

Saquinavir, an HIV Protease Inhibitor, Is Transported by P-Glycoprotein

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ABSTRACT

Saquinavir, a peptidomimetic HIV protease inhibitor, has been shown to be effective in reducing patient viral load and reducing mortality. In this report we investigated whether saquinavir is a substrate for the multidrug resistance transporter P-glycoprotein (P-gp), which may reduce the effective intracellular concentration of the drug. G185 cells, which highly express P-gp, are resistant to saquinavir-mediated cytotoxicity, and co-administration of cyclosporine reversed this resistance. Saquinavir and saquinavir mesylate inhibited basolateral to apical transport of the fluorescent dye rhodamine 123 in a polarized epithelial transport assay, a result that suggests competition of

these drugs for the P-gp transporter. Finally, we measured specific, directional transport of saquinavir and saquinavir mesylate in an epithelial monolayer model. Transport in the basolateral to apical direction was 3-fold greater than apical to basolateral flux for both saquinavir and saquinavir mesylate and was blocked by co-incubation with the established P-gp reversal agents cyclosporine and verapamil. These data provide evidence that saquinavir is a substrate for the P-gp transporter and suggest that this protein may affect intracellular accumulation of the drug and contribute to its poor oral bioavailability.

The recent discovery of HIV-1 protease inhibitors has introduced a new class of first-line drug therapies for mid-stage and advanced-stage HIV patients. Saquinavir mesylate (Invirase, originally Ro 31-8959) is one such agent and was the first to become clinically available in the United States to HIV patients (fig. 1). In infected cells, the integrated HIV viral DNA is translated into a polyprotein that requires cleavage by the HIV-1 protease for activation. *In vitro* studies show that active site mutations in the HIV-1 protease have resulted in immature and non-infectious viral products. Further, 3 of the 9 HIV-1 protease cleavage sites are in Phe-Pro and Tyr-Pro sequences not targeted by mammalian proteinases, which suggests that inhibition at this site will be specific for viral enzymes (Noble and Faulds, 1996). Saquinavir mesylate relies on this selectivity to function as a transition state analog peptidomimetic inhibitor of the HIV-1 protease. Clinical trials have shown that saquinavir mesylate monotherapy administered p.o. at 600 mg three times per day is effective in both raising CD4⁺ cell counts and reducing HIV viral load (Vella, 1995; Noble and Faulds, 1996). Also, both *in vitro* assays and clinical experience suggest that combination therapy of saquinavir with reverse transcriptase inhibitors is effective in the treatment of patients infected with HIV.

P-gp is an ATP-dependent drug efflux pump typically associated with MDR in cancer chemotherapy. This 170-kDa

transmembrane protein is an ATP-dependent transporter of a wide range of compounds, including anticancer drugs, peptides, steroids, calcium channel blockers and antihistamines (Endicott and Ling, 1989; Borst *et al.*, 1993; Gottesman and Pastan, 1993). Compounds that interact with P-gp are structurally and mechanistically diverse; however, they tend to be large, amphipathic and aromatic. P-gp-mediated efflux reduces the intracellular accumulation of these compounds, thereby diminishing drug efficacy. In the case of cytotoxic drugs, this leads to enhanced cell survival. P-gp is normally expressed in a large number of tissues, including the intestine, the liver, the brain, and the immune system (Fojo *et al.*, 1987; Thiebaut *et al.*, 1987, 1989; Borst *et al.*, 1993). Its localization in the epithelial cells of those organs has led to the hypothesis that a physiologic function of this protein is to prevent the accumulation of toxic substances or to serve as a protective barrier against the entry of xenobiotics.

P-gp is also expressed in peripheral blood cells. Pluripotent CD34⁺ hematopoietic stem cells express P-gp, which may serve a protective role for those important cells (Chaudhary and Roninson, 1991). These cells accumulate increased amounts of the fluorescent dye R123, a P-gp substrate, in the presence of P-gp inhibitors and were recognized by two P-gp-specific monoclonal antibodies. Decreased R123 accumulation attributable to expression of P-gp was also observed in CD56⁺, CD8⁺ and CD20⁺ cells and to a lesser extent in a subset of CD4⁺ cells (Chaudhary *et al.*, 1992). Flow cytomet-

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ABBREVIATIONS: CsA, cyclosporin A; MDR, multidrug resistance; P-gp, P-glycoprotein, R123, rhodamine 123.

