

## Perspective

# Drug Transporters in HIV Therapy

*Drug transporter proteins play a crucial role in drug disposition. The P-glycoprotein drug efflux transporter is a determinant of oral bioavailability and central nervous system (CNS) penetration of protease inhibitors (PIs), and may affect drug penetration to other tissue compartments that can serve as sanctuaries for HIV. Potent and selective inhibitors of P-glycoprotein can dramatically increase PI CNS penetration. Polymorphisms in the MDR1 gene regulating P-glycoprotein expression are associated with differences in drug disposition, with some data indicating that different genotypes are associated with differences in plasma PI levels and magnitudes of CD4+ cell count recovery under therapy. The activity of drug transporters in modulating antiretroviral drug effects and the potential for exploiting this activity to maximize therapeutic benefit and minimize drug toxicity are the subjects of ongoing study. In addition, inhibition of transporter proteins may increase the risk of hepatotoxicity and other adverse drug effects and is being investigated. This article summarizes a presentation given by Richard B. Kim, MD, at the March 2003 International AIDS Society–USA course in Atlanta.*

Drug disposition and drug interactions are important components of the activity of and response to antiretroviral drugs. Determinants of drug disposition include the transporter proteins active in cellular uptake and efflux of drug molecules. Considerable attention has recently been given to understanding the role of the P-glycoprotein (P-gp), or *MDR* (multidrug resistance)-1, efflux transporter in modulating drug levels in cells and tissues and to delineating the genetic variation in this transporter that may account for differences in individual responses to drug therapy.

### Characteristics of the P-gp Transporter

The P-gp transporter was initially studied in the setting of anticancer treatment and was identified as the means by which a number of drugs were removed from cells, resulting in what has been termed multidrug resistance in tumor cells. This transporter is expressed in cells in a number of locations in the body that are important to drug absorption and disposition, including the same cells in the intestine, liver, and kidney that express cytochrome

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P450 3A enzymes. They are also present at the level of the blood-brain barrier and act to limit central nervous system (CNS) penetration of many drugs and toxins. The transporters exhibit a broad substrate specificity, including hormones, plant-derived chemicals, and many drugs in clinical use. In the gastrointestinal tract, P-gp is exclusively expressed on the brush border, or apical domain, of the intestinal enterocyte. Its activity there limits intestinal absorption and thus oral bioavailability of a number of drugs. Expression of the transporter in the liver and kidney enhances elimination of drugs. The

transporters are exclusively expressed in the liver on the canalicular membrane domain of hepatocytes and in the kidney on the luminal side of the proximal tubular cells, where they act to increase excretion of drugs into the bile and urine, respectively. Drugs cross the blood-brain barrier via either passive diffusion or active transport from capillary endothelial cells. The P-gp transporter is expressed on the luminal side of these endothelial cells and acts to pump drug molecules back into the blood. Given the great variability of CNS penetration of many drugs and the fact that the CNS may act as a sanctuary for HIV in the absence of drug activity in this compartment, the role of the P-gp transporter in limiting antiretroviral drug CNS penetration has attracted particular attention.

### Effects of the P-gp Transporter on PI Concentrations

HIV protease inhibitors (PIs) are substrates for the P-gp transporter. In initial studies of the interaction of P-gp expression and PI disposition, Dr Kim's group assessed plasma and brain concentrations of nelfinavir in P-gp (*mdr1a*) wild-type mice. Plasma drug levels were

