. Cells were fixed and permeabilized using the IntraPrep permeabilization reagent kit (Beckman Coulter, Miami, FL). A fluorescein isothiocyanate (FITC)-labeled monoclonal antibody to the HIV-1 p24 core antigen (KC-57 monoclonal antibody; Beckman Coulter) was added to the permeabilized cells and allowed to incubate at room temperature for 15 min in the dark. Unbound antibody was removed by centrifugation at 400 × *g*, and the percentage of FITC-positive cells was ascertained using the Cytomics FC 500 flow cytometry system (Beckman Coulter).

Dose-ranging studies in the HFIM system. The HFIM system has been extensively described elsewhere (14–16). For these studies, H9 cells were infected with the IIIB strain of HIV-1 by incubating viral stocks with H9 cells for 1 week. Briefly, H9 cells were infected by mixing 10^6 cells with a 1:2 dilution of the virus stock in cell growth medium. Cell infections were carried out in a volume of 0.5 ml for 2 h at 37°C with 5% CO_2. After 2 h of incubation, cells were washed with cell growth medium, resus-

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Address correspondence to Ashley N. Brown, Ashley.Brown@medicine.ufl.edu.

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TABLE 1 PK parameters from dose fractionation studies in the HFIM system

Simulated half-life (h)	Dosing interval	PK parameters at steady state			
		$AUC_{0\text{-}24}$[a] (mg · h/liter)	C_{max} (mg/liter)	Trough (mg/liter)	$T>EC_{90}$ (%)[b,c]
8	q24	1.967	0.187	0.025	100.0
	q12	1.930	0.124	0.049	100.0
4	q24	0.984	0.159	0.003	56.9
	q12	0.965	0.088	0.014	85.4
3	q24	0.738	0.153	0.001	42.8
	q12	0.724	0.080	0.007	61.8
2	q24	0.492	0.144	0.000	29.1
	q12	0.482	0.072	0.002	40.6

[a] Free drug $AUC_{0\text{-}24}$.
[b] $T>EC_{90}$ for 24 h.
[c] EC_{90} value for raltegravir against the IIIB strain of HIV was 0.018 mg/liter.

pended in 10 ml of complete medium, and placed into a T-25 flask. T-25 flasks were incubated at 37°C with 5% CO_2 for 3 days. After the third day, the cells were diluted into new T-25 flasks at a 1:10 ratio to maintain active cell growth. After 7 days in tissue culture, the cells were inoculated into the HFIM system. At the time of inoculation, the H9 cells were ~50% infected. A total of 10^6 H9 cells infected with IIIB virus were mixed with 10^8 uninfected CEM-SS cells and inoculated into the extracapillary space (ECS) of six polysulfone hollow-fiber cartridges (FiberCell Systems, Frederick, MD). Raltegravir was administered into five hollow-fiber cartridges as a continuous infusion at concentrations ranging from 1.5 to 24 ng/ml. One cartridge did not receive the drug and served as a no-treatment control. The ECS of each cartridge was sampled daily from both the left and right ports for 6 days. The percentage of HIV antigen-positive cells was determined by flow cytometry analysis. The effective 50% and 90% concentration (EC_{50} and EC_{90}) values for raltegravir against the IIIB strain of HIV were calculated by fitting a four-parameter inhibitory sigmoid E_{max} model to the flow cytometry data generated from the dose-ranging study on day 4 (the day of peak viral infection in the no-treatment control experimental arm) using S-Adapt (version 1.56) software. Two independent dose-ranging studies were performed.

