Drug Resistance and Predicted Virologic Responses to Human Immunodeficiency Virus Type 1 Protease Inhibitor Therapy

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The extent to which human immunodeficiency virus (HIV) type 1 drug resistance compromises therapeutic efficacy is intimately tied to drug potency and exposure. Most HIV-1 protease inhibitors maintain in vivo trough levels above their human serum protein binding-corrected IC50 values for wild-type HIV-1. However, these troughs are well below corrected IC50 values for protease inhibitor–resistant viruses from patients experiencing virologic failure of indinavir and/or nelfinavir. This suggests that none of the single protease inhibitors would be effective after many cases of protease inhibitor failure. However, saquinavir, amprenavir, and indinavir blood levels are increased substantially when each is coadministered with ritonavir, with 12-h troughs exceeding corrected wild-type IC50 by 2-, 7-, and 28–79-fold, respectively. These indinavir and amprenavir troughs exceed IC50 for most protease inhibitor–resistant viruses tested. This suggests that twice-daily indinavir-ritonavir and, to a lesser extent, amprenavir-ritonavir may be effective for many patients with viruses resistant to protease inhibitors.

The emergence of resistance to antiretroviral drugs is one of the greatest obstacles to the successful long-term treatment of human immunodeficiency virus (HIV) disease. Incomplete suppression of viral replication during therapy fosters the emergence of resistant virus variants, leading to a resurgence of high-level replication and virologic failure. If rebound of virus occurs, the clinician is faced with a complex and constantly changing list of potential therapeutic options for salvage, but often no clear rationale exists to choose among them. Treatment decisions are often based on mutational patterns associated with resistance to specific drugs, but, with very few exceptions (notably the nonnucleoside reverse-trancriptase inhibitors and lamivudine), these relationships are still poorly understood. For that reason, viral resistance phenotypes cannot usually be inferred reliably from genotypic (sequence) information.

Alternatively, drug susceptibilities of patients’ virus isolates may be measured directly in vitro. The advent of recombinant virus assays has significantly improved the accuracy and reproducibility of phenotypic determinations, and these assays are now available commercially. By measuring viral phenotypes directly, these assays eliminate the need to interpret complex genotypic data, to infer viral phenotypes.

Even if genotypic and phenotypic data are available, there still exists no clearly defined relationship between viral drug resistance and therapeutic response. To place the consequences of drug resistance into proper context, it is necessary to consider the biologic basis of resistance, the ways it is measured, and the pharmacologic factors that influence antiviral drug efficacy.

Virologic treatment failure may not always require that failing drugs be substituted by others. Recent reports of virologic failure without resistance [1–4] have demonstrated that rebound in virus load during therapy may occur without genotypic or phenotypic resistance to all components of a combination regimen. In such cases, therapeutic intensification may provide sufficient drug exposure to regain control of rebounding wild-type viruses. Additionally, even if some level of resistance is manifest, it may be possible to overcome that resistance by providing greater drug exposure.

To understand the rationale for this latter prediction, it is important to consider how resistance is defined. In the practical context of antiretroviral therapy, the term “resistance” may be misleading. It is more accurately described as a reduction in drug susceptibility: the concentration of drug needed to achieve an arbitrary degree of inhibition of viral replication in cell culture. Thus, an IC50 or IC95 represents the concentration of a drug required to inhibit spread of virus in cell culture by 50% or 95%, respectively. Reduced susceptibility (i.e., resistance) to that drug is reflected as an increase in that inhibitory concentration to a level convincingly higher than that of wild-type viruses.
Given that working definition of resistance, it should also be possible to overcome reduced drug susceptibility, if sufficient drug potency and exposure are achieved. For example, 10-fold resistance to a drug signifies that a 10-fold–higher drug exposure would be necessary to inhibit viral replication to any given extent. A logical approach to dealing with resistance, then, would be to use simple “brute force” by increasing drug exposure to a level sufficient to inhibit any resistant viruses that may be present.

