INVITED REVIEW

MDR- and CYP3A4-Mediated Drug–Drug Interactions

Dhananjay Pal · Ashim K. Mitra

Abstract P-glycoprotein (P-gp), multiple drug resistance associated proteins (MRPs), and cytochrome P450 3A4 together constitute a highly efficient barrier for many orally absorbed drugs. Multidrug regimens and corresponding drug–drug interactions are known to cause many adverse drug reactions and treatment failures. Available literature, clinical reports, and *in vitro* studies from our laboratory indicate that many drugs are substrates for both P-gp and CYP3A4. Our primary hypothesis is that transport and metabolism of protease inhibitors (PIs) and NNRTIs will be altered when administered in combination with azole antifungals, macrolide, fluroquinolone antibiotics, statins, cardiovascular agents, immune modulators, and recreational drugs [benzodiazepines, cocaine, lysergic acid dithylamide (LSD), marijuana, amphetamine (Meth), 3,4-methylene-dioxymethamphetamine (MDMA), and opiates] due to efflux, and/or metabolism at cellular targets. Therefore, such drug combinations could be a reason for the unexpected and unexplainable therapeutic outcomes. A number of clinical reports on drug interaction between PIs and other classes (macrolide antibiotics, azole antifungals, cholesterol lowering statins, cardiovascular medicines, and immunomodulators) are discussed in this article. MDCKII-MDR1 was employed as an *in vitro* model to evaluate the effects of antiretrovirals, azole antifungals, macrolide, and fluroquinolone antibiotics on efflux transporters. Ketoconazole (50 μM) enhanced the intracellular concentration of ^{3}H ritonavir. The inhibitory effects of ketoconazole and MK 571 on the efflux of ^{3}H ritonavir were comparable. An additive effect was observed with simultaneous incorporation of ketoconazole and MK 571. Results of ^{3}H ritonavir uptake studies were confirmed with transcellular transport studies. Several fluroquinolones were also evaluated on P-gp-mediated efflux of ^{3}H cyclosporin and 14C erythromycin. These *in vitro* studies indicate that grepafloxacin, levofloxacin, and sparfloxacin are potent inhibitors of P-gp-mediated efflux of 14C erythromycin and ^{3}H cyclosporin. Simultaneous administration of fluoroquinolones and macrolides could minimize the efflux and metabolism of both of the drugs. Effects of erythromycin and ketoconazole on carbamazepine metabolism were examined. Formation of 10,11-epoxy carbamazepine, a major CBZ metabolite, was significantly inhibited by these agents. Therefore, drug efflux proteins (P-gp, MRPs) and metabolizing enzyme (CYP450) are major factors in drug interactions. Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles of multidrug regimens. Drug–drug interactions with PIs and other coadministered agents for human immunodeficiency virus (HIV) positive population have been discussed in light of efflux transporters and metabolizing enzymes. This article provides an insight into low and variable oral bioavailability and related complications leading to loss of therapeutic activity of MDR and CYP 450 substrates.

Key words MDR · CYP3A4 · drug–drug interactions · protease inhibitors

D. Pal · A. K. Mitra (✉)
Division of Pharmaceutical Sciences, School of Pharmacy,
University of Missouri–Kansas City,
5005 Rockhill Road,
Kansas City, MO 64110-2499, USA
e-mail: mitraa@umkc.edu

Abbreviations

ABC	ATP binding cassette
AF	activation function
AIDS	acquired immunodeficiency syndrome
AUC	areas under curves

BCRP	breast cancer resistance protein
BSEP	bile salt export pump
CAR	constitutive androstane receptor
CYP	cytochrome P450
GHB	gamma hydroxybutyrate
GSTs	glutathione-S-transferases
HAAT	highly active antiretroviral therapy
HIV	human immunodeficiency virus
LBD	ligand binding domain
LSD	lysergic acid dithylamide
Meth	amphetamine
MDR	multidrug resistance
MDMA	3,4-methylenedioxymethamphetamine
MRPs	multiple drug resistance associated proteins
NRTIs	nucleoside and nucleotide reverse transcriptase inhibitors
NNRTIs	nonnucleoside reverse transcriptase inhibitors
OIs	opportunistic infections
PIs	protease inhibitors
P-gp	P-glycoprotein
PXR	pregnane X receptor
RXR	retinoid X receptor
UGT	uridine 5′-diphosphate glucuronosyltransferase
VRD	vitamin D response

Introduction

The advent of highly active antiretroviral therapy (HAART) has not only reduced the mortality rate of human immunodeficiency virus (HIV)-positive individuals, but also vastly improved their quality of life. However, treatments of acquired immunodeficiency syndrome (AIDS) and its associated conditions remain very complex. Several protease inhibitors, drug combinations (antibacterial and antifungal) indicated for opportunistic infections (OIs), medicines for depression, cardiovascular problems, and other associated conditions such as hyperlipidemia are recommended for the treatment of AIDS. These medications are generally taken orally and during combinations are absorbed from the gastrointestinal tract. Any agent that alters the absorption and transport of these medications can affect therapy. Therefore, multidrug regimens and corresponding drug–drug interactions may cause potential adverse drug reactions and ultimately treatment failures. Absorption of many oral drugs involves efflux and metabolism in the enterocytes and liver. Drug efflux proteins [P-glycoprotein (P-gp) and multiple drug resistance associated proteins (MRPs)] and metabolizing enzyme cytochrome P450 3A4 (CYP3A4) are the main factors leading to such interactions (Patel and Mitra 2001). Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic pro-

files of multidrug regimens. In this report, we discuss the mechanisms by which drugs in selected drug combinations interact with these efflux proteins (P-gp, MRP-1, and MRP-2) and metabolizing enzymes, CYP450. The entire discussion is based on clinical reports and some *in vitro* results from our laboratory. Our primary hypothesis is that the kinetics of these drug classes—protease inhibitors (PIs), azole antifungals, macrolide antibiotics, lipid lowering drugs, anticonvulsing agents will be altered when given in combinations due to efflux, and/or metabolism at cellular targets. Therefore, these drug combinations may cause unexpected and unexplainable therapeutic outcomes. Moreover, botanicals in combination therapy can also potentially change clinical outcomes (Pal and Mitra 2006). These hypotheses are based on the following observations. Saquinavir undergoes efflux by MRP-2 and P-gp, because PIs are substrates of both P-gp and MRPs. Erythromycin and ketoconazole are found to interact with MRPs and/or P-gp. Our studies, along with other published reports, indicate that herbal products also interact with P-gp and possibly with MRPs. These therapeutic agents in the presence of herbals such as St John's Wort and garlic) can undergo P-gp- and/or CYP3A4-mediated interactions (Pal and Mitra 2006). Because multidrugs resistance (MDR) and CYP-mediated drug–drug interactions involve many agents, it is not possible here to provide comprehensive listing of all possible drug interactions; rather, we restrict our discussion to antiretroviral agents and their interactions with other combination therapy in AIDS treatment. This review attempts to mechanistically predict drug–drug interactions that may lower the risk of treatment failure in AIDS patients. It will also allow clinicians and pharmacists to recommend doses in binary/ternary combinations of drug therapy to achieve maximum effectiveness with minimum toxicity.

General basis of drug interactions

Drug–drug interactions comprise two broad categories: pharmacodynamic and pharmacokinetic. Many drug interactions involve the effect of one drug on the absorption or disposition of another. Pharmacodynamic interactions involve two or more drugs having additive, synergistic, or inhibitory effects. In Kaletra, lopinavir and ritonavir act together to produce maximum efficacy in reducing viral load. Ritonavir causes higher area under curve (AUC) of lopinavir, possibly through the inhibition of efflux protein, P-gp, and metabolizing enzyme, CYP3A4. For treating OIs, saquinavir and ketoconazole administered together can elevate AUC of saquinavir as a result of inhibition of both P-gp and CYP3A4. Several antimycobacterial agents, i.e., clarithromycin and refabutin, inhibit CYP3A4, but rifam-

picin induces CYP3A4. In both cases, any combination therapy with PIs requires dosage adjustment.

Role of efflux proteins and metabolizing enzymes

Both active efflux and metabolism in the small intestine result in poor drug absorption. CYP450 enzymes are highly expressed on enterocytes that can metabolize xenobiotics during their transit across intestinal epithelium (Shen et al. 1997). The most abundant isozyme of CYP present in intestinal enterocytes is CYP3A4 (Kolars et al. 1992; Watkins et al. 1987). In addition to these metabolic enzymes, MDR-1 gene product P-gp and MRPs are also highly expressed on the brush border membrane of enterocytes. MDR gene products are "primary active" drug efflux pumps that bind and transport drug molecules against a concentration gradient by an energy-dependent process. This process plays an important role in the efflux of xenobiotics back to intestinal lumen, thereby exposing the drug to further metabolism (Gottesman and Pastan 1993; Ambudkar et al. 1999). A recent review by Katragadda et al. (2005) from our laboratory has described in details the expression of efflux proteins and their role in oral drug delivery. CYP450 enzymes and P-gp/MRPs proteins act synergistically to reduce the oral bioavailability of their substrates (Parkinson 1996).

Efflux proteins

P-gp, a product of MDR gene, was first characterized as the ATP-dependent transporter responsible for the efflux of chemotherapeutic agents from resistant cancer cells (Endicottal and Ling 1989). P-gp is an integral membrane protein (170 kDa) having two homologous halves, each consisting of one hydrophobic domain with six transmembrane segments and one hydrophilic nucleotide-binding domain. Two halves are connected by a short flexible linker polypeptide to form the functionally active 1280-amino-acid protein. P-gp encoding genes are found in hamster, mice, human, and other species. P-gp is a transporter protein, encoded by a small multigene family, described by MDR I, II, and III. P-gps from all three MDR genes are present in rodents, whereas human cells express P-gp belonging to class I and III only. P-gp was the first ATP-dependent transporter found in the liver, and represents the most studied member of the ATP binding cassette (ABC) family of transporters. MDR proteins in humans and other species play an important role in protecting cells from cytotoxic drugs (Borst et al. 2000). These efflux pumps exhibit a versatile ability to efflux xenobiotics from cells. These proteins cover a broad range of substrates such as cyclosporin A, taxol, dexamethasone, li-

docaine, erythromycin, protease inhibitors, and many anti-cancer agents (Borst et al. 1993; Gottesman and Pastan 1993; Hofsliand and Nissen-Meyer 1989; Ueda et al. 1987, 1992; Cole et al. 1992; Deeley and Cole 1997). Nearly 200 proteins are involved in the active transport of substances across biological membranes. Most of these proteins belong to an ABC superfamily. One or more ATP binding sites characterizes this family of transmembrane proteins. This includes P-gp, MRPs, breast cancer resistance protein (BCRP), bile salt export pump, sodium bile acid cotransporter, and the organic anion transporter (Cvetkovic et al. 1999). Localization of P-gp, MRPs and BCRP in the intestinal epithelium is depicted in Fig. 1.

