Pharmacodynamics of Human Immunodeficiency Virus Type 1 Protease Inhibitors

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Many factors are involved in the success or failure of antiretroviral therapy. Recent data suggest that there are significant differences in drug absorption and disposition for the protease inhibitor class of antiretroviral drugs, and relationships between plasma concentrations and their antiviral effect have been described. Consequently, the issue of whether therapeutic drug monitoring should be employed for patients receiving treatment with these drugs has arisen. Several criteria must be met before a drug is considered a candidate for therapeutic drug monitoring. These criteria include pharmacological, clinical, and analytic components. Although not all the necessary criteria have yet been met, some of these components have been defined, and additional data are being generated. However, prospectively designed clinical trials must be completed to determine if monitoring protease inhibitor plasma concentrations provides additional clinical benefit to the patient.

Heterogeneity in the response to antiretroviral agents has been attributed to pharmacological, virological, immunologic, and behavioral differences among patients. Quantifying the pharmacological contribution to differences in response is an important objective, since between-patient pharmacokinetic variability is uniformly present for all antiretroviral agents and data on dose- or concentration-effect relationships continue to accumulate. For the nucleoside reverse transcriptase inhibitors, establishing relationships between plasma concentrations and antiviral effect has been elusive because measurement of the active moiety, the intracellular triphosphate, is analytically challenging. Data now exist, however, to indicate that the anti-HIV effect can be better explained by intracellular triphosphate concentrations than by plasma concentrations of the parent compound [1, 2]. For further information, the reader is referred to a recent review of nucleoside intracellular phosphorylation [3]. This article will review the pharmacodynamics of HIV type 1 (HIV-1) protease inhibitors and discuss the clinical implications of these data, including consideration of therapeutic drug monitoring (TDM) for this class of drugs. Some of the data presented here are still in abstract form, and results must be viewed as preliminary until the complete study is reported.

General Pharmacodynamic Principles

Pharmacodynamics is the study of drug concentration and effect relationships. Whereas pharmacokinetics describes what the body does to the drug, pharmacodynamics describes what the drug does to the body. The general relationship between pharmacokinetics and pharmacodynamics is depicted in figure 1. Several factors are important with respect to pharmacokinetic and pharmacodynamic relationships. First, a given dose of a drug does not always produce the same blood concentration. Although other factors are involved, this variability in blood concentrations is primarily a result of between-patient differences in drug absorption and clearance. Second, variability in blood concentrations leads to variability in concentrations at the site of action. Consequently, variability at the site of action ultimately contributes to heterogeneity in response to therapy.

The mean (±SD) of various pharmacokinetic parameters for the HIV-1 protease inhibitors are listed in table 1; the magnitude of the SDs illustrates the variability observed with each drug following the same dose [4–9]. Typically, concentration and effect relationships are modeled mathematically. The most common model used is the maximum effect (E_{max}) model, which is derived from classic drug receptor theory. The simplest form of this model is illustrated in figure 2. There are 2 important properties that distinguish this model from a linear one: it predicts the maximum effect a drug can produce, and it predicts no effect when no drug is present. It is clear from figure 2 that as the drug concentration continues to increase, beyond a certain point further increases produce little additional effect.

When drug concentrations from 20% to 80% of the E_{max} are obtained, the concentration-response relationship may appear to be linear. This relationship may occur because the analytical
techniques used to detect low drug concentrations might not be available and high concentrations are avoided to prevent toxicities. In the case of therapy for HIV infection, technology to quantify very low levels of plasma HIV RNA may not have been used or available, which would prevent the absolute magnitude of change from being observed, thereby limiting the data set to the linear range of values.

Confounding Factors in Describing Concentration and Effect Relationships

Crucial to establishing concentration and effect relationships is the ability to quantify drug at the intended site of action (usually assumed to be the plasma) and to relate these concentrations to some marker of clinical efficacy. In current therapy for HIV infection, changes in plasma HIV RNA levels are typically used to assess outcome, although CD4 cells are also a marker of an anti-HIV effect. Because patients are usually receiving multiple drugs to treat HIV infection and to prevent or treat opportunistic infections, several factors that can complicate these relationships need to be addressed. In vitro data suggest that antagonism, additivity, or synergy can exist between combinations of nucleoside reverse transcriptase inhibitors and protease inhibitors [10]. If antagonism exists between classes of drugs, then theoretically the concentration required to produce the $E_{\text{max}}$ would increase, and higher doses would have to be administered. The opposite would occur if additivity or synergy exists between drug classes: lower concentrations would be required to produce the same $E_{\text{max}}$. Clearly, this theory needs to be confirmed in vivo; however, it may imply that if a protease inhibitor monotherapy regimen was designed to maintain plasma concentrations above a certain threshold (e.g., the 95% inhibitory concentration [IC95]), this concentration might be lowered as a result of combination therapy.

