

The Drug Transporter P-glycoprotein Limits Oral Absorption and Brain Entry of HIV-1 Protease Inhibitors

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Abstract

Currently available HIV-1 protease inhibitors are potent agents in the therapy of HIV-1 infection. However, limited oral absorption and variable tissue distribution, both of which are largely unexplained, complicate their use. We tested the hypothesis that P-glycoprotein is an important transporter for these agents. We studied the vectorial transport characteristics of indinavir, nelfinavir, and saquinavir in vitro using the model P-glycoprotein expressing cell lines L-MDR1 and Caco-2 cells, and in vivo after intravenous and oral administration of these agents to mice with a disrupted *mdr1a* gene. All three compounds were found to be transported by P-glycoprotein in vitro. After oral administration, plasma concentrations were elevated 2–5-fold in *mdr1a* (–/–) mice and with intravenous administration, brain concentrations were elevated 7–36-fold. These data demonstrate that P-glycoprotein limits the oral bioavailability and penetration of these agents into the brain. This raises the possibility that higher HIV-1 protease inhibitor concentrations may be obtained by targeted pharmacologic inhibition of P-glycoprotein transport activity. (*J. Clin. Invest.* 1998. 101:289–294.) Key words: P-glycoprotein • HIV-1 protease inhibitors • AIDS • membrane transport • pharmacokinetics

Introduction

The development of inhibitors of HIV-1 protease has been a major advance in the clinical management of HIV-1 infection, and their use has resulted in dramatic reductions in viral load in the plasma and many tissues of infected patients (1, 2). However, currently available drugs have a number of limitations, related in many instances to poor and/or variable transport across important biological membranes (3). For example, oral absorption is often low and variable and drug penetration into the brain is poor, a potential therapeutic limitation with

respect to the neurologic manifestations of late-stage HIV-1 infection. The factor(s) responsible for these characteristics is not well understood.

P-glycoprotein is an ATP-dependent, efflux membrane transporter with broad substrate specificity for a number of structurally diverse drugs (4, 5). It is distributed in normal tissues, particularly epithelial cells of importance in drug disposition such as the gastrointestinal tract, the liver, and the kidney (4). Apical expression of P-glycoprotein in such tissues results in reduced drug absorption (gastrointestinal tract), and enhanced elimination into the bile (liver) and urine (kidney) (4). In addition, the expression of P-glycoprotein at the level of the blood brain barrier (6, 7) has been shown to be a critical factor in preventing the entry of some drugs into the central nervous system (8–10). Accordingly, it was hypothesized that the limited membrane permeability of HIV-1 protease inhibitors could be explained if they were substrates of P-glycoprotein and that the tissue expression of this transporter may be a determinant of whether therapeutic drug levels are attained.

Methods

Transport in cultured LLC-PK1, L-MDR1, and Caco-2 cells. LLC-PK1 and L-MDR1 cells were grown under identical conditions to those described by Schinkel et al. (9). Caco-2 cells were grown with DMEM (high glucose) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% nonessential amino acids (GIBCO BRL, Gaithersburg, MD), and incubated at 37°C in 5% CO₂. LLC-PK1 and L-MDR1 cells were plated at a density of 4×10^5 cells/12-mm well on porous (3.0 µm) polycarbonate membrane filters (Transwell™; Costar Corp., Cambridge, MA), while the Caco-2 cells were plated at a density of 10^5 cells/12-mm well on 0.4-µm polycarbonate membrane filters (Transwell™). Cells were supplemented with fresh media every 2 d and used in the transport studies on the fourth day after plating. Transepithelial resistance was measured in each well using a Millicell ohmmeter (model ERS; Millipore Corp., Bedford, MA); wells registering a resistance of 200 Ω or greater, after correcting for the resistance obtained in control blank wells, were used in the transport experiments.

About 1–2 h before the start of the transport experiments, the medium in each compartment was replaced with Optimem (GIBCO BRL), a serum-free medium. Then, the transport of indinavir, nelfinavir, and saquinavir was measured after replacing the medium in each compartment with 700 µl Optimem (GIBCO BRL) serum free medium with and without [¹⁴C]-radiolabeled drug (5 µM). Radioactivity appearing in the opposite compartment after 1, 2, 3, and 4 h was measured in 25-µl aliquots taken from each compartment, after the addition of 5 ml of ScintiVerse BD scintillation fluid (Fisher Scientific Co., Fairlawn, NJ). Inhibition of transport was determined in a similar manner after the addition of quinidine (5 µM) or PSC-833 (1 µM) to both the apical and basal compartments, before the addition of radiolabeled drug.