P-gp is expressed in a broad spectrum of tissues including the adrenals, cells of the blood–brain barrier, kidney, liver, lungs, and pancreas (Gottesman and Pastan 1993; Borst et al. 1993; Gottesman and Pastan 1993). Mammalian P-gps display approximately 60–65% homology with most P-gps from other species, suggesting that their role in drug trafficking is highly conserved throughout evolution. Transfection studies have shown that class I and II isoforms confer MDR, whereas class III isoform represents phosphatidylcholine translocation responsible for the secretion of phospholipids into bile (Gottesman et al. 1996; Sharom 1997). Evidence accumulated to date suggests that the transporter interacts directly with nonpolar substrates within the membrane environment, and may act as a drug flippase, transferring drugs from the inner to the outer leaflet of the bilayer, although a clear mechanism of translocation has not yet been established. Chemosensitizers that block the action of P-gp/MRPs appear to act as alternate substrates (Gottesman et al. 1996; Sharom 1997). MRPs, glycophosphoproteins belonging to the same ABC superfamily, are 190-kDa proteins and responsible for transport of drugs across lipid membranes from inside to outside the cell (Zaman et al. 1994; Krishnamachary and Center 1993). Although the role of P-gp in this drug interaction process has received some attention, the actual participation of MRPs and BCRP in this cascade also deserves further consideration. Unlike P-gp, MRPs primarily transport conjugated organic anions including cysteinyl leukotriene (LTC) (Xiao et al. 2005; Larkin et al. 2004). So far, eight different MRPs have been reported for the ABC
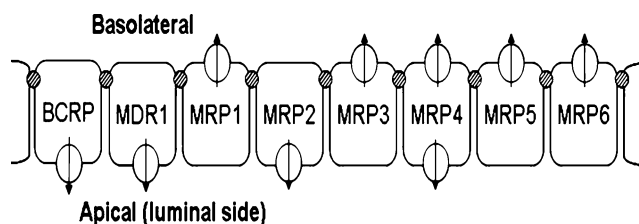

Fig. 1 Localization of efflux transporters in the intestine. Arrows indicate the direction of efflux.

proteins (Table 1). Human MRP-1, MRP-2, and MRP-3 are involved in the efflux of many therapeutic agents, i.e., antineoplastic drugs and their conjugated metabolites, floroquinoles, PIs (Letourneau et al. 2005; Bakos et al. 2000; Naruhashi et al. 2002; Hulot et al. 2005; Huisman et al. 2002). BCRP (ABCG2) is considered as ABC half transporter having only six transmembrane domains, extrudes a variety of unrelated compounds (anthracyclines, donarubicins, doxorubicins, campothecins, eporubicin) (Litman et al. 2000, 2001). A recent report suggests that BCRP imparts resistance to HIV-1 NRTI (zidovudine). Figure 2 diagrammatically represents the structural differences of P-gp, MRP, and BCRP.

Metabolizing enzymes

Role of CYP450 in drug–drug interactions

The most versatile enzyme system involved in the metabolism of xenobiotics is cytochrome P450. This enzyme system is responsible for the oxidative metabolism of a wide variety of compounds including PIs, nonnucleoside reverse transcriptase inhibitors (NNRTIs), cholesterol-lowering drugs, cardiovascular agents, steroids, psychotropic agents, antibiotics, azoles, and toxins. Enhanced metabolism and biliary clearance may affect drug efficacy, but delayed and lowered metabolism can lead to toxicity and other therapeutic interactions. These drugs can act as CYP inducers or inhibitors, or simply substrates. Depending on how the drugs interact with this enzyme system, it can be beneficial by enhancing blood levels or detrimental leading to therapeutic failure. Drug–drug interactions can become very complex in multidrug regimen when all three combinations (inducers, inhibitors, and substrates) are administered such as Kaletra + atorvastatin, Kaletra + ketoconazole, or Kaletra + rifampin. A recent review from our laboratory has discussed this enzyme system and its role in drug delivery (Katragadda et al. 2005). The CYP3A

Table 1 Human MRP gene family

Gene	Protein	Other names used
ABCC1	MRP1	ABCC: MRP:GS-X:
ABCC2	MRP2	cMOAT.cMRP
ABCC3	MRP3	MOAT-D: cMOAT-2
ABCC4	MRP4	MOAT-B
ABCC5	MRP5	MOAT-C: pABC11
ABCC6	MRP6	MOAT-E: MLP-1:ARA
ABCC10	MRP7	
ABCC11	MRP8	

MOAT: multiorganic anion transporter.

family of enzymes constitutes the most predominant phase I drug metabolizing enzymes and accounts for approximately 30% of hepatic CYP and more than 70% of intestinal CYP activity. CYP3A4 is a major congener of the CYP family known to metabolize more than 50% of currently administered drugs (Shen et al. 1997). This CYP3A4 enzyme is abundant in the hepatocytes and enterocytes (Guengerich et al. 1986; Katragadda et al. 2005; Parkinson 1996). A recent study indicates the expression of the polymorphic form of CYP3A5 in the small intestine, which significantly contributes to drug metabolism (Lown et al. 1994). Although hepatic biotransformation can make a major contribution to systemic drug elimination, a combination of hepatic and intestinal drug metabolism may cause significant presystemic or first-pass drug loss.

Because HIV protease inhibitors, macrolide antibiotics, and azole antifungals along with many therapeutic agents are substrates of CYP3A4, these compounds can alter the oral bioavailability of one another upon coadministration. Depending on the mechanisms of drug–drug interactions, multidrug regimens could lower blood levels of anti-HIV drugs, thus possibly placing people at risk for the development of drug resistance.

A significant amount of CYP3A4 is expressed in the enterocytes to metabolize xenobiotics during their transit across the intestinal epithelium (Shen et al. 1997). Although hepatic metabolism makes a major contribution to systemic drug elimination, the combination of hepatic and intestinal drug metabolism appears to have a significant effect on presystemic or first-pass drug loss. Saquinavir undergoes extensive first-pass metabolism by CYP3A4. Ketoconazole (selective CYP3A4 inhibitor) can inhibit the formation of all saquinavir metabolites. Also, saquinavir inhibits the metabolism of terfenadine and the formation of 6-β-hydroxylation products of testosterone (Vella and Floridia 1998). Metabolism of ritonavir, on the other hand, is caused by CYP3A4 and CYP2D6. It significantly inhibits the metabolism of CYP3A4 substrates such as nifedipine and CYP2D6 substrates such as dextromethorphan (Gottesman and Pastan 1993; Hsu et al. 1998). The major isozyme responsible for indinavir metabolism is CYP3A4, whereas the metabolism of nelfinavir is caused by several isozymes including CYP3A4, CYP2C19, CYP2D6, and possibly CYP2C9 and CYP2E1 (Williams and Sinco 1999; Malaty and Kuper 1999; Li and Chan 1999). In addition to oxidative metabolism, conjugation reactions may play an important role in detoxification of xenobiotics in the small intestine. Transporters responsible for cellular efflux of the conjugated metabolites and organic anions has been characterized recently (Leslie et al. 2001).
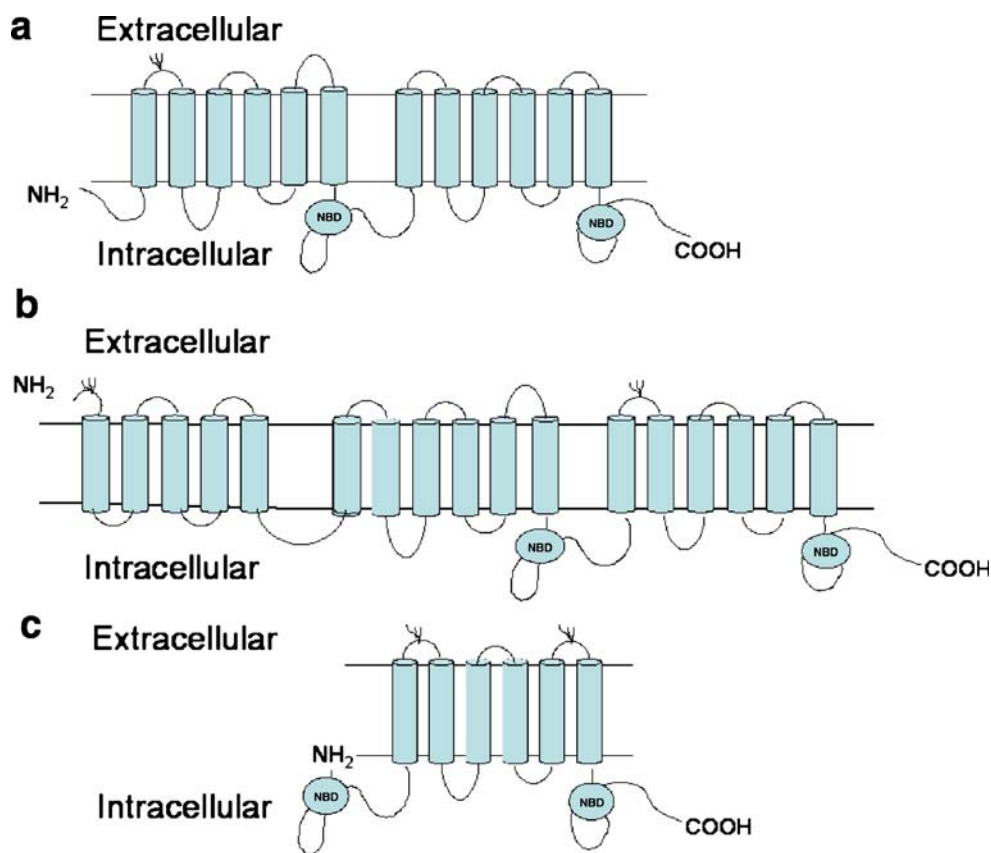
A combination of metabolic enzymes and efflux proteins play an important role in determining the bioavailability of xenobiotics from the intestinal lumen into the systemic

Fig. 2 Diagrammatic representation of ABC transporters: (a) P-glycoprotein, (b) MRP, and (c) BCRP. NBD: nucleotide binding domain.

circulation (Katoh et al. 2001). It is also true that most compounds, which are substrates for P-gp, are also substrates for CYP3A4. Because of a similarity in substrate specificity for both CYP3A4 and P-gp, these proteins appear to act synergistically (Chiou et al. 2000). Such coordinated function of active efflux and metabolism in the small intestine result in poor absorption of xenobiotics (Wacher et al. 1995, 2001; Watkins 1997).

