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# MINIREVIEW

# P Glycoprotein in Human Immunodeficiency Virus Type 1 Infection and Therapy

Sanjay U. C. Sankatsing,[1,2] Jos H. Beijnen,[3] Alfred H. Schinkel,[4] Joep M. A. Lange,[1,2] and Jan M. Prins[1]*

*Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam,[1] International Antiviral Therapy Evaluation Center,[2] Department of Pharmacy & Pharmacology, Slotervaart Hospital,[3] and Division of Experimental Therapy, The Netherlands Cancer Institute,[4] Amsterdam, The Netherlands*

With the advent and widespread use of potent antiretroviral therapy in the mid-1990s, the clinical course of human immunodeficiency virus (HIV) type 1 (HIV-1) infection has changed dramatically in a substantial proportion of HIV-1-infected individuals. This has led to a significant decline in the incidence of AIDS and AIDS-related morbidity and mortality in the developed world (30, 84, 96, 102). Protease inhibitors in combination with inhibitors of HIV-1 reverse transcriptase cause a dramatic reduction in plasma viremia, with the plasma HIV-1 RNA load being below the limit of detection in many patients (52, 56). However, with the currently available drugs, complete eradication of HIV-1 from an infected person is not achieved because of the persistence of latently infected, resting $CD4^+$ T cells harboring replication-competent HIV-1 and because of ongoing low-level viral replication (23, 24, 33, 42, 45, 117, 150, 153). One cause of ongoing viral replication can be suboptimal penetration of drugs into anatomical sanctuary sites like the central nervous system. It has been suggested that drug transporters like P glycoprotein (P-gp) may contribute to this suboptimal penetration. Such drug transporters might also lower intracellular drug levels, thereby limiting the therapeutic efficacies of antiviral drugs in peripheral blood mononuclear cells (PBMCs) (15), including $CD4^+$ T cells (68).

P-gp, a plasma membrane protein encoded by the multidrug resistance (MDR) gene, was discovered in 1976 (71) and functions as an ATP-dependent drug efflux pump (118). It is a transporter of a wide range of compounds, including hydrophobic amphipathic drugs, calcium channel blockers, antihistamines, peptides, and steroids. The function of P-gp is thoroughly investigated in the oncology field because of its ability to induce resistance to anticancer therapy by pumping the drugs out of tumor cells (50, 78). In this minireview we summarize the possible roles of P-gp in HIV-1 infection and therapy.

* Corresponding author. Mailing address: Academic Medical Center, Division of Infectious Diseases, Tropical Medicine and AIDS, Department of Internal Medicine, Room F4-217, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Phone: 31 (0)20 5664479. Fax: 31 (0)20 6972286. E-mail: J.M.Prins@amc.uva.nl.

## TISSUE DISTRIBUTION OF P-gp

P-gp expression on cell membranes can be detected with monoclonal antibodies like MRK16 and UIC2 (17, 18, 128). P-gp function can be studied with the fluorescent dye rhodamine 123 (Rh 123). Cells expressing functional P-gp have less intracellular accumulation of rhodamine because of increased efflux of the dye by P-gp (17, 18, 94, 99). The level of intracellular Rh 123 accumulation can be measured by flow cytometry.

P-gp is expressed at high levels in tissues like the gastrointestinal tract, liver, and kidney and on capillary endothelial cells of the brain and on PBMCs (17, 26, 44, 53, 61, 94, 128, 129). P-gp was found on freshly isolated $CD4^+$ and $CD8^+$ T cells. P-gp expression increased on $CD4^+$ and $CD8^+$ T cells after stimulation with phytohemagglutinin, with almost 100% of $CD8^+$ T cells expressing P-gp after stimulation, suggesting that the level of P-gp expression on these cells increased upon immune activation (54). An overview of the distribution of P-gp in tissue is given in Table 1.

## P-gp FUNCTION

It is not clear whether P-gp expression is necessary for a normal life. P-gp-knockout mice do not express P-gp but still have normal viability and fertility and a normal life span (9, 61, 107, 109, 111, 112). It has been suggested that in vivo P-gp protects cells against toxic substances by efflux of these compounds. In the gastrointestinal tract (duodenum, jejunum, ileum, cecum, and colon), high levels of P-gp are located only on the mucosal surfaces of these tissues. This suggests that the role of P-gp is to prevent the uptake of toxic substrates and perhaps to facilitate excretion across the mucosa of the gastrointestinal tract (122, 128, 138). The P-gp expressed in the liver and kidney could be responsible for the secretion of xenobiotics into the bile and urine (112, 128). The role of P-gp in the pancreas and lungs is not known so far.