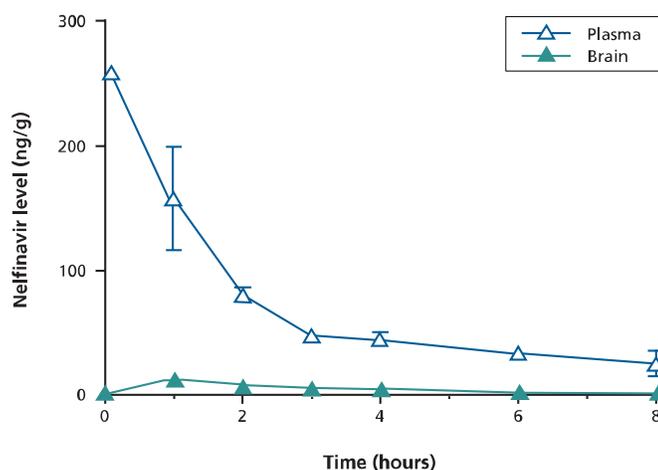


Figure 1. Nelfinavir concentrations in plasma and brain tissue of *mdr1a* wild-type mice after intravenous administration. Adapted with permission from Choo et al, *Drug Metab Dispos*, 2000.

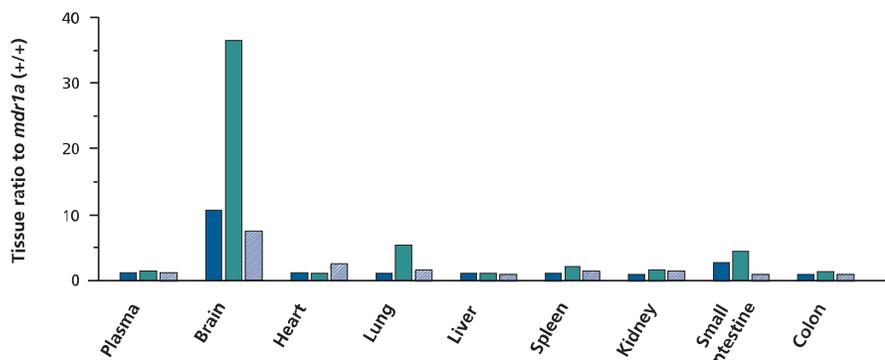


Figure 2. Ratio of plasma and tissue levels of indinavir (blue bar), nelfinavir (green bar), and saquinavir (striped bar), in *mdr1a*-knockout mice to levels in *mdr1a* wild-type mice after intravenous drug administration. Adapted with permission from Kim et al, *J Clin Invest*, 1998.

markedly higher than brain levels, which had a minimal peak and were virtually negligible from 2 hours after an intravenous (IV) dose (Figure 1). Comparison of tissue levels of indinavir, saquinavir, and nelfinavir after IV dosing in *mdr1a* wild-type mice and in *mdr1a*-knockout mice in which the gene regulating transporter expression is deleted, showed that brain levels of the drugs were dramatically increased (approximately 7- to 40-fold) in the *mdr1a*-knockout mice (Figure 2). In subsequent studies, Dr Kim's group sought to determine whether CNS entry of PIs could be selectively increased by pharmacologic inhibition of the transporter. Use of the potent P-gp inhibitor LY335979 in mice resulted in an approximate doubling of plasma nelfinavir levels but a dramatic increase in brain drug levels after IV nelfinavir dosing (Figure 3), with the increase in brain-to-plasma drug ratio being LY335979 dose-dependent. Cells in the testes are also known to express P-gp, and these studies likewise showed a dose-dependent increase in testes-to-plasma nelfinavir concentration ratio. Testing of other compounds and drugs selected on the basis of known effects on transporter inhibition showed markedly reduced ability to increase nelfinavir brain-to-plasma ratios compared with LY335979. These studies have suggested that there may be a number of tissue compartments protected from adequate PI levels by function of the transporter, and that potency and selectivity of P-gp inhibition are

necessary for adequate reduction of activity at blood-tissue barriers.

These issues of drug disposition and adequate penetration to tissue compartments are not limited to PIs. The P-gp transporter may be the most important transporter identified to date in terms of clinically relevant effects on drug disposition. Table 1 provides a list of drugs known to be substrates, inhibitors, or inducers of the transporter, a list that is similar to that of drugs known to affect CYP3A4 metabolism. In some cases, drug interactions are known to be completely accounted for on the basis of P-gp activity—eg, in the case of interaction of digoxin (a transporter substrate that is not metabolized) and quinidine (a transporter inhibitor).