Dose fractionation studies. Dose fractionation studies were performed as previously described (17, 18). Briefly, 10^6 H9 cells infected with IIIB were mixed with 10^8 uninfected CEM-SS cells and inoculated into the ECS of nine polysulfone hollow-fiber cartridges. The protein binding of raltegravir to human plasma proteins is ~83% (19). In the HFIM system, only free-drug concentrations are simulated. Thus, raltegravir was delivered into eight of the hollow-fiber cartridges via programmable syringe pumps over a 1-h infusion to achieve a free-drug area under the concentration–time curve from 0 to 24 h ($AUC_{0\text{-}24}$) that is associated with the mean free-drug clinical exposure of raltegravir ($AUC_{0\text{-}24}$ = 1.98 mg · h/liter). Four cartridges received the total $AUC_{0\text{-}24}$ exposure once daily (q24h), and four cartridges received half the daily $AUC_{0\text{-}24}$ exposure twice daily (q12h). One cartridge did not receive drug and served as a no-treatment control. Raltegravir was removed from the HFIM system at rates to mimic half-lives of 8, 4, 3, and 2 h for each dosing interval. The PK-PD parameters for all experimental arms are shown in Table 1. Hollow-fiber cartridges were sampled daily from the left and right sampling ports for 7 days, and the percentage of p24 antigen-positive cells was determined by flow cytometry. Three independent dose fractionation studies were conducted.

Pharmacokinetic analysis. Medium from the central reservoir of each hollow-fiber system was sampled at various time points post-drug infusion during the first 48 h of the study. Samples were diluted with water and analyzed by high-pressure liquid chromatography (HPLC)-tandem mass spectrometry (LC-MS/MS; ABSciex, Foster City, CA) for raltegravir concentrations. The HPLC conditions were as follows: the column used was a Phenomenex Kinetex C_{18}-XB (50 by 2.1 mm, 2.6-μm particle size), with a guard column; the mobile phases A and B were 2 mM ammonium acetate with 0.1% formic acid in water and 0.1% formic acid in methanol, respectively, and run in isocratic mode at 73% buffer B with a flow rate of 0.300 ml/min. Raltegravir concentrations were measured using MS/MS transition m/z 445 → m/z 109. The analysis run time was 3.00 min, and the target concentration range was 0.500 to 200 ng/ml (r^2 = 0.998). Quality control samples (1, 10, and 100 ng/ml) had coefficients of variation (CVs) ranging from 5.5% to 11.1% and accuracy (percent recovery) values between 99.8% and 110.6%. All measured raltegravir concentrations from studies performed in the HFIM system were within 10% of the targeted values (data not shown), indicating that the desired PK profiles were achieved in the system.

Statistical analysis and simulation. Area under the percentage of HIV antigen-positive cells–time curves ($AUC_{\%HIV+\ cells}$) for the left and right hollow-fiber ports were calculated using Prism software version 6.01 (GraphPad Software, Inc., La Jolla, CA). An unpaired *t* test with Welch's correction (GraphPad Prism) was used to identify significant differences between $AUC_{\%HIV+\ cells}$ values. For PK-PD analyses of data generated from dose fractionation studies, the $AUC_{\%HIV+\ cells}$ for each regimen was graphed against C_{max}/EC_{90}, AUC/EC_{90}, $trough/EC_{90}$, and the percentage of time above EC_{90} ($\%T>EC_{90}$). An inhibitory sigmoid E_{max} model was fitted to the $trough/EC_{90}$ and $\%T>EC_{90}$ data using GraphPad Prism software. PK simulations were conducted using a 5,000-subject Monte Carlo simulation with Adapt V software.

RESULTS

Susceptibility of IIIB HIV-1 to raltegravir in the HFIM system. Dose-ranging studies were performed in the HFIM system to evaluate the antiviral activity of raltegravir against the IIIB strain of HIV. The IIIB virus spread efficiently from cell to cell in the HFIM system (Fig. 1). The percentage of HIV-infected cells rose steadily in the no-treatment control arm through day 4, peaking at 52.8%, and then sharply declined. This decline in the percentage of HIV-infected cells was due to the killing of infected cells by the virus and the lack of fresh target cells in the system to propagate the infection. Similar kinetics of spread were observed in the experimental arms receiving raltegravir therapy. Raltegravir inhibited cell-to-cell viral spread in a dose-dependent manner for up to 4 days. The

