Perspective

Advances in HIV Pharmacology: Protein Binding, Pharmacogenomics, and Therapeutic Drug Monitoring

Developing better antiretroviral drugs, individualizing therapy through patient genetic profiling, and maintaining effective drug concentrations with therapeutic drug monitoring (TDM) represent 3 current areas of interest in the field of HIV pharmacology. This article first examines antiretroviral drug binding to plasma proteins, a factor that affects the amount of free drug available to enter cells. Protein binding influences drug development, raising questions about whether the drug levels required for appropriate therapeutic effect can be achieved at tolerable doses. Second, individualized antiretroviral therapy has generated considerable interest, but much work remains in the area of pharmacogenomics before this strategy finds a place in clinical practice. Finally, studies are mixed on the benefits of TDM; although such monitoring may be appropriate in some settings, such as pregnancy and pediatrics, data are currently lacking to support its routine use in HIV care. Although data on these pharmacologic strategies do not currently support their widespread clinical application, ongoing research of such strategies offers hope for future improvement of the efficacy of antiretroviral therapy. This article summarizes a presentation given by Charles W. Flexner, MD, at the November 2002 International AIDS Society–USA course in San Diego.

Developing antiretroviral therapy that is more potent, safer, and better tolerated by patients requires consideration of several factors, including drug binding to plasma proteins, which affects the amount of free drug available in the body. Individualizing drug therapy through patient genetic profiling and therapeutic drug monitoring (TDM) are also areas of interest in HIV pharmacology. Each of these topics is discussed below.

Plasma Protein Binding

Antiretroviral drugs differ in the degree to which they are bound to plasma proteins. Plasma protein binding is a concern in drug development because, in general, only free drug can penetrate cells or tissues and exert its therapeutic effect. In the case of antiretroviral drugs, any factor that reduces free-drug concentrations could in theory reduce drug activity and thus promote HIV resistance.

For the most part, studies of plasma protein binding assess binding to alpha$_1$-acid glycoprotein (AAG) or albumin. AAG, which accounts for only about 1% to 3% of plasma proteins, binds drug molecules with low capacity but high affinity, with the latter characteristic making dissociation of the drug molecule from AAG more difficult than from albumin. Although albumin is a major protein component of plasma, it is a high-capacity but low-affinity binder. Studies of protease inhibitors (PIs) that test how much the inherent fluorescence of AAG is quenched by binding to drug molecules have shown a wide range of drug binding affinities for AAG (Bakker et al, 12th World AIDS Conf, 1998). Of PIs tested in these studies, indinavir had the lowest affinity for AAG, with an equilibrium association constant of less than 1×10^{1} M^{-1}, followed by ritonavir at 1×10^{4} M^{-1}, nelfinavir at 2×10^{5} M^{-1}, saquinavir at 8×10^{5} M^{-1}, and the investigational drug SC-52151 at 2×10^{6} M^{-1}. SC-52151 thus had a binding affinity approximately 2 million times greater than indinavir and approximately 200 times greater than ritonavir. The potential effect of greater binding affinity on activity against HIV is indicated by studies showing that the 50% inhibitory concentrations (IC_{50}) of PIs in the presence of AAG in vitro were correlated with reported binding affinities: those drugs with higher AAG binding affinity showed less potent inhibition in the presence of that protein (Lazdins et al, *J Infect Dis,* 1997; Zhang et al, *J Infect Dis,* 1999). Albumin also was found to increase PI IC_{90} values, but to a lesser degree than AAG (Molla, *Virology,* 1998).

Given the inverse correlation between binding affinity and drug activity in vitro, the question is whether protein binding affects in vivo performance of highly protein-bound drugs. There are some direct consequences of protein binding in terms of clinical use. For example, cerebrospinal fluid concentrations of many highly protein-bound drugs, including highly bound PIs, correlate better with plasma concentrations of free drug than with total drug plasma concentrations. For a few highly protein-bound drugs, such as phenytoin, procainamide, and lidocaine, free drug concentration correlates better with activity than does total plasma concentration. However, for a number of reasons, plasma protein binding does not generally need to be compensated for in clinical use. These reasons include the fact that many drugs can be dosed high enough to achieve therapeutic levels of free drug even if they are highly protein-bound, and that drug concentrations are significantly affected by other aspects of drug pharmacokinetics.