Antiretroviral agents

Antiretroviral agents may be classified into three broad groups: (A) nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), (B) NNRTIs, and (C) PIs. Their affinities for efflux protein, P-gp, and metabolizing enzyme CYP3A4 are depicted in Table 2. This classification is based on available information relating to substrate specificities of antiretroviral agents. Accordingly, NRTIs belong to class A, which indicates that these drugs do not posses affinity for either P-gp or CYP3A4. NNRTIs are placed in class B, because these compounds are metabolized by CYP3A4 enzymes. PIs, which belong to class C, are substrates for both P-gp and CYP3A4. Thus, in multidrug regimens, drugs from class A may not influence the absorption of any drugs from other two groups, but it can

influence the pharmacodynamics of drug action. However, interaction of NRTIs can occur as a result of competition for nucleoside transporter during influx and competitive inhibition of efflux by efflux proteins (MRPs) (Patel et al. 2004). Drugs belonging to class B (NNRTIs) and C (PIs) will interact, possibly as a result of competition for metabolic enzyme, CYP3A4. Therefore, combining drugs from class B and C may result in higher bioavailability because of metabolic inhibition leading to toxicity. Similar results are also expected if two drugs are selected from class C in multidrug therapy such as Kaletra where ritonavir and lopinavir both compete for P-gp and CYP3A4, thereby altering plasma concentrations. Appropriate addition of modulating agents, which are substrates for either CYP3A4 or, efflux proteins, or both, can result in higher oral bioavailability. In fact, interaction between two protease

Table 2 Absorption–metabolism classification of antiretroviral agents

Class	Efflux (P-gp)	Metabolism (CYP3A4)	Antiretroviral agents
A	–	–	NRTIs
B	–	+	NNRTIs
C	+	+	PIs

NRTIs: nucleoside and nucleotide reverse transcriptase inhibitors; NNRTI: nonnucleoside reverse transcriptase inhibitors.

inhibitors is not always nonproductive; instead, it may be beneficial in some cases. Thus, ritonavir's unique interaction allows the drug to be a boosting master. Lopinavir's antiviral property is profoundly enhanced after a low dose combination with ritonavir in Kaletra, which inhibits lopinavir's efflux and metabolism. Currently, ritonavir is combined with all other PIs for its boosting effect, which resulted in the production of new Kaletra (ritonavir + tipranavir)—the most potent anti-HIV drug. Enfuvirtide (Fuzeon) is a new class of anti-HIV agent, known as fusion inhibitor. This drug needs to be administered by injection because it is degraded in the low stomach pH. So far, enfuvirtide has not produced any interaction with other antiretroviral medications.

In general, NRTIs have mostly pharmacodynamic interactions because of their additive/synergistic effects. Because NRTIs are not substrates for CYP3A4 and are eliminated by kidney, these drugs have little kinetic impact on PIs and NNRTIs. In contrast, all NNRTIs are either inducer or inhibitors of CYP3A4. Nevirapine is a CYP3A4 inducer, whereas delavirdine serves as an inhibitor for the same enzyme and efavirenz has both inducer/inhibitor properties. Therefore, selection of NNRTIs in HAART regiment requires more attention because some combinations can lead to low bioavailability and others may exert toxicity of PIs. Dosages of PIs need to be carefully considered, because these drugs can induce/inhibit both P-gp and CYP3A4. Therefore, PIs cause a maximum number of drug–drug interactions, particularly if the other drugs are also substrate for P-gp and/or CYP3A4.

Interactions between PIs and drugs for opportunistic infections

HIV patients are highly susceptible to OIs caused by both bacteria and fungi and require antimycobacterial antibiotics and azole antifungal agents. Several antimycobacterials (clarithromycin, erythromycin, rifabutin, and rifampin) have been reported to interact with PIs (Table 3). In most cases, coadministration of clarithromycin with PIs caused the inhibition of CYP3A4 and possibly P-gp, resulting in higher AUC of PIs and clarithromycin (Table 3). Similarly, the AUC of saquinavir was elevated by 99% in the presence of erythromycin (Grub et al. 2001). In contrast, another macrolide, azithromycin (zithromax), has shown minimal effect on CYP3A4 and did not alter the blood level of indinavir (Foulds et al. 1999), and may serve as a better alternative to erythromycin. Rifabutin interacted with PI in a different manner (Table 3). Ritonavir caused a 3-fold elevation in rifabutin blood level due to inhibition of CYP3A4 (Cato et al. 1998; Norvir 2005) and possibly P-gp. In general, PIs elevated blood concentration of rifabutin

with simultaneous alteration in PI levels. Coadministration of rifabutin and PI caused the reduced blood level of amprenavir, fosamprenavir, indinavir, nelfinavir, and saquinavir possibly as a result of the induction of CYP3A4 causing rapid metabolism. The possibility of higher efflux due to induction of P-gp cannot be ruled out. Interactions between PIs and antituberculosis drug rifampin are a growing concern, because rifampin is an inducer of both P-gp and CYP3A4 (Katragadda et al. 2005). Consequently, coadministration of rifampin with PIs constitutively decreased the blood level of PIs because of higher efflux and metabolism (Table 3). Thus, coadministration of PIs with rifampin may lead to subtherapeutic level of PIs.

Several azole antifungals are administered to treat OIs, and these agents are potent inhibitors of CYP3A4 as well as P-gp. Therefore, coadministration of azole with PIs will result in higher AUC of PIs due to decreased efflux and metabolism. This combination may cause drug toxicity unless the dosage regimen is properly adjusted. Table 4 summarizes the clinical interactions between PIs and ketoconazole, and itraconazole and fluconazole, respectively. An elevated blood concentration of PIs (amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir) was evident, possibly as a result of inhibition of both efflux and metabolism. In contrast, coadministration of ketoconazole with Kaletra resulted in lower blood concentration of lopinavir, whereas ketoconazole level was elevated. As a result, Kaletra effect was diminished, but ketoconazole effect was enhanced significantly (Table 4) (Kaletra [package insert] 2005; CDC 2000a, b). Mechanism of interactions between Kaletra and ketoconazole is unknown. In the case of itraconazole, the blood levels of indinavir and ritonavir were elevated possibly as a result of inhibition of CYP3A4 (Albengres et al. 1998, Crixivan [package insert] 2005). When saquinavir was administered with itraconazole, saquinavir level remained unchanged but the half-life of itraconazole was prolonged (Table 4). However, interaction between fluconazole and PIs are similar to that of ketoconazole. Although blood concentration of indinavir was depressed, levels of ritonavir, saquinavir, and tipranavir were elevated possibly as a result of inhibition of CYP3A4 (Table 4). The role of efflux protein in these interactions is unknown.

Cholesterol lowering drugs and PIs

Although HAART extends the life of HIV-infected population, controlling elevated blood cholesterol levels associated with PIs is a challenging tusk. Because high cholesterol and triglyceride levels are connected to cardiovascular problem, patients are instructed to take prescription for cholesterol-lowering drugs, i.e., the statins. This group of drugs is also metabolized by CYP3A4 and may therefore cause higher PIs levels in the blood. However, all

Table 3 Drug–drug interactions among antimycobacterials and protease inhibitors

Protease inhibitors	Effect on antimycobacterial levels	Effect on PI levels	Mechanism of interaction	References
Clarithromycin				
Amprenavir	AUC (NS)	AUC ↑	Inhibition of CYP3A4 by APV	Agenerase [packageinsert] 2004; Mummaneni et al. 2002; Brophy et al. 2000
Atazanavir	AUC ↑	AUC ↑	Inhibition of CYP3A4 by ATV	Mummaneni et al. 2002; Norvir [package insert] 2005
Fosamprenavir	No Change	AUC ↑	Inhibition of CYP3A4 by clarithromycin	Frotovase [package insert] 2003
Indinavir	AUC ↑	AUC ↑	Inhibition of CYP3A4 by both drugs	Boruchoff et al. 2000; Foulds et al. 1999
Lopinavir + Ritonavir	May increase	–	Inhibition of CYP3A4 by Kaletra	Kaletra [package insert] 2005
Nelfinavir				
Ritonavir	AUC ↑	AUC (NS)	Inhibition of CYP3A4 by RTV	Fiske et al. 1998; Ouellet et al. 1998
Saquinavir	AUC ↑	AUC ↑	Inhibition of CYP3A4 by clarithromycin	Frotovase [package insert] 2003
Tipranavir	AUC ↑	AUC ↑	Induction of CYP3A4 by TPV	Aptivus [package insert] 2005
Rifabutin				
Amprenavir	AUC ↑	AUC ↓	Inhibition of CYP3A4 by APV	Agenerase [package insert] 2004; Decker et al. 1998; DHHS 2001
Atazanavir	AUC ↑	AUC ↑	Inhibition of CYP3A4 by ATV	Reyataz [package insert] 2006; Agarawala et al. 2002
Fosamprenavir	AUC ↑	AUC ↓	Induction of CYP3A4 by rifabutin	Frotovase [package insert] 2003
Indinavir	AUC ↑	AUC ↓	Inhibition of CYP3A4 by IDV, induction of CYP3A4 by rifabutin	CDC 2000a, b; Crixivan [package insert] 2005; Hamzeh et al. 2000
Lopinavir + Ritonavir	AUC ↑	AUC ↑	Inhibition of CYP3A4 by Kaletra	Kaletra [package insert] 2005
Nelfinavir	AUC ↑	AUC ↓	Inhibition of CYP3A4 by NFV, induction of CYP3A4 by rifabutin	Viracept [package insert] 2004; CDC 2000a, b
Ritonavir	AUC ↑↑↑	–	Inhibition of CYP3A4 by RTV	Cato et al. 1998; Norvir [package insert] 2005
Saquinavir	–	AUC ↓	Induction of CYP3A4 by rifabutin	Frotovase [package insert] 2003
Tipranavir	AUC ↑	NS	Induction of CYP3A4 by TPV	Norvir [package insert] 2005
Rifampin				
Amprenavir	AUC (NS)	AUC ↓	Induction of CYP3A4 by rifampin	Agenerase [package insert] 2004
Atazanavir	No change	AUC ↓	Induction of CYP3A4 by rifampin	Burger et al. 2005
Fosamprenavir	No change	AUC ↓	Induction of CYP3A4 by rifampin	Frotovase [package insert] 2003
Indinavir	–	AUC ↓	Induction of CYP3A4 by rifampin	Crixivan [package insert] 2005
Lopinavir Ritonavir	–	AUC ↓	Induction of CYP3A4 by rifampin	Kaletra [package insert] 2005
Nelfinavir	No change	AUC ↓	Inhibition of CYP3A4 by NFV; induction of CYP3A4 by rifampin	Viracept [package insert] 2004; CDC 2000a, b
Ritonavir	AUC ↑	AUC ↓	Inhibition of CYP3A4 by RTV	Cato et al. 1998; Norvir [package insert] 2005
Saquinavir	–	AUC ↓	Induction of CYP3A4 by rifampin	Frotovase [package insert] 2003