Obviously, the ability of resistance to compromise therapy is intimately tied to in vivo drug efficacy. This is influenced by many factors, including pharmacologic variables (intrinsic drug potency, drug absorption, clearance, metabolism, protein binding, and drug distribution), virologic factors (drug susceptibility of the virus, replication kinetics, and distribution), and patient factors (adherence to therapy). The complex interplay of these many variables has limited the ability of practitioners to interpret the results of resistance tests, either to identify regimens likely to be effective or to rule out those with less therapeutic potential. Consequently, there is a need to define ways to predict virologic responses to drug treatment and to integrate data from in vitro susceptibility and pharmacologic studies of drug exposure.

Here, we examine the interrelationships of drug potency, drug exposure, and drug resistance. In so doing, we provide a theoretical framework in which these factors can be considered together, in an effort to predict more accurately how drug resistance may affect virologic responses to therapy.

Materials and Methods

HIV-1 protease inhibitor–resistant viruses. These were derived from clinical studies of indinavir-treated patients—Merck protocols 004 [5], 006 [6], 010 [7], 018 (unpublished), and 008 (unpublished)—or from patients experiencing nelfinavir failure (Merck protocol 075 [8]). RNA from patient serum or plasma specimens was amplified by use of reverse transcription–polymerase chain reaction, and recombinant viruses were constructed, as described elsewhere [9].

Phenotypic and genotypic analyses. Phenotypic susceptibilities were determined by the PhenoSense assay [9] (ViroLogic), and amino acid substitution mutations in the viral protease coding region were determined, as described elsewhere [10]. Susceptibilities relative to wild type are expressed relative to recombinant virus from HIV-1 NL4-3 determined within the same assay.

Results

Protein binding and antiviral efficacy. The efficacy of a drug is a function of its antiviral potency; however, substantial in vitro potency does not necessarily translate into clinical efficacy. Because of limitations of bioavailability, rapid metabolism, or high protein binding, drugs that are quite potent in cell culture may have limited or no antiviral activity in vivo. For example, although the investigational HIV-1 protease inhibitor SC-52151 had initially shown promising antiviral activity in vitro and achieved mean plasma levels in patients above the IC95 for viral replication, it had no significant antiviral activity in patients, because of its extensive human serum protein binding [11, 12].

This attenuation of antiviral activity by protein binding has been demonstrated directly in vitro. In the presence of human serum, the activities of all the available HIV-1 protease inhibitors are reduced significantly [13]. Table 1 compares the measured in vitro potencies of the available HIV-1 protease inhibitors against wild-type HIV-1 in a recombinant virus assay in the absence of human serum and after correction for loss of activity by human serum protein binding, as described by Molla et al. [13]. We have independently verified this inhibition of protease inhibitor activity by IC95 increases in the presence of human serum (data not shown). These data illustrate that protein binding limits the antiviral activities of all the HIV-1 protease inhibitors to varying degrees, and the greatest effects are observed with nelfinavir, saquinavir, and ritonavir, which are the most extensively bound by human serum proteins, especially α-1 acid glycoprotein and/or serum albumin [13–15]. These attenuation values may be underestimates of the actual effects in vivo, because, for practical reasons, they were measured in 50% human serum rather than in 100% human plasma. These data show that, unless they are corrected for protein binding effects, in vitro measurements of drug potencies may greatly overestimate their activities in vivo.

Plasma drug levels. A second parameter commonly used to predict efficacy is the plasma drug concentration. These measurements usually express total drug levels, without considering the fraction bound by protein. During the dosing interval, drug clearance and metabolism reduce the concentrations of available drug, and the greatest risk of residual viral replication and the selection of resistance occurs during these drug troughs between doses. Therefore, the trough drug level can be used as a conservative indicator of the minimal in vivo drug exposure achieved during the dosing interval; thus, it is useful to examine how the plasma drug concentration (Cmin) compares with its (protein binding–corrected) IC95 for inhibition of virus in vitro.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC95 (nM)</th>
<th>Fold attenuation by 50% human serum</th>
<th>IC95 (nM), corrected for attenuation by 50% human serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaprenavir</td>
<td>92</td>
<td>7</td>
<td>644</td>
</tr>
<tr>
<td>Indinavir</td>
<td>34</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>27</td>
<td>37</td>
<td>999</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>105</td>
<td>20</td>
<td>2100</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>16</td>
<td>26</td>
<td>416</td>
</tr>
</tbody>
</table>