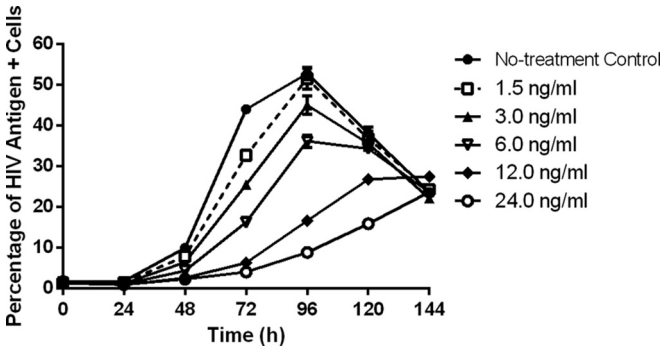

FIG 1 Dose-ranging studies with raltegravir against the IIIB strain of HIV-1 in the HFIM system. Raltegravir was administered into hollow-fiber cartridges as a continuous infusion at concentrations ranging from 0 to 24 ng/ml. Hollow-fiber cartridges were sampled from the left and right ports every 24 h for 144 h. Flow cytometry was performed daily on cells harvested from the HFIM system as described in Materials and Methods to determine the inhibitory effect of raltegravir on the cell-to-cell spread of HIV IIIB. Symbols, mean of two independent samples from the HFIM system; error bars = 1 SD. The data in the figure are representative of two independent dose-ranging experiments.

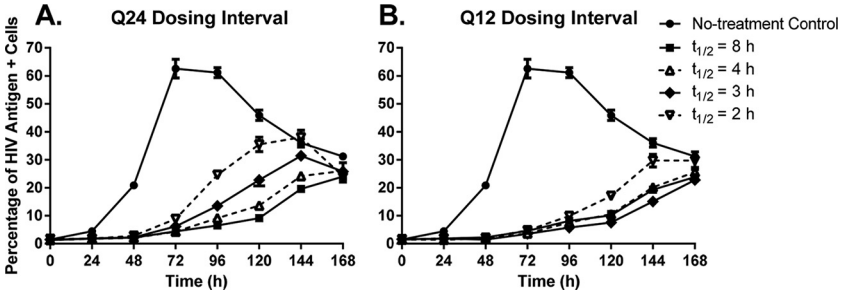

FIG 2 Dose fractionation studies with raltegravir against the IIIB strain of HIV-1 in the HFIM system. The free $AUC_{0\text{-}24}$ exposure associated with the clinical dose of raltegravir was administered into hollow-fiber cartridges as a 1-h infusion at dosing intervals of q24h (A) or q12h (B) and washed out at specific rates to simulate half-lives of 8, 4, 3, and 2 h, as described in Materials and Methods. Hollow-fiber cartridges were sampled every 24 h for 168 h. Two independent samples were harvested from each cartridge via the left and right ports, and cells in the sample were evaluated for the HIV p24 antigen by flow cytometry. Symbols, mean of the two independent samples; error bars = 1 SD. The data in the figure are representative of three independent dose fractionation experiments.

highest concentration evaluated in this study (24 ng/ml) delayed viral spread but was unable to completely suppress it, as HIV-positive cell populations peaked at 23.7% on day 6. EC_{50} and EC_{90} values were calculated for raltegravir on day 4, the day of near-peak viral infection in the no-treatment control arm. The EC_{50} for raltegravir against the IIIB strain of HIV in the HFIM system was 7.43 ng/ml (95% confidence interval [CI], 6.756 to 8.098 ng/ml), and the EC_{90} value was 17.54 ng/ml (95% CI, 15.95 to 19.12 ng/ml). The EC_{50} and EC_{90} values were corrected for protein binding and were similar to values reported by others (20–22).