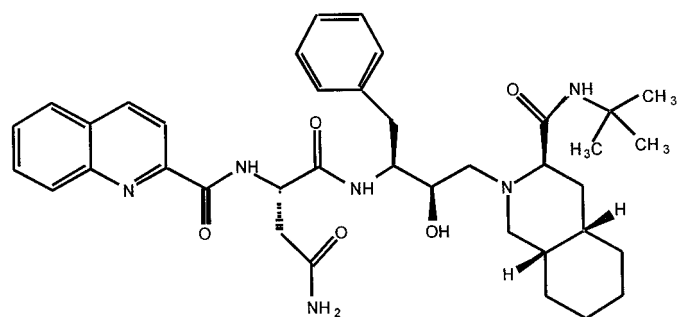


Fig. 1. Chemical structure of saquinavir.

ric analysis and decreased R123 retention have more recently confirmed significant P-gp expression in both CD4+ and CD8+ cells (Gupta *et al.*, 1992; Gupta and Gollapudi, 1993). Infection of H9 T cells or U937 monocytic cells with HIV-1 resulted in enhanced levels of P-gp expression (Gollapudi and Gupta, 1990; Antonelli *et al.*, 1992; Dianzani *et al.*, 1994). Significantly, administration of the reverse transcriptase inhibitor azidothymidine (AZT) to HIV-infected T cells also resulted in elevated expression of P-gp (Dianzani *et al.*, 1994). AZT and other nucleoside analog drugs have been observed to be substrates for P-gp-mediated efflux (Antonelli *et al.*, 1992). Increased expression of P-gp may, therefore, be an additional mechanism leading to resistance to nucleoside analogs.

Expression of P-gp in these immune cells suggests that other HIV drug therapies that target these cells may also be subject to P-gp transport. Saquinavir mesylate and a number of other peptidomimetic protease inhibitors display several of the structural characteristics common to P-gp substrates, having several planar aromatic rings and basic nitrogen groups. In the experiments presented here, we investigate whether saquinavir mesylate and its free base, saquinavir, are substrates of P-glycoprotein. These drugs were less cytotoxic to P-gp-expressing cells and decreased the transport of R123 across an epithelial cell monolayer. Saquinavir and saquinavir mesylate were also specifically transported across an epithelial cell monolayer, and this flux was inhibited with established P-gp-reversal agents. These data suggest that P-gp may limit the intracellular accumulation of peptidomimetic drugs in cells that express this protein.

Materials and Methods

Cell culture. The parental drug-sensitive NIH3T3 Swiss mouse embryo cell line was purchased from American Type Culture Collection (ATCC, Rockville, MD) and was grown in 150-cm² culture flasks (Costar Corporation, Cambridge, MA) in Dulbecco's Modified Eagles Medium (Biowhittaker, Walkersville, MD) supplemented with 4.5 g/l glucose, 10% fetal bovine serum (Hyclone Laboratories, Logan, Utah), 2 mM L-glutamine (Advanced Biotechnologies Incorporated (ABI), Columbia, MD) and 0.01 mg/ml gentamicin (ABI). The drug-resistant line NIH-MDR-G185, expressing P-gp, was obtained from M. M. Gottesman (NCI, NIH) and was maintained in similar medium supplemented with 60 ng/ml of colchicine (Sigma Chemical Co., St. Louis, MO) (Currier *et al.*, 1992). HCT-8 cells (ATCC), derived from a human ileocecal adenocarcinoma cell line, were cultured in RPMI 1640 medium (Biowhittaker) supplemented with 10% horse serum (Biowhittaker), 1 mM sodium pyruvate (Gibco BRL, Grand Island, NY) and 0.01 mg/ml gentamicin. All cells were maintained in a humidified atmosphere with 5% CO₂ at 37°C.

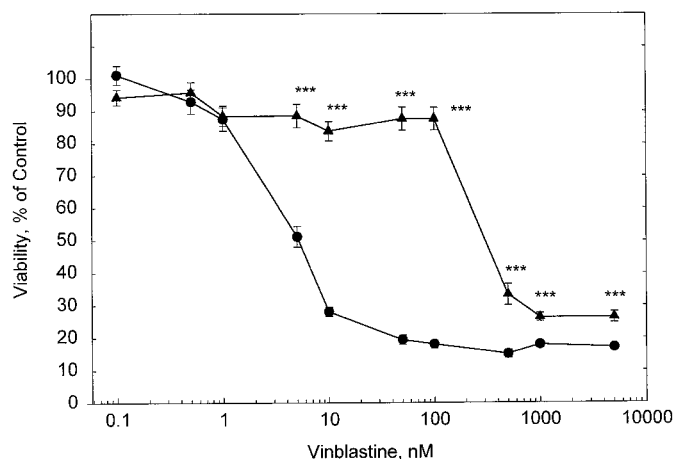
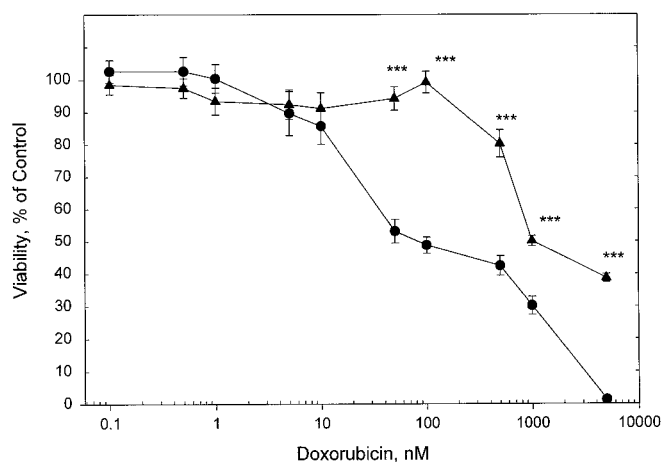