Another factor that can complicate concentration and effect relationships is cross-resistance, which has been documented among drugs in the same class [11]. If present, treatment with a second or third protease inhibitor or protease inhibitor combination will probably be less effective [12]. As a result, increased plasma concentrations may be required to produce the same $E_{\text{max}}$, if this $E_{\text{max}}$ can be achieved.

Variable adherence patterns may also make establishing pharmacodynamic relationships difficult. If, for example, a patient misses one-third of their prescribed regimen, they will probably not have the same response to treatment over time as a completely adherent patient. Calculated pharmacokinetic parameters derived from a single visit may be similar between both patients after administration of the same observed dose. However, a variable adherence pattern will likely obscure any concentration and effect relationships over the course of therapy; the pharmacokinetic parameters are similar, but the response is different. For adherent individuals, plasma concentrations of the drug may be much more predictable. Data presented in figure 3 demonstrate plasma concentration-time curves and data on random samples obtained from a patient in one of our ongoing trials; this figure illustrates the predictability of indinavir plasma concentrations over a 72-week period in this patient. Importantly, the low variability for this individual may not be representative of all adherent patients, nor is the magnitude of within-patient pharmacokinetic variability of indinavir representative of all protease inhibitors.

Protease inhibitors are moderately to highly bound to plasma proteins, specifically α1-acid glycoprotein (AAG). The percent of each drug bound to AAG is shown in table 1. Protein binding is an important characteristic among the protease inhibitors, since by pharmacological theory only the free drug fraction is able to elicit its pharmacological action, and the greater the free fraction the better it can distribute into other tissues (such as the CNS). In general, free fractions of drugs >90% bound are susceptible to fluctuations in AAG concentrations; a small increase in the AAG concentration can cause substantial transient decreases in the free drug concentration. This susceptibility to changes in AAG concentrations is important for drugs bound primarily to AAG, since there is a large degree of within-patient variability in AAG concentrations [13]. In addition, because AAG is an acute-phase reactant protein, concomitant disease states such as cirrhosis, cancer, and HIV infection [14] can alter AAG concentrations.

Protein binding must be taken into consideration when determining in vivo inhibitory concentrations (i.e., IC90, IC50, or IC95). For example, if a drug is 98% protein bound and has an in vitro IC50 of 0.10 μM (determined with no AAG present), then the in vivo adjusted IC50 must take into account physiological concentrations of AAG. In this case, the in vitro IC50 of 0.10 μM becomes 5.0 μM in vivo (0.10 divided by 98% free fraction). The impact of protein binding on in vivo inhibitory
concentrations is illustrated in figure 4. A study of this shift in susceptibility as a result of protein binding has been reported for the protease inhibitors [15]. The IC₉₀ of saquinavir (98% bound) in lymphocytes, for example, increases >10-fold when 2 mg/mL AAG is added to the cellular system. The IC₉₀ of indinavir, which is 60% protein bound, increased 3.3-fold.

Additional characteristics to be considered when assessing the in vivo effect of protein binding include multiple binding sites, different binding proteins (e.g., albumin), the binding affinity of the drug to the protein, and whether concentration-dependent binding is present. Clearly, there are still other factors that can affect concentration and effect relationships in vivo. Some of these include baseline plasma HIV RNA levels and CD4 cell counts and initial susceptibility to the drugs.

**Concentration-Effect Relationships for Protease Inhibitors**

**Saquinavir.** Changes in plasma HIV RNA levels have been correlated with systemic concentrations of saquinavir [16]. Monotherapy with high dosages (7200 mg/d) or low dosages (3600 mg/d) of saquinavir was evaluated for 16 HIV-infected protease inhibitor–naive patients over 24 weeks. The 24-h area under the concentration-time curve (AUC₂₄) after 4 weeks of therapy was related to the decrease in viral load from baseline to week 4 (r = .80). From this relationship, a saquinavir AUC₂₄ of 10,000 ng · h/mL would be required to produce the maximum decrease in the plasma HIV RNA level. Although the baseline plasma HIV RNA level was lower in the 20 patients receiving low doses of saquinavir (4.86 vs. 3.21 log₁₀ copies/mL), key resistance mutations developed in 9 patients (45%) in the low-dose group but only 4 (20%) of 20 patients in the high-dose group.