The influence of PK variability on the efficacy of the raltegravir dosing interval. Dose fractionation studies were conducted in the HFIM system to identify the PD index that was best linked with viral suppression when various half-lives were simulated for raltegravir. All dosage regimens suppressed the cell-to-cell spread of HIV relative to the no-treatment control (Fig. 2A and B). Raltegravir administered q24h inhibited viral spread in a half-life–dependent manner, where the 8-h half-life provided the greatest viral inhibition and the 2-h half-life suppressed viral spread the least (Fig. 2A). In contrast, all q12h dosage regimens provided similar levels of viral suppression, with the exception of the 2-h half-life, which had higher percentages of HIV antigen-positive cells compared to the other regimens beginning at 120 h (Fig. 2B). $AUC_{\%HIV+\ cells}$ values were calculated for all therapeutic arms evaluated in the dose fractionation study (Table 2), and these values signified the degree of viral suppression for each dosage regimen relative to the no-treatment control. For the q24h dosing interval, the $AUC_{\%HIV+\ cells}$ values increased steadily as the raltegravir half-life changed from 4 h to 3 h to 2 h (Fig. 3A). The observed increase in $AUC_{\%HIV+\ cells}$ values was substantial between the 2- and 4-h half-lives, as the 2-h half-life yielded an $AUC_{\%HIV+\ cells}$ value that was ~77% greater than that reported for the 4-h half-life. In contrast, the differences between $AUC_{\%HIV+\ cells}$ values for the 4- and 8-h half-lives were less dramatic at ~26% (Fig. 3A). For the q12h dosing interval, $AUC_{\%HIV+\ cells}$ values were less variable with change in half-life (Fig. 3B), as maximal viral suppression was achieved at a 3-h half-life. Additionally, $AUC_{\%HIV+\ cells}$ values between the 2- and 8-h half-lives were only 38% different for the q12h regimen compared to the 122% difference in those values for the q24h regimen (Table 2).

TABLE 2 Area under the percentage of HIV antigen-positive cells–time (from 0 to 168 h) curve for each dosage regimen evaluated in dose fractionation studies

Simulated half-life of raltegravir (h)	$AUC_{\%HIV+\ cells}$ (% HIV antigen$^+$ cells · h)[a]		
	q24 dosing interval	q12 dosing interval	*P* value (q24 vs q12)
8	1,348 ± 61.5	1,413 ± 87.7	0.487
4	1,692 ± 119.5	1,409 ± 79.9	0.126
3	2,199 ± 108.2	1,139 ± 52.3	0.019
2	2,988 ± 169.7	1,944 ± 113.1	0.027

[a] $AUC_{\%HIV+\ cells}$ values were generated from the flow cytometry data. They were calculated independently for samples harvested from the left and right sampling ports of each hollow-fiber cartridge. The means ± SD for the two $AUC_{\%HIV+\ cells}$ values are shown. The $AUC_{\%HIV+\ cells}$ value for the no-treatment control arm was 5,935 ± 196.6% HIV antigen$^+$ cells · h.

We also compared the antiviral activities of the q24h and q12h dosing intervals for each half-life that was simulated for raltegravir in this study. The q24h and q12h dosage regimens achieved similar levels of viral suppression when half-lives of 8 and 4 h were simulated (Fig. 4A and B). The $AUC_{\%HIV+\ cells}$ values were not statistically significantly different between the q24h and q12h dosing intervals for the 8-h ($P = 0.487$) or 4-h ($P = 0.126$) half-life (Table 2). In contrast, the q12h dosing interval yielded the greatest inhibition of viral spread compared to the q24h dosing interval for the 3- and 2-h half-lives (Fig. 4C and D). Differences in the percentage of HIV-positive cells between the q24h and q12h regimens were first observed at 96 h for the 3-h half-life and at 72 h for the 2-h half-life. The $AUC_{\%HIV+\ cells}$ values between the q24h and q12h dosing intervals were significantly different for the 3-h half-life ($P = 0.019$, $AUC_{\%HIV+\ cells}$ values between q24h and q12h regimens) and the 2-h half-life ($P = 0.027$, $AUC_{\%HIV+\ cells}$ values between q24h and q12h regimens) (Table 2). These data show that q24h dosing for raltegravir is effective at half-lives of 8 and 4 h but not at half-lives of 3 and 2 h, which require q12h dosing for maximal antiviral activity.