Table 4 Drug–drug interactions among antifungals and protease inhibitors

Protease inhibitors	Effect on antifungal levels	Effect on PI levels	Mechanism of Interaction	References
Ketoconazole				
Amprenavir	AUC ↑	AUC ↑	Inhibition of CYP3A4 and P-gp by APV and CYP3A4 by ketoconazole	Agenerase [package insert] 2004; Decker et al. 1998; Polk et al. 1999
Atazanavir	No change	Unknown	Unknown	
Fosamprenavir	Unknown	Unknown	Unknown	
Indinavir	Unknown	AUC ↑	Inhibition of CYP3A4 by ketoconazole	Agenerase [package insert] 2004
Lopinavir + Ritonavir	AUC ↑↑	LPV-AUC ↓	Increased ketoconazole effect, but decreased LPV/r; Mechanism unknown	Kaletra [package insert] 2005; CDC 2000a, b
Nelfinavir	Unknown	AUC ↑	Inhibition of CYP3A4 by ketoconazole	Viracept [package insert] 2004
Ritonavir	Unknown	AUC ↑	Inhibition of CYP3A4 by ketoconazole	Albengers et al. 1998
Saquinavir	No change	AUC ↑	Inhibition of CYP3A4 by ketoconazole	Grub et al. 2001
Itraconazole				
Indinavir	Unknown	AUC ↑	Inhibition of CYP3A4 by itraconazole	Crixivan [package insert] 2005
Lopinavir + Ritonavir	Unknown	Unknown	Inhibition of CYP3A4 by both drugs	Kaletra [package insert] 2005; Sporonax [package insert] 2000
Ritonavir	Unknown	AUC ↑	Inhibition of CYP3A4 by itraconazole	Albengres et al. 1998
Saquinavir	Half-life increased	No change	Inhibition of CYP3A4 by itraconazole	Cardiello et al. 2003
Fluconazole				
Indinavir	No change	AUC ↓	Unknown	Crixivan [package insert] 2005; Bellibas 1999; DeWit 1998
Ritonavir	Unknown	AUC ↑	Inhibition of CYP3A4 by fluconazole	Cato et al. 1997
Saquinavir	Unknown	AUC ↑	Inhibition of CYP3A4 by fluconazole	Koks et al. 2001
Tipranavir	No change	AUC ↑	Inhibition of CYP3A4 by fluconazole	Aptivus [package insert] 2005

of these medicines do not interact with all the PIs. Atorvastatin (Lipitor) has no significant effect on the blood level of fosamprenavir (Lexiva [package insert] 2005), lopinavir/ritonavir (Kaletra [package insert] 2005; Carr et al. 2000), and tipranavir(Aptivus [package insert] 2005). Similarly, pravastatin (Pravachol) and fluvastatin (Lescol) appears to have lower interactions with most PIs (Kaletra [package insert] 2005; Carr et al. 2000). However, PIs can have adverse effect on these statins. For example, blood levels of simvastatin were dangerously elevated when concurrently taken with indinavir, Kaletra, nelfinavir, and saquinavir (Kaletra [package insert] 2005; Hsyu et al. 2001; Viracept [package insert] 2004; Fortovase [package insert] 2003).

Cardiovascular medicines and antiretrovirals agents

Cardiovascular drugs (i.e., amiodarone, bepridil, diltazem, digoxin, dofetilide, ergotamine, and nifedipine) interact with PIs, and NNRTIs possibly result from inhibition of CYP3A4 by PIs. Digoxin is known to interact with efflux proteins. Profound drug–drug interactions are evident upon concomitant therapy with digoxin and rifampin. Plasma concentration of digoxin after oral administration was significantly diminished in combination with rifampin. Rifampin is a potent CYP3A4 inducer (Kolars et al. 1992). This antibiotic appeared to induce intestinal P-gp by 3.5-fold and CYP3A4 by 4.4-fold (Greiner et al. 1999; Schuetz et al. 1996). Therefore, rifampin caused a higher efflux of digoxin because of induction of P-gp expression in the intestine and higher metabolism by induced CYP3A4 both in the intestine and liver. Both effects reduced the oral bioavailability of digoxin. However, blood concentration of digoxin was elevated only by 29% following the coadministration of ritonavir possibly as a result of inhibition of P-gp-mediated efflux and CYP3A4-mediated metabolism (Norvir [package insert] 2005; Penzak et al. 2003).

Immune modulators and antiretrovirals agents

The immune modulator drugs are not free from adverse interaction when coadministered with antiretroviral agents. Several immune modulators such as cyclosporin, interleukin-2, mycophenolate, rapamycin, sirolimus, and tacrolimus are prescribed in conjunction with anti-HIV medications. Among these, the most interacting drug is cyclosporine. The simultaneous administration of saquinavir with cyclosporin resulted in a 300% increase in blood

Table 5 Drugs interact with P-glycoprotein

P-glycoprotein

| Substrate	Inhibitor	Inducer
Anti-HIV drugs		
Aprenavir	Nelfinavir	Amprenavir
Atazanavir	Ritonavir	Nelfinavir
Fosamprenavir	Saquinavir	Ritonavir
Indinavir		
Lopinavir		
Nelfinavir		
Ritonavir		
Saquinavir		
Tipranavir		
Cardiac drugs		
Digoxin	Quinidine	Amiodarone
Quinidine	Verapamil	Diltiazem
Lovastatin	Amiodarone	Nicardipine
Celiprolol	Atorvastatin	Nifedipine
Digoxin	Diltiazem	Verapamil
Diltiazem	Felodipine	
Talinolol	Lidocaine	
Verapamil	Nicardipine	
	Nitrendipine	
	Terfenadine	
Anticancer drugs		
Doxorubicin	Cisplatin	Tamoxifen
Daunorubicin	Daunorubicin	Vinblastine
Vinblastine	Doxorubicin	
Vincristine	Etoposide	
Actinomycin D	Fluorouracil	
Etoposide	Methotrexate	
Cisplatin	Tamoxifen	
Paclitaxel	Vinblastine	
Docetaxel	Vincristine	
Teniposide		
Cytarabine		
Fluorouracil		
Methotrexate		
Mitomycin		
Vindesine		
Antibiotics		
Grepafloxacin	Clarithromycin (I)	Erythromycin
Erythromycin	Erythromycin	Rifampicin
Rifampicin		
CNS drugs		
Levomeprazine		
Protriptylene		
Ripseridone		
Hormonal drugs		
Aldosterone	Cortisol	Dexamethasone
Cortisol	Hydrocortisone	Estradiol
Dexamethasone	Progesterone	Insulin
Estradiol	Testosterone	
Hydrocortisone		
Anxiolytics, sedatives		
Amitriptyline	Midazolam	Phenobarbital
Midazolam	Nefazodone	
Nefazodone		

Table 5 (continued)

P-glycoprotein

Substrate	Inhibitor	Inducer
Immunosuppressive agents		
Cyclosporin A	Cyclosporine A	Cyclosporine
FK506	Tacrolimus	Prednisolone
Methylprednisolone	Sirolimus	Sirolimus
Prednisolone		Tacrolimus
Tacrolimus		
Opiates		
Fentanyl	Methadone	Morphine
Loperamide		
Methadone		
Morphine		
Antifungals		
Itraconazole	Ketoconazole	
Sparfloxacin	Clotrimazole	
	Itraconazole	
Antihistamines		
Terfenadine	Astemizole (I)	
Domperidone	Chlorpromazine (I)	
Antiemetic		
Ondansetron		

levels of cyclosporin with increased immunomodulating effect and high renal toxicity (Brinkman et al. 1998). Similar elevation in cyclosporin have been reported when it was coadministered with Kaletra or nelfinavir (Kaletra [package insert] 2005; Viracept [package insert] 2004). Cyclosporin is a well-known substrate for both P-gp and CYP3A4. The enhanced immunomodulatory effect and flux of cyclosporin are attributable to competitive inhibition of intestinal P-gp and CYP3A4 and hepatic CYP3A4 by the antiretrovirals.

Recreational drugs and antiretroviral agents

Interactions between recreational drugs [e.g., benzodiazepines, cocaine, lysergic acid dithylamide (LSD), marijuana, amphetamine (Meth), 3,4-methylenedioxymethamphetamine (MDMA), and opiates] and anti-HIV medications can cause drug toxicity, and influence clinical outcomes of HAART, mostly as a result of altered metabolism. In a recent a review, Wynn et al. (2005) have nicely described the interactions of antiretrovirals and drugs of abuse. Many benzodiazepines (midazolam, trizolam, and alprazolam) are substrates and inhibitor for P-gp and metabolized by CYP3A4. During coadministration of ritonavir with alprazolam, initial clearance of alprazolam diminished leading to enhancement of alprazolam toxicity (Greenblatt et al. 2000).

Fig. 3 Uptake of ^{3}H ritonavir across MDCK-MDR1 cell monolayers in the presence of Ketoconazole (50 μM) and MK571 (25 μM). $p < 0.05$; $n = 3$–5; ±1 SD.
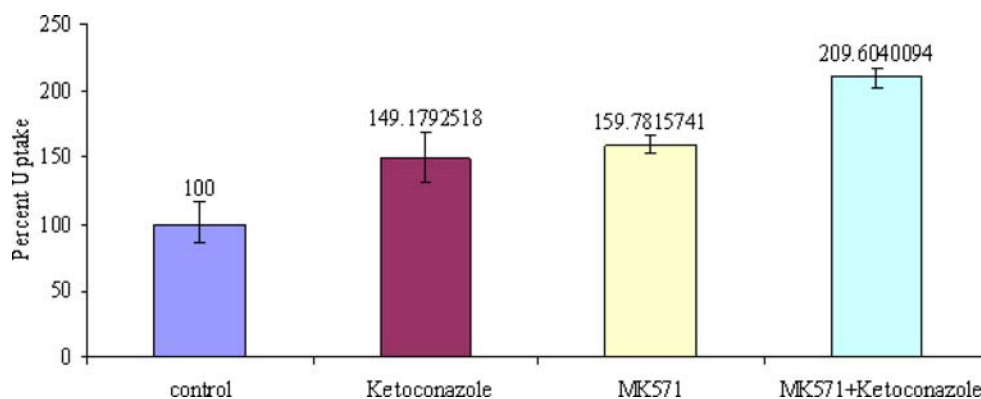
Such effect was reversed following prolonged coadministration (12 days) of these two drugs. In the later case, alprazolam AUC diminished significantly (Venkatakrishnan et al. 1998). Such discrepancy is possibly attributable to ritonavir's initial inhibitory effect on CYP3A4 followed by induction of CYP3A4 with chronic dosing. Such effects can result from both short-term inhibition and long-term induction of P-gp by ritonavir. Inhibition of CYP3A4 and/or P-gp may cause alprazolam, midazolam, and trizolam levels to rise, and will therefore cause toxicity, whereas induction of CYP3A4 and/or P-gp will lead to withdrawal symptoms and therapeutic failure. Flunitrazepam is primarily metabolized by CYP3A4 and 2C19. Ritonavir coadministration may result in flunitrazepam toxicity including hypotension, confusion, and aggressive behavior (Smith et al. 2002). Other benzodiazepines, such as lorazepam, oxazepam, temazepam, and diazepam, are metabolized by non-CYP3A4 enzymes such as uridine 5′-diphosphate glucuronosyltransferase (UGT), UGT1A1, UGT1A3, and UGT2B7, but can also undergo drug interactions with antiretrovirals.