P-gp in the blood-brain barrier could prevent the uptake of toxic substances into the brain (10, 112–114, 137), and the absence of P-gp in mice resulted in increased levels of accumulation of many drugs in the brain (10, 66, 112–114). Therefore, the absence of P-gp or the inhibition of P-gp might increase the central nervous system toxicities of some drugs. This was indeed demonstrated for ivermectin, domperidone, loper-

TABLE 1. Localization and function of P-gp

| Tissue or cell | Site | (Possible) function |
|---|---|---|
| Jejunum, ileum, colon | Mucosal surface (44, 128)[a] | Prevention of uptake and facilitation of excretion across the mucosa (122) |
| Liver | Biliary canalicular surfaces of hepatocytes and apical surfaces of cells lining the small biliary ductules (128) | Excretion of xenobiotics in bile (112) |
| Kidney | Apical surfaces of epithelial cells of the proximal tubulus (128) | Excretion of xenobiotics in urine (112) |
| Pancreas | Apical surfaces of epithelial cells lining the small ductules (128) | Unknown |
| Lung | Capillary endothelium (6) | Unknown |
| Heart | Endothelial luminal membranes of cardiac arterioles and capillaries (6, 93) | Protection against cardiac toxicity of certain drugs (93) |
| Adrenal gland | Surfaces of cells in the cortex and medulla (128) | Secretion of cortisol and aldosterone in the cortex (136) |
| Brain | Capillary endothelium (6, 26, 94) | Protection against toxic substances (26) |
| Testes | Capillaries (26) | Protection against toxic substances (26) |
| Uterus | Placenta (97) | Protection of the fetus against toxic substances (81, 97, 120) |
| Bone marow | Hematopoietic stem cells (18) | Protection against toxic substances (18); a role in differentiation and proliferation of stem cells by influencing regulatory substances (18); secretion of certain growth factors and cytokines (53) |
| Immune system cells | Natural killer cells (53)<br>B lymphocytes (53)<br>$CD4^+$ T cells (54)<br>$CD8^+$ T cells (54) | NK cell-mediated toxicity (21, 53, 152)<br>Unknown<br>Secretion of cytokines (53)<br>Secretion of cytotoxic molecules (54) |

[a] Reference numbers are provided in parentheses.

amide, and ritonavir in mice (66, 112, 113). P-gp expressed in the placenta plays an important role in the protection of the developing fetus against toxic substances (81, 120).

P-gp expression on bone marrow stem cells might protect them from toxic substances and could also be responsible for the transport of certain growth factors and cytokines produced by stem cells (53). It is unclear whether P-gp also has this function in other cell types.

It has been suggested that NK-cell cytotoxicity requires P-gp function for the efflux of lytic products. Inhibition of P-gp resulted in a decreased cytotoxicity of NK cells (21, 152). The decrease in the lytic activities of NK cells in the presence of *R*-verapamil, an inhibitor of P-gp, was dose dependent (89). However, no difference in cytotoxic T-cell function was found between wild-type mice and P-gp-knockout mice (39). It is possible that P-gp-knockout mice develop some compensation mechanism for the lack of P-gp.

## POLYMORPHISM OF P-gp

The *MDR-1* (or *ABCB1*) gene encodes P-gp. So far, 28 single nucleotide polymorphisms have been found at 27 positions (60, 108), and these are sometimes linked to each other (76). A C/T polymorphism in exon 26 (C3435T) correlates in a significant manner with P-gp expression and activity in the duodenum (60). The polymorphism in exon 26 appears to be linked to single nucleotide polymorphisms in exon 12 (C1236T) and exon 21 (G2677T) (76). Individuals with the homozygous T genotype at position 3435 showed a P-gp function higher than that of individuals with the homozygous C genotype (76), but this is in contrast to the findings of another study, which demonstrated the contrary (60). It has been suggested that the discrepancy between these studies might reflect linkage to an as yet undefined promoter or enhancer region polymorphism(s) or nucleotide sequences that are important for mRNA processing (76).