* Determined by ViroLogic (South San Francisco, CA) PhenoSense assay of strain NL4-3.

b Reported in [13]; represents mean of determinations for strains HXB2, IIIB, and NL4-3.

c Corrected IC95 = IC95 × fold attenuation by 50% human serum.
The mean trough concentrations of HIV-1 protease inhibitors in the most commonly used single-protease-inhibitor dosing regimens are shown in Table 2, along with their protein binding-corrected IC₉₅ values for inhibition of viral replication. It can be seen from the ratios of Cₘᵦ to IC₉₅ that, at trough, all the individual HIV-1 protease inhibitors (except saquinavir) reach concentrations approaching or exceeding their protein binding-adjusted IC₉₅ values, which is consistent with their documented clinical efficacies against wild-type viruses.

However, although most viral replication can be inhibited by that drug level, it is still incomplete. By definition, at the IC₉₅, 5% residual viral replication remains. Because even low-level viral replication ultimately may lead to the emergence of resistance, maximizing drug exposure, particularly the Cₘᵦ, is likely to increase the long-term durability of therapy. Accordingly, it is important to maximize the suppression of viral replication, and this can be achieved by combination therapy.

**Protease inhibitor intensification.** Substantial intensification of HIV-1 protease inhibitors can be accomplished by using them in combination, taking advantage of a favorable pharmacologic interaction that occurs between these drugs. All available HIV-1 protease inhibitors—indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir—are inhibitors (to varying degrees) of the cytochrome P450 3A4 (CYP3A4), which is primarily responsible for their metabolism [21]. Among these, ritonavir is the most potent CYP3A4 inhibitor. In addition, they are all substrates for the multidrug resistance-1 multidrug transporter system [22–24], which mediates their efflux from susceptible cells. This system also has been shown to be inhibited by ritonavir [25]. The net result of these effects is a pronounced increase in the levels (primarily Cₘᵦ) and flattening of the pharmacokinetic curves of the protease inhibitors when they are combined with ritonavir. As shown in Table 3, in the presence of ritonavir, the steady-state 12-h trough levels of saquinavir, amprenavir, or indinavir exceed the protein binding-corrected IC₉₅ values for wild-type virus by 2-fold, 7-fold, or 28–79-fold, respectively. Accordingly, combination therapy with protease inhibitors may achieve far more efficient suppression of replication of wild-type viruses than can be achieved by the individual drugs.

Because these intensified regimens use 2 protease inhibitors, it is useful to compare the relative contributions of the individual drugs to the overall antiviral effect. As can be seen from the corrected Cₘᵦ to IC₉₅ ratios in Table 3, the trough contributions of saquinavir and ritonavir, when used in combination, are comparable (1.7 vs. 1.1). However, in the presence of ritonavir, the contribution of indinavir to the antiviral effect at trough, as measured by the ratio of Cₘᵦ to IC₉₅, exceeds that of ritonavir by 10–66-fold at all dosages examined. On the basis of these same pharmacokinetic studies [27], the ratios of 24-h area under the curve (AUCₜₐₐ) to corrected IC₉₅ for indinavir exceed those for ritonavir by 13–136-fold, and the ratios of maximum concentration (Cₘᵦ) to corrected IC₉₅ for indinavir exceed those for ritonavir by 14–177-fold (data not shown). Thus, on the basis of Cₘᵦ, Cₘᵦ, and AUC, the antiviral effect of indinavir-ritonavir combination therapy appears to be driven primarily by indinavir levels, and the relative antiviral contribution of ritonavir is minimal.