Protein Binding In Vivo

Drugs that bind to plasma proteins bind to and dissociate from those proteins at particular rates, termed association and dissociation rates. At equilibrium, as much drug is associating with protein as is dissociating at any given time, and there is a constant concentration of free drug. However, protein binding is not the sole determinant of the amount of free drug that is available for therapeu-

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tic activity. For example, a highly protein-bound drug may be as potent in vivo as a drug that is less protein-bound, since greater amounts of the latter may be available for elimination or for entering therapeutically irrelevant sites (eg, molecules, cells, or organs other than the target sites).

With regard to the impact of metabolism and elimination on therapeutic activity as it relates to protein binding, the average time to circulate plasma through the liver of an adult is 9 seconds. In an average individual, every molecule of a drug, even drugs that are highly protein-bound, is estimated to be free in the liver every few minutes, providing ample opportunity to clear free drug for those agents that are metabolized. In short, determining clinical potency of a drug is much more complicated than would be represented by consideration of protein binding alone.

Modulating Protein Binding

Some drugs are known to affect AAG levels (eg, phenobarbital increases AAG levels in cats), and it is possible that antiretroviral protein binding could be modulated through use of drugs that upregulate or downregulate AAG. Studies to determine whether PIs that are cytochrome P450 (CYP 450) inducers affect AAG levels found that, after 5 weeks of treatment (during which steady state was achieved in all patients), neither nelfinavir nor ritonavir altered AAG levels in HIV-infected patients (Flexner et al, 12th World AIDS Conf, 1998). There was some variability in AAG response among patients, with levels increasing in some and decreasing in others; however, no supraphysiologic AAG levels that might have substantially reduced free drug were observed in any patients. Other studies of the effects of drugs or HIV disease on AAG levels similarly suggest that in most cases the impact is not sufficient to substantially alter free drug concentrations long term.

For the most part, the problem of protein binding is solved during clinical drug development, by ascertaining whether therapeutically meaningful drug levels and good therapeutic effect are achieved at tolerable doses. Development of the investigational PI SC-52151, for example, was stopped not because of the drug's high degree of protein binding but because the drug could not be dosed to achieve an adequate anti-HIV effect in vivo. This was largely due to the drug's poor water solubility, which required SC-52151 to be administered in an elixir that contained large amounts of ethanol and thus limited dose (Fischl et al, *J Acquir Immune Defic Syndr Hum Retrovirol,* 1997).

Individualizing Treatment: Pharmacogenomics

There is considerable enthusiasm about the prospect of individualizing antiretroviral therapy based on genetic profiling of patients. However, much research remains to be done before this prospect becomes reality. In a recent study performed by Mallal and colleagues (*Lancet,* 2002) in a relatively homogeneous population of individuals of mostly English or Irish descent in Western Australia, 14 (78%) of 18 HIV-infected patients with hypersensitivity to abacavir had HLA type HLA-B5701, compared with only 4 (2.4%) of 167 patients with abacavir tolerance, yielding an odds ratio for sensitivity among the former of 117. The combination of the 3 genetic markers HLA-B5701, HLA-DR7, and HLA-DQ3 was present in 13 (72%) of the abacavir-sensitive patients and in none of the abacavir-tolerant patients, yielding an odds ratio for the former group of 822. This association is similar in strength to the link between the HLA-B27 marker and ankylosing spondylitis, which is one of the strongest recognized genetic associations for a common disease. However, another study that was performed in a larger and more heterogeneous population, reported at about the same time, found that only about 45% of patients with abacavir sensitivity had the HLA-B5701 marker (Hetherington et al, *Lancet,* 2002).