These genetic differences might explain the differences in P-gp expression between individuals and, thereby, the differences in individual drug responses in patients (12, 76, 80). There is an ethnic difference in allele frequencies. Africans or people of African descent have a lower frequency of the T/T genotype and a higher frequency of the C/C genotype than Caucasians (2, 76, 110).

## MECHANISM OF ACTION

The most prevalent theory of the mechanism of action of P-gp favors a direct pump mechanism (13, 14, 27, 51, 105). The P-gp pump recognizes substrates through a complex substrate recognition region and directly pumps drugs out of the cell by

TABLE 2. Examples of P-gp modulators

| Modulator | Modulator |
|---|---|
| First group[a] | Second group[b] |
| Amiodarone (19, 20)[d] | Dexverapamil (103) |
| Bepridil (116) | Emopamil (103) |
| Caroverine (134) | Gallopamil (103, 130) |
| Clomipramine (134) | PSC 833 (valspodar) (8) |
| Cyclosporine (100, 135) | Ro11-2933 (1) |
| Diltiazem (132) | |
| Felodipine (62) | Third group[c] |
| Isradipine (62) | GF120918 (67) |
| Nicardipine (132) | LY-335979 (29) |
| Quinidine (131) | OC144-093 (98) |
| Quinine (121) | VX-710 (48) |
| Tri fluoperazine (46, 134) | XR9051 (28) |
| Verapamil (133, 134) | |

[a] Therapeutic agents with a P-gp inhibitor effect at concentrations higher than those required for therapeutic activity.
[b] Analogues of the first group of modulators.
[c] Modulators developed and targeted against specific MDR mechanisms.
[d] Reference numbers are provided in parentheses.

using molecular mechanisms that are not yet well understood. ATP hydrolysis by P-gp provides the energy for this active process. The question is, how are the drugs actually pumped out of the cell? One proposed mechanism is that P-gp detects drugs and ejects them before they reach the cytoplasm by removing the drugs directly from the plasma membrane (50, 63, 106, 125). Another possibility is that P-gp acts as a flippase, carrying its substrate from the inner leaflet of the lipid bilayer to the outer leaflet (58).

## P-gp INHIBITION

P-gp inhibitors, also called reversal agents or P-gp modulators, inhibit the efflux of P-gp-transported drugs in vitro and in mice (22, 90, 139).

P-gp inhibitors modulate P-gp function by competing with the binding and transport of the drug or through noncompetitive binding by binding either to the drug interaction site or to another modulator binding site, which leads to allosteric changes (31, 47). This is consistent with the finding that P-gp has at least two independent drug-binding sites (31).

There are three groups of P-gp inhibitors or modulators (Table 2). The first group of inhibitors are therapeutic agents. In vivo they function as a P-gp inhibitor only at concentrations higher than those required for therapeutic activity. Therefore, these agents cannot be used as P-gp inhibitors in vivo because of their potential toxic effects. The second group of P-gp modulators are analogues of the first group of modulators. They are more potent and less toxic. For example, emopamil, gallopamil, and Ro11-2933 are analogues of verapamil; and PSC 833 is a nonimmunosuppressive cyclosporine analogue. The third group of modulators are developed and targeted against specific MDR mechanisms (78). Clinical trials have demonstrated the beneficial effect of P-gp inhibitors for the treatment of cancer. One trial with breast cancer patients and one trial with lung cancer patients demonstrated a survival benefit with the addition of the P-gp inhibitor verapamil to chemotherapy (7, 95). In another study, the combination of the P-gp inhibitor cyclosporine with chemotherapy statistically improved the relapse-free survival rate and the overall survival rate (85). The proposed mechanism for this beneficial effect was reversal of the P-gp-induced drug efflux out of tumor cells. P-gp inhibitors can cause side effects. The first group of P-gp inhibitors must be used at high doses to accomplish the inhibition of P-gp and can therefore cause side effects. In addition, side effects may be caused by the accumulation of substrates of P-gp. The second and third groups of P-gp inhibitors are used at much lower doses to accomplish the desired effect, and the side effects are probably mainly caused by the accumulation of P-gp substrates (127).