Fig. 2. Cytotoxicity of doxorubicin (top) and vinblastine (bottom) in parental NIH3T3 cells (circles) and drug-resistant NIH 3T3 G185 cells (triangles). Cells were treated with the indicated concentrations of drugs, and the viability of the cells was measured as described in "Materials and Methods." Data are expressed as a percentage of untreated control cells; presented are the mean (\pm S.E.M.) of data averaged from four independent experiments each performed in quadruplicate. *** denotes data significantly different from parental cells using Student's unpaired *t* test, $P < .001$.

Cytotoxicity assay. Cells were plated at a density of 3×10^3 cells/well for NIH3T3 cells, and 2.5×10^3 cells/well for NIH-MDR-G185 cells, in 96-well microtiter plates (PGC, Gaithersburg, MD). Cells were exposed to the indicated concentrations of saquinavir or saquinavir mesylate for 72 hr. Cell viability was determined with the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazo-

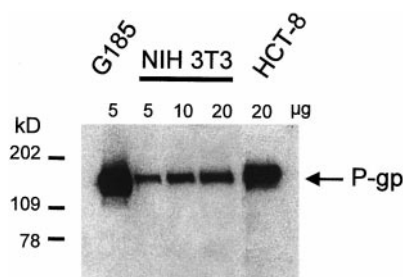


Fig. 3. Western blot analysis. Cell membranes were isolated and separated by SDS/PAGE as described in "Materials and Methods." P-glycoprotein was visualized using the C219 antibody and chemiluminescence with Kodak X-AR film. Membranes were from the following cells: G185, 5 μ g, lane 1; NIH3T3, 5 μ g, lane 2; 10 μ g, lane 3; 20 μ g, lane 4; HCT-8, 20 μ g, lane 5. Location of molecular weight standards are indicated.