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Figure 1. Immunoblot analysis of P-glycoprotein expression in plasma membrane enriched vesicles derived from L-MDR1, LLC-PK1, and Caco-2 cells. Membrane vesicle preparation (10 μ g) was added to each lane, and after incubation with the primary antibody (C219) and appropriate washes, the blot was exposed to a standard Kodak X-omat AR film for 15 s.

Immunoblot analysis of P-glycoprotein expression. The LLC-PK1, L-MDR1, and Caco-2 cellular P-glycoprotein content was assessed by immunoblot analysis of plasma membrane enriched cell preparations as described previously (11). Briefly, 10 ml of lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1 mM MgCl₂, 1 μ g/ml leupeptin, 1 μ g/ml pepstatin A, 50 μ g/ml phenylmethylsulfonyl fluoride) was added to the cells. These were disrupted with a tightly fitting Dounce homogenizer. Cellular and nuclear debris were removed by centrifugation at 400 g for 10 min and the pellet was discarded. The supernatant was centrifuged at 30,000 g for 30 min and the pellet containing the membrane vesicles was resuspended in 200 μ l of lysis buffer. Protein content of the extracted membrane vesicles was determined using Coomassie assay reagent (Pierce Chemical Co., Rockford, IL). Subsequently, Western blots for the immunodetection of P-glycoprotein were obtained using 10 μ g of extracted membrane proteins which