## RELATION OF P-gp AND CYP3A4

Cytochrome P450 (CYP) mixed-function oxidases account for the majority of oxidative biotransformations of xenobiotics and endogenous compounds. More than 30 different human CYP enzymes have been identified, of which CYP3A4 appears to be one of the most important, as it contributes to the biotransformation of approximately 60% of therapeutic drugs. Most of the activity of CYP3A4 is located in the liver and in the small intestine, and CYP3A4 is responsible for the first-pass drug metabolism (34). Some drugs, for example, vinblastine, are substrates for both CYP3A4 and P-gp. Other drugs, for example, verapamil, are substrates for CYP3A4 and inhibitors of P-gp. Yet other drugs, for example, ketoconazole, inhibit both CYP3A4 and P-gp (144, 154). St John's wort contains compounds that are substrates for both P-gp and CYP3A4 and, in addition, induce intestinal P-gp and intestinal and hepatic CYP3A4 in humans (36, 57).

P-gp might enhance the metabolic effect of CYP3A4 in the small intestine in three ways. First, P-gp can limit the uptake of substrates in the small intestine, thereby limiting the amount of substrate that needs to be transformed by CYP3A4. Second, P-gp can increase the extent of metabolism by CYP3A4 through repeated cycles of intracellular uptake and efflux, thereby increasing the level of exposure of a drug to CYP3A4 before absorption in the systemic circulation. Finally, P-gp might preferentially remove drug metabolites catalyzed by CYP3A4, thereby limiting competitive inhibition (147).

## P-gp EXPRESSION AND FUNCTION DURING HIV-1 INFECTION

The level of P-gp expression on T-cell and monocytic cell lines increases upon infection with HIV-1 (49). By using monoclonal antibody MRK-16, an increased level of P-gp expression was established on $CD4^+$ T cells from HIV-1-infected patients (3, 53). The level of P-gp expression on $CD4^+$ T cells increased with the progression of HIV-1 infection (3, 53). Later studies did not confirm these findings, however (86, 88, 91). In cells with increased levels of P-gp expression, one would expect a lower level of accumulation of Rh 123. However, $CD4^+$, $CD8^+$, $CD16^+$, and $CD19^+$ cells from HIV-1-infected patients accumulated more Rh 123 than cells from healthy controls (3, 86). In the presence of cyclosporine, a P-gp inhibitor, the level of intracellular accumulation of Rh 123 increased in $CD4^+$ and $CD8^+$ T cells from healthy controls, but in HIV-1-infected cells, the level of intracellular accumulation of Rh 123 did not increase further (3). This suggests that even when the level of

P-gp expression is increased in HIV-1-infected patients, the pump function of P-gp is decreased.

A significantly reduced P-gp function was found in $CD16^+$ NK cells from HIV-1-infected patients compared with that found in cells from healthy individuals. This reduced P-gp function became more prominent with the progression of the HIV infection and was significantly correlated with a decreased NK-cell cytotoxic function (86, 87). This is consistent with the decreased NK-cell cytotoxicity in HIV-1-infected patients (11). In vitro the reduced level of P-gp expression and function of NK cells of HIV-1-infected individuals could be recovered by stimulating them with interleukin 15 (16).

## P-gp EXPRESSION AND ANTIRETROVIRAL AGENTS

There is clear evidence for interactions between P-gp activity and antiretroviral agents.

Only a few studies have addressed the effect of P-gp on the behaviors of nucleoside reverse transcriptase inhibitors (NRTIs). Early in vitro studies demonstrated a decreased level of accumulation of the NRTI zidovudine in HIV-1-infected cells compared to that in uninfected cells, which was correlated with an increased level of P-gp expression on HIV-1-infected cells (49). Zidovudine was less effective in inhibiting HIV-1 replication in cells expressing P-gp (4). It may be possible that this effect was mediated by another drug transporter, such as MRP4, which was discovered later (115). Zidovudine, didanosine, and zalcitabine do not appear to induce P-gp expression or function in the cells analyzed (3, 86).

The nonnucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, efavirenz, and delavirdine are not substrates for transport by P-gp in Caco-2 cell lines. All are able to induce P-gp expression and function, resulting in increases in the levels of P-gp expression of 3.5-, 1.75-, and 2.35-fold, respectively (126), and reduced levels of accumulation of Rh 123 by 72, 81, and 85%, respectively (126). Delavirdine is not only an inducer of P-gp but also an inhibitor of P-gp (126).