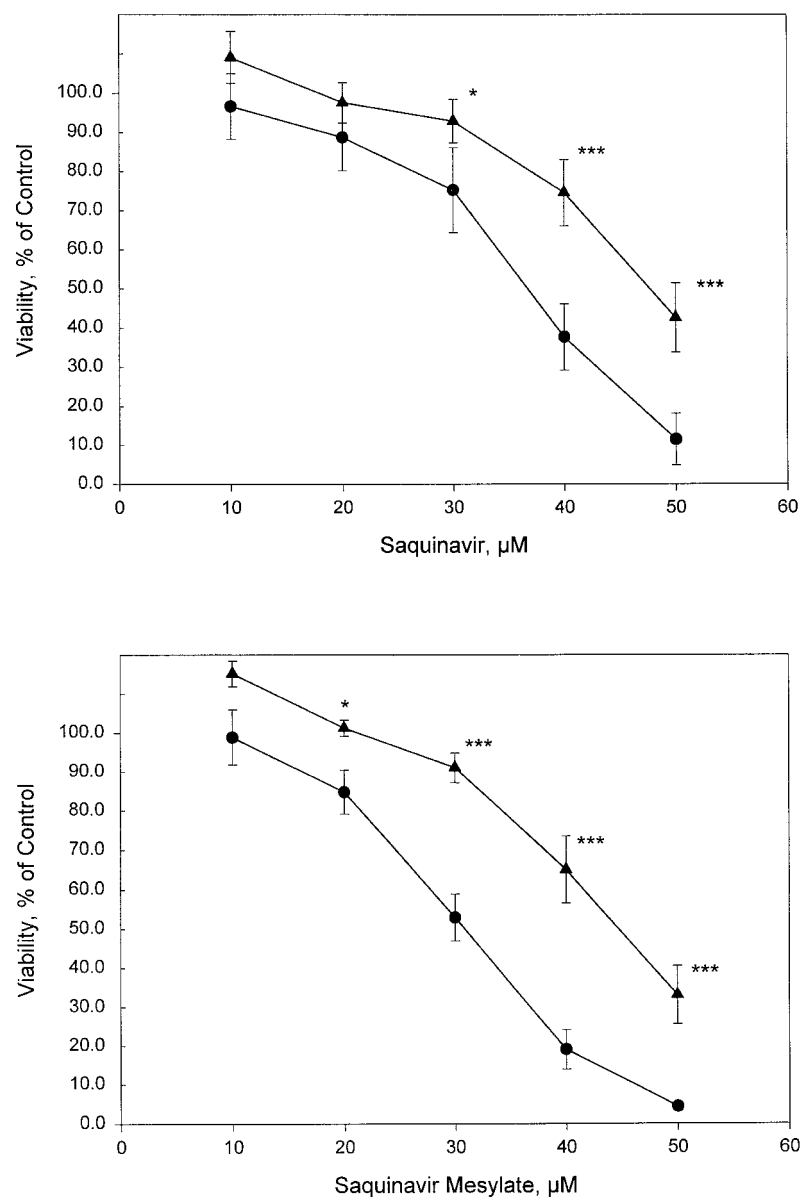


Fig. 4. Cytotoxicity of saquinavir and saquinavir mesylate. Parental NIH3T3 cells (circles) and drug-resistant NIH 3T3-G185 cells (triangles) were exposed to increasing concentrations of saquinavir (top) or saquinavir mesylate (bottom). Cytotoxicity was measured by the MTT assay as described in "Materials and Methods." Data are expressed as a percentage of untreated control cells; presented are the mean (\pm S.E.M.) of data averaged from three independent experiments each performed in quadruplicate. * and *** denote data significantly different from parental cells using Student's unpaired *t* test, $P < .05$ and $.001$, respectively.

lium, Sigma] assay as previously described (Mosmann, 1983; Hansen *et al.*, 1989), and the resulting absorbance was measured with a Dynex MRX Microplate Reader (Chantilly, VA) at 570 nm.

Western blot. Twenty micrograms of membrane proteins was separated on an 8% SDS polyacrylamide gel and transferred to a 0.45- μ m nitrocellulose membrane as described previously (Gant *et al.*, 1991). The blots were blocked in TBS-T containing 5% skim milk for 1 hr and then probed with 1 μ g/ml of C219 antibody (Signet Laboratories, Dedham, MA) in TBS-T for 2 hr. The blots were visualized by enhanced chemiluminescence according to the manufacturer's instructions (Pierce, Rockford, IL).

R123 transport. Inhibition of R123 (Sigma) transport was examined as previously described (Hunter *et al.*, 1991) using a HCT-8 monolayer system. Briefly, R123 was added at a final concentration of 5 μ g/ml (13 μ M) to the basal or apical compartments, and 200- μ l samples were taken at the indicated times from the opposite chamber. Saquinavir or saquinavir mesylate was added to both compartments as an inhibitor. Media aliquots were taken at the indicated times, and the fluorescence of R123 was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm with a Biotek FL500 Fluorescence Plate Reader (Winooski, VT).

Saquinavir transport assay. HCT-8 cells were plated at a density of 3 to 4 $\times 10^5$ cells/cm² on Transwell polyester membranes 24 mm in diameter and 4.0 μ m in pore size (Corning, Corning, NY). Culture medium was replaced every 2 days until a cell monolayer was formed and verified by transepithelial electrical resistance using the EVOM Epithelial Volt-ohmmeter (World Precision Instruments Incorporated, Sarasota, FL). Once the monolayer was established (300–500 mohms), saquinavir or saquinavir mesylate was added to either the basal or the apical side, and 200- μ l aliquots were taken every hour for 6 hr from the opposite chamber. Drug concentrations were measured by HPLC analysis. The permeability coefficients (P_e) were calculated from the following equation:

$$P_e = \frac{1}{AC_0} \cdot \frac{dQ}{dt}$$

where A is the surface area of the membrane, C_0 is the initial drug concentration and dQ/dt is the drug flux across the membrane (Artursson, 1990; Artursson and Karlsson, 1991).

HPLC analysis. Sample aliquots, 200 μ l, were precipitated with an equal volume of acetonitrile containing diltiazam as an internal

standard and were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was then analyzed on a 15-cm, 5- μ m particle size Microform-MV Phenyl Column (Rainin Instrument Company Inc., Woburn, MA). The column temperature was maintained at 40°C. Separation was achieved with an isocratic solvent system composed of 74:26 (v:v) methanol/aqueous ammonium hydroxide (0.9%) with a flow rate of 1.0 ml/min. The absorbance of the samples was monitored at 238 nm.