> A problem in translating genetic findings into clinical practice is that many associations do not pinpoint a single gene responsible for a biologic effect

Other studies of genetic markers have indicated weaker associations and yielded findings that are more difficult to interpret than those in the Mallal study, which may prove to be more typical of data emerging in this field. One study examined the association of mutations in the gene encoding the P-glycoprotein drug transporter (the gene associated with multidrug resistance in cancer chemotherapy) with outcomes of antiretroviral treatment in HIV-infected patients (Fellay et al, *Lancet,* 2002). The investigators found that having the thymidine-thymidine (TT) genotype at position 3453 of the gene, rather than cytidine-thymidine or cytidine-cytidine (CC), was associated with lower trough concentrations of nelfinavir and efavirenz but higher CD4+ cell counts after 6 months of treatment. The TT genotype is found in 25% of white patients and in 13% of African-American patients, and a smaller study (Wegner et al, 9th CROI, 2002) suggested that efavirenz, one of the drugs affected by the TT genotype, may be less effective in African-American patients than in white patients. African-American patients receiving efavirenz had a significantly less durable plasma HIV-1 RNA response and a 2- to 3-fold higher risk of relapse than did white patients, with the time to treatment failure being approximately 400 days versus 1400 days. The study concluded that these disparities were probably not associated with differences in drug concentrations or adherence, and no comparable racial differences were observed with nelfinavir or indinavir.

Several factors may make it difficult to assess the effect of the TT genotype on antiretroviral treatment. This genotype is associated with lower efavirenz concentrations but better CD4+ cell count responses. It is linked with lower concentrations of some drugs (eg, fex-

ofenadine, as well as efavirenz) but higher concentrations of others (eg, digoxin). Further, the TT/CC polymorphism is "silent" in that it does not affect the sequence or structure of the protein produced. Finally, the odds ratio for the impact of the TT mutation on the anti-HIV effect of efavirenz is weak, suggesting a weak association; indeed, a number of studies that have yet to be published have not found an association between this genotype and antiretroviral drug concentrations.

A major problem in translation of genetic findings into clinical practice is that many associations do not pinpoint a single gene that is responsible for a biologic effect. Rather, they represent an association between a previously identified marker and a biologic effect; it often remains to be determined whether the marker is linked to another locus that is actually responsible for the biologic effect. Figure 1 shows a comparison of all marker variants in the abacavir-sensitive and abacavir-tolerant patients studied by Mallal and colleagues. The study found a fair amount of overlap and lack of specificity between these 2 patient groups in the HLA-B5701 locus. However, in another part of this immune response region of the chromosome, encoding genes for heat shock proteins, there was no overlap, suggesting that the gene responsible for abacavir hypersensitivity actually resides in this region. Thus, HLA-B5701 is tightly linked to the trait for abacavir hypersensitivity but is not the gene responsible for this biologic effect. The specific causative gene remains to be identified.

Guaranteeing Success: TDM

There is considerable interest in monitoring antiretroviral drug levels in HIV-infected patients to maintain concentrations that provide maximal therapeutic effect with the least possible toxicity. Particularly with regard to PIs, trough serum concentrations are often predictive of virologic outcome. There is a clear rationale for TDM for PIs, since they are highly metabolized by the CYP 450 system, particularly CYP 3A4, with some PIs being CYP 450 inducers, some inhibitors, and some both. Levels of PIs can be affected by the many other drugs metabolized via the 3A4 enzyme system and by the inherent variability of metabolism via this route.

A number of studies have evaluated use of TDM in patients receiving PI-based antiretroviral therapy. In the PharmAdapt study, 256 treatment-experienced patients were randomized in unblinded fashion to HIV genotyping or genotyping plus pharmacokinetic analysis (Clevenbergh et al, 8th CROI, 2001; Clevenbergh et al, 41st ICAAC, 2001). Genotyping and pharmacokinetic analysis were performed at week 4, with treatment modified on the basis of this information at week 8. Overall, there was no difference between the 2 groups with regard to virologic response at 12 weeks, with plasma HIV-1 RNA levels below assay detection limits in 43% of patients in the genotyping/TDM arm and in 50% of patients in the genotyp-