All presently available HIV-1 protease inhibitors are substrates for P-gp (22, 25, 55, 66, 74, 75, 82, 104, 139, 146) and interact with P-gp with an affinity in the order ritonavir > nelfinavir > amprenavir > indinavir > saquinavir (15, 124). CEM cells expressing P-gp have reduced intracellular concentrations of the protease inhibitors ritonavir, indinavir, saquinavir, and nelfinavir compared with those in CEM cells not expressing P-gp (68, 69). The intracellular accumulation of saquinavir and other protease inhibitors increased in the presence of the P-gp inhibitors verapamil and GF120918 (68). In patients, the concentration of protease inhibitors in PBMCs was inversely correlated with the amount of *MDR-1* mRNA expression (15). This explains why the protease inhibitors ritonavir, indinavir, and saquinavir are less effective against HIV-1 replication in cells expressing P-gp (82), although this effect of P-gp was refuted by other investigators (124). The different cell lines used in those studies might explain this difference.

Further in vitro studies demonstrated that ritonavir, indinavir, saquinavir, and nelfinavir have weak inhibitory effects on P-gp, with ritonavir being the most potent one (22, 55, 66, 94, 104, 119, 124, 146). One study reported that ritonavir is a sixfold more potent P-gp inhibitor than the cyclosporine analog SDZ PSC 833, which is assumed to be one of the most potent inhibitors (35). However, that study used cultured pig brain endothelial cells, in which the transporter(s) affected was not unambiguously identified. On the other hand, the simultaneous administration of more than one protease inhibitor did not result in a decreased P-gp efflux function in contrast to that after exposure to LY-335979, a more potent P-gp inhibitor (139). This suggests that ritonavir is only a moderate P-gp inhibitor (66). Nelfinavir and, to a lesser extent, its metabolite M8 are also inhibitors of P-gp function on $CD4^+$ and $CD8^+$ T cells (32). Protease inhibitors are also able to increase the level of P-gp expression. Lopinavir, for instance, is both an inhibitor and an inducer of P-gp, but the overall effect of lopinavir seemed to be induction (142).

Given the tissue distribution of P-gp (Table 1), it might lower the bioavailability of protease inhibitors and could be responsible for the existence of sanctuary sites, such as the brain and the testes, by limiting the levels of accumulation of protease inhibitors in these tissues. In P-gp-knockout mice, the levels of indinavir, saquinavir, and nelfinavir in plasma were 2- to 5-fold higher after oral administration compared with the levels in wild-type mice, and the concentrations in the brain were 7- to 36-fold higher after intravenous administration and 10-fold higher after oral administration compared with the levels in wild-type mice (75, 146). In wild-type mice intravenous administration of the potent P-gp inhibitor LY-335979 resulted in a dose-dependent increase in the $^{14}$C-labeled nelfinavir, amprenavir, indinavir, and saquinavir concentrations in the brain and an increase in the $^{14}$C-labeled nelfinavir concentration in the testes (22). Likewise, in the same model, an increase in plasma saquinavir concentrations and improved penetration into the brain and testes were seen after coexposure to the potent P-gp inhibitor GF120918 (65). The penetration of amprenavir into the brains of rats increased in the presence of GF120918 (38).

These data support the idea that P-gp expression in the gastrointestinal tract can limit the bioavailabilities of these drugs and that P-gp can contribute to the decreased concentrations of these drugs in sanctuary sites like the brain and the male genital tract (141, 146).

In vivo, the addition of low-dose ritonavir increased plasma indinavir trough concentrations and indinavir concentrations in seminal plasma and cerebrospinal fluid (141). Inhibition of CYP3A4 by ritonavir decreases the metabolism of indinavir, but this could not fully explain the increased indinavir concentrations in seminal plasma and cerebrospinal fluid (141). Therefore, ritonavir might also influence the blood-brain and blood-testis barrier by its P-gp-inhibitory function (55), even though ritonavir is only a moderate P-gp inhibitor (66, 72).

## EFFECT OF P-gp ON HIV-1 REPLICATION

Finally, P-gp can influence the infectivity and replication of HIV-1.