Cocaine is metabolized to nor-cocaine primarily by CYP3A4 (Ladona et al. 2000; LeDuc et al. 1993). Therefore, concomitant administration of cocaine with any PIs, particularly ritonavir, will result in overdose effect (acute response) or withdrawal symptom (prolonged response) due to inhibition and induction of CYP3A4 by ritonavir, respectively. Coadministration of cocaine with PIs, efavirenz, ketoconazole, nefazodone, erythromycin, and clarithromycin, i.e., the CYP3A4 inhibitors, will result in cocaine toxicity. In contrast, drugs (nevirapine, rifampin) that induce CYP3A4 may shift the metabolism from hydroxylation to N-demethylation, thereby producing higher levels of toxic metabolites (Bornheim 1998; Pellinen et al. 1994). Many HIV-infected patients use gamma hydroxybutyrate (GHB). However, its metabolism is not established in human. Harrington et al. (1999) reported GHB toxicity in HIV-positive individuals on ritonavir and saquinavir therapy, also consuming MDMA regularly. The authors concluded that PI-mediated inhibition of CYP3A4 was likely the cause of such toxicity.

Both amphetamine and MDMA have potential interactions with ritonavir and delaviridine (Pau 2002; Henry and Hill 1998). MDMA is demethylated to 3,4- dihydroxymethamphetamine primarily by CYP2D6 with minor contributions from CYP1A2, CYP2B6, and CYP3A4 (Lin et al. 1997; Maurer et al. 2000). Besides CYP3A4, ritonavir can inhibit CYP2D6, CYP2C9, and CYP2C19 (Harrington et al. 1999). Fetal drug–drug interactions were observed in HIV patients receiving MDMA with zidovudine, lamivudine, and ritonavir in their treatment regimen (Henry and Hill 1998). Inhibition of CYP2D6 by ritonavir is likely the cause of enhanced MDMA toxicity. A nonlinear MDMA pharmacokinetic pattern was observed in a study on 14 patients with CYP2D6 genotype (de la Torre et al. 2000). Metabolism of amphetamine and MDMA, although not completely understood, CYP2D6 genetic variability, and possibly P-gp effect may cause adverse toxicity, taking CYP2D6 inhibitors such as ritonavir, bupropion, fluxetine, and quinidine (Ketabi-Kiyanvash et al. 2003; Wynn et al. 2005). Although marijuana, with its active ingredient identified as tetrahydrocannabinol (THC), is metabolized by CYP3A4 and CYP2C9 (Matsunaga et al. 2000), no clinically significant drug interaction was observed in two separate studies (Kosel et al. 2002; Abrams et al. 2003).

A detailed review of opiate interactions with other drugs has been reported by Armstrong and Cozza (2003a, b). Narcotic analgesics [phenylpiperidines, meperidines, fentanyl, pseudopiperidines (methadone), and propoxyphen] and alkaloids (natural: heroin, morphine, and codeine; semisynthetics: hydromorphone, oxymorphone, hydrocodone, oxycodone, dihydrooxycodeine, and buprenorphine) are widely indicated in AIDS patients. Many of these opiates are metabolized by CYP3A4 and substrates/inhibitors/inducers for P-gp (Table 5), posing a serious risk of causing potential drug–drug interactions with multidrug regimens in HAART. Ritonavir dosing in healthy subjects caused a decrease in AUC of meperidine along with a rise in normeperidine levels indicating enhanced metabolism (Piscitelli et al. 2000). Lopinavir + ritonavir (Kaletra), ritonavir, nevirapine, and efavirenz were found to cause
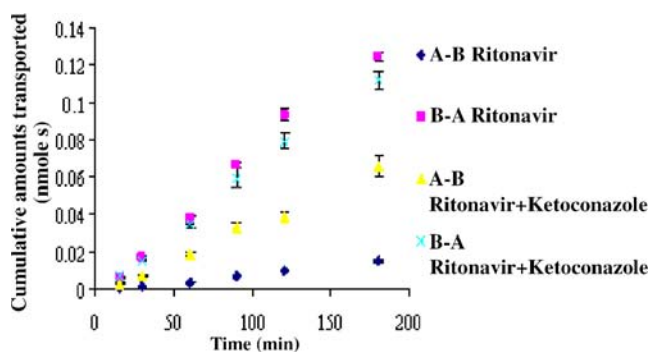
Fig. 4 Transport of ^{3}H ritonavir across MDCK-MDR1 cell monolayers in Transwell® inserts in the presence of ketoconazole. [3]H ritonavir permeability in apical to basolateral (A–B) direction was significantly enhanced ($p < 0.05$) in the presence of ketoconazole (50 μM). P_{app} (A–B) cm/s $\times 10^{-6}$, control (3.0 ± 0.12), and ketoconazole (12.7 ± 1.31); $n = 4–5$; ±1 SD.

opiate withdrawal when given in combination with methadone (Altice et al. 1999; Beauverie et al. 1998; Pinzani et al. 2000; McCance-Katz et al. 2000; Bart et al. 2001). Methadone is primarily metabolized by CYP3A4 with minor contributions from CYP2D6, CYP2C9, and CYP2E1. It is a substrate and inducer of P-gp. The mechanisms behind this opiate withdrawal may involve drug–drug interaction due to induction of P-gp and CYP3A4 by antiretrovirals resulting in enhanced efflux and metabolism of methadone. Metabolisms of heroin, morphine, and codeine occur independently of CYP 450 system. These alkaloids are first metabolized by liver and plasma esterases, and then by UGT2B7 and UGT1A3. Also, several sedatives (hydromorphone, oxymorphone, and dehydrocodeine) are mainly metabolized by UGT2B7 and UGT1A3 with a minor contribution from CYP2D6. Therefore, these alkaloids are unlikely to interact with antiretrovirals. In contrast, semisynthetic opiates (hydrocodone, oxycodone, and buprenorphine) are mainly metabolized by CYP3A4 and CYP2D6. Therefore, inhibition or induction of CYP2D6 and CYP3A4 by retrovirals is likely to produce variable analgesic effects of these agents.

PIs and NNRTIs are either substrate/inducer/inhibitor for CYP3A4 or P-gp or both and any drug that interacts with this efflux proteins and metabolizing enzyme will generate drug–drug interactions resulting in altered bioavailability and lead to synergism or antagonism. A list of these drugs was presented in our earlier review (Katragadda et al. 2005).

In vitro testing for drug–drug interactions

The effect of MRPs in drug efflux is yet to be established. In clinical settings, it is difficult to determine the interactions of efflux proteins with multidrug regimens. Therefore, we performed in vitro experiments to determine the

role of efflux pump in drug absorption. By using MDR-transfected MDCK cell line, several experiments were designed to evaluate such drug–drug interactions particularly for their effects on absorption. Uptake of ritonavir was examined in the presence of ketoconazole, a potent inhibitor of P-gp. Such uptake of ritonavir was challenged with FK571, which is potent inhibitor of MRP. A significant elevation in ritonavir uptake was evident in the presence of both Ketoconazole and MK571 (Fig. 3). This higher uptake was probably caused by inhibition of P-gp and MRP with ketoconazole and MK571, respectively. When both ketoconazole and MK571 were added, uptake of ritonavir was further enhanced compared to ketoconazole alone indicating that ritonavir is effluxed by both P-gp and MRP. Once such efflux was stopped by the inhibitors, higher intracellular uptake of ritonavir was observed. Another experiment was conducted to establish P-gp-mediated ritonavir efflux. This study was conducted in a Transwell plate, where cells were grown on semipermeable membrane. Polarized MDCK-MDR-1 cells express P-gp on the apical surface. During a transport run, ritonavir was added either onto the apical or basolateral surface, and samples were collected from the opposite side. Figure 4 shows that ritonavir transport was significantly enhanced in the presence of ketoconazole. Similar profile is expected in clinincal setting, if ketoconazole is administered with ritonavir, it will inhibit the P-gp-mediated efflux of PI resulting in higher transport. We next examined the transport interaction between erythromycin and saquinavir. Both erythromycin and saquinavir are substrates for P-gp and MRP. Thus, uptake of erythromycin was augmented by
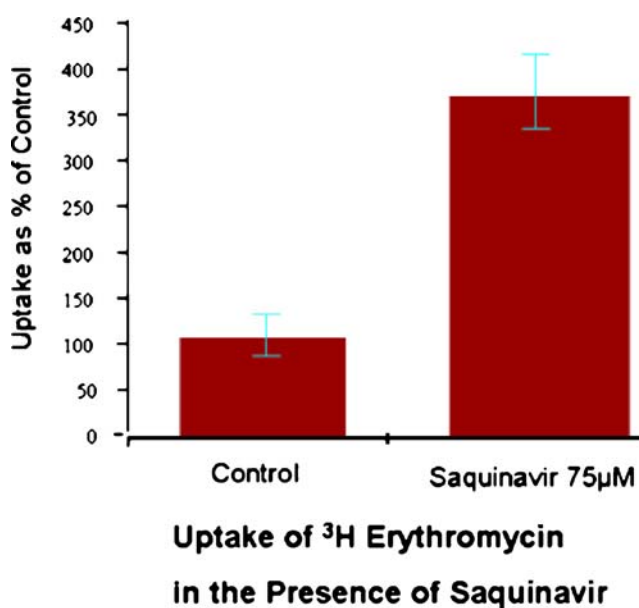

Fig. 5 Uptake of ^{3}H erythromycin in the presence of saquinavir (75 μM) across MDCK-MDR1 cell monolayers. Erythromycin uptake was significantly elevated ($p < 0.05$) due to inhibition of efflux proteins (P-gp and MRP) by saquinavir. $n = 4$; ±1 SD.
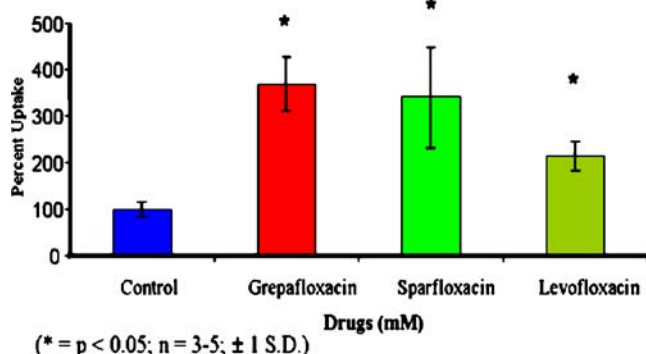
Fig. 6 Uptake of ¹⁴C erythromycin across MDCK-MDR1 cell monolayers in the presence of various fluoroquinolones. Inhibition of P-gp/MRP-mediated efflux by grepafloxacin (1 mM), sparfloxacin (1 mM), and levofloxacin (1 mM) enhanced erythromycin absorption significantly. $p < 0.05$; $n = 3–5$; ±1 SD.