### Polymorphisms Affecting P-gp Function

Dr Kim's group and others have identified a number of polymorphisms in the *MDR1* gene (Kim, *Clin Pharmacol Ther*, 2001). One of the most common haplotypes is the *MDR1*\*2 haplotype (ie, 2 synonymous mutations in exons 12 and 26 that do not change the encoded amino acid flanking a mutation in exon 21 that does change the amino acid [ie, non-synonymous]) involving an Ala 893Ser substitution (Kim, *Clin Pharmacol Ther*, 2001). Work is ongoing to identify polymorphisms that have functional consequences in humans. Hoffmeyer and colleagues (*Proc Natl Acad Sci U S A*, 2000) have reported that some individuals naturally express low amounts of duodenal P-gp. This low expression phenotype is determined by natural polymorphisms within the P-gp gene (those with a TT genotype have low levels of P-gp while those with a CC or CT genotype have higher levels). Fexofenadine is a terminal metabolite of terfenadine that is a high-affinity substrate of P-gp and that can be used safely as a probe drug to study transporter function in humans. Studies using fexofenadine have found that there are marked differences in plasma drug concentrations according to *MDR1* haplotypes (Figure 4). With regard to HIV PI activity, Fellay and colleagues (*Lancet*, 2002) reported marked differences in

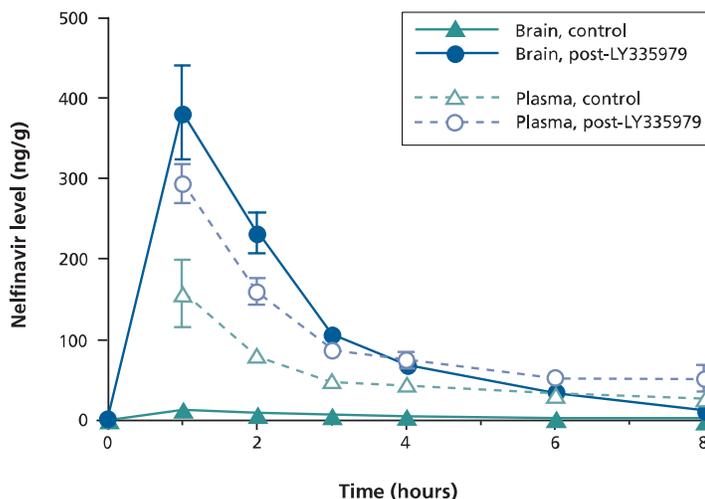


Figure 3. Plasma and brain levels of nelfinavir in control animals and after administration of P-glycoprotein inhibitor LY335979. Adapted with permission from Choo et al, *Drug Metab Dispos*, 2000.

**Table 1.** P-glycoprotein Substrates, Inhibitors, and Inducers**Substrates**

- Cyclosporine
- Digoxin
- Erythromycin
- Fexofenadine
- HIV protease inhibitors
- Mibefradil
- Paclitaxel
- Quinidine
- Tacrolimus
- Terfenadine
- Verapamil (but not nifedipine or felodipine)

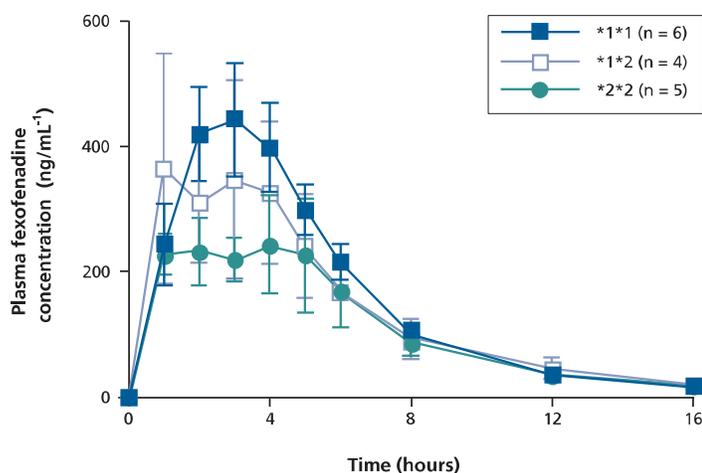
**Inhibitors**

- Clarithromycin
- Cyclosporine
- Erythromycin
- HIV protease inhibitors
- Itraconazole
- Ketoconazole
- Mibefradil
- Quinidine
- Terfenadine
- Verapamil