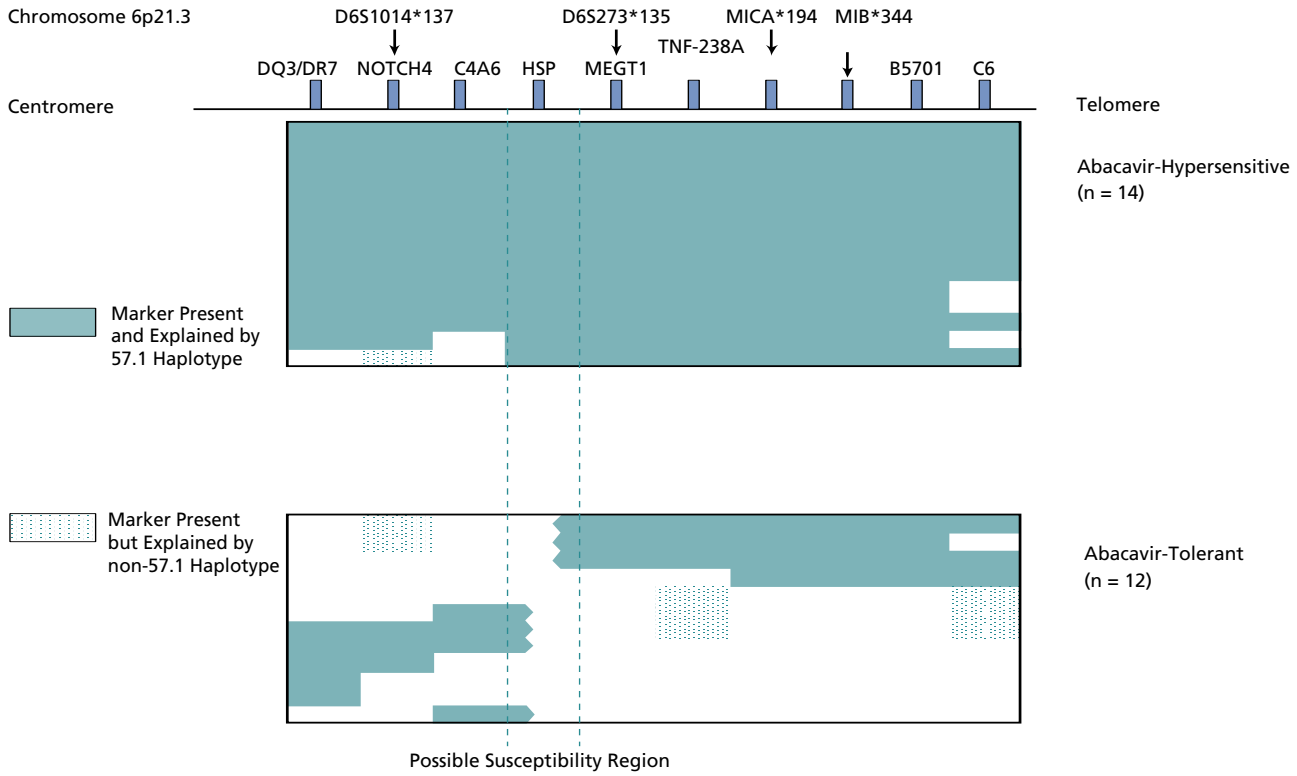

Figure **1.** Genetic mapping of the abacavir hypersensitivity region in abacavir-treated patients with polymorphisms as indicated. Adapted with permission from Mallal et al, *Lancet,* 2002.

ing-only arm. However, a number of factors make these findings difficult to interpret. First, the target drug concentrations were relatively low, equivalent to the protein-adjusted IC_{50}. In addition, about 60% of patients in both arms were receiving ritonavir, which acts pharmacokinetically to boost levels of other PIs. Finally, since 8 weeks of treatment elapsed prior to changes based on the pharmacokinetic analysis, modifications based on this information may have been made too late to prevent development of viral resistance. It should also be noted that there was intrasubject variability with regard to drug levels, with some patients moving from "suboptimal" to "optimal" concentrations between week 4 and week 8 with no change in drug dose.