PK-PD analysis of dose fractionation studies. We graphed the $AUC_{\%HIV+\ cells}$ values calculated for each experimental arm in the dose fractionation studies against the PD indices C_{max} (maximum concentration of drug in serum)/EC_{90}, AUC/EC_{90}, trough/EC_{90}, and $T>EC_{90}$. There was no observable correlation between C_{max}/EC_{90} and raltegravir antiviral activity (Fig. 5A). The AUC/

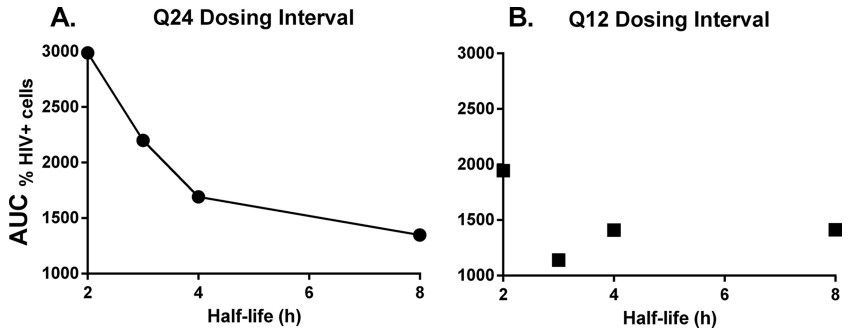

FIG 3 $AUC_{\%HIV+\ cells}$ as a function of the simulated half-life for raltegravir in the HFIM system. Raltegravir was administered into hollow-fiber cartridges as a 1-h infusion at dosing intervals of q24h (A) and q12h (B), and half-lives of 8, 4, 3, and 2 h were simulated for raltegravir in the HFIM system. Hollow-fiber cartridges were sampled every 24 h for 168 h. Two independent samples were harvested from each cartridge via the left and right ports, and cells in the sample were evaluated for the HIV p24 antigen by flow cytometry. $AUC_{\%HIV+\ cells}$ values were quantified by calculating the area under the percentage of HIV antigen-positive cells–time curve (Fig. 2) for each dosage regimen from the dose fractionation studies. The data in the figure are representative of three independent dose fractionation experiments.

EC_{90} graph showed that the discordance in activity became greater as half-life became shorter, despite similar AUC/EC_{90} values between q24h and q12h dosing intervals for each half-life (Fig. 5B). An inhibitory sigmoid E_{max} model was not fitted to these data due to the clear separation between the q24h and q12h dosing intervals at the shorter half-lives, as two models would be more appropriate to fit the q24h and q12h dosing intervals individually. In contrast, there was a clear association between raltegravir antiviral activity and the PD indices trough/EC_{90} and $\%T>EC_{90}$ (Fig. 5C and D). It should be noted that $T>EC_{90}$ and trough/EC_{90} map 1:1 until 100% $T>EC_{90}$ is achieved. Trough/EC_{90} is not bound from above. sigmoid E_{max} models were fitted to these data, and the model fits are represented in the figures by a solid black line (Fig. 5C and D). The model fit the data well, yielding r^2 values of 0.931 for trough/EC_{90} and 0.933 for $\%T>EC_{90}$. These data imply that raltegravir trough concentrations must be ≥3 ng/ml (adjusted for protein binding; equivalent to a total drug concentration of 17.65 ng/ml) or concentrations must be above the EC_{90} for at least 57% of a 24-h period in order for a dosage regimen to be therapeutically successful. In Fig. 5D, the lines connect the effects achieved by q12h versus q24h dosing for different half-lives.