Relationships between saquinavir plasma concentrations and changes in plasma HIV RNA levels have also been explored for patients receiving routine clinical care in an outpatient setting [17]. More than 500 random saquinavir plasma concentration determinations were obtained for 130 patients over a 1.5-year period. Patients were also receiving concomitant nucleoside therapy. Measured saquinavir concentrations were related to an average saquinavir concentration-time profile derived from data for 20 previously studied patients. The ratio of an observed saquinavir concentration to that of the average saquinavir profile was used to represent saquinavir exposure in each patient. The saquinavir concentration ratio was compared with changes in plasma HIV RNA levels at weeks 12, 24, 36, and 48 using linear regression. Results demonstrated a relationship between the saquinavir concentration ratio and a decline in the plasma HIV RNA level at each observation time point (P = .024). The likelihood of maintaining a decline of >2 log₁₀ copies/mL in plasma HIV RNA levels after 48 weeks was significantly greater for patients with estimated saquinavir trough concentrations >50 ng/mL (P = .008).

This finding is consistent with concentration-effect relationships described for HIV-infected children receiving soft-gel capsules of saquinavir with nucleoside agents [18]. Fourteen protease inhibitor–naive children, 3–13 years of age, participated in this evaluation of the pharmacokinetics, safety, and tolerance of saquinavir. The initial saquinavir dosage was 33 mg/kg

**Table 1. Pharmacokinetic and pharmacodynamic parameters of HIV type 1 protease inhibitors.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC₅₀ (µg/mL)</th>
<th>Adult dose</th>
<th>tₘₐₓ (h)</th>
<th>Cₘₐₓ (µg/mL)</th>
<th>Cₘᵢₙ (µg/mL)</th>
<th>AUCₚₜ (µg ⋅ h/mL)</th>
<th>tₜₕ/2 (h)</th>
<th>% PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amprenavir</td>
<td>IC₅₀ 0.006-0.040</td>
<td>1200 mg b.i.d.</td>
<td>1.9 ± 1.0</td>
<td>5.36 ± 3.32</td>
<td>0.28 ± 0.15</td>
<td>18.5 ± 11.7</td>
<td>8.9 ± 1.8</td>
<td>~90</td>
</tr>
<tr>
<td>Indinavir</td>
<td>IC₅₀ 0.015-0.061</td>
<td>800 mg q8h</td>
<td>0.8 ± 0.3</td>
<td>7.75 ± 2.48</td>
<td>0.15 ± 0.11</td>
<td>18.8 ± 7.0</td>
<td>1.8 ± 0.4</td>
<td>~60</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>IC₅₀ 0.004-0.111; ICₐₕ 1.0b</td>
<td>750 mg t.i.d.</td>
<td>2.8 ± 1.0</td>
<td>2.9 ± 1.3</td>
<td>1.3 ± 0.7</td>
<td>16.3 ± 7.7</td>
<td>4.0 ± 0.6</td>
<td>~98</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>IC₅₀ 0.003-0.110; ICₐₕ 2.1b</td>
<td>600 mg b.i.d.</td>
<td>3.3 ± 2.2</td>
<td>11.2 ± 3.6</td>
<td>3.03 ± 2.13</td>
<td>60.8 ± 23.4</td>
<td>4.0 ± 1.0</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>IC₅₀ 0.003-0.054</td>
<td>1200 mg t.i.d.</td>
<td>2.5 ± 0.5</td>
<td>2.5 ± 1.9</td>
<td>0.16 ± 0.16</td>
<td>7.2 ± 6.2</td>
<td>ND</td>
<td>~97</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean or mean ± SD unless indicated otherwise. AUCₚₜ, area under the curve for the dosing interval specified under adult dose; Cₘₐₓ, maximum concentration of drug; Cₘᵢₙ, minimum concentration of drug; IC₅₀, concentration of drug needed to inhibit 50% of viral replication in vitro; % PB, percentage of drug bound to plasma protein (primarily α₁-acid glycoprotein); ND, no data available; tₜₕ/2, time to reach maximum concentration after drug administration; tₘₐₓ, elimination half-life.

a Cₘᵢₙ is commonly considered to occur immediately before administration of the next dose; however, the presence of an absorption lag phase may result in minimum concentrations actually occurring after the dose is given. This phenomenon has been observed with nelfinavir.

b Adjusted for plasma protein binding in vitro.
Figure 3. Indinavir concentration-time profiles. Serial indinavir concentrations were measured at week 2 ( ), week 30 ( ), and week 56 ( ) after administration of an observed dose to an HIV-infected patient in one of our ongoing clinical trials. Circles ( ) indicate single measured concentrations at weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 60, 64, 68, and 72 that were determined by using patient-reported information at the time of the last dose. Each sample was obtained at approximately the same time after administration of the dose. The consistency of the serial indinavir concentrations and clustering of the single time points provide evidence of low within-patient variability in indinavir concentrations in this case. The clustered single time points also indicate the ability of a single, timed sample to provide pharmacokinetic and adherence information.