The GENOPHAR study had a design similar to PharmAdapt (eg, genotyping and pharmacokinetic analysis of treatment-experienced patients at week 4 and change in regimen at week 8), although it was conducted in blinded fashion (Bossi et al, 9th CROI, 2002). This study also showed no difference in virologic outcome between genotyping/TDM and genotyping alone; as with the PharmAdapt study, however, target drug levels may have been too low and changes at week 8 based on pharmacokinetic analysis may have been made too late. A third study performed in treatment-experienced patients, the GART study, also showed no benefit of treatment based on optimal PI levels. (Baxter et al, *AIDS*, 2000)

The ATHENA study has provided some evidence of benefit of TDM in treatment-naive patients. In this "blinded" study, all patients underwent TDM, with their physicians given either dosing advice based on monitoring or no advice (Burger et al, 1st IAS Conf, 2001). Advice resulted in significant decreases in discontinuation rates at 1 year among patients receiving nelfinavir (2.4% vs 17.6% with no advice) or indinavir (9.5% vs 40.0%). However, results of this study are also difficult to interpret, since many practitioners who received advice based on TDM did not institute it. Further, while virologic response improved among patients receiving nelfinavir, no virologic benefit of TDM was observed among patients receiving indinavir, and no benefits of TDM at all were observed in patients receiving indinavir/ritonavir.

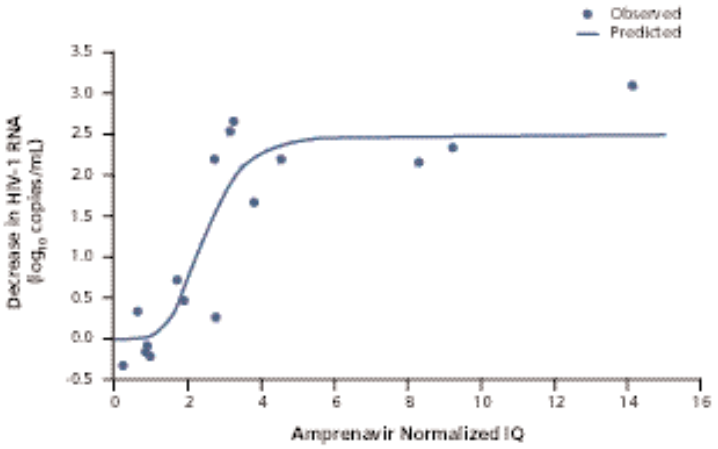

Figure **2.** Association of decrease in plasma HIV-1 RNA levels with amprenavir normalized inhibitory quotient (IQ) in patients receiving amprenavir. Adapted with permission from Piscitelli, ECCATH, 2001.

On balance, the available data suggest that a somewhat different approach to TDM is needed in treatment-experienced patients. Three recent studies reported a better correlation between drug concentrations and treatment outcome in treatment-experienced patients if correction was made for the level of drug resistance in the patient's viral population. Methods by which this correction can be achieved include measurement of the inhibitory quotient (IQ), which is the trough concentration divided by the IC_{50} for the drug; the virtual IQ, which is the trough concentration divided by the virtual IC_{50} derived from a virtual phenotype database; and the normalized IQ, which is the patient's virtual IQ divided by a population mean virtual IQ for a patient with drug-sensitive virus. Figure 2 shows the correlation between amprenavir normalized IQ and decrease in plasma HIV-1 RNA level found by Piscitelli and colleagues (ECCATH, 2001) indicating greater decreases in viral load with higher IQ values.

Much work remains to be done before clinical guidelines for TDM can be developed. Until then, such monitoring may be of benefit in some clinical situations. These include the settings of pregnancy and pediatrics, in which drug concentrations can change rapidly and are difficult to predict; use with phenotyping to determine optimal drug concentrations in salvage treatment, as noted above; in patients with renal or hepatic dysfunction; and in documentation of adequate drug levels in the presence of other drugs known to induce or inhibit the CYP 450 system.

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Suggested Reading

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