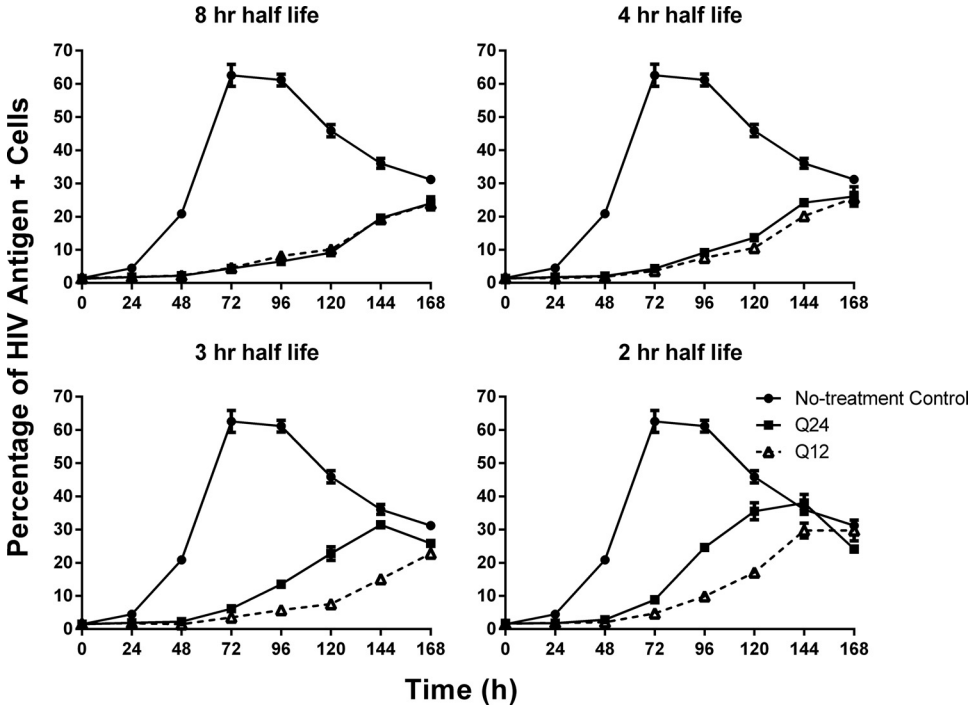

FIG 4 The effect of half-life on the efficacy of raltegravir dosing intervals. Raltegravir was administered into hollow-fiber cartridges as a 1-h infusion at dosing intervals of q24h and q12h. Half-lives of 8 h, 4 h, 3 h, and 2 h were simulated for raltegravir in the HFIM system. Hollow-fiber cartridges were sampled every 24 h for 168 h. Two independent samples were harvested from each cartridge via the left and right ports, and cells in the sample were evaluated for the HIV p24 antigen by flow cytometry. Symbols, mean of the two independent samples; error bars = 1 SD. The data in the figure are representative of three independent dose fractionation experiments.