Fig. 8 Erythromycin and ketoconazole resulted in a decrease of carbamazepine metabolism due to inhibition of microsomal CYP3A activity. $p < 0.05$; $n = 5$; ±1 SD.

3-fold in the presence of saquinavir possibly as a result of competitive inhibition of both P-gp and MRP (Fig. 5). A recent study from our laboratory revealed that erythromycin flux was enhanced in the presence of some fluoroquinolones (sparfloxacin, levofloxacin, and grepafloxacin) possibly as a result of competitive inhibition of P-gp and MRP (Fig. 6). This result led us to propose that the simultaneous administration of fluoroquinolones and macrolides could minimize the efflux and metabolism of both drugs. Such interaction would result in reduction of the required dose, thus preventing dose-related side effects and development of resistance by gram-positive bacteria such as *Streptococcus pneumoniae* and *Legionella pneumoniae* (Sikri et al. 2004). Several fluoroquinolones, such as norfloxacin, ofloxacin, grepafloxacin, enoxacin, and lomefloxacin, were evaluated for P-gp-mediated efflux of cyclosporin (Fig. 7). These *in vitro* studies indicate that grepafloxacin, levofloxacin, and sparfloxacin are potent inhibitors of P-gp-mediated efflux. We have also examined the CYP3A4-mediated drug–drug interactions by using an *in vitro* system. Carbamazepine is a substrate for CYP3A4. Both erythromycin and ketoconazole caused significant reduction of carbamazepine metabolism (Fig. 8).
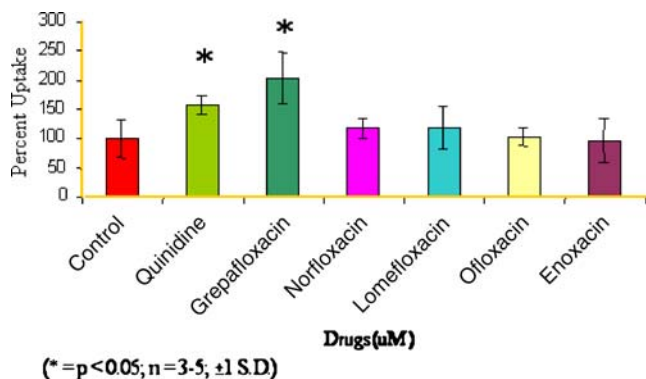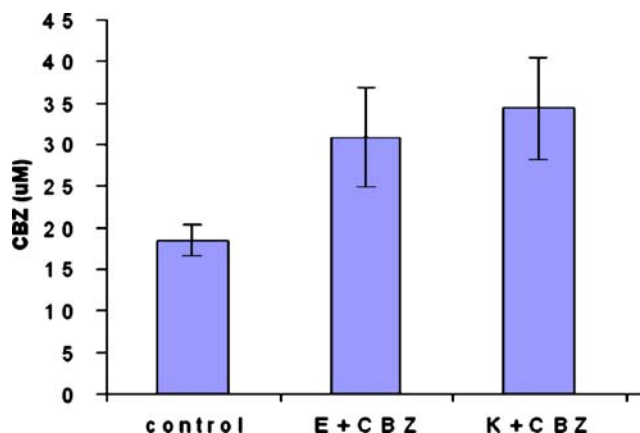
Integrated functions of efflux and metabolism

P-gp is a robust efflux transporter and there are so far no structure delineations for its substrate specificity, but a number of drugs are found to interact with this transporter. CYP3A4 is the major phase 1 drug-metabolizing enzyme in human that metabolizes approximately 70% of xenobiotics. Therefore, when two drugs are taken orally, it is very likely that they will interact via metabolism. In human, CYP3A4 is the key enzyme responsible for intestinal and hepatic metabolism, and both P-gp and CYP3A4 have overlapping substrate specificity. Also, both proteins coexpress in the intestine. As a result, the concomitant administration of cyclosporine with rifampin, an inducer of both proteins, lowers the oral bioavailability (C_{max}) of cyclosporine. Similarly, saquinavir concentration was reduced by 80% after coadministration with rifampin. Conversely, ketoconazole, an inhibitor of P-gp/CYP3A4, caused the elevation of cyclosporine levels (C_{max}) in the blood resulting in higher toxicity (Wacher et al. 1998). However, the concomitant administration of ritonavir with saquinavir led to 5- to 6-fold increase of plasma concentration of saquinavir (C_{max}) as a result of inhibition of both CYP3A4 and P-gp by ritonavir (Wacher et al. 1998). In addition to oxidative metabolism, conjugation reactions may play a crucial role in detoxification of xenobiotics from the small intestine. Many drug molecules are effluxed into intestinal lumen after being conjugated with a glucuronide or sulfate moiety. Transport mechanism for the cellular extrusion of the conjugated metabolites and organic anions has been described recently (Leslie et al. 2001). Several antiretroviral agents and other drugs coadministered in AIDS patients can pharmacokinetically act as inhibitors or inducers. However, coadministration of clarithromycin, an inhibitor of CYP3A4 with indinavir, did not alter the blood levels of indinavir (Boruchoff et al. 2000). Administration of antiretrovirals

Fig. 7 Uptake of cyclosporin A in the presence of various fluoroquinolones across MDCK-MDR1 cells.

335

concurrently with other therapeutic agents may lead to increased absorption as a result of inhibition of P-gp-mediated efflux and CYP-mediated metabolism leading to potential toxicity. The chronic administration of certain PIs will enhance the production of MDR proteins (P-gp) and CYP enzymes, resulting in lower bioavailability and subtherapeutic plasma concentrations of coadministered agents. If the coadministered drug is a strong inhibitor or inducer of P-gp/CYP3A4, results will be variable. For example, the concomitant administration of PIs and clarithromycin resulted in higher AUC of both drugs because of the strong inhibition of CYP3A4 (Table 3). In contrast, opposite results were observed during the coadministration of PIs + rifampin due to heavy induction of CYP3A4 and P-gp (Table 3). Therefore, it is conclusively evident that integrated function of CYP3A4 and P-gp can drastically alter the oral bioavailability and may lead to the emergence of drug resistance viral strains. An understanding of the increased/decreased bioavailability of one drug in the presence of another may greatly aid in the design of appropriate drug regimen. Figure 9 depicts the coordination of efflux and metabolism in GI tract.

Activation of mammalian xenobiotic-sensing nuclear receptors PXR and CAR

P-gp/MDR1 is regarded as one of the major factors in the development of cellular drug resistance. Up-regulation of CYP enzymes in response to chronic administration of therapeutic agents may also play an important role in lowering their blood levels. A close chromosomal location of P-gp (MDR gene locus 7q21–7q21*7q21.1–7q21.1) and
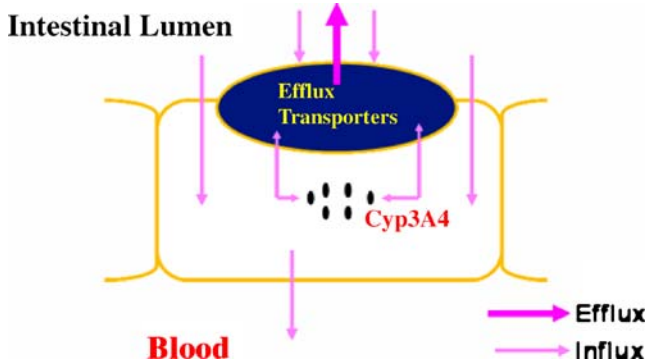

Fig. 9 Mechanism of drug interactions with efflux transporters and metabolizing enzyme CYP3A4 in enterocytes. Drug absorption is regulated by coordinated functions of both efflux transporters and CYP3A4. Because of efflux pump, limited amounts of drugs may enter the cell and then get metabolized by CYP3A4. Some metabolites can be pumped out of the cell into the lumen. Ultimately, very limited amounts drugs are absorbed and reach the blood for further hepatic metabolism. Therefore, any induction or inhibition of these two key factors results in altered bioavailability of drugs in a multidrug regimen.
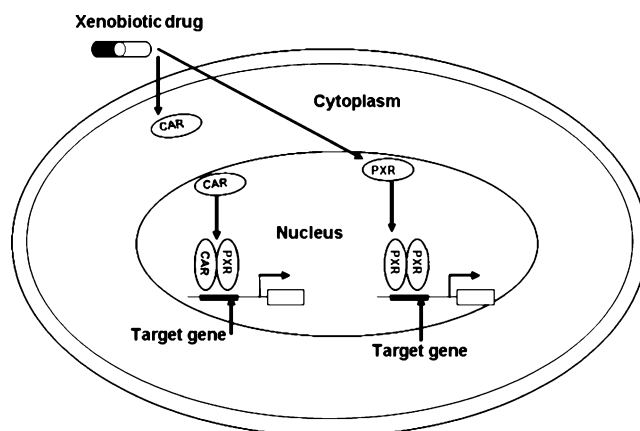

Fig. 10 Activation of mammalian xenobiotic-sensing nuclear receptors PXR and CAR. Xenobiotic agent after entering the cell, triggers cytoplasmic CAR for nuclear translocation by an unknown mechanism, or directly activates PXR. Then, both CAR and PXR subsequently heterodimerize with RXR and bind to specific response elements leading to transcription.

CYP3A4 (CYP3A4 gene locus 7q21.3–7q22.1*) genes, their expression in mature enterocytes, and similar substrate specificities indicate that the function of these two proteins are complementary in nature and may form a coordinated intestinal barrier (Katragadda et al. 2005; Patel et al. 2004).

