CONCISE COMMUNICATION

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 a half-life that allows for once-daily dosing. A concentration of 4 times the viral 50% effective concentration (EC50 [i.e., ~EC95]) administered as a continuous infusion in vitro provides virtually complete suppression of viral replication. This exposure, modeled in vitro as once-daily administration with oral absorption, allows ongoing viral replication. An exposure 4 times as large was calculated to be necessary to provide virus suppression equivalent to the continuous-infusion exposure. These experiments demonstrated that concentration above a threshold (time > 4 × EC50) is the pharmacodynamically linked variable for this HIV-1 protease inhibitor. Protein-binding experiments demonstrated that the EC50 was increased 13.4 times by the addition of human binding proteins. Monte Carlo simulation of protein binding–adjusted pharmacokinetic data from volunteers demonstrated that 64%–70% of a simulated population (n = 3000) would achieve virus suppression with 400–600 mg of BMS-232632 given once daily, if the viral EC50 were ≈1 nM.

BMS-232632 is a new 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 to attain virus suppression across a range of viral susceptibility targets.

Methods

Viral 50% effective concentration (EC50) determination. The susceptibility of the HIV isolate (HIVIIIb) 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 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

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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 \(\text{BMS-232632}\) derived from the hollow-fiber unit experiments described above), 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 \(\text{EC}_{50}\) and \(\text{EC}_{95}\), using data from the protein-binding effect on \(\text{EC}_{50}\) determination experiments. For theoretical virus isolate \(\text{EC}_{50}\) 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 \((\pm SD)\) of the proportion was determined.

Results

The mean \((\pm SD)\) values for clearance/bioavailability \((F)\), volume/\(F\), and absorption first order rate constant \((K_a)\) from the population analysis were 31.9 \((\pm 14.5)\) L/h, 134.6 \((\pm 77.9)\) L, and 0.541 \((\pm 0.274)\) h\(^{-1}\), respectively.

The addition of AAG to the medium increased the \(\text{EC}_{50}:\text{EC}_{95}\) from 6.9 nM:17.4 to 67 nM:144 nM. Further addition of albumin increased these values to 93 nM:241 nM. The \(\text{EC}_{95}\) values were 2.1–2.63 times greater than the \(\text{EC}_{50}\) values. A correction factor of 34.88 was derived from the product of these values \((2.6 \times 13.4)\) and was used to correct theoretical \(\text{EC}_{95}\) values from growth medium–only experiments to protein binding–adjusted \(\text{EC}_{95}\) values.

The first hollow-fiber unit experiments demonstrated that \(4 \times \text{EC}_{50}\) 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 \(4 \times \text{EC}_{50}\) 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 \(4 \times \text{EC}_{50}\) continuous-infusion regimen also completely controlled viral growth for the duration of the experiment \((4 \times 4 \times \text{EC}_{50}\) 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 approximately the same state of growth as the control unit 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 recapitulated 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 \(\text{EC}_{50}\) from 1 to 10 nM. The results are displayed in figure 2. For a theoretical isolate with an \(\text{EC}_{50}\) 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](https://example.com/figure1.png)
Figure 2. Percentage of simulated subjects with target attainment (maximal antiviral response) for BMS-232632, a human immunodeficiency virus (HIV) type 1 protease inhibitor. A Monte Carlo simulation of 1000 subjects was used 3 times to estimate the fraction of these subjects whose concentration-time curve would produce maximal virus suppression on the basis of the data presented. Evaluation was done for 400- and 600-mg doses of BMS-232632, administered once daily by mouth. Forty-three isolates from a clinical trial of BMS-232632 were tested by use of the Virologics Phenosense assay (Virologics). EC$_{50}$, 50% effective concentration.

Discussion

Protein binding increases the EC$_{50}$ 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$_{50}$, 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$_{max}$), is the dynamically linked variable. If AUC (or, more specifically, the AUC:EC$_{50}$ 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$_{max}$/EC$_{50}$ 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 $>$ 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$_{50}$ 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$_{50} \times 34.88$) was determined for theoretical EC$_{50}$ 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$_{50}$, 82.6% ± 3.3% of 3000 simulated subjects exceeded the target value. This target attainment rate declined to <20% when the beginning EC$_{50}$ values were 10 nM. The 600-mg dose was more successful for the higher EC$_{50}$ values, with attainment of the target at 0.2 nM and with 34.9% ± 2.0% 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$_{50}$ 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 \text{nM}, and none was as high as 2 \text{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