Results

Saquinavir cytotoxicity. We first examined whether expression of P-gp confers resistance to saquinavir-mediated cytotoxicity using the drug-resistant, MDR1-transfected NIH3T3-G185 (G185) cells (Currier *et al.*, 1992; Cardarelli *et al.*, 1995). Exposure of these cells to vinblastine or doxorubicin demonstrates a 27-fold resistance to vinblastine and an 11-fold resistance to doxorubicin (fig. 2), which is consistent with previous observations for these cells (Currier *et al.*, 1992; Cardarelli *et al.*, 1995). Western immunoblot analysis of membranes isolated from these cells confirms high expression of P-gp in the G185 cells compared with the parental NIH3T3 cells (fig. 3). Treatment of these cells with increasing concentrations of saquinavir and saquinavir mesylate revealed that the NIH3T3 cells were more sensitive to the cytotoxicity of these drugs, with an LD₅₀ of approximately 37 μ M for saquinavir and 31 μ M for saquinavir mesylate (fig. 4). The LD₅₀ values in the MDR1-transfected G185 cells were approximately 47 and 45 μ M for these compounds, respectively. Thus the relative resistance of the G185 cells to saquinavir, 25% to 45%, is modest when compared with cytotoxic anticancer drugs. This is probably because the specificity of saquinavir for viral proteases results in low toxicity to mammalian cells. Despite the small degree of resistance conferred by P-gp, these results suggest that this drug may be a substrate for P-gp-mediated transport. Additional low-toxicity or noncytotoxic drugs have previously been observed to be substrates for P-gp-mediated transport (Yang *et al.*, 1989, 1990; Schinkel *et al.*, 1996). It is worth noting that because of the low cytotoxicity of saquinavir, these LD₅₀ concentrations are approximately 1000 to 5000-fold higher than that necessary to produce 50% viral inhibition (Noble and Faulds, 1996).

Effect of P-glycoprotein reversal agents. The potential interaction of P-gp with saquinavir was further investigated by determining the effect of the established P-gp-reversal agent CsA on the cytotoxicity of this drug. The G185 cells were treated with increasing concentrations of saquinavir or saquinavir mesylate in the presence of CsA. A dose-dependent increase in toxicity was observed, which indicates that this agent was a potent reversal agent of cellular resistance to saquinavir and saquinavir mesylate (fig. 5). Addition of 5 μ g/ml CsA reduced the LD₅₀ of saquinavir and saquinavir mesylate to approximately 27 μ M in drug-resistant G185 cells. The effect of CsA on saquinavir-mediated toxicity in parental NIH3T3 cells was modest, which is consistent with their low level of P-gp expression (data not shown; fig. 3). Similarly, addition of verapamil, another P-gp-reversal agent, also increased saquinavir and saquinavir mesylate cytotoxicity in G185 cells (data not shown).

R123 transport. Polarized drug transport across an epithelial cell monolayer was used to determine specific P-gp-mediated flux. As an established P-gp substrate, the fluores-