**Overcoming resistance with potency.** The foregoing analyses have considered the activities of protease inhibitors against wild-type viruses. However, protease inhibitor intensification may also be capable of suppressing the replication of resistant viruses.

To assess the virologic potential of this approach, we have examined a diverse panel of 20 protease inhibitor–resistant primary HIV-1 isolates. These were derived from patients in clinical studies of virologic failure during prolonged indinavir therapy.
Interpreters were observed for most of the isolates (figure 1). Levels of phenotypic resistance to the available protease inhibitors are expressed relative to the wild-type IC_{95} for the drug in question, as determined in a standardized test system. By comparison with the wild-type control, substantial suppression of most of these virus variants.

This suggests that the potency of saquinavir-ritonavir combination therapy would be inadequate to control the replication of many protease inhibitor–resistant viruses.

By this analysis, however, the relationships between C_{min} and IC_{95} for either amprenavir-ritonavir or indinavir-ritonavir regimens suggest that either of these combinations would be able to achieve more efficient suppression of many protease inhibitor–resistant viruses. Interestingly, in comparing both types of regimens, this may occur for different reasons. Although the protein binding–corrected amprnavir C_{min} in the presence of ritonavir is calculated to be only ~7 times the amprnavir IC_{95}.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Protease inhibitor therapy</th>
<th>Amino acid differences from clade B consensus protease sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indinavir (36 weeks)</td>
<td>L10V, T12I, L24I, N37D, M46I, L63P, A71V, V82T, I84V</td>
</tr>
<tr>
<td>3</td>
<td>Indinavir (52 weeks)</td>
<td>L10I, K20M, L24I, E35D, M36I, R41K, I54V, L63P, A71V, V82A</td>
</tr>
<tr>
<td>5</td>
<td>Indinavir (60 weeks)</td>
<td>L10I, M46I, L63P, V77I, V82F, I84V, N88T, I93L</td>
</tr>
<tr>
<td>6</td>
<td>Indinavir (48 weeks)</td>
<td>L10I, I12S, I13V, L33I, M36I, I54V, I64V, I84V, L90M</td>
</tr>
<tr>
<td>8</td>
<td>Indinavir</td>
<td>L10R, M46I/L, R57K, L63P, H69R, L90M</td>
</tr>
<tr>
<td>11</td>
<td>Indinavir</td>
<td>K20I, M46I, L63P, A71V, I12E, G73S, V77I, L90M, I93L</td>
</tr>
<tr>
<td>12</td>
<td>Indinavir (44 weeks)</td>
<td>V32I, M46I/L, L63A/P, I64I/M, A71V, I72I/V, V82A</td>
</tr>
<tr>
<td>13</td>
<td>Indinavir (36 weeks)</td>
<td>I13V, V32I, M46I/L, I47V, L63P, I64V, Q92K, I93L</td>
</tr>
<tr>
<td>18</td>
<td>Nelfinavir</td>
<td>I13V, K20I, M46I, Q58E, L63P, A71V, G73S, V77I, L90M</td>
</tr>
<tr>
<td>19</td>
<td>Nelfinavir</td>
<td>I13V, D30N, L63P</td>
</tr>
</tbody>
</table>

NOTE. Genotypes are listed relative to clade B HIV-1 protease consensus sequence [29]. Samples were derived from patients in clinical studies of indinavir monotherapy for indicated periods of time (Merck protocols 004, viruses 1–4, 006, viruses 5–7, 010, viruses 12 and 13, 018, virus 14) or from patients who had experienced prior virologic failure of either indinavir (Merck protocol 008, viruses 8–11) or nelfinavir (075, viruses 15–20) as their first protease inhibitor.
Figure 1. Relationship between IC<sub>95</sub> for protease inhibitor–resistant viruses (table 4) and steady-state protein binding–corrected trough concentrations (C<sub>min</sub>) in commonly used single–protease-inhibitor regimens (determined with ViroLogic [South San Francisco, CA] PhenoSense assay [9]). Data are fold resistance relative to matched wild-type control IC<sub>95</sub> values. Horizontal line, steady-state C<sub>min</sub> reported for each regimen, corrected for attenuation of activity by 50% human serum (table 2). For saquinavir<sub>sgc</sub> (F), line is superimposed over horizontal axis and is not visible. IC<sub>95</sub> values above upper assay range. For those determinations, bar heights represent highest measurable levels of resistance and should be considered minimum estimates. Note that vertical axes are scaled differently for different regimens, to facilitate visualization of data. bid, 2×/day; tid, 3×/day; q8h, every 8 h.