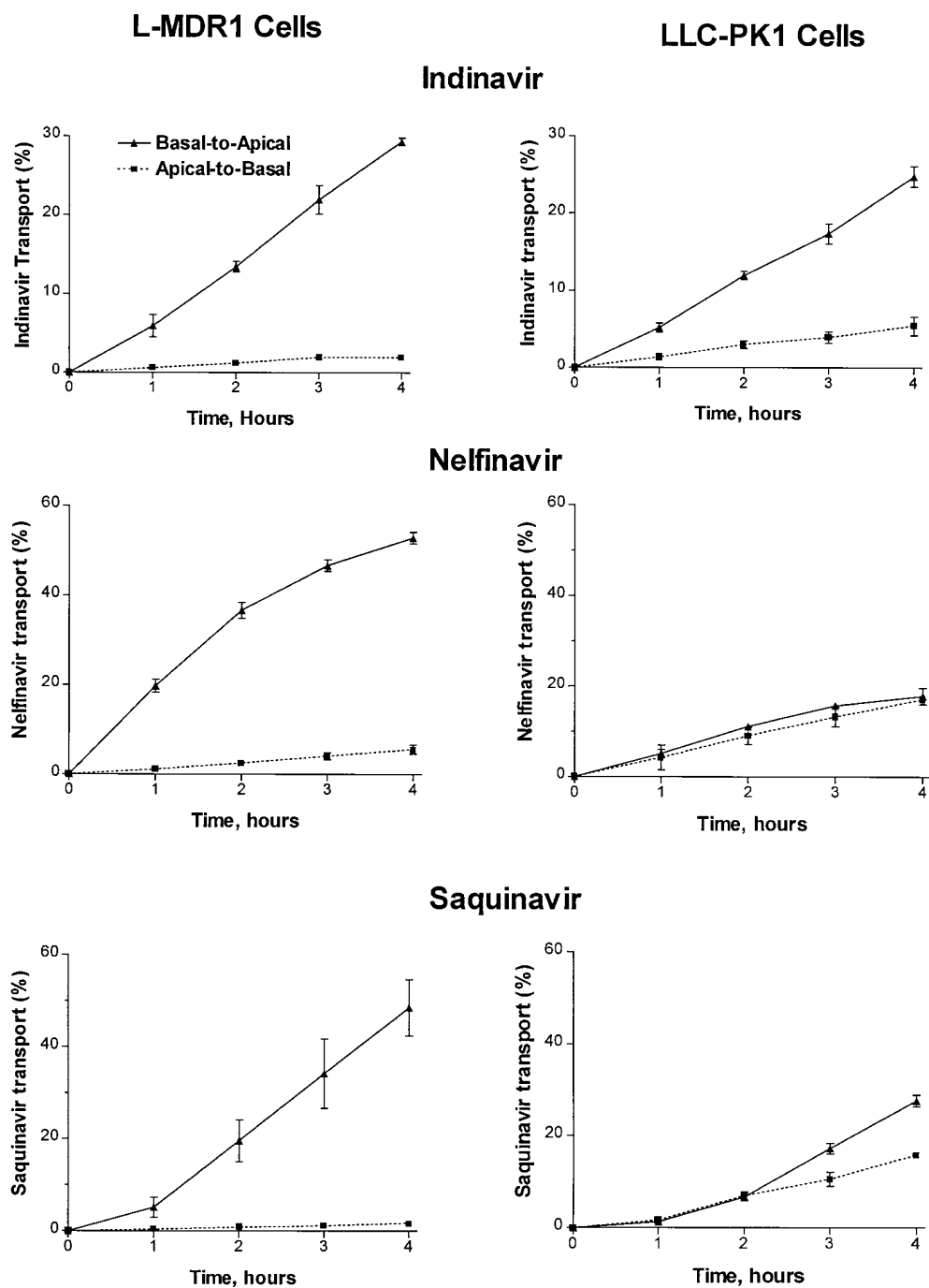


Figure 2. Transepithelial transport of [¹⁴C]-indinavir (5 μ M), -nelfinavir (5 μ M), and -saquinavir (5 μ M) across L-MDR1 and LLC-PK1 cell culture monolayers. Labeled drug was added to either the basal or apical compartment, and at the indicated time points, samples were obtained from both the apical and basal compartments. Appearance of radioactivity in the opposite compartment was measured and represented as the fraction (shown in percent) of total radioactivity added at the beginning of the experiment. Translocation from basal to apical compartments, (filled triangles) solid line; translocation from apical to basal compartments, (filled squares) dotted line. Data are mean \pm standard error from three or more experiments.

were resolved using 12% SDS-PAGE. After the transfer of the proteins to a nitrocellulose membrane, the membrane was placed in blocking buffer (100 mM NaCl, 20 mM KH_2PO_4 , 80 mM K_2HPO_4 , 0.1% Tween 20, 10% dry milk) for 2 h. Then the blot was incubated for 2 h with the mAb C219 (Signet Laboratories Inc., Dedham, MA) (2 $\mu\text{g}/\text{ml}$ in 100 mM NaCl, 20 mM KH_2PO_4 , 80 mM KH_2PO_4 , 0.1% Tween 20). After washing for 5 min, the membrane was incubated with anti-mouse IgG horseradish peroxidase conjugate (1:3,000) for 1 h. After four additional washes (5 min per wash), the proteins were visualized using the enhanced chemiluminescence detection system (Amersham Corp., Arlington Heights, IL).

Determination of tissue distribution in *mdr1a* (+/+) and (-/-) mice. Male *mdr1a* (-/-) mice, (FVB/TacBR-[KO]mdr1aN7), 6–12 wk of age and genetically matched male *mdr1a* (+/+) mice (FVB/NTacBR) were obtained from Taconic Farms, Inc. (Germantown, NY). Radiolabeled indinavir (1.5 mg/kg, sp act 8.5 mCi/mmol), nelfinavir (3 mg/kg, sp act 34 mCi/mmol), or saquinavir (2 mg/kg, sp act

20.3 mCi/mmol) dissolved in ethanol/0.9% saline solution in a total volume of 2.1 $\mu\text{l}/\text{g}$ of body weight was separately injected intravenously, over 10 min into the tail vein of groups of three mice. After 4 h, the animals were anesthetized using isoflurane (IsoFlo; Abbott Laboratories, Abbott Park, IL) and blood was completely removed by orbital bleeding. The mice were then killed and the harvested tissues were weighed and homogenized with 4% (wt/vol) bovine serum albumin. Total radioactivity was determined after the addition of 100 μl of plasma or tissue homogenate (500 μl) to vials containing 4 ml of ScintiVerse scintillation fluid (Fisher Scientific Co.). The contents of the small intestine and colon were removed before homogenizing and were blotted with filter paper to remove any blood. After oral administration (6 mg/kg) of each radiolabeled drug using gastric intubation (vol 3.5 $\mu\text{l}/\text{g}$ of body weight), an orbital blood sample was obtained at 4 h and plasma radioactivity was determined as described. The protocols for the animal experiments were approved by Vanderbilt University Animal Care Committee, and they were cared for in accor-