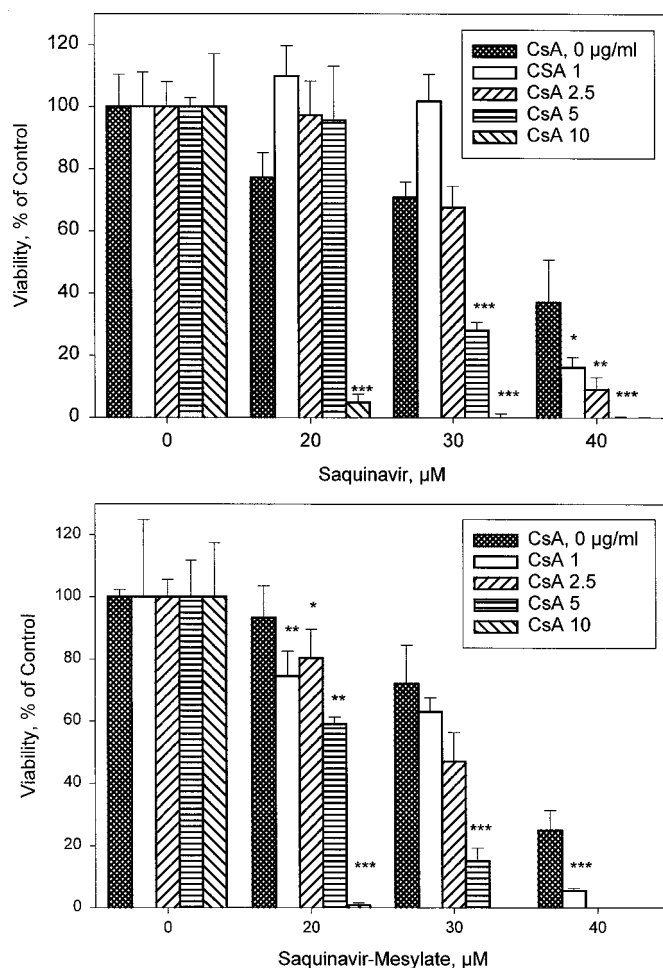


Fig. 5. Effect of CsA on the cytotoxicity of saquinavir and saquinavir mesylate. Drug-resistant NIH 3T3-G185 cells were exposed to the indicated concentrations of saquinavir (top) or saquinavir mesylate (bottom) in the presence of 0, 1, 2.5, 5 or 10 μ g/ml (0.83, 2.1, 4.2 and 8.4 μ M) CsA. Cytotoxicity was measured by the MTT assay as described in "Materials and Methods." Values are expressed as a percentage of untreated control cells, are the mean \pm S.D. of quadruplicate samples and are representative of at least two independent experiments. *, ** and *** denote data significantly different from control using Student's unpaired *t* test, $P < .05$, $.01$ and $.001$, respectively.

cent dye R123 is rapidly removed from drug-resistant cells that overexpress P-gp (fig. 6). Further, P-gp inhibitors such as CsA and verapamil block R123 efflux and increase intracellular accumulation of this dye (Neyfakh, 1988; Kessel *et al.*, 1991). The HCT-8 human intestinal adenocarcinoma cell line is well documented as having high levels of P-gp, which is polarized to the apical membrane (Hunter *et al.*, 1991). Western blot analysis with the C219 antibody confirmed the high expression of P-gp in these cells (fig. 3). HCT-8 cells readily display directional, basolateral to apical transport of P-gp substrates such as vinblastine (Zacherl *et al.*, 1994). Therefore, we used these cells to measure the transport of R123 in the presence and absence of saquinavir and saquinavir mesylate. Figure 5 demonstrates time-dependent, polarized transport of R123 in these cells from the basolateral to the apical compartments and shows that the addition of 5 μ M CsA effectively inhibits this P-gp-mediated dye flux. Interestingly, apical to basolateral, absorptive flux of R123 did not increase in the presence of CsA. This may suggest the pres-

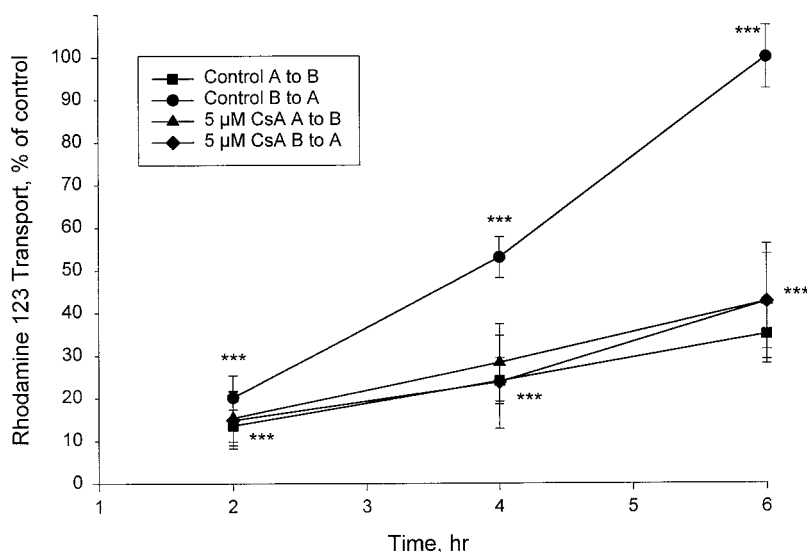


Fig. 6. R123 transport. HCT-8 cells were grown in Transwell dishes until a complete monolayer was formed. R123, 5 $\mu\text{g/ml}$ (13 μM), was placed in either the apical or the basolateral chamber in the absence or presence of 5 μM CsA in the medium in both chambers. Media aliquots were taken at the indicated times. The data represent the mean \pm S.D. of three independent measurements. For the control cells, *** denotes data significantly different from apical to basolateral flux, $P < .001$. For the CsA-treated cells, *** denotes data significantly different from the basolateral to apical transport in control cells, $P < .001$.

ence of an additional, yet-unidentified transporter(s) in these cells. These data are consistent with previous investigations and support the use of these cells as a model for P-gp-mediated transport (Zacherl *et al.*, 1994). Addition of 5 to 20 μM saquinavir resulted in a dose-dependent reduction of the amount of R123 transported across the membrane (fig. 7). Thus these data support the hypothesis that saquinavir interacts with P-gp to reduce the transport of an established substrate, R123, in MDR1-expressing cells.