**Inducers**

- Rifampin
- St John's wort

nelfinavir plasma concentrations according to genotype in patients with *MDR1* exon 26 3435C/T polymorphisms (Figure 5, left). The finding of genetic differences that affect plasma drug levels suggests the possibility that these differences can alter drug effectiveness. Indeed, these investigators found that the TT genotype was associated with a significantly greater CD4+ cell count recovery under antiretroviral therapy including nelfinavir (Figure 5, right). Analysis of a number of factors associated with CD4+ cell count recovery showed that the TT genotype was among the best predictors (odds ratio, 3.0) of cell count, after baseline plasma HIV-1 RNA level. A subset of CD4+ cells expresses P-gp. It is thus possible that the transporter could affect levels of CD4+ cells, the cellular targets of HIV, by altering the amount of drug within these cells that is available for intracellular antiviral effect. It should be noted that the study by Fellay et al also



**Figure 4.** Fexofenadine concentrations in healthy humans according to type of *MDR1* polymorphisms. Subjects with *MDR1* \*1\*1, \*1\*2, and \*2\*2 were given a single dose of fexofenadine, and plasma levels for the drug were determined. Those with the *MDR1*\*2 alleles tended to have lower fexofenadine levels. Adapted with permission from Kim et al, *Clin Pharmacol Ther*, 2001.

observed an *MDR1* genotype-dependent effect for efavirenz, a drug which has not been shown to be a substrate of this transporter. Accordingly, many aspects of the Fellay et al study remain controversial.

Despite the promising findings indicating an association of particular *MDR1* polymorphisms with alterations in P-gp function, a significant amount of debate remains regarding the actual impact of these mutations. A number of groups, including HIV researchers, are currently attempting to better define the relevance of *MDR1* polymorphisms to effects of drug therapies.

### Drug Transporters and Drug Toxicity

The prospect of maximizing therapeutic effectiveness of drug therapy by modulating activity of drug transporters is an attractive one. It should also be recognized that modulation of transporter function can result in drug toxicity and drug-drug interactions. The endothelial receptor antagonist bosentan furnishes one example of drug inhibition of transport function that appears to account for toxic effects. Initial trials of bosentan in the settings of chronic heart failure and hypertension showed an increasing frequency of elevated liver transaminas-

es with increasing bosentan doses. Bosentan is an inhibitor of the bile salt export pump (BSEP) transporter located on the canalicular membrane of hepatocytes (Fattinger et al, *Clin Pharmacol Ther*, 2001). Since inhibition of this transporter may result in cholestasis and hepatocellular damage, it is possible that the drug produces liver toxicity via this mechanism. Similar considerations apply to the drug troglitazone, which was withdrawn from the market on the basis of hepatotoxicity in 2000. Although it was initially unclear why some patients appeared to be more susceptible to liver toxicity associated with troglitazone use, it has been shown that the sulfate metabolite of troglitazone is a particularly potent inhibitor of the BSEP transporter (Funk et al, *Mol Pharmacol*, 2001) and may also cause hepatotoxicity through this mechanism. These and other findings suggest that this transporter may be susceptible to inhibition by many drugs that cause hepatotoxicity. Indeed, it may turn out to be the case that a substantial proportion of otherwise unexplained liver toxicity and toxicity in other organs is explained by inhibition of drug transporters. A number of groups currently are investigating the potential role of drug transporters in hepatotoxicity associated with antiretroviral drugs.