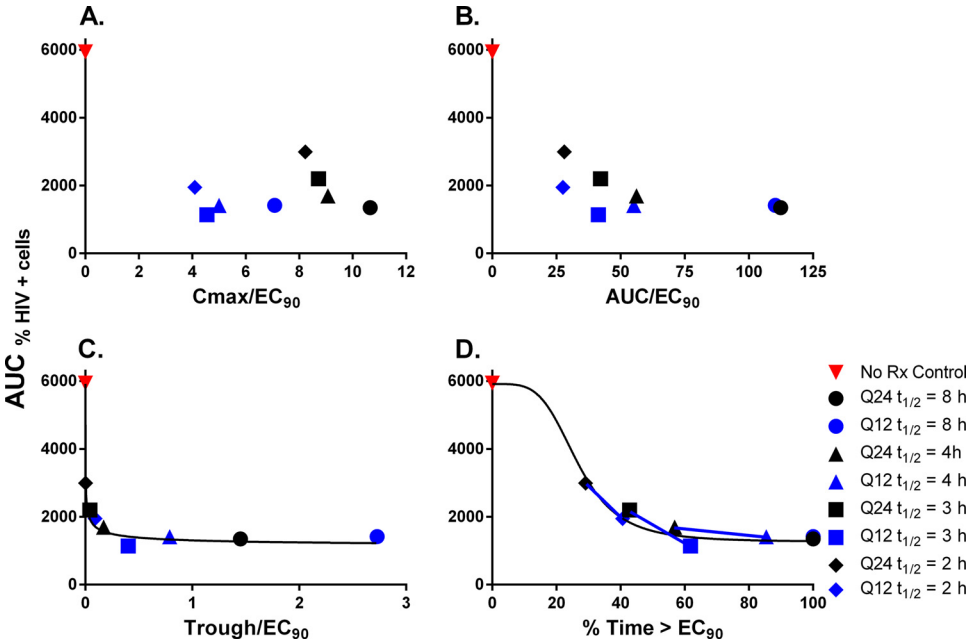

FIG 5 PK-PD analysis of raltegravir dose fractionation experiments. $AUC_{\%HIV+\ cells}$ values were calculated for each experimental arm evaluated in the dose fractionation studies. $AUC_{\%HIV+\ cells}$ were graphed against the PD indices of C_{max}/EC_{90} (A), AUC/EC_{90} (B), $trough/EC_{90}$ (C), and $\%T>EC_{90}$ (D). Each data point represents the mean $AUC_{\%HIV+\ cells}$ value calculated from samples harvested from the left and right sampling ports. Red symbols, no-treatment control; black symbols, q24h dosing interval; blue symbols, q12h dosing interval. A sigmoid E_{max} model was fitted to the data illustrated in the $trough/EC_{90}$ and $\%T>EC_{90}$ graph, and the model fit is designated by the black curve. Blue lines connect the q24h and q12h dosing intervals for each half-life.

DISCUSSION

The main objective of this investigation was to explain the discordance in conclusions between experiments conducted in an *in vitro* setting and the clinical trial. Results from previous *in vitro* studies indicated that once-daily dosing was as efficacious as twice-daily dosing for raltegravir against HIV-1 in the HFIM system when a half-life of 9 h was simulated (11). However, these results were not validated in a human clinical trial. Although QD and BID dosing both yielded overall high response rates (83.2% for QD and 88.9% for BID) in a phase 3 noninferiority study conducted by Merck, the QD regimen failed to meet the −10% noninferiority margin (difference, −5.7%; 95% CI, −10.7 to −0.8) (12). The failure of the 800-mg QD dosage arm was attributed to lower trough values for raltegravir in these patients (23). Previous studies demonstrated that human PK profiles for raltegravir are characterized by a high degree of variability (19, 24, 25). To that end, we hypothesized that the success or failure of QD dosing for raltegravir is largely dependent on the PK profiles for the drug in patients.

Interpatient variability for raltegravir PK can be attributed to a variety of factors, including variations in absorption rate, bioavailability, volume of distribution, and clearance. For this study, we chose to simulate interpatient PK variability in the HFIM system by altering clearance (and thus half-life) for raltegravir. Results from the dose fractionation studies showed that raltegravir antiviral activity was similar between q24h and q12h dosing intervals when PK profiles with longer half-lives (smaller clearance values) were simulated, but more frequent dosing (shorter dosing intervals) was required to provide maximal viral suppression for PK profiles with shorter half-lives/larger clearance. These findings are similar to those reported for our previous work with the influenza antiviral zanamivir, in which we showed that the PD index linked to antiviral effect can change as a function of the PK parameters (e.g., shorter half-life/larger clearance driving a switch from AUC/EC_{90} to $T>EC_{90}$) (26). PK-PD analyses revealed that $trough/EC_{90}$ and $\%T>EC_{90}$ values correlated with antiviral efficacy. This finding was not surprising, since these two PD indices map one to one until 100% $T>EC_{90}$ is achieved. Since maximal activity for raltegravir in this study was achieved at 57% $T>EC_{90}$, it is not possible to definitively distinguish between $trough/EC_{90}$ and $\%T>EC_{90}$ as the PD index linked with maximal virological response.

In order to determine the percentage of the population for which QD dosing would be efficacious, we ran a 5,000-patient simulation to estimate the proportion of patients that would exhibit raltegravir half-lives of <4 h, assuming a mean terminal half-life of 11 h and an interpatient CV of 61% for half-life (Matthew Rizk, Merck Research Laboratory, personal communication). Our simulations revealed that 93.4% of the population would exhibit half-lives that were ≥4 h, and 6.6% were <4 h. These data show that 6.6% of the total patient population is at risk of not achieving near-maximal viral suppression, suggesting that the daily raltegravir regimen is at a higher risk for failure in these patients. Interestingly, the difference in efficacy between the QD and BID raltegravir regimens in the phase 3 clinical trial was ~6% (12), strikingly similar to our estimation of 6.6% for individuals who are predicted to exhibit shorter raltegravir half-lives (<4 h). Thus, our finding that failure of daily therapy was linked to the variability of raltegravir PK (i.e., shorter half-life/higher clearance) is a plausible explanation for the initial discordance in conclusions between the *in vitro* HFIM system studies and the human clinical trial.