for wild-type virus (table 3), this is offset by the relatively modest reductions in susceptibility to amprenavir manifested by this virus panel, which was derived from virologic failures of indinavir and/or nelfinavir (figure 2C). These results suggest that some cases of resistance to either indinavir or nelfinavir may be salvageable by amprenavir-ritonavir combination therapy. However, because none of these viruses was derived by selection with amprenavir and none carries the 150V amino acid substitution commonly observed in amprenavir failure [31], these data do not address how viruses selected during amprenavir treatment might respond to ritonavir-amprenavir combination therapy.

The data in figure 2 also show that indinavir-ritonavir combination therapy may achieve blood levels of indinavir sufficient to suppress many viruses expressing resistance to indinavir or to other protease inhibitors. Because this panel was derived largely from patients who had developed high-level indinavir resistance mediated by many different mutational combinations, it is noteworthy that any possible bias in selection of viruses for this panel would be expected to mitigate strongly against successful suppression by indinavir. Nonetheless, at either 400 mg of indinavir and 400 mg ritonavir twice daily (figure 2D) or 800 mg of indinavir and 100 mg of ritonavir twice daily (figure 2F), indinavir 12-h trough levels exceed the corrected IC<sub>95</sub> values for about half the resistant variants tested, and at 800 mg of indinavir and 200 mg of ritonavir twice daily the 12-h trough level of indinavir exceeds the IC<sub>95</sub> values of at least 18 of the 20 protease inhibitor–resistant viruses tested (figure 2G). In contrast, even at the highest ritonavir dosage combined with indinavir (400 mg of indinavir and 400 mg of ritonavir twice daily), the ritonavir 12-h trough level remains well below its IC<sub>95</sub> for 19 of these 20 virus variants (figure 2E). This suggests that the indinavir exposure achievable by indinavir-ritonavir combination therapy may be capable of suppressing many viruses that are resistant to protease inhibitors, including those that were selected by and exhibit high-level resistance to indinavir.

Discussion

These analyses illustrate that a meaningful assessment of HIV-1 resistance to antiviral therapies must consider not only
Figure 2. Relationship between IC₉₅ for protease inhibitor–resistant viruses (table 4) and steady-state protein binding–corrected trough concentrations (Cmin) in commonly used dual–protease-inhibitor regimens (determined with ViroLogic [South San Francisco, CA] PhenoSense assay [9]). Data are fold resistance relative to matched wild-type control IC₉₅ values. Horizontal line, steady-state Cmin reported for each regimen, corrected for attenuation of activity by 50% human serum (table 3). ≥ IC₉₅ values above upper assay range. For those determinations, bar heights represent highest measurable levels of resistance and should be considered minimum estimates. Note that vertical axes are scaled differently for different regimens, to facilitate visualization of data. bid, 2×/day.

the potency of that therapy in vitro but also the drug exposure that can be achieved in vivo. In particular, by its very definition, resistance to existing antiretroviral therapies is a continuum, rather than a discrete threshold phenomenon. This, in turn, predicts that drug exposure is the primary determinant of virologic response to therapy, and it provides a firm foundation for the concept of therapeutic intensification.