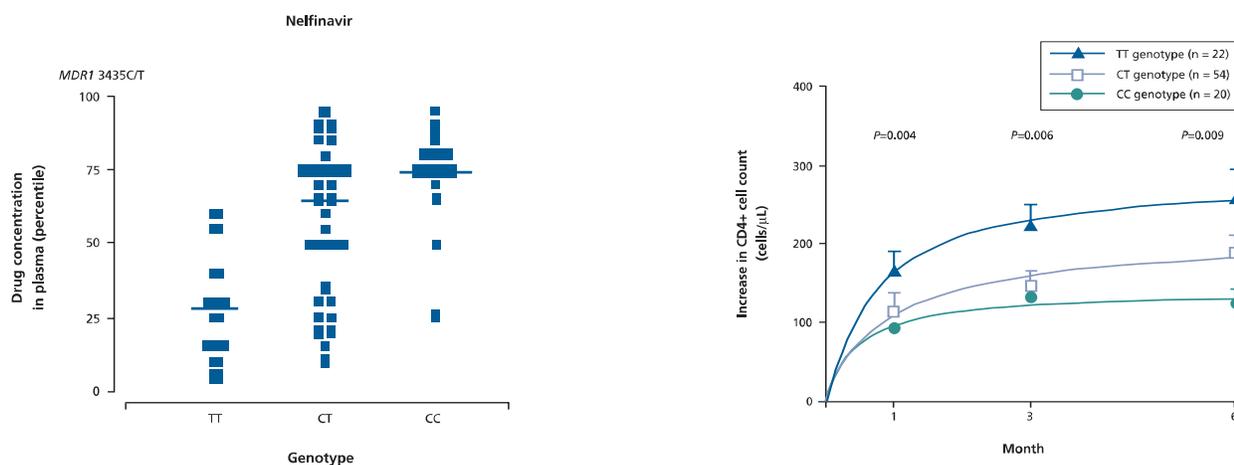


Figure 5. Effect of genotype in patients with *MDR1* 3435C/T polymorphism on nelfinavir plasma concentrations (left) and CD4+ cell count recovery during antiretroviral therapy including nelfinavir (right). Adapted with permission from Fellay et al, *Lancet*, 2002.

## Conclusions

Drug transporter proteins play a significant role in drug disposition and tissue

### Transporter Terminology

**CYP3A4:** A drug-metabolizing enzyme in the cytochrome P450 superfamily. This CYP enzyme is involved in the metabolism of nearly 50% of all the drugs in clinical use. It is involved in the metabolism of nearly all the HIV protease inhibitors.

**Efflux pumps:** Family of cell membrane expressed transporters which pump drugs from intracellular to extracellular compartment.

**MDR efflux system:** This typically refers to the P-glycoprotein transporter. However, there are other transporters capable of mediating MDR (multidrug resistance) phenomena.

**MDR1:** This is the gene encoding for human P-glycoprotein.

**P-glycoprotein (P-gp):** This is a transmembrane protein found on many cell types; it mediates transport of certain molecules out of cells.

**Polymorphisms:** Refers to naturally occurring nucleotide substitutions in the genomic DNA.

penetration. The P-gp transporter is a key determinant of oral bioavailability and CNS penetration of PIs and also appears to affect drug penetration of other tissues that could act as sanctuaries for HIV. Specific inhibitors of P-gp can dramatically increase PI penetration of the CNS. Available data indicate *MDR1* polymorphisms can significantly alter drug disposition. In the case of PIs, mutations affecting transporter expression may also affect HIV infectivity in CD4+ cells by modulating intracellular drug levels. Inhibition of drug transporters may increase risk of hepatotoxicity and other adverse drug effects. Investigation is ongoing into the activity of transporters in modulating antiretroviral drug effects and into the possibility of exploiting this activity to maximize therapeutic benefit and minimize drug toxicity.

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### Suggested Reading

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