In vitro, P-gp expression on T cells inhibited HIV-1 fusion with the plasma membrane and also inhibited virus replication at a later step in the viral life cycle. This reduction in the level of HIV-1 replication correlated with the level of P-gp expression (83, 123). Mutations of P-gp at the ATP utilization site, which thereby inactivated ATP hydrolysis and resulted in an

inactive P-gp pump function, still resulted in decreased HIV-1 infectivity. This suggests that the P-gp function is not necessary to block the infectivity of HIV-1 (83). On the other hand, when $CD4^+$ T cells were incubated with quinidine or PSC 833 to inhibit the P-gp function but not P-gp expression, the levels of HIV-1 production in these cells increased. In summary, P-gp expression and function can inhibit HIV-1 infectivity and replication capacity.

## P-gp POLYMORPHISMS IN HIV-1

Polymorphisms in *MDR-1* alleles might be of clinical importance in HIV treatment, although controversy remains about the effects of the different polymorphisms on P-gp expression and function. As discussed earlier, it is not clear whether a C or a T allele at position 3435 in exon 26 is associated with a higher level of P-gp function. Patients with a homozygous T genotype at exon 26 had, on average, a lower concentration of nelfinavir in plasma compared with that in the plasma of patients with the homozygous C genotype (41). This finding suggests that the T allele is associated with a higher level of P-gp function, but this was difficult to reconcile with the fact that the level of P-gp mRNA transcription in PBMCs was lower in these patients. In that study plasma efavirenz levels were also lower in patients with the homozygous T genotype, although efavirenz is not a substrate for P-gp. The patients with the homozygous T genotype had a greater rise in $CD4^+$ T-cell counts (41). This was explained by the protective function of P-gp on pluripotent stem cells (18).

## P-gp AND CYP3A4 DURING HIV TREATMENT

Antiretroviral drugs can not only influence and be influenced by P-gp, but they may also be substrates for and influenced by CYP3A4 activity. The resulting effect is not always predictable.

The NNRTI delavirdine, which is an inducer and inhibitor of P-gp, is also an inhibitor of CYP3A4 (126). Likewise, nevirapine and efavirenz are inducers not only of P-gp but also of CYP3A4 (40, 143).

Not only are the protease inhibitors substrates for P-gp, but all of them are also substrates for CYP3A4 in the liver and small intestine, where they are metabolized by at least 80 to 95% (5). In vitro studies revealed, in addition, the CYP3A4-inhibitory capacities of the protease inhibitors, with ritonavir being the most potent inhibitor, followed by indinavir, nelfinavir, amprenavir, and saquinavir (37, 43, 73, 77, 79, 151).

In vitro, P-gp facilitated the removal of the metabolites of indinavir, thereby preventing competition for CYP3A4 by the metabolites and indinavir itself (59). It has been suggested that this might increase the level of metabolism by CYP3A4 (147), but that result could not be confirmed (59). That study (59) supported the suggestion that the mechanism of increased drug metabolism by CYP3A4 is through repeated cycles of intracellular uptake and efflux by P-gp. In vivo, low-dose ritonavir increased the bioavailability of indinavir by inhibiting metabolism by CYP3A4 and probably by inhibiting drug transport by P-gp. This combination proved to be clinically relevant (140, 141).

## OTHER DRUG TRANSPORTERS RELEVANT FOR HIV THERAPY

Members of the MDR protein (MRP) family, which belongs to the group of ATP-binding cassette drug transporters, have also been recognized as transporters of nucleoside-based antiretroviral drugs (115, 148) and protease inhibitors (94, 109, 115, 139, 149). For example, MRP4 overexpression impairs the antiviral efficacies of adefovir and zidovudine in vitro (115), and MRP5 overexpression in vitro results in the efflux of adefovir from cells (148). In vitro studies demonstrated that cells expressing MRP1 have reduced intracellular concentrations of saquinavir and ritonavir compared with those in cells not expressing MRP1, but the intracellular concentrations of nelfinavir and indinavir were not influenced by MRP1 expression (69, 149). Also, saquinavir, ritonavir, and indinavir are effectively transported in vitro by MRP2 (64) but not by MRP1, MRP3, or MRP5. The conflicting data regarding the effect of MRP1 on saquinavir, ritonavir, and indinavir might be explained by the different cell lines used in these studies. MRP drug transporters seem to play a minor role in the transport of ritonavir and indinavir across the blood-brain barrier (139). In vivo, expression of MRP1 on lymphocytes was correlated with lower levels of accumulation of saquinavir and ritonavir in these lymphocytes (92). Furthermore, saquinavir and ritonavir are also inhibitors of MRP1- and MRP2-mediated drug transport (55, 94, 101, 124). It seems likely that there is a combined effect of P-gp and the MRP-drug transporter family on the concentrations of antiretroviral drugs that results in the decreased bioavailabilities of these drugs.

