Hollow-Fiber Unit Evaluation of a New Human Immunodeficiency Virus Type 1 Protease Inhibitor, BMS-232632, for Determination of the Linked Pharmacodynamic Variable

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BMS-232632 is a potent human immunodeficiency virus type 1 (HIV-1) protease inhibitor with potent activity and activity against some isolates already resistant to older members of this class. To optimize the dose and schedule of administration, it is important to delineate which portion of the concentration-time curve outcome is most directly related. Our group has previously used the hollow-fiber system to examine these dose and schedule issues [1, 2] for drugs of different classes. In each instance, predictions of effect, schedule, and dose have been validated.

We initiated this study to delineate the pharmacodynamically linked variable for BMS-232632, to calculate a regimen that would be effective on a once-daily basis, and to test it in the hollow-fiber system. Monte Carlo simulation was employed to examine the relative ability of different doses of BMS-232632 against some isolates already resistant to older members of this class. To optimize the dose and schedule of administration, it is important to delineate which portion of the concentration-time curve outcome is most directly related. Our group has previously used the hollow-fiber system to examine these dose and schedule issues [1, 2] for drugs of different classes. In each instance, predictions of effect, schedule, and dose have been validated.

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Methods

Viral 50% effective concentration (EC50) determination. The susceptibility of the HIV isolate (HIV susceptible) was determined by using the Department of Defense/AIDS Cooperative Treatment Group consensus assay [3], with and without supplementation of the human binding proteins α-1 acid glycoprotein (AAG; 1.5 mg/mL) and albumin (4 mg/dL). EC50 was determined by calculation from the model that was fitted to the primary data (sigmoid-Emax model with a nonzero intercept).

Hollow-fiber study methodology. The techniques that we used have been published elsewhere [1, 2]. The study design in hollow-fiber units first elucidated an exposure-response curve, with BMS-232632 being given as a continuous infusion. This was followed by an experiment in which 4 hollow-fiber units were simultaneously evaluated. All units were challenged with chronically infected cells at time zero, such that 1% of the total cell population was chronically infected with HIV. One unit served as the negative control (an infected hollow-fiber unit where only growth medium was circulated). The second unit served as the positive control (the infected unit was treated with BMS-232632 by continuous infusion at 4 × EC50). The third unit was exposed to BMS-232632, with a peak concentration of 56.7 nM at 2 h (with a 5.5-h terminal half-life), which produced a 24-h concentration of 3.55 nM. The area under the curve (AUC) that was the same as that for the continuous-infusion unit. The fourth unit was exposed to BMS-232632 at a regimen calculated to cause equivalent suppression to the 4 × EC50 continuous-infusion unit. This required a 4-fold higher AUC than that used in the third unit. The intermittent administrations of drug (third and fourth units) were performed daily for the duration of the experiment. This experiment was done once.

Population pharmacokinetic modeling of BMS-232632. Plasma concentration–time data and assay performance data were provided by S.K (Bristol-Myers Squibb). Doses of 400 and 600 mg
were modeled. The studies were conducted in normal volunteers (n = 12). All subjects were male and white, with an average weight of 75.3 kg, an average age of 29.2 years, and an average height of 177.3 cm. The data were modeled by using a nonparametric expectation maximization approach [4]. Models were discriminated by using the Akaike information criterion [5].

**Monte Carlo simulation.** Monte Carlo simulation was performed by ADAPT II [6] with the population simulation option without noise, employing a log-normal distribution. Three 1000-subject Monte Carlo simulations were done, using the full covariance matrix.

**Regimen evaluation.** The goal for therapy with BMS-232632 was total suppression of viral replication and was set from the data derived from the hollow-fiber unit experiments described above. Once the goal was set (amount of time of free drug [BMS-232632] >EC50 in a steady-state dosing interval), the concentration-time data were imported into a statistical package (SYSTAT for Windows, version 9.0; SPSS). Plasma concentration-time data for each of the simulated subjects at the goal-defined time were transformed from nanograms per milliliter to nanomolar concentrations and were corrected for protein binding and the difference between EC50 and EC95, using data from the protein-binding effect on EC50 determined experiments. For theoretical virus isolate EC50 of 0.2–10 nM, the proportion of subjects attaining the therapeutic target among each of the 3 1000-subject simulations was determined. The mean (±SD) of the proportion was determined.