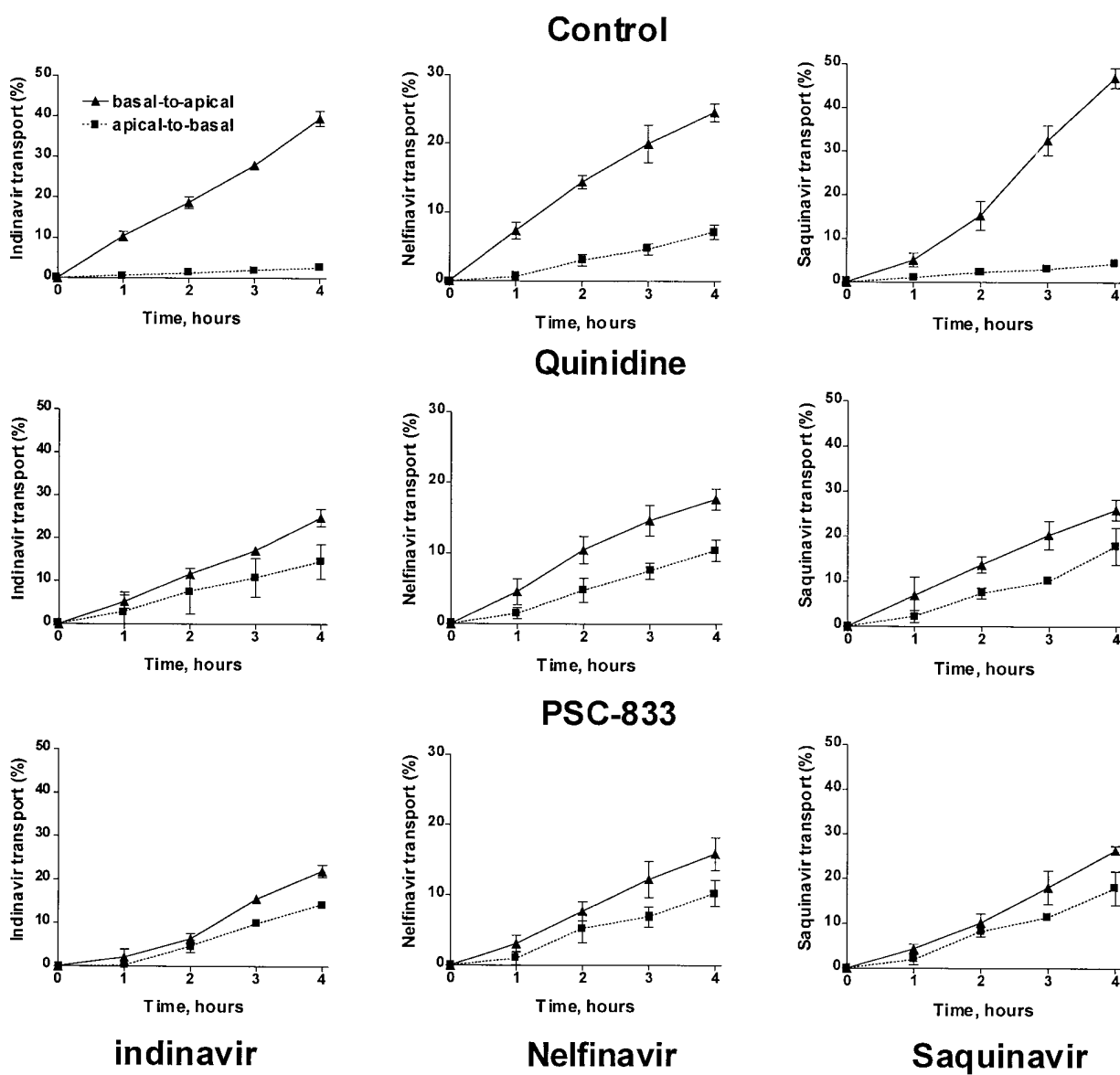


Figure 3. Transepithelial transport of [^{14}C]-indinavir (5 μM), -nelfinavir (5 μM), and -saquinavir (5 μM) across a Caco-2 cell culture monolayer in the absence, or presence of 5 μM quinidine, or the presence of 1 μM PSC-833. Translocation from basal to apical compartments, (filled triangles) solid line; translocation from apical to basal compartments, (filled squares) dotted line. Data are mean \pm standard error from three or more experiments.