Molecular mechanism of P-gp and CYP induction

Molecular mechanisms of P-gp and CYP induction are still being investigated by researchers. Figure 10 depicts a diagrammatic representation of molecular mechanism of such induction. The role of two members of the nuclear receptor superfamily of transcription factors, the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) have been recently reported. These receptors act as sensors for drugs including xenobiotics. The nuclear receptor superfamily consists of four modular domains: (1) a highly variable N-terminal region with an activation function (AF-1); (2) a DNA-binding domain with two zinc-finger motifs, a flexible domain, and a ligand binding domain (LBD) that also have an AF-2. After cellular entry, xenobiotics and other activators either trigger cytoplasmic-nuclear translocation of CAR by promoting the release of unknown proteins, or directly activate PXR in the nucleus. Subsequently, both PXR and CAR heterodimerize with retinoid X receptor (RXR) bind to their respective response elements on target gene and increase the transcription of those genes. In the flanking regions of several genes, response elements have been found that are activated by both PXR and CAR, thus allowing direct cross-communication between these two receptors. New insights into the transcriptional regulation of CYP3A4 revealed that vitamin D and its derivatives regulate the transcription by interact-

ing with vitamin D response (VDR) element. This regulation of intestinal CYP3A4 by VDR is complementary to the apparent role of PXR in regulating hepatic CYP3A4 (Ambudkar et al. 2003; Handschin and Meyer 2003; Synold et al. 2001).

Conclusion

Drug–drug interactions may occur when more than one drug is indicated for concomitant therapy, particularly if overlapping mechanisms are involved in the absorption and/or metabolism of these agents. Drug efflux proteins and metabolizing enzymes are two central factors controlling the oral bioavailability of drugs and subsequently their therapeutic efficacy, and are therefore responsible for many drug–drug interactions. This review outlines such interactions that commonly occur in AIDS patients receiving concomitant multiple drugs in HAART and also other recreational drugs. In this review, we briefly discussed drug interactions of antiretrovirals with other agents causing alteration of oral bioavailability of each another. These compounds include drug classes comprising antimicobacterial, azole antifungals, statins, cardiovascular agents, immune modulators, and recreational drugs (benzodiazepines, cocaine, LSD, marijuana, Meth, MDMA, and opiates). A list of drugs that are substrates for P-gp is presented in Table 5. A number of these agents may interact with several PIs. Most clinical studies report primarily metabolism-mediated drug interactions. Interestingly, it appears that any agent that induces CYP may also simultaneously activate P-gp. So these two barriers act in harmony to eliminate xenobiotics and the magnitude of P-gp-mediated efflux on the coordination of drug metabolism seems to be dependent on the spatial relationship between P-gp and CYP3A4. Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles of multidrug regimens. Therefore, a clear understanding of drug–drug interactions particularly with efflux transporters and metabolizing enzymes is pivotal to our understanding of such variable oral bioavailability and subsequent development of drug resistance as well as loss of anti-HIV activity from these antiretroviral drugs. Adverse drug interactions may be avoided simply by adjusting doses or substituting with drugs which are less or noninteracting.

References

Abrams DI, Hilton JF, Leiser RJ, Shade SB, Elbeik TA et al (2003) Short-term effects of cannabinoids in patients with HIV-1 infection: a randomized, placebo-controlled clinical trial. Ann Intern Med 139:258–266

Agarwala S, Mummaneni V, Randall D et al (2002) Pharmacokinetic effect of rifabutin on atazanavir with and without ritonavir in healthy subjects [abst #445]. 9th Conference on Retroviruses and Opportunistic Infections; Feb 24–28; Seattle, Washington

Agenerase [package insert]. Research Triangle Park, NC: Glaxo Wellcome Inc; 2004

Albengres E, Le Louet H, Tillement JP (1998) Systemic antifungal agents. Drug interactions of clinical significance. Drug Saf 18:83–97

Altice F, Friedland G, Cooney E (1999) Nevirapine induced opiate withdrawal among injection drug users with HIV infection receiving methadone. AIDS 13:957–962

Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 39:361–398

Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM (2003) P-glycoprotein: from genomics to mechanism. Oncogene 22:7468–7485

Aptivus [package insert] (2005) Ridgefield, CT: Boehringer Ingelheim Pharmaceuticals, Inc.; Nov

Armstrong SC, Cozza KL (2003a) Pharmacokinetic drug interactions of morphine, codeine, and their derivatives: theory and clinical reality, part I. Psychosomatics 44:167–171

Armstrong SC, Cozza KL (2003b) Pharmacokinetic drug interactions of morphine, codeine, and their derivatives: theory and clinical reality, part II. Psychosomatics 44:515–520

Bakos E, Evers R, Sinko E et al (2000) interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. Mol Pharmacol 57:760–768

Bart PA, Rizzardi PG, Gallant S, Golay KP, Baumann P, Pantaleo G, Eap CB (2001) Methadone blood concentrations are decreased by the administration of abacavir plus amprenavir. Ther Drug Monit 2001:23:553–555

Beauverie P, Taburet AM, Dessalles MC, Furlan V, Touzeau D (1998) Therapeutic drug monitoring of methadone in HIV-infected patients receiving protease inhibitors (letter). AIDS 12:2510–2511

Bellibas SE (1999) Indinavir–fluconazole interaction. [letter] Antimicrob Agents Chemother 43:432–433

Bornheim L (1998) Effect of cytochrome P450 inducers on cocaine-mediated hepatotoxicity. Toxicol Appl Pharmacol 150:158–165

Borst P, Schinkel AH, Smit JJ, Wagenaar E et al (1993) Classical and novel forms of multidrug resistance and the physiological functions of P-glycoproteins in mammals. Pharmacol Ther 60:289–299

Borst P, Evers R, Kool M, Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 92:1295–1302

Boruchoff SE, Sturgill MG, Grasing KW et al (2000) The steady-state disposition of indinavir is not altered by the concomitant administration of clarithromycin. Clin Pharmacol Ther 67:351–359

Brinkman K et al (1998) Pharmacokinetic interaction between saquinavir and cyclosporine. Ann Intern Med 129:914–915

Brophy DF, Israel DS, Pastor A et al (2000) Pharmacokinetic interaction between amprenavir and clarithromycin in healthy male volunteers. Antimicrob Agents Chemother 44:978–984

Burger D, Agarwala S, Child M et al (2005) Effect of rifampin on steady-state pharmacokinetics of atazanavir and ritonavir in healthy subjects [abst #657]. 12th Conference on Retroviruses and Opportunistic Infections; February 22–25; Boston, Massachusetts

Cardiello PG, Samor T, Burger D et al (2003) Pharmacokinetics of lower doses of saquinavir soft-gel caps boosted with itraconazole in HIV-1 positive patients. Antivir Ther 8:245–249

Carr RA, Andre AK, Bertz RJ et al (2000) Concomitant administration of ABT-378/ritonavir. Results in a clinically important pharmacokinetic interaction with atorvastatin but not pravastatin [abst #1644]. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–20; Toronto, Canada

Cato A, Cao G, Hsu A et al (1997) Evaluation of the effect of fluconazole on the pharmacokinetics of ritonavir. Drug Metab Dispos 25:1104–1106

Cato A, Cavanaugh J, Shi H et al (1998) The effect of multiple doses of ritonavir on the pharmacokinetics of rifabutin. Clin Pharmacol Ther 63:414–421

CDC (2000a) Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Jan 28, [AIDS Treatment Information Service: Current Treatment], Available at: http://www.hivatis.org/trtgdlns.html

CDC (2000b) Notice to readers: updated guidelines for the use of rifabutin or rifampin for the treatment and prevention of tuberculosis among HIV-infected individuals taking protease inhibitors or nonnucleoside reverse transcriptase inhibitors. MMWR 49:185–189

Chiou WL, Chung SM, Wu TC (2000) Potential role of P-glycoprotein in affecting hepatic metabolism of drugs. Pharm Res 17:903–905

Cole SP, Bhardwaj G, Gerlach JH et al (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258:1650–1654

Crixivan [package insert](2005) Whitehouse Station, NJ: Merck & Co., Inc.; Oct

Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, Kim RB (1999) OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. Drug Metab Dispos 27:866–871

Decker CJ, Laitinen LM, Bridson GW et al (1998) Metabolism of amprenavir in liver microsomes: role of CYP3A4 inhibition for drug interactions. J Pharm Sci 87:803–807

Deeley RG, Cole SP (1997) Function, evolution and structure of multidrug resistance protein (MRP). Semin Cancer Biol 8:193–204

de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R et al (2000) Non-linear pharmacokinetics of MDMA ("Ecstasy") in humans. Br J Clin Pharmacol 49:104–109

Department of Health and Human Services (2001) Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. August 13

DeWit S, Debier M, DeSmet M et al (1998) Effect of fluconazole on indinavir pharmacokinetics in human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 42:223–227

Endicottaland JA, Ling V (1989) The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu Rev Biochem 58:137–171

Fiske W, Benedek IH et al (1998) Pharmacokinetics of efavirenz (EFV) and ritonavir (RIT) after multiple oral doses in healthy volunteers [abst. #42269]. 12th International Conference on AIDS; June 28–July 3; Geneva, Switzerland

Foulds G, LaBoy-Goral L, Wei GCG et al (1999) The effect of azithromycin on the pharmacokinetics of indinavir. J Clin Pharmacol 39:842–846

Fortovase [package insert] (2003) Basel, Switzerland: F. Hoffman-La Roche Ltd

Gottesman MM, Pastan I (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 62:385–427

Gottesman MM, Pastan I' Ambudkar SV (1996) P-glycoprotein and multidrug resistance. Curr Opin Genet Dev 6:610–617

Greenblatt DJ, von Moltke LL, Harmatz JS, Durol AL, Daily JP, Graf J, Mertzanis P, Hoffman JL, Shader RI (2000) Alprazolam–ritonavir interaction: implications for product labeling. Clin Pharmacol Ther 67:335–341

Greiner B, Eichelbaum M, Fritz P (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 104:147–153

Grub S, Bryson H, Goggin T et al (2001) The interaction of saquinavir with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. Eur J Clin Pharmacol 57:115–121

Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ (1986) Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J Biol Chem 261:5051–5060

Hamzeh F, Benson C, Gerber J et al (2000) Steady-state pharmacokinetic interaction of modified-dose indinavir and rifabutin [abst #90]. 7th Conference on Retroviruses and Opportunistic Infections; Jan 30–Feb 2; San Francisco, California

Handschin C, Meyer UA (2003) Induction of drug metabolism: the role of nuclear receptors. Pharmacol Rev 55:649–673

Harrington RD, Woodward JA, Hooton TM, Horn JR (1999) Life-threatening interactions between HIV-1 protease inhibitors and the illicit drugs MDMA and gamma-hydroxybutyrate. Arch Intern Med 159:2221–2224

Henry J, Hill I (1998) Fatal interaction between ritonavir and MDMA. Lancet 352:1751–1752

Hofsliand E, Nissen-Meyer J (1989) Reversal of drug resistance by erythromycin: erythromycin increases the accumulation of actinomycin D and doxorubicin in multidrug-resistance cells. Int J Cancer 44:149–154

Hsu A, Granneman GR, Bertz RJ, Ritonavir (1998) Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 35:275–291