3 times daily; subsequently, the dose was changed as necessary to achieve a saquinavir AUC₄₈ > 10,000 ng · h/mL. The change in the plasma HIV RNA level from baseline to week 4 was related to the saquinavir concentration 8 h after administration of the dose (C₈h, trough). Children with a C₈h < 50 ng/mL had an average drop of 1.7 log₁₀ copies/mL in plasma HIV RNA levels compared with an average decrease of 2.3 log₁₀ copies/mL in those with trough concentrations > 50 ng/mL.

Exposure-response relationships for saquinavir, zidovudine, and zalcitabine in combination were evaluated in AIDS Clinical Trials Group Study 229 [19]. Relationships between changes in CD4 cell counts, peripheral blood mononuclear cell titer, and plasma HIV RNA levels were compared with saquinavir concentrations for ~200 patients receiving treatment with saquinavir (Invirase; Roche Laboratories, Nutley, NJ; 600 mg 3 times daily) with zidovudine and/or zalcitabine. Linear regression of saquinavir concentrations on the 3 covariates demonstrated 2 important characteristics: first, the change in CD4 cell counts and plasma HIV RNA levels was significantly related to saquinavir concentrations, and second, a clear trend toward synergy between the different classes of drugs was apparent in the treatment arm containing all 3 compounds.

**Ritonavir.** An initial safety and efficacy study evaluated 4 ritonavir regimens over 32 weeks for 84 patients previously naive to protease inhibitor therapy [4]. The regimens included a placebo group and twice-daily dosing of ritonavir mono-therapy at 300, 400, 500, and 600 mg. Pharmacokinetic evaluations were completed in each group after 3 weeks of treatment for 48 patients; trough concentrations were also determined at random time points during the study. The 500-mg and 600-mg dose groups had a more pronounced and sustained decrease in plasma HIV RNA levels through the course of study. Although all groups had initial declines in plasma HIV RNA levels, only the subgroups that maintained trough concentrations above the IC₉₀ (2.1 µg/mL) had more durable increases in CD4 cell counts and decreases in plasma HIV RNA levels.

A correlation between the number of in vivo drug-resistant mutations and ritonavir plasma concentrations has been observed [20]. Thirteen patients with > 4 available viral sequences determined over an 8-week period were evaluated. Patients received ritonavir monotherapy (600–1200 mg daily, divided into 2, 3, or 4 doses). Pharmacokinetic evaluations were performed at day 21 of therapy. An estimate of the in vivo mutation selection rate for each patient was determined by plotting the number of consensus resistance mutations for each sequence vs. time. The mutation selection rate was inversely related to both ritonavir trough concentrations (coefficient of determination [R²] = 0.58) and AUC₁₂ (R² = 0.51). The dose of ritonavir, however, did not correlate with the mutation selection rate. It is interesting that several patients receiving 600 mg of ritonavir twice daily had lower trough values and a higher selection rate than did 3 patients receiving 500 mg twice daily, and no consensus mutations emerged in the patient with the highest trough concentration.

A multiple-dose pharmacokinetic study of ritonavir including 46 HIV-infected patients provides evidence for relationships between ritonavir concentrations and adverse effects [21]. Patients received ritonavir monotherapy at 200, 300, 400, or 500 mg every 12 h and underwent pharmacokinetic evaluations on days 1 and 17 of treatment. An increase in ritonavir concentrations was observed that was greater than the dose propor-

![Increasing total drug concentration](image-url)
tional, confirming the nonlinear disposition of the drug. Plasma triglyceride concentrations were also obtained throughout the study, and relationships between the triglyceride concentrations and various pharmacokinetic parameters (AUC, maximum concentration of drug \(C_{\text{max}}\), and minimum concentration of drug \(C_{\text{min}}\)) were evaluated by means of analysis of variance. The increase in triglyceride levels was found to be dose-dependent; statistically significant trends with dose were observed during the 2-week dosing period. All 3 pharmacokinetic parameters were also correlated with triglyceride changes \((R^2, 0.28–0.45; \text{significance not stated})\). A relationship between gastrointestinal and/or neurological side effects and high plasma concentrations of ritonavir has been shown for a group of 15 patients [22]. Patients with severe side effects had a higher plasma concentrations of ritonavir has been shown for a group of 15 patients [22]. Patients with severe side effects had a higher mean \(C_{\text{max}}\) \((P < .02)\) and a longer mean time to reach maximum concentration after drug administration \((t_{\text{max}}; P < .02)\) than did patients without these complaints.