MRP1 also directly influences HIV-1 replication. Overexpression of MRP1 on CEM cells resulted in an increase in the level of HIV-1 replication by a factor 160 compared to that in control CEM cells (123). The mechanism is unknown so far.

Breast cancer resistance protein (BCRP) is another drug transporter with the ability to influence intestinal (re)uptake and hepatobiliary excretion of transported drugs (70). Preliminary data showed that BCRP is not an efficient transporter of the protease inhibitors saquinavir, ritonavir, and indinavir (64) but can transport zidovudine (145).

## SUMMARY AND CONCLUSIONS

Many of the data generated to date are from in vitro studies with different cell lines. The use of different cell lines might explain in part why some of the data conflict, and the observed effects might be different for different tissues in vivo. Nevertheless, some aspects of P-gp relevant for HIV infection and therapy have become clear. The potential effects of P-gp activity relevant for HIV-1 treatment include decreasing the uptake of protease inhibitors together with CYP3A4 in the small intestine, decreasing the entry of protease inhibitors into the central nervous system, decreasing the entry of protease inhibitors into the testis, and decreasing the intracellular accumulation of protease inhibitors and protecting against HIV-1 infectivity and replication in $CD4^+$ T cells; P-gp activity is also potentially necessary for the function of natural killer cells.

By limiting the penetration of protease inhibitors into anatomical sites like the central nervous system, P-gp contributes to the maintenance of sanctuary sites. P-gp function also re-

duces intracellular protease inhibitor concentrations and could therefore result in intracellular concentrations too low to block HIV-1 replication completely. These suboptimal concentrations in sanctuary sites and in cells could contribute to ongoing low-level HIV-1 replication. Moreover, P-gp can be an important limiting factor in the oral bioavailabilities of protease inhibitors.

It must be kept in mind that P-gp is not the only drug transporter of protease inhibitors. Other drug transporters, especially transporters of the MRP drug transport family, have been identified. The relative contribution of each of the transporters to the overall effect needs to be clarified.

Furthermore, the additional effect of CYP3A4 on protease inhibitor concentrations is also important, although the resulting effect is not always predictable.

The clinical relevance of P-gp during treatment with NNRTIs is less clear. However, it is conceivable that in a regimen consisting of protease inhibitors and NNRTIs the induction of P-gp expression and function by the NNRTIs might contribute to lower concentrations of the protease inhibitors in plasma and cells.

There are variations in P-gp expression and function among patients. These variations are explained at least in part by polymorphisms in the *MDR-1* gene encoding P-gp, and this could contribute to the interpatient variabilities in plasma protease inhibitor concentrations. There is still controversy about the exact effects of the different polymorphisms on P-gp function. Insight into the significance of these polymorphisms might be of clinical use. If the *MDR-1* genotype in patients is known before the start of treatment with protease inhibitors, one could predict which patients are at risk for having low plasma protease inhibitor levels. This could justify a dose adjustment at the start of treatment.

The bioavailability and the intracellular concentrations of protease inhibitors can be increased in the presence of potent P-gp inhibitors. Therefore, a logical step in order to increase the concentrations of protease inhibitors in plasma, cells, and sanctuary sites is to inhibit P-gp function, which can be done with available potent P-gp inhibitors. Inhibition of P-gp function might, however, lead to increased toxicities of other drugs, for example, loperamide and domperidone. Moreover, P-gp expression appears to decrease HIV-1 infectivity and replication in T cells, and P-gp inhibition might therefore be counterproductive.

Before manipulation of P-gp function is considered in anti-HIV-1 therapy, the question of which effect of P-gp is clinically more important must be answered: P-gp as a drug efflux pump or P-gp as a protector against HIV infectivity and replication.

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