Table I. Tissue Levels of Radioactivity (ng/g Tissue) in *mdr1a* (+/+) and (-/-) Mice at 4 h after Intravenous Injection of [¹⁴C]-Indinavir (1.5 mg/kg), [¹⁴C]-Nelfinavir (3 mg/kg), or [¹⁴C]-Saquinavir (2 mg/kg)

Tissue	Indinavir			Nelfinavir			Saquinavir		
	<i>mdr1a</i> (+/+)	<i>mdr1a</i> (-/-)	Ratio (-/-)/(+/+)	<i>mdr1a</i> (+/+)	<i>mdr1a</i> (-/-)	Ratio (-/-)/(+/+)	<i>mdr1a</i> (+/+)	<i>mdr1a</i> (-/-)	Ratio (-/-)/(+/+)
Plasma	19±6.7	21±2.0	1.13	14±0.5	17±1.0 [‡]	1.26	31±4.2	34±3.4	1.05
Brain	1.6±0.6	17±6.2*	10.6	1.2±0.5	45±4.3 [‡]	36.3	4.1±0.6	30±4.9 [‡]	7.37
Heart	15±5.2	16±1.7	1.01	9.6±2.8	12±3.3	1.22	31±4.4	79±12 [‡]	2.51
Lung	61±16	63±1.2	1.04	108±82	582±755	5.38	85±21	135±51	1.59
Liver	397±102	458±93	1.15	854±0.8	904±131	1.06	186±15	149±90	0.80
Spleen	20±5.3	21±9.2	1.03	42±17	89±91	2.13	37±8.3	52±8.4	1.39
Kidney	135±93	126±18	0.94	25±3.4	36±9.6	1.47	113±14	149±43	1.32
Small intestine	344±105	925±263 [‡]	2.69	120±23	533±393	2.43	221±99	413±328	1.87
Colon	1382±761	1127±128	0.82	555±140	764±541	1.38	954±192	707±533	0.74

Data are shown as mean±SD. Statistical difference in radioactivity between the two groups of mice (three per group) was assessed by a two-sided Student's *t* test, with *P* < 0.05 as the limit of significance. **P* < 0.05. [‡]*P* < 0.01.

dance with the U.S. Public Health Service Policy for the Care and Use of Laboratory Animals.

Results

Immunoblot analysis of P-glycoprotein expression using the mAb anti-P-glycoprotein C219 revealed that L-MDR1 cells expressed high levels of P-glycoprotein, while the parental cell line, LLC-PK1, showed little if any detectable P-glycoprotein (Fig. 1). Caco-2 cells, however, had a readily detectable, but significantly lower amount of expressed P-glycoprotein than L-MDR1 cells (Fig. 1).

Indinavir, nelfinavir, and saquinavir cellular translocation was markedly greater when [¹⁴C]-labeled drug was administered to the basal side of cultured, L-MDR1, and Caco-2 cells and its appearance measured on the apical side (basal-apical) compared to addition of drug to the opposite compartment (apical-basal) (Figs. 2 and 3). In LLC-PK1 cells, such apical-basal and basal-apical transport differences were absent for nelfinavir and saquinavir; however with indinavir, a significantly greater basal-apical versus apical-basal transport was observed in LLC-PK1 cells, although the net basal-apical transport was greater in the L-MDR1 cells. Collectively, these data suggest that the HIV-1 protease inhibitors are substrates of P-glycoprotein. Furthermore, the addition of quinidine or

PSC-833 to Caco-2 cells markedly reduced the directional transport difference (Fig. 3).

To determine the importance of P-glycoprotein in the tissue distribution of the HIV-1 protease inhibitors in vivo, total radioactivity was determined 4 h after intravenous or oral administration of radiolabeled drugs to wild-type mice [*mdr1a* (+/+)] and mice in which the *mdr1a* gene had been disrupted [*mdr1a* (-/-)] (8). After intravenous administration, there was a similar pattern of tissue radioactivity for all three agents, with extensive localization in the gastrointestinal tract, liver, kidney, and to a lesser extent in the lung. Also, the brain concentrations of these agents were lower than in any other tissue, and more importantly, were almost 10-fold below those of plasma (Table I and Fig. 4). Of particular interest, and confirming the role of P-glycoprotein-mediated translocation of the drugs across membranes, was the markedly higher levels of radioactivity of all three drugs seen in the brains of *mdr1a* (-/-) mice. This result is noteworthy since plasma radioactivity levels after intravenous administration were similar to those in the control animals. For indinavir and saquinavir, brain levels were 8–10-fold higher than in *mdr1a* (+/+) mice, while in the case of nelfinavir, an almost 40-fold increase was observed (Fig. 4).