**Indinavir.** The initial dose-ranging indinavir trial investigated the safety, tolerability, and activity of 2 indinavir regimens, 200 mg every 6 h (800 mg/d; 23 patients) and 400 mg every 6 h (1600 mg/d; 25 patients) [5]. Both indinavir treatment arms were compared with zidovudine (200 mg every 8 h; 25 patients); all patients were naive to antiretroviral therapy. For patients in the indinavir treatment arms, the dose was increased to 600 mg every 6 h (2400 mg/d) at some point between week 12 and week 16. All regimens resulted in decreased plasma HIV RNA levels over the 24-week study period, with the greatest average drop \((0.79 \log_{10} \text{copies/mL})\) in the group that received 400 mg of indinavir every 6 h. The antiretroviral effect of indinavir in this study was dose-related, and dosages of <2400 mg/d were deemed suboptimal in comparison with the results of other studies. Suboptimal dosing led to a loss of antiretroviral effect over the 24 weeks of study and resulted in the development of increased viral resistance. In addition, the antiretroviral effect for patients initially treated with <2400 mg/d whose dosages were increased to 2400 mg/d was not the same as that for those patients initially treated with 2400 mg/d.

Indinavir concentrations were prospectively determined at steady state for 95 patients treated with 800 mg 3 times daily with concomitant nucleoside therapy [23]. Random postdose samples were collected; no correlation was observed between measured indinavir concentrations and changes in plasma HIV RNA levels or increases in bilirubin levels. Low concentrations, however, did retrospectively confirm poor adherence to the regimen. Although no correlation was observed for these patients, the results do not preclude the existence of a concentration-effect relationship. Collecting blood samples at various times after drug intake from different patients and attempting to relate the resultant drug concentrations to an effect is not likely to reveal any underlying relationship. If random but timed samples are collected, then a pharmacokinetic model should be imposed on the data to determine the pharmacokinetic parameters. Relationships between those pharmacokinetic parameters and measures of effect can then be sought.

Pooled concentration data collected for patients receiving indinavir monotherapy (1800–3200 mg/d) during phase I and II studies were compared with changes in plasma HIV RNA levels at week 4 and/ or week 24 of therapy [24]. The median plasma HIV RNA level at baseline for 95 eligible patients was 4.87 \(\log_{10}\) copies/mL. No significant correlations were found between changes in the plasma HIV RNA level and daily AUC (\(\text{AUC}_{24}\)) or \(C_{8h}\) at week 4 or week 24. All linear correlation coefficients were between –0.15 and 0.1 \((P = .15)\). In addition, any differences between \(\text{AUC}_{24}\) and \(C_{8h}\) were not distinguishable between those patients with plasma HIV RNA levels of >500 copies/mL and those with levels of <500 copies/mL. As discussed previously, relationships between drug concentrations and anti-HIV effects are inherently nonlinear. Relationships between AUC and \(C_{8h}\) and changes in the plasma HIV RNA level at week 4 may have been observed in this study if a nonlinear model were applied to the data set (such as that depicted in figure 2). Furthermore, these data were derived from monotherapy studies, and resistance has been shown to develop with indinavir monotherapy over a 24-week period, especially when this agent is administered suboptimally [25]. Finally, variable adherence to the study regimens may also contribute to the development of resistance. Therefore, the antiviral effect at week 24 is confounded by varying degrees of antiviral resistance among the study population, which will obscure any effect relationship.

Several investigations have found relationships between indinavir concentrations and effect. Indinavir plasma concentrations have previously been correlated with changes in plasma HIV RNA levels in a small cohort of 5 patients [26]. Changes in plasma HIV RNA levels from baseline were significantly related to the 6-h AUC (\(\text{AUC}_6\)) and the 6-h minimum concentration (\(C_{6h}\)). For example, response to indinavir therapy for these individuals varied from no measurable effect at a \(C_{6h}\) of 0.2 \(\mu\)M and an \(\text{AUC}_6\) of 16 \(\mu\)M · h to the \(E_{\max}\) at a \(C_{6h}\) of 0.4 \(\mu\)M and an \(\text{AUC}_6\) of 20 \(\mu\)M · h.

Two investigations have studied the combination of indinavir and nevirapine with a concomitant nucleoside regimen. When administered prospectively in combination with nevirapine and lamivudine, indinavir trough concentrations were related significantly to the cumulative treatment effect over 24 weeks (plasma HIV RNA normalized area under the curve [\(\text{NAUC}\)] [27]). \(\text{NAUC}\) is derived by dividing the AUC of the marker (plasma HIV RNA) by the baseline value multiplied by time. In this regard, \(\text{NAUC} > 1\) indicates a net increase in the marker over time; \(\text{NAUC} < 1\) signifies a net decrease, and \(\text{NAUC}\) equal to 1 indicates no change. In this study, 17 patients underwent peak-trough pharmacokinetic evaluations after 6 weeks of therapy; standard dosing was used for all 3 compounds. No correlation was found between nevirapine pharmacokinetic parameters or with the indinavir peak concentration. Indinavir
trough concentrations, however, were correlated with the 24-week cumulative antiviral effect (r = .53; P = .03).