A second factor needing consideration for the difference in

conclusions between the *in vitro* and clinical trial lies with the endpoint used to describe therapeutic success. In the phase 3 trial, the endpoint was defined as achieving plasma HIV RNA levels that were <50 copies/ml. Patients with HIV RNA levels of >50 copies/ml after 48 weeks of therapy were classified as having virologic failure (12). Use of these criteria results in a purely dichotomous outcome, treatment is either successful or not successful. Patients might achieve a burden of 60 copies/ml and still be considered a failure. It is clear that the lower the viral burden, the less likely the emergence of viral resistance. However, it is reasonable that a rational index of viral effect for a regimen would be the absolute fall in viral burden from baseline.

The trial noted in a subgroup analysis that daily raltegravir also was not as efficacious in patients with a higher baseline viral burden (>100,000 copies/ml) (12). This is understandable. If the patient had a higher baseline viral burden and a shorter half-life, the near maximal viral effect achieved in this subgroup would have a lower likelihood of reaching the goal of <50 copies/ml and would be considered a virological failure.

A major benefit to the HFIM system is that PK parameters can be carefully controlled in an *in vitro* setting. The studies performed in this investigation showed that raltegravir concentrations must remain above the EC_{90} value for at least 57% of the time for a 24-h period or that free-drug trough concentrations must be >3 ng/ml in order to achieve maximal viral suppression. Raltegravir regimens with PK profiles in the HFIM system that did not achieve this standard, including the 3-h half-life q24 and the 2-h half-life q24 and q12 regimens, ultimately drove significantly less antiviral effect (Fig. 2 and 4). Considering only the $\%T>EC_{90}$ breakpoint for therapeutic success ($T>EC_{90}$ of 57%), this value is reasonable when considering the long occupancy time of raltegravir in the integrase binding pocket. Raltegravir displays a dissociative half-life of ~8 h (9, 10). These experiments were conducted under static conditions, meaning that raltegravir was allowed to bind to the viral integrase for a specified period before all traces of the drug were washed away. Following drug removal, the dissociation of raltegravir from the integrase was measured (9, 10). In the HFIM system, drug concentrations are not static, resulting in an always present but declining concentration of raltegravir throughout a 24-h period, with the rate of decline dependent on the half-life. This continuing but declining drug pressure impedes the dissociation of raltegravir from the integrase binding pocket, thereby elongating the dissociation half-life. In our study, regimens that yielded maximal viral suppression had raltegravir concentrations that were above the EC_{90} for ~14 h (equivalent to 57% $T>EC_{90}$ for a 24-h period). The constant drug pressure in the HFIM system likely produces a dissociation half-life that is >8 h, resulting in near-complete binding to the integrase active site for the entire 24-h period. Dosage regimens that do not achieve the 57% $T>EC_{90}$ parameter allow for raltegravir dissociation from the viral integrase sometime during the 24-h period. Once the drug is unbound from the integrase, the virus can replicate freely and spread from cell to cell. For monitoring purposes, a therapeutic target would be to achieve free-drug trough/EC_{90} values approximating 0.5, as maximal antiviral effect was achieved when free-drug trough/EC_{90} values were at or above this level (Fig. 5C).

In summary, these studies suggest that patient PK profiles for raltegravir are likely to play a major role in the success or failure of QD regimens. Our findings indicate that the PD indices $T>EC_{90}$ (percent of 24 h) and trough/EC_{90} are associated with optimal therapeutic outcomes for raltegravir when clearance variability is evaluated *in vitro*.

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