In contrast to the results with intravenous administration, the plasma levels of radioactivity after oral administration

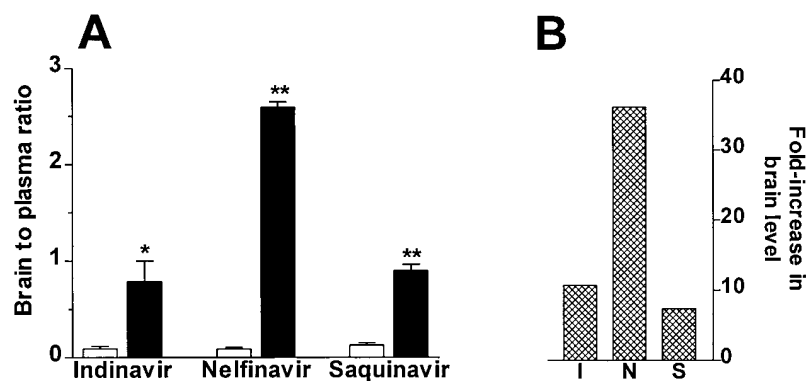


Figure 4. Brain to plasma radioactivity ratios (A) 4 h after intravenous administration of [¹⁴C]-indinavir (1.5 mg/kg), -nelfinavir (3 mg/kg), or -saquinavir (2 mg/kg). The ratios (mean±standard error) observed in the *mdr1a* (+/+) (open bar), and *mdr1a* (-/-) (solid bar) mice are shown. Three mice were analyzed in each group; **P* < 0.05, and ***P* < 0.01, comparing *mdr1a* (-/-) to corresponding *mdr1a* (+/+) mice. (B) The corresponding fold-increase in brain tissue levels in *mdr1a* (-/-) mice compared to those in *mdr1a* (+/+) mice; I, indinavir; N, nelfinavir; and S, saquinavir.

Table II. Plasma Radioactivity (ng/ml Tissue) in *mdr1a* (+/+) and (-/-) Mice at 4 h after Oral Administration of [¹⁴C]-Indinavir (6 mg/kg), [¹⁴C]-Nelfinavir (6 mg/kg), or [¹⁴C]-Saquinavir (6 mg/kg)

Drug	Plasma level (ng/ml)		Ratio (-/-)/(+/+)
	<i>mdr1a</i> (+/+)	<i>mdr1a</i> (-/-)	
Indinavir	34±7.4	69±11*	2.03
Nelfinavir	2.3±0.8	11±4.4*	4.78
Saquinavir	5.4±1.6	22±2.1 [‡]	4.07

Data are shown as mean±SD. Statistical difference in radioactivity between the two groups of mice was assessed by a two-sided Student's *t* test, with *P* < 0.05 as the limit of significance. **P* < 0.05, [‡]*P* < 0.01.

were two- to fivefold greater in the *mdr1a* (-/-) mice than in *mdr1a* (+/+) mice (Table II). This result strongly suggests that the limitation of systemic availability of these agents is attributable to P-glycoprotein-mediated transport (at the intestinal and/or hepatic level).

Discussion

This study provides two lines of evidence indicating an important role for transporter proteins, and in particular P-glycoprotein, in the disposition of HIV-1 protease inhibitors. First is the observation that their translocation across polarized cells, where the transporter is expressed on the apical surface, is dependent on the direction of vectorial movement, i.e., basal-apical transport is greater than apical-basal. The data using the L-MDR1 cell line are particularly relevant in this respect since these cells differ from the parent and non-P-glycoprotein-containing LLC-PK1 cells (Fig. 1) only by the stable overexpression of the human MDR1 gene. With all three drugs, the difference in transport according to which cell surface was initially exposed was markedly greater when P-glycoprotein was expressed. In the case of nelfinavir and saquinavir transport in LLC-PK1 cells was the same regardless of direction. On the other hand, with indinavir a difference, although reduced, was still present, suggesting the additional involvement of a transporter other than P-glycoprotein. A similar differential transport phenomenon was also observed using a Caco-2 cell line in which P-glycoprotein was constitutively expressed but at a lower level than in L-MDR1 cells (Fig. 1). Moreover, quinine and PSC-833, which are well-established inhibitors of P-glycoprotein function at concentrations used in these studies (12, 13), reduced the magnitude of the directional transport difference (Fig. 3). Since Caco-2 cells exhibit many of the morphological and biochemical characteristics of the human small intestine (14), these findings suggest that, along with CYP3A-mediated metabolism by the enterocyte (15), P-glycoprotein limits the oral bioavailability of HIV-1 protease inhibitors by transporting absorbed drug back into intestinal lumen (16). The two- to fivefold higher plasma levels of HIV-1 protease inhibitor radioactivity after oral administration in *mdr1a* (-/-) mice support this hypothesis.