**Results**

The mean (±SD) values for clearance/bioavailability (F), volume/F, and absorption first order rate constant (Ka) from the population analysis were 31.9 (±14.5) L/h, 134.6 (±77.9) L., and 0.541 (±0.274) h⁻¹, respectively.

The addition of AAG to the medium increased the EC50/EC95 from 6.9 nM:17.4 nM to 67 nM:144 nM. Further addition of albumin increased these values to 93 nM:241 nM. The EC95 values were 2.1–2.63 times greater than the EC50 values. A correction factor of 34.88 was derived from the product of these values (2.6 × 13.4) and was used to correct theoretical EC50 values from growth medium–only experiments to protein binding–adjusted EC95 values.

The first hollow-fiber unit experiments demonstrated that 4 × EC50 in a continuous infusion would completely suppress viral replication for the duration of the experiment (data not shown).

In the second experiment, the virus within the untreated fiber unit grew, as expected (figure 1). The 4 × EC50 continuous-infusion unit showed complete control of viral replication (figure 1). The matching AUC experiment, with the same exposure as the continuous-infusion experiment but with the in vitro profile of drug concentration simulating an oral administration profile, demonstrated breakthrough viral growth. Finally, the prospectively designed regimen that would be equivalent in effect to the 4 × EC50 continuous-infusion regimen also completely controlled viral growth for the duration of the experiment (4 × 4 × EC50 as daily dosing in figure 1). This regimen covered 85% of the dosing interval (i.e., dropped to <22.7 nM at 20.4 h). This required percentage of the dosing interval was estimated from other data derived from previous experiments with another protease inhibitor. The unit being treated with once-daily dosing at the same daily AUC as the continuous infusion loses control between days 9 and 11 and is in approximatively the same state of growth as the control unit is on day 3. This implies that a less-than-optimal regimen still will offer some measure of control of viral replication.

The 1-compartment model was chosen for simulation. Monte Carlo simulation, using a log normal distribution for both 400- and 600-mg doses, accurately recaptured the starting mean parameters.

After using the 34.88-fold correction factor, we tested the three 1000-subject simulations to determine whether the 20.4-h concentration was above the corrected concentrations for theoretical EC50 from 1 to 10 nM. The results are displayed in figure 2. For a theoretical isolate with an EC50 of 1 nM, 63.8% ± 1.28% of simulated subjects receiving the 400-mg dose attained the target, whereas, for a 10-nM isolate, there was a target attainment rate of 18.7% ± 1.52%. For the 600-mg dose, these values were 70.4% ± 1.88% and 34.9% ± 2.03%, respectively.

![Figure 1. Effect of BMS-232632, a human immunodeficiency virus (HIV) type 1 protease inhibitor, on HIV replication: dose and schedule comparison in the hollow-fiber system. Three infected hollow-fiber units were treated with BMS-232632. One tube was treated with a concentration 4 times the 50% effective concentration (EC50) as a continuous infusion. This produced a 24-h area under the curve (AUC) of 4 × 24 × EC50. The second tube received the same 24-h AUC but was given in a peak-and-valley mode once daily (QD). The third tube received an exposure calculated a priori, to provide a time >EC50 that would give essentially the same suppression as the continuous infusion of 4 × EC50.](https://academic.oup.com/jid/article-abstract/183/7/1126/860240/1032141)
Discussion

Protein binding increases the EC\textsubscript{50} for BMS-232632. This finding is consistent with previous work from our laboratory regarding protein binding for protease inhibitors [7]. The final value for EC\textsubscript{50}, with both AAG and albumin considered, was 13.4 times the original value.

The hollow-fiber unit model demonstrates that time greater than threshold, not AUC or maximum concentration (C\text{max}), is the dynamically linked variable. If AUC (or, more specifically, the AUC:EC\textsubscript{50} ratio) were the linked variable, the continuous-infusion unit and the once daily–dosing unit at a matched AUC would have provided equivalent outcomes. The 2 outcomes were not equivalent, since breakthrough growth was seen with the latter. If peak concentration (actually, the C\text{max}:EC\textsubscript{50} ratio) were the linked variable, this unit would have been at least equivalent but was not.