Saquinavir transport by P-glycoprotein. Finally, to determine whether saquinavir is actually a substrate for P-gp-mediated transport, we measured the specific, directional flux across HCT-8 cell monolayers. Saquinavir or saquinavir mesylate (data not shown) was placed on the apical or basal side of the monolayer, and drug transport was quantified over 6 hr. For saquinavir, 4.6 nmol, 7% of the initial drug concentration, was transported from the basolateral to the apical compartment, whereas 1.6 nmol, 3% of the initial

drug, was transported in the reverse direction (fig. 8). The basolateral to apical P_e was 1.83×10^{-6} cm/sec, whereas in the reverse direction, the P_e was 6.24×10^{-7} cm/sec, for a $P_{e,\text{basal}}/P_{e,\text{apical}}$ ratio of 2.9. Addition of CsA or verapamil reduced the transepithelial flux of saquinavir approximately 5-fold so that 1.4% of the initial drug concentration was transported into the apical compartment (fig. 8). These data demonstrate that saquinavir is vectorially transported across the epithelial monolayer and suggest that this flux is mediated by P-gp.

Discussion

Our results demonstrate for the first time that saquinavir, an important new drug for treatment of HIV infections, is a

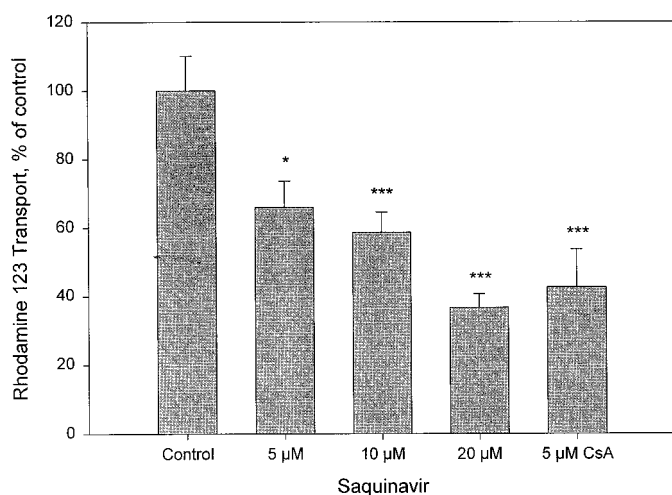


Fig. 7. Saquinavir inhibition of R123 transport. HCT-8 cells were grown in Transwell dishes until a complete monolayer was formed. R123, 5 $\mu\text{g/ml}$ (13 μM), was placed in the basolateral chamber with 0, 5, 10 or 20 μM saquinavir, and media aliquots were taken from the apical compartment at the indicated times. The data presented are the mean \pm S.D. of three independent experiments. * and *** denote data that are significantly different from the control cells, $P < .05$ and $.001$, respectively.

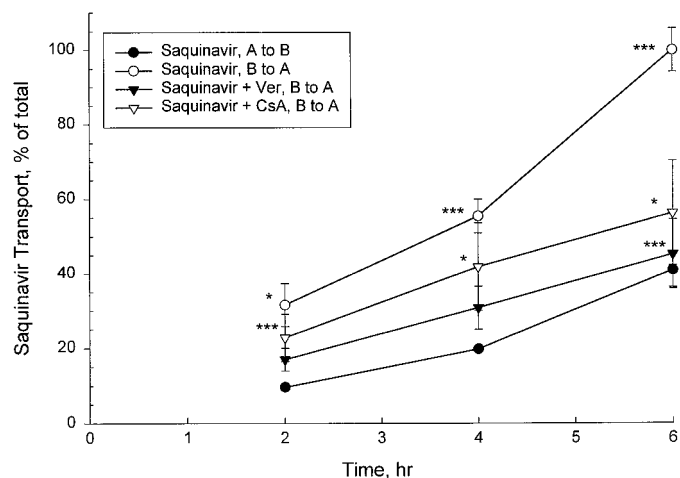


Fig. 8. Transport of saquinavir. HCT-8 cells were grown in Transwell dishes until a complete monolayer was formed. Saquinavir, 10 μM , was added to the apical chamber and aliquots were taken at the indicated times from the basolateral chamber, or drug was added to the basolateral chamber and samples were drawn from the apical compartment. Drug concentrations were measured by HPLC as described in "Materials and Methods." CsA, 4 μM or verapamil (Ver), 5 μM , was added as a P-glycoprotein inhibitor. The data represent the mean of six independent wells \pm S.E.M. For the control cells, * and *** denote data that are significantly different from apical to basolateral flux in these cells. For the verapamil- and CsA-treated cells, * and *** denote data that are significantly different from basolateral to apical flux in the control cells, $P < .05$ and $.001$, respectively.