Hsyu PH, Schultz-Smith MD, Lillibridge JH et al (2001) Pharmacokinetic interactions between nelfinavir and 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors atorvastatin and simvastatin. Antimicrob Agents Chemother 45:3445–3450

Huisman MT, Smit JW, Crommentuyn KM et al (2002) Multidrug resistance protein 2 (MRP2) transport HIV protease inhibitors, and transport can be enhanced by other drugs. AIDS 16:2295–2301

Hulot JS, Villard E, Maguy A et al (2005) A mutation in the drug transporter gene ABCC2 associated with impaired methotrexate elimination. Pharmacogenet Genomics 15:277–285

Kaletra [package insert] (2005) North Chicago, IL: Abbott Laboratories; Oct

Katoh M, Nakajima M, Yamazaki H, Yokoi T (2001) Inhibitory effects of CYP3A4 substrates and their metabolites on P-glycoprotein-mediated transport. Eur J Pharm Sci 12:505–513

Katragadda S, Budda B, Anand BS, Mitra AK (2005) Role of efflux pumps and metabolizing enzymes in drug delivery. Expert Opin Drug Deliv 2:683–705

Ketabi-Kiyanvash N, Weiss J, Haefeli WE, Mikus G (2003) P-glycoprotein modulation by the designer drugs methylenedioxymethamphetamine, methylenedioxyethylamphetamine, and paramethoxyamphetamine. Addict Biol 8:413–418

Koks CH, Crommentuyn KM, Hoetelmans RM et al (2001) The effect of fluconazole on ritonavir and saquinavir pharmacokinetics in HIV-1 infected individuals. Br J Clin Pharmacol 51:631–635

Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB (1992) Identification of rifampin-inducible P540IIIA4 (CYP3A4) in human small bowel enterocytes. J Clin Invest 90:1871–1878

Kosel BW, Aweeka FT, Benowitz NL, Shade SB et al (2002) The

effects of cannabinoids on the pharmacokinetics of indinavir and nelfinavir. AIDS 16:534–550

Krishnamachary N, Center MS (1993) MRP gene associated with a non-P-glycoprotein multidrug resistance encode a 190-kDa membrane bound glycoprotein. Cancer Res 53:3658–3661

Ladona MG, Gonzalez ML, Rane A, Peter RM, de la Torre R (2000) Cocaine metabolism in human fetal and adult liver microsomes is related to cytochrome P450 3A expression. Life Sci 68:431–443

Larkin A, O'Driscol L, Kennedy S, Purcell R et al (2004) Investigation of MRP-1 protein and MDR-1 P-glycoprotein expression in invasive breast cancer: a prognostic study. Int J Cancer 112:286–294

LeDuc BW, Sinclair PR, Shuster L, Sinclair JF, Evans JE, Greenblatt DJ (1993) Norcocaine and N-hydroxynorcocaine formation in human liver microsomes: role of cytochrome P-450 3A4. Pharmacology 46:294–300

Letourneau IJ, Bowers RJ, Deeley RG, Cole SP (2005) Limited modulation of the transport activity of the human multidrug resistance proteins MRP1, MRP2 and MRP3 by nicotine glucuronide metabolites. Toxicol Lett 157:9–19

Leslie EM, Deeley RG, Cole SP (2001) Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. Toxicology 167:3–23

Lexiva [package insert] (2005) Research Triangle Park, NC: Glaxo Wellcome Inc.; Nov

Li X, Chan WK (1999) Transport, metabolism and elimination mechanisms of anti-HIV agents. Adv Drug Deliv Rev 39:81–103

Lin LY, Di Stefano EW, Schmitz DA, Hsu L et al (1997) Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. Drug Metab Dispos 25:1059–1064

Litman T, Brangi M, Hudson E et al (2000) Multidrug-resistant phenotype associated with over expression of the new ABC half transporter, MXR (ABCG2). J Cell Sci 113:2011–2021

Litman T, Druley TE, Stein WD, Bates SE (2001) From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci 58:931–959

Lown KS, Kolars JC, Thummel KE et al (1994) Inter-patient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction by the erythromycin breath test. Drug Metab Dispos 22:947–955

Malaty LI, Kuper JJ (1999) Drug interactions of HIV protease inhibitors. Drug Saf 20:147–169

Matsunaga T, Kishi N, Higuchi S, Watanabe K, Ohshima T, Yamamoto I (2000) CYP3A4 is a major isoform responsible for oxidation of 7-hydroxy-Δ^8-tetrahydrocannabinol to 7-oxo-Δ^8-tetrahydrocannabinol in human liver microsomes. Drug Metab Dispos 28:1291–1296

Maurer HH, Bickeboeller-Friedrich J, Kraemer T, Peters FT (2000) Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ("Ecstasy"). Toxicol Lett 112/113:133–142

McCance-Katz EF, Farber S, Selwyn PA, O'Connor A (2000) Decrease in methadone levels with nelfinavir mesylate (letter). Am J Psychiatry 157:481

Mummaneni V, Randall D, Chabuel D et al (2002) Steady state pharmacokinetic (PK) interaction study of atazanavir (ATV) with clarithromycin (CLR) in healthy subjects [abs. #H-1717]. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy; September 27–30; San Diego, California

Naruhashi K, Tamai I, Inoue N et al (2002) Involvement of multidrug resistance associated protein 2 in intestinal secretion of grepafloxacin in rats. Antimicrob Agents Chemother. 46:344–349

Norvir [package insert] (2005) Abbott Laboratories, North Chicago, IL

Ouellet D, Hsu A, Granneman GR et al (1998) Pharmacokinetic interaction between ritonavir and clarithromycin. Clin Pharmacol Ther 64:355–362

Pal D, Mitra AK (2006) MDR- and CYP3A4-mediated drug–herbal interactions. Life Sci 78:2131–2145

Parkinson A (1996) An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. Toxicol Pathol 24:48–57

Patel J, Mitra AK (2001) Strategies to overcome simultaneous P-glycoprotein mediated efflux and CYP3A4 mediated metabolism of drugs. Pharmacogenomics 2:401–415

Patel J, Buddha B, Dey S, Pal D, Mitra AK (2004) In vitro interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. Am J Ther 11:262–277

Pau AK (2002) Polypharmacy problems: Drug interactions in multidrug therapy of HIV infection. www.hiv-druginteractions.org

Pellinen P, Honkakoski P, Stenbeck F et al (1994) Cocaine N-demethylation and the metabolism-related hepatotoxicity can be prevented by cytochrome P450 3A inhibitors. Eur J Pharmacol 270:35–43

Penzak S, Shen J, Alfar R, Remaley A, Falloon J (2003) Influence of low-dose ritonavir on the pharmacokinetics of the P-glycoprotein (P-gp) substrate digoxin. Program and abstracts of the 4th International Workshop on Clinical Pharmacology of HIV Therapy; March 27–29, Cannes, France. Abstract 2.6

Pinzani V, Faucherre V, Peyreire H, Blayac JP (2000) Methadone withdrawal symptoms with nevirapine and efavirenz (letter). Ann Pharmacother 2000 34:405–407

Piscitelli SC, Kress DR, Bertz RJ, Pau A, Davey R (2000) The effect of ritonavir on the pharmacokinetics of meperidine and norme-peridine. Pharmacotherapy 20:549–553

Polk RE, Crouch MA, Israel DS et al (1999) Pharmacokinetic interaction between ketoconazole and amprenavir after single doses in healthy men. Pharmacotherapy 19:1378–1384

Reyataz [package insert] (2006) Princeton, NJ: Bristol-Myers Squibb Company; Jan

Schuetz EG, Schinkel AH, Relling MV, Schuetz JD (1996) P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P450 3A4 in mice and humans. Proc Natl Acad Sci 93:4001–4005

Sharom FJ (1997) P-glycoprotein efflux pump: how does it transport? J Membr Biol 160:161–175

Shen DD, Kunze KL, Thummel KE (1997) Enzyme-catalyzed process of first-pass hepatic and intestinal drug extraction. Adv Drug Delivery Rev 27:99–127

Sikri V, Pal D, Jain R, Kalyani D, Mitra AK (2004) Cotransport of macrolide and fluoroquinolones, a beneficial interaction reversing P-glycoprotein efflux. Am J Ther 11:433–442

Smith KM, Larive LL, Romanelli F (2002) Club drugs: methylenedioxymethamphetamine, flunitrazepam, ketamine hydrochloride, and gamma-hydroxybutyrate. Am J Health Syst Pharm 59:1067–1076

Sporanox [package insert] (2000) Titusville, NJ: Janssen Pharmaceutica

Synold TW, Dussault I, Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat Med 7:584–590

Ueda K, Cardarelli C, Gottesman MM, Pastan I (1987) Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc Natl Acad Sci USA 84:3004–3008

Ueda K, Okamura M, Hirai M, Tanigawara T et al (1992) Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. J Biol Chem 267:24248–24252

Vella S, Floridia M (1998) Saquinavir: clinical pharmacology and efficacy. Clin Pharmacokinet 34:189–201

Venkatakrishnan K, Greenblatt DJ, von Molkte LL et al (1998) Alprazolam is another substrate for human cytochrome P450-3A isoforms. J Clin Psychopharmacol 18:256

Viracept [package insert] (2004) La Jolla, CA: Agouron Pharmaceuticals, Inc.; Sept

Wacher VJ, Wu CY, Benet LZ (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol Carcinog 13:129–134

Wacher VJ, Silverman JA, Zhang Y, Benet LZ (1998) Role of P-glycoprotein and cytochrome P450 3A4 in limiting oral absorption of peptides and peptidomimectics. J Pharm Sci 87:1322–1330

Wacher VJ, Salphati L, Benet LZ (2001) Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv Drug Deliv Rev 46:89–102

Watkins PB (1997) The barrier functions of CYP3A4 and P-glycoprotein in the small bowel. Adv Drug Deliv Rev 27:161–170

Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, Guzelian PS (1987) Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. J Clin Invest 80:1029–1036

Williams GC, Sinco PJ (1999) Oral absorption of the HIV protease inhibitors: a current update. Adv Drug Deliv Rev 39:211–238

Wynn GH, Cozza KL, Zapor MJ, Wortmann GW, Armstrong SC (2005) Antiretrovirals: Part III. Antiretrovirals and drugs of abuse. Psychomatics 46:79–87

Xiao JJ, Foraker AB, Swaan PW, Liu S et al (2005) Efflux of Depsipeptide FK228 (FR091228, NSC-630176) is mediated by both P-glycoprotein and MRP1. J Pharmacol EXP Ther 268–276

Zaman Gj, Flens MJ, Van Leusden MR et al (1994) Human drug resistance associated protein MRP is a plasma membrane drug-efflux pump. Prod Natl Acad Sci USA 91:8822–8826