The second major line of evidence is based on differences in tissue distribution of the drugs in *mdr1a* (-/-) mice compared to syngeneic animals. This gene knock-out model has

provided considerable insight into the role and importance of P-glycoprotein in the disposition of several other drugs (8–10). In particular, it has been shown that P-glycoprotein is a critical component of the blood-brain barrier which prevents the ready access of systemic drug to the central nervous system. For example, markedly higher brain levels of ivermectin (100-fold), digoxin (35-fold), and cyclosporine (17-fold) have been found in *mdr1a* (-/-) mice compared to *mdr1a* (+/+) animals, after their intravenous administration (8, 9). Accordingly, the 7–36-fold differences observed with the HIV-1 protease inhibitors, in the absence of any differences in their plasma concentration, strongly suggest that P-glycoprotein also plays a major role in limiting the drugs' access to the brain. This appears particularly to be the case with nelfinavir. P-glycoprotein is also present throughout the intestinal tract (17) and, therefore, may function to secrete drug in systemic blood into the intestinal lumen (16). It would therefore be expected that intestinal tissue levels would be elevated in mice with a disrupted *mdr1a* gene. This probably accounts for the observed trend in the results for the small intestine which, in the case of indinavir, were statistically significant.

In vitro and in vivo studies have indicated that the ability of HIV protease inhibitors to suppress virus replication is dependent on the drug's concentration and this relationship is relatively steep (18–20). The demonstrated presence of a substantial barrier to the drug's distribution into brain caused by P-glycoprotein suggests that the ability to achieve therapeutic brain concentrations is limited, creating a potential sanctuary for viral replication. The persistence of central nervous system virus activity may contribute to the adverse neurologic outcome in HIV infection even after effective systemic HIV protease inhibitor administration (21–23). Of additional interest is the fact that P-glycoprotein is expressed in ~ 5% of CD4 T lymphocytes, a major cellular target for HIV-1 protease inhibitor effect (24).

An approach to overcoming tumor cell resistance secondary to the overexpression of P-glycoprotein in tumor cells is the use of a chemomodulator to inhibit the efflux pump's activity (12, 13). In principle, a similar strategy could be applied to HIV protease inhibitor therapy in order to enhance distribution into sites such as the brain and CD4 T lymphocytes, as well as to increase oral absorption. In fact, enhanced brain penetration of the P-glycoprotein substrates, ivermectin and digoxin, after pretreatment with PSC-833 have been reported recently in mice (25, 26). A complicating factor in the case of protease inhibitors is the likelihood that available modulators of P-glycoprotein activity also have the potential to inhibit the metabolism of HIV protease inhibitors themselves and therefore alter their systemic availability and elimination characteristics.