The second study evaluated indinavir in combination with nevirapine and concomitant nucleoside analogues as treatment of 24 asymptomatic HIV-infected individuals [28]. The first 7 days of treatment were with indinavir monotherapy, and then nevirapine was added to the regimen for the duration of the study; samples for pharmacokinetic evaluations were obtained on days 7 and 36 (with nevirapine). Both the indinavir AUC<sub>8h</sub> (r = .58; P = .02) and C<sub>8h</sub> (r = .56; P = .03) were significantly related to decreases in plasma HIV RNA levels after 7 days of therapy. For 19 patients who completed the 36 days of treatment, nevirapine therapy reduced the indinavir C<sub>max</sub>, by 11%, the C<sub>8h</sub> by 38% (P = .001), and the AUC<sub>8</sub> by 28% (P < .001). Results from these studies indicate that establishing concentration and effect relationships for a particular drug is feasible during monotherapy or combination therapy; concomitant antiretroviral therapy does not necessarily mask underlying pharmacodynamic relationships for a particular drug in a given drug combination.

A single-center study designed to assess the between-patient variability of indinavir pharmacokinetics and to explore concentration-effect relationships has been completed [29]. Forty-three patients underwent a formal indinavir pharmacokinetic evaluation. Twenty-three of these patients were receiving indinavir (with concomitant nucleoside agents) as their first protease inhibitor; at the time of evaluation, 14 had undetectable plasma HIV RNA levels (<500 copies/mL), and 9 had detectable plasma HIV RNA levels. Significant variability in indinavir systemic exposure was observed between patients who received the same dose; the AUC<sub>8h</sub> ranged from 5.4 to 67.9 μM·h. The indinavir AUC<sub>8</sub> was statistically higher for patients with undetectable plasma HIV RNA levels (31.2 μM·h) than for those with detectable plasma HIV RNA levels (21.5 μM·h; P = .035). Similarly, the indinavir C<sub>8h</sub> was significantly higher in the group with undetectable levels than in the group with detectable levels (0.55 μM vs. 0.07 μM, respectively; P = .007).

Low plasma concentrations of indinavir as a cause of virological treatment failure have been prospectively evaluated for 65 patients [30]. Outpatients prescribed treatment with 800 mg of indinavir 3 times daily were included; 35% of patients were pretreated with a different protease inhibitor. Virological treatment failure was defined as a plasma HIV RNA level >200 copies/mL after 24 weeks of treatment. Multivariate regression analysis demonstrated that a high plasma HIV RNA level at baseline, pretreatment with a protease inhibitor, and low indinavir plasma concentrations (<75% of the average concentration following the same dose) were significant factors predicting treatment failure.

Relationships between indinavir plasma concentrations and adverse effects have recently been demonstrated [31]. Fifteen patients with urologic complaints (flank pain, renal colic, dysuria, or hematuria) during standard indinavir therapy were included. Concentrations for these patients were compared with concentrations for a control group (14 patients). Plasma samples for determination of indinavir concentrations were randomly obtained 1.5–8 h after dosing from patients presenting with symptoms. The difference in indinavir concentrations between groups was expressed as a ratio. The ratios ranged from 0.55 to 11.5, and concentrations for all but one patient were above the mean concentration for the control group. Indinavir concentrations for 12 (80%) of the patients were above the upper 95% confidence limit for the patients. These results suggest that elevated indinavir plasma concentrations may be a causative factor in the development of urologic symptoms.

**Nelfinavir.** Dose-response relationships have been demonstrated for nelfinavir. In a phase III study, 65 patients naive to protease inhibitor therapy received nelfinavir monotherapy in dosages of 500, 600, or 750 mg twice daily or 500, 750, or 1000 mg 3 times daily [6]. Ten patients completed each treatment arm, except the 750-mg twice-daily group, which had 15 patients. After 2 weeks of treatment, plasma HIV RNA levels in those patients assigned to the twice-daily regimens gradually returned toward baseline, whereas virus suppression in the 3-times-daily groups was better maintained. Fifty percent of patients in the 3-times-daily group had plasma HIV RNA levels of <500 copies/mL at day 28, compared with just 26% of the twice-daily groups (P = .043). In addition, at day 28, 60% of the patients in the 750-mg and 1000-mg 3-times-daily groups had plasma HIV RNA levels <500 copies/mL, compared with only 30% in the 500-mg 3-times-daily group (significance not stated).