substrate for P-gp-mediated drug efflux. Cells that express large amounts of this protein have a small selective growth advantage over parental cells in the presence of these drugs. Furthermore, addition of the P-gp-reversal agent CsA sensitizes the MDR1-transfected G185 drug-resistant cells. Saquinavir and saquinavir mesylate were also able to block P-gp-mediated flux of R123 across an epithelial cell monolayer and to increase R123 retention in G185 cells (data not shown). Whereas these assays suggest the possibility of P-gp-mediated transport, saquinavir and saquinavir mesylate transport by P-gp was confirmed by measurement of specific and directional flux in an HCT-8 epithelial cell monolayer system. This transport was inhibited by the addition of the established P-gp-reversal agents CsA and verapamil.

HIV infection of either T cell or monocytic cell lines resulted in increased P-gp expression and decreased levels of accumulation of 3' azido-3'-deoxythymidine (AZT) (Gollapudi and Gupta, 1990). Additional investigations demonstrated that MDR-expressing cells are also resistant to AZT and 2',3'-dideoxycytidine (DDC) (Yusa *et al.*, 1990). Freshly isolated CD4+ and CD8+ T cells express low levels of P-gp; however, activation of these cells significantly increases the level of expression (Gupta *et al.*, 1992). These data suggest that anti-HIV nucleoside analogs may be transported by P-gp. In contrast, induction of drug resistance by selection with increasing concentrations of AZT resulted in cells that were resistant to AZT but did not express detectable amounts of P-gp (Yusa *et al.*, 1990). Clearly, expression of P-gp is but one of several mechanisms that contribute to resistance to nucleoside analog drugs. Resistance to AZT develops over a long time and is primarily associated with mutations in HIV reverse transcriptase at several positions (Mayers, 1996). Similarly, reduced sensitivity to saquinavir is associated with two independent mutations in the HIV protease (Roberts, 1995). One effect of the action of P-gp, or other drug exporters, may be to reduce the intracellular level of antiviral drugs and their potential antiviral effect.

Although saquinavir treatment has proved effective in combating HIV, the low oral bioavailability of saquinavir remains a significant obstacle to drug delivery (Vella, 1995; Noble and Faulds, 1996). Hepatic, and more recently intestinal, metabolism are most often assumed to limit oral bioavailability (Fitzsimmons and Collins, 1997). The interaction of saquinavir with P-gp in the current investigation, however, suggests that active drug efflux may also adversely affect its bioavailability. P-gp may be one of several cellular transporters that limit the absorption of pharmaceuticals and xenobiotics. Recently it has been recognized that intestinal CYP3A and P-gp may act together to limit drug absorption (Wacher *et al.*, 1995, 1996). Expressed in the small intestine, P-gp may function as a barrier against entry of potentially toxic compounds. Indeed, recent investigations with *mdr1a* knockout mice have suggested such a role for this protein (Schinkel *et al.*, 1994). In mice, *mdr1a* encodes the only drug-transporting P-gp in the intestine. The p.o. administration of paclitaxel to these mice resulted in a 6-fold increase in the area under the plasma concentration *vs.* time curve compared with wild-type mice (Sparreboom *et al.*, 1997). Also, after i.v. administration, these mice excreted significantly less drug into the intestinal lumen than wild-type mice, 3% *vs.* 11%. These data indicate that P-gp may limit the oral bioavailability and the intestinal excretion of

this drug. The functional efflux of saquinavir by P-gp suggests that this transporter may also contribute to the poor oral bioavailability of this drug by lowering the amount of drug that crosses the intestinal epithelium. Additionally, P-gp-mediated transport of saquinavir back into the lumen may permit the drug to be cyclically reabsorbed, thereby increasing its exposure to intestinal drug-metabolizing enzymes, notably cytochrome P450 3A (Wacher *et al.*, 1996; Fitzsimmons and Collins, 1997).

In conclusion, saquinavir was used as a model protease inhibitor because it shares structural characteristics common to this class of drugs that suggest they may be substrates for the MDR transporter. These experiments support the hypothesis that saquinavir is a substrate for P-gp. Transport of peptides and peptidomimetic drugs by P-gp may diminish the intracellular accumulation of many novel compounds currently being developed for therapy of cancer, arthritis, viral and fungal infections and many other diseases. P-gp in the intestinal epithelium may also limit the oral bioavailability of saquinavir and other peptidomimetic drugs administered p.o. and may provide a rational approach to developing improved formulations. These data suggest that further investigations utilizing *in vivo* models are warranted to determine whether and to what extent P-gp affects oral bioavailability of this important class of drugs.

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