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References

1. Carpenter, C.C.J., M.A. Fischl, S.M. Hammer, M.S. Hirsch, D.M. Jacobsen, D.A. Katzenstein, J.S. Montaner, D.D. Richman, M.S. Saag, R.T. Schooley, et al. 1996. Antiretroviral therapy for HIV infection in 1996. Recommendations of an international panel. *J. Am. Med. Assoc.* 276:146–154.
2. Collier, A.C., R.W. Coombs, D.A. Schoenfeld, R.L. Bassett, J. Timpone, A. Baruch, M. Jones, K. Facey, C. Whitacre, V.J. McAuliffe, et al. 1996. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *N. Engl. J. Med.* 334:1011–1017.
3. Deeks, S.G., M. Smith, M. Holodniy, and J.O. Kahn. 1997. HIV-1 protease inhibitors. A review for clinicians. *J. Am. Med. Assoc.* 277:145–153.
4. Gottesman, M.M., and I. Pastan. 1993. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* 62:385–427.
5. Levêque, D., and F. Jehl. 1995. P-glycoprotein and pharmacokinetics. *Anticancer Res.* 15:331–336.
6. Cordon-Cardo, C., J.P. O'Brien, D. Casals, L. Rittman-Grauer, J.L. Biedler, M.R. Melamed, and J.R. Bertino. 1989. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc. Natl. Acad. Sci. USA.* 86:695–698.
7. Tsuji, A., T. Terasaki, Y. Takabatake, Y. Tenda, I. Tamai, T. Yamashima, S. Moritani, T. Tsuruo, and J. Yamashita. 1992. P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. *Life Sci.* 51:1427–1437.
8. Schinkel, A.H., J.J.M. Smit, O. van Tellingen, J.H. Beijnen, E. Wagenaar, L. van Deemter, C.A.A.M. Mol, M.A. van der Valk, E.C. Robanus-Maandag, H.P.J. Riele, et al. 1994. Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell.* 77:491–502.
9. Schinkel, A.H., E. Wagenaar, L. van Deemter, C.A.A.M. Mol, and P. Borst. 1995. Absence of the *mdr1a* P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J. Clin. Invest.* 96:1698–1705.
10. Schinkel, A.H., E. Wagenaar, C.A.A.M. Mol, and L. van Deemter. 1996. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J. Clin. Invest.* 97:2517–2524.
11. Bain, L.J., and G.A. LeBlanc. 1996. Interaction of structurally diverse pesticides with the human MDR1 gene product P-glycoprotein. *Toxicol. Appl. Pharmacol.* 141:288–298.
12. Ferry, D.R., H. Traunecker, and D.J. Kerr. 1996. Clinical trials of P-glycoprotein reversal in solid tumours. *Eur. J. Cancer.* 32A:1070–1081.
13. Ford, J.M. 1996. Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. *Eur. J. Cancer.* 32A:991–1001.
14. Meunier, V., M. Bourrié, Y. Berger, and G. Fabre. 1995. The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications. *Cell Biol. Toxicol.* 11:187–194.
15. Kolars, J.C., P. Schmiedlin-Ren, J.D. Shuetz, C. Fang, and P.B. Watkins. 1992. Identification of rifampin-inducible P450III_{A4} (CYP3A4) in human small bowel enterocytes. *J. Clin. Invest.* 90:1871–1878.
16. Sparreboom, A., J. van Asperen, U. Mayer, A.H. Schinkel, J.W. Smit, D.K. Meijer, P. Borst, W.J. Nooijen, J.H. Beijnen, and O. van Tellingen. 1997. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc. Natl. Acad. Sci. USA.* 94:2031–2035.
17. Fojo, A.T., K. Ueda, D.J. Slamon, D.G. Poplack, M.M. Gottesman, and I. Pastan. 1987. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci. USA.* 84:265–269.
18. Stein, D.S., D.G. Fish, J.A. Bilello, S.L. Preston, G.L. Martineau, and G.L. Drusano. 1996. A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). *AIDS.* 10:485–492.
19. Rusconi, S., D.P. Merrill, and M.S. Hirsch. 1994. Inhibition of human immunodeficiency virus type 1 replication in cytokine-stimulated monocytes/macrophages by combination therapy. *J. Infect. Dis.* 170:1361–1366.
20. Craig, J.C., I.B. Duncan, D. Hockley, C. Grief, N.A. Roberts, and J.S. Mills. 1991. Antiviral properties of Ro 31-8959, an inhibitor of human immunodeficiency virus (HIV) proteinase. *Antivir. Res.* 16:295–305.
21. Bagasra, O., E. Lavi, L. Bobroski, K. Khalili, J.P. Pestaner, R. Tawadros, and R.J. Pomerantz. 1996. Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: identification by the combination of in situ polymerase chain reaction and immunohistochemistry. *AIDS.* 10:573–585.
22. Wiley, C.A., E. Masliah, and C.L. Achim. 1994. Measurement of CNS HIV burden and its association with neurologic damage. *Adv. Neuroimmunol.* 4:319–325.
23. Achim, C.L., R. Wang, D.K. Miners, and C.A. Wiley. 1994. Brain viral burden in HIV infection. *J. Neuropathol. Exp. Neurol.* 53:284–294.
24. Lucia, M.B., R. Cauda, A.L. Landay, W. Malorni, G. Donelli, and L. Ortona. 1995. Transmembrane P-glycoprotein (P-gp/P-170) in HIV infection: analysis of lymphocyte surface expression and drug-unrelated function. *AIDS Res. Human Retroviruses.* 11:893–901.
25. Didier, A.D., and F. Loor. 1995. Decreased biotolerability for ivermectin and cyclosporin A in mice exposed to potent P-glycoprotein inhibitors. *Int. J. Cancer.* 63:263–267.
26. Mayer, U., E. Wagenaar, B. Dorobek, J.H. Beijnen, P. Borst, and A.H. Schinkel. 1997. Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier p-glycoprotein by oral treatment of mice with PSC833. *J. Clin. Invest.* 100:2430–2436.