Information on nelfinavir concentration and effect relationships have recently emerged. Results from a phase III study were analyzed to explore relationships between nelfinavir exposure and response [32]. The regimens included zidovudine plus lamivudine plus either 500 or 750 mg of nelfinavir 3 times daily. Response to treatment was defined as an undetectable plasma HIV RNA level (at both 400 and 50 copies/mL) after 48 weeks of treatment. Nelfinavir plasma concentrations were obtained before dosing (broadly defined as 6–12 h after the previous nelfinavir dose) and 2 h after dosing (within 4 h after the current dose). A stepwise multivariate regression analysis was used to identify significant covariates. The probability of maintaining a plasma HIV RNA level <50 copies/mL at 48 weeks was significantly dependent on the baseline plasma HIV RNA level, as well as the predose nelfinavir concentration (P = .035; 79 patients) and the 2-h postdose nelfinavir concentration (P = .002; 115 patients). The predose and 2-h postdose concentrations of the nelfinavir metabolite M8 were not significant predictors of response.

**Amprenavir.** The pharmacokinetics of amprenavir monotherapy have been evaluated in a cohort of 22 patients [33]. Amprenavir was administered to 4 groups of patients at dosages of 300 mg every 12 h, 300 mg every 8 h, 900 mg every 12 h,
and 1200 mg every 12 h. Plasma HIV RNA levels were determined at baseline and 5 times during the subsequent 4 weeks of therapy. Measures of drug exposure were linked to changes in viral load by using an $E_{\text{max}}$ model. $C_{\text{max}}$, AUC, and $C_{\text{min}}$ were the steady-state pharmacokinetic parameters used, and significant relationships were observed for all 3 parameters ($R^2$, 0.35, 0.54, and 0.6, respectively; $P < .05$ for all). In addition, amprenavir susceptibilities (IC$_{50}$) for viruses recovered from a subset of 10 patients were determined. When the $C_{\text{min}}$ was normalized to the IC$_{50}$ ($C_{\text{min}}$/IC$_{50}$), the relationship with the plasma HIV RNA level improved ($R^2$, 0.35 vs. 0.50, respectively). The investigators concluded that baseline susceptibility is important in determining the magnitude of change in the plasma HIV RNA level following exposure to amprenavir.

**Clinical Implications**

Available data suggest that the protease inhibitors exhibit significant between-patient plasma concentration variability. They also exhibit within-patient variability, due in part to non-adherence with rather strict drug regimens. Compelling data are accumulating that provide evidence for concentration and effect relationships for protease inhibitors; there are also data showing that variability in plasma concentrations contributes to variability in therapeutic response. Therefore, the existence of data that support a pharmacological contribution to therapeutic success or failure allows the question of whether protease inhibitors are suitable candidates for TDM.

TDM is a concept that has been applied to drugs used for the treatment of disease states such as epilepsy, cardiovascular therapy, immunosuppressive therapy, treatment of asthma, and antimicrobial treatment. TDM, also known as applied or clinical pharmacokinetics, uses drug concentrations, pharmacokinetics, and pharmacodynamics to individualize and optimize drug therapy. Probably the most important characteristic of a candidate drug is its established therapeutic range. The therapeutic range is a probabilistic concept and not an absolute; it is a range of concentrations within which the probability of an efficacious response is greater than the probability of unwanted toxicity. Accordingly, drug concentration information cannot be used alone in guiding therapy, and other clinical data must be considered. The characteristics common among drugs considered to be candidates for TDM are listed in table 2 [36].

Taken together, the data presented indicate that concentration and effect relationships exist for HIV-1 protease inhibitors. In particular, the AUC and $C_{\text{min}}$ appear to be most closely related to changes in plasma HIV RNA levels. The potential clinical significance of not maintaining adequate plasma concentrations has recently been exemplified (PRNewswire, New York, NY, 18 September 1998). Indinavir, when administered as 1200 mg twice daily, produced virological results compared with 800 mg every 8 h (64% vs. 91% of patients, respectively, had plasma HIV RNA levels <400 copies/mL at 24 weeks). Additional criteria, however, must be met before TDM of this

**Combination Protease Inhibitor Therapy**

Drug exposure and effect relationships have been observed in patients receiving 600 mg of saquinavir twice daily with 600 mg of ritonavir twice daily [34]. All patients were protease inhibitor-naive; patients were classified as responders or nonresponders according to whether their plasma HIV RNA level had decreased by 1 log$_{10}$ copies/mL after 5 weeks of therapy. The study duration was 16 weeks, and most of the patients had previously received extensive nucleoside reverse transcriptase inhibitor therapy (median duration, 35 months). Plasma concentration data for 16 patients (11 responders and 5 nonresponders) were evaluable. Plasma samples were obtained from 10 patients before dosing and 1, 2, and 4 h after dosing. Random samples were obtained from 7 patients. Responders had significantly higher median steady-state plasma concentrations of both ritonavir and saquinavir ($P = .04$ and $P < .01$, respectively). The median ritonavir concentration for the responders was 5.6 mg/L and 2.6 mg/L for the nonresponders. Median saquinavir concentrations were 1.5 mg/L for the responders and 0.4 mg/L for the nonresponders.