Furthermore, these observations also make clear and testable predictions about the relationship between the results of genotypic or phenotypic drug resistance tests and virologic responses to therapy. Marginally effective antiviral therapies, because of limitations of either drug potency, exposure, or both, would be expected to be compromised easily by even small reductions in viral drug susceptibility. Because individual amino acid substitu-

tions generally make relatively small (<2–10-fold) contributions to protease inhibitor resistance, it follows that the effects of such substitutions will be most pronounced when small phenotypic shifts are sufficient to compromise the antiviral effect of therapy. Accordingly, it is not surprising that the failure of saquinavir-ritonavir salvage therapy has been associated with small shifts in viral susceptibility [32] and that an assessment of genotypic changes may facilitate the prediction of virologic responses to saquinavir-ritonavir salvage therapies [33].

However, the data presented here also suggest that, for more potent regimens, this relationship may not apply. If drug exposure greatly and consistently exceeds the IC₉₅ of the virus in question, viruses manifesting significant drug resistance may still be suppressed, and only in the case of very high-level re-
sistance would the efficacy of therapy be expected to be compromised. Because of their quantitative nature, phenotypic assays may be able to determine the extent to which $C_{\text{min}}$ exceeds viral IC$_{50}$, as we have shown here. However, because of the relatively small contributions of individual mutations toward phenotypic resistance to indinavir and the still-obscure relationship between genotype and phenotype, genotypic resistance to indinavir may not predict virologic failure of indinavir-ritonavir therapy. The viruses used in our test panel had been chosen on the basis of the presence of multiple resistance-associated substitutions. Despite these many substitutions (table 4) and significant phenotypic resistance to multiple protease inhibitors (figure 1), indinavir or amprenavir exposures in the presence of low-level ritonavir are predicted to exceed the IC$_{50}$ values for most, if not all, of the protease inhibitor-resistant viruses tested.

Whereas our analysis is based on virologic considerations, these predictions are directly amenable to clinical testing. Although no data from prospective clinical salvage trials of indinavir-ritonavir therapy are yet available, the results of 2 retrospective studies of this regimen in salvage therapy for patients experiencing protease inhibitor failure have been reported recently. In both studies, favorable virologic responses to indinavir-ritonavir–based therapy were observed in most patients who had previously experienced failure of multiple protease inhibitor–containing regimens [34, 35], despite clear baseline genotypic and high-level phenotypic (≥35-fold) resistance to indinavir and ritonavir [34]. Therefore, the available clinical data support the interpretation that increased indinavir exposure may be sufficient to overcome many cases of indinavir resistance.

The foregoing analyses have examined the relationship between drug exposure and antiviral efficacy. Although we have considered in vitro viral drug susceptibilities in light of protein binding and mean trough blood levels achieved by different regimens, these results are still rough approximations of the effects that would be expected in vivo.

We have not attempted to consider the issues of safety or tolerability of these intensified regimens, and clinical experience must provide the ultimate test of their practical utility. Neither have we considered the issue of interpatient variability in protease inhibitor blood levels [36]. Because of this variability, virologic responses of individual patients to the therapeutic regimens described may be greater or less than suggested by the values described here. In this regard, the variability of indinavir levels has been reported to be reduced substantially by the coadministration of ritonavir [37].

Furthermore, our analyses do not consider the relative merits of comparing peak ($C_{\text{max}}$) drug levels or integrals of drug levels over time (AUC) as possible alternatives to $C_{\text{min}}$ as indices of drug exposure. In the present analysis, we judged the $C_{\text{min}}$ to be the most conservative of the 3 measures and the parameter most likely to be associated with the risk of virologic breakthrough during the dosing interval.

Our estimates of in vivo drug exposures and therapeutic efficacies could be improved by considering other factors not addressed here, including the distribution of virus and virus-susceptible cells in different compartments and differences in drug distribution, protein binding, and pharmacokinetics in those compartments, as well as viral drug susceptibility, host cell tropism, replication kinetics, and pathogenicity. However, these parameters are difficult to quantify with available data, and no straightforward method currently exists to apply such measurements to the analyses presented here. Nonetheless, as our understanding of HIV-1 disease and its treatment improve, we will be able to apply more sophisticated methods to predict more accurately how individual patients may respond to alternative strategies of antiretroviral therapy.

References