Finally, we could predict prospectively an exposure profile that would match the HIV inhibitory effect of the continuous-infusion regimen. Such an exposure profile required that an AUC, which was 3.9 times larger than the AUC of the continuous-infusion experiment, maintain a concentration \( \geq 4 \times EC_{50} \) for 85% of the dosing interval (20.4/24 h). This is to be expected, since time greater than threshold increases as the log\(_{10}\) (dose), whereas AUC and Peak increase linearly with dose.

From this experiment, it is known that the concentration of nonprotein-bound (free) drug must exceed the EC\textsubscript{50} of the virus for the majority of the dosing interval for maximal virus suppression to occur.

For 400- and 600-mg doses of BMS-232632 administered once daily, 2 population pharmacokinetic analyses and subsequent 1000-subject Monte Carlo simulations, which were repeated 3 times, were done. The fraction of the population whose plasma concentration exceeded the target value (EC\textsubscript{50} × 34.88) was determined for theoretical EC\textsubscript{50} values in the range of 0.2–10 nM. As can be seen in figure 2, for the 400-mg dose, at 0.2 nM EC\textsubscript{50}, 82.6\% \pm 3.3\% of 3000 simulated subjects exceeded the target value. This target attainment rate declined to <20\% when the beginning EC\textsubscript{50} values were 10 nM. The 600-mg dose was more successful for the higher EC\textsubscript{50} values, with 84.8\% \pm 2.0\% attaining the target at 0.2 nM and with 34.9\% \pm 2.03\% still attaining the target at 10 nM.

It is important to place such a finding in proper clinical perspective. The target attainment rate here is for maximal effect of a single agent. For application to a study of BMS-232632 in which patients will receive monotherapy for 2 weeks, it is likely that the largest decrease in HIV load that can be observed from baseline is \( \sim 2 \log_{10} \) U. For a theoretical group of 10 patients, all of whom had a virus isolate with an EC\textsubscript{50} of 1 nM and who took the 400-mg dose, \( \sim 6 \) of 10 patients would have a maximal decrease (2 logs) in virus load, whereas the other 4 would have a lesser decrease (<2 logs and log-normally distributed, because the plasma concentrations were log-normally distributed). For the other 4 patients, it is likely that 2 would have <2 log change but >1.5 log decrease: one probably would have between 1 and 1.5 log decrease, and one probably would have <1 log decrease. Consequently, for the 400-mg dose,
the median virus load change would be 2 logs, and the mean would be slightly less (~1.6–1.7 logs). For larger doses, the median change cannot exceed the maximal change and therefore must be between the maximum change and the median change (2.0 and 1.6 log_{10}).

Patients naive to previous antiretroviral therapy were studied in a phase I/II clinical trial of BMS-232632 [8]. Forty-three of these patients had EC_{50} values determined for the drug. Of these, 37 were \leq 1 nM, and none was as high as 2 nM (data on file, Bristol-Myers Squibb). The distribution is shown at the bottom of figure 2. It is obvious from figure 2 that major differences in virus load decline over a short period of monotherapy (e.g., 2 weeks) would be difficult to discriminate by dose.

Performance of an expectation operation over the viral distribution (multiplying the target attainment fraction times the fraction of the viral distribution at a specific EC_{50} and then summing over all products) of EC_{50} values shows that the 400-mg dose will generate a probability of 69.1% of attaining a maximal response, whereas the 600-mg dose produces a 74.4% probability. Examination of the dose dependence of effect by Monte Carlo simulation (figure 2) indicates that higher doses (perhaps 800-mg daily) might be required to show a difference of effect, but this would be the case only if more resistant virus isolates are encountered. Choice of the higher doses will become more important with larger patient numbers (more likely to encounter more resistant viruses) and as length of therapy exceeds 2 weeks. Since the duration of monotherapy is limited by ethical concerns, this analysis points up problems with dose choice made by examining such time-limited data.

References