Twenty-nine patients naive to antiretroviral therapy who were treated with nelfinavir (750 mg 3 times daily), saquinavir (600 mg 3 times daily), stavudine (40 mg twice daily), and lamivudine (150 mg twice daily) had plasma HIV RNA levels and protease inhibitor concentrations determined at weeks 0, 1, 2, 4, and 8 after therapy [35]. The slope of the initial viral decay curve (from baseline to week 2) was related to the median nelfinavir and saquinavir concentration ratio for each patient that was determined at the 5 visits. The concentration ratio was obtained by comparing each measured concentration with a predicted value derived from data for a reference population previously studied. Concentration ratios were compared with the slope of the curve of the decline in the plasma HIV RNA level by using univariate and multivariate regression analyses. Univariate regression indicated that both saquinavir and nelfinavir concentration ratios were positively related to the slope of the curve of the decline in the plasma HIV RNA level ($P = .001$ and .016, respectively). However, multiple regression revealed that only the nelfinavir concentration ratio was a significant variable ($P = .01$). Therefore, the higher the measured nelfinavir concentration (relative to an average concentration-time profile), the greater the initial decline in the plasma HIV RNA level.

Results from these 2 studies suggest that systemic exposure to protease inhibitors, even when administered in combination, may play an important role in achieving a specific antiviral response. These results also suggest that establishing concentration-effect relationships may be feasible even when 2 compounds with similar mechanisms of action are administered concomitantly.
Table 2. Characteristics of drugs applicable to therapeutic drug monitoring.

<table>
<thead>
<tr>
<th>Pharmacological</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetic data concerning the drug are available</td>
<td>Clinical studies have documented the therapeutic range of the drug</td>
</tr>
<tr>
<td>Plasma concentration of the drug reflects the concentration at the site of action</td>
<td>Significant between-patient variability in drug absorption and disposition</td>
</tr>
<tr>
<td>Narrow range between therapeutic and toxic concentrations</td>
<td>Lack of an effect may be detrimental to the patient</td>
</tr>
<tr>
<td>Pharmacological effect persists for a relatively long period</td>
<td>Clinical studies have documented the therapeutic range of the drug</td>
</tr>
<tr>
<td>Pharmacological effect is related to the drug concentration</td>
<td>Significant between-patient variability in drug absorption and disposition</td>
</tr>
<tr>
<td>Analytical</td>
<td>Lack of an effect may be detrimental to the patient</td>
</tr>
<tr>
<td>A drug assay is available</td>
<td>Clinical studies have documented the therapeutic range of the drug</td>
</tr>
<tr>
<td>The assay has acceptable accuracy and precision with high specificity</td>
<td>Significant between-patient variability in drug absorption and disposition</td>
</tr>
<tr>
<td>Analysis time is short, required sample volume is small, and cost is minimal</td>
<td>Lack of an effect may be detrimental to the patient</td>
</tr>
</tbody>
</table>

class of drugs becomes incorporated into routine patient care. Of the characteristics listed in table 2, protease inhibitors have several: pharmacokinetic data are available, significant between-patient variability exists in plasma concentrations following the same dose, concentration-effect relationships are present, lack of effect (or toxicity) is detrimental to the patient, and analytical techniques are available. Also important to the protease inhibitors is that the observed effect cannot be rapidly evaluated, and by the time the effect occurs or the lack of effect is observed, resistant isolates may have already developed. The therapeutic concentration for each drug (and possibly each drug combination) has yet to be defined.

Clearly, there are other confounding factors that must also be understood, including additive or synergy between classes of drugs, cross-resistance among classes of drugs, and relationships between adherence and drug exposure. The use of protease inhibitor susceptibility testing for viruses, similar to antibiotic susceptibility testing for bacteria, also needs to be explored. This information may enhance the clinical utility of using pharmacokinetic parameters to increase efficacy. Most importantly, well-designed prospective clinical trials must demonstrate that monitoring protease inhibitors to achieve a desired target plasma concentration provides clinical benefit through the reduction of between-patient pharmacokinetic variability, prevention of unwanted toxicities, and/or improved therapeutic response.

Perspectives

The introduction of HIV-1 protease inhibitors has contributed enormously to the delay in disease progression and death in HIV-infected persons [37]. Unfortunately, not all patients have an optimal, uniform response to treatment. TDM seeks to increase the probability of an optimum response for all patients. However, definitive studies evaluating TDM of protease inhibitors for the treatment of HIV infection are not available. Therefore, TDM cannot be recommended for routine clinical care at this time. Nonetheless, monitoring protease inhibitor concentrations may play an important role in other aspects of patient management, such as evaluating drug-drug interactions and perhaps periodically assessing adherence. Prospectively designed studies to determine a role for TDM are necessary and important to continue to advance the considerable progress made in pharmacotherapy for HIV infection.

References


