**ABSTRACT**

Caco-2 cells grown in the presence of \(1\alpha,25\)-di-OH vitamin \(D_3\) (di-OH \(D_3\)) were used as a model to evaluate the effects of P-glycoprotein (Pgp) efflux on CYP3A4-mediated metabolism of indinavir during intestinal absorption. Caco-2 cells grown under these conditions demonstrated significant CYP3A4 activity and maintained Pgp-mediated directional transport of indinavir. Metabolism of indinavir in the di-OH \(D_3\)-treated cells correlated with the level of CYP3A activity and generated metabolites consistent with CYP3A4-mediated metabolism. During transport experiments, indinavir metabolites are selectively secreted into the apical compartment, consistent with Pgp-mediated efflux. Using formation of the most abundant metabolite, M6, as a marker for indinavir metabolism, we observed that the extent of indinavir metabolism is not significantly affected by the direction of indinavir transport or by inhibition of Pgp with cyclosporin A. However, because Pgp efflux results in higher indinavir transport in the basolateral-to-apical direction than in the apical-to-basolateral direction, the ratio of M6 produced normalized to the amount of drug transported across the monolayer was higher for apical-to-basolateral transport. Thus, Pgp efflux in a direction opposite to absorptive transport results in more metabolite produced per mole of drug that is absorbed. In summary, the results support a role of Pgp in increasing intestinal presystemic metabolism and in removal of CYP3A4-generated metabolites from the intracellular compartment.

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**ABBREVIATIONS:** Pgp, P-glycoprotein; EHS, Engelbreth-Holmes-Swarm; LC/MS, liquid chromatography/mass spectrometry; CYP3A4, cytochrome P-450 3A4; di-OH \(D_3\), \(1\alpha,25\)-di-OH vitamin \(D_3\); CsA, cyclosporin A; HBSS, Hanks’ balanced salt solution.
tobalism (Gan et al., 1996; Watkins, 1997; Wacher et al., 1998; Ito et al., 1999). According to these models, increased intestinal residence time resulting from Pgp efflux and prevention of product inhibition by the removal of primary metabolites from the cell interior result in more extensive intestinal metabolism by CYP3A4. Support for these models largely comes from circumstantial evidence citing overlapping substrate specificity for the two proteins (Wacher et al., 1995), similarities in the gene regulation and tissue distribution for CYP3A4 and Pgp (Wacher et al., 1995; Schuetz et al., 1996; Watkins, 1997; Salphati and Benet, 1998b), and the close intracellular spatial proximity of CYP3A4 to the apical membrane where Pgp is expressed (Watkins, 1997). However, direct evidence to support synergism between the two proteins has been elusive due to the lack of potent inhibitors specific to only CYP3A4 or Pgp that can be used in in vivo studies and the failure to identify an appropriate in vitro model expressing both CYP3A4 and Pgp.

The colon cancer cell line Caco-2 (Pinto et al., 1983) has been used extensively as a model to study drug transport across the intestinal epithelium and the role of Pgp in restricting drug absorption (for a review, see Artursson et al., 1996; Bailey et al., 1996; Hidalgo and Li, 1996). However, under conventional growth conditions, Caco-2 cells do not express significant levels of CYP3A4 (Prueksaritanont et al., 1996). Recently, Schmiedlin-Ren et al. (1997) reported that Caco-2 cells grown in the presence of 1,25-di-OH vitamin D₃ (di-OH vit D₃) expressed high levels of CYP3A4 activity. In this study, we applied this culture technique to study the effects of Pgp and CYP3A4 on indinavir metabolism during drug transport. The results presented demonstrate the use of this culture system for studying Pgp-CYP3A4 interactions and provide evidence to support a synergistic role of Pgp efflux in increasing the extent of intestinal metabolism of drugs that are substrates for both proteins.

Materials and Methods

Engelbreth-Holme-Swarm (EHS) extracellular matrix was purchased from Promega Corp. (Madison, WI). Testosterone, 6β-OH-testosterone, di-OH vit D₃, and cyclosporin A (CsA) were purchased from Sigma Chemical Co. (St. Louis, MO). FBS, glutamine, trypsin-EDTA solution, penicillin-streptomycin solution, nonessential amino acids, Hanks’ balanced salt solution (HBSS), and HEPES buffer were purchased from Life Technologies (Grand Island, NY). Dulbecco’s modified Eagle’s medium with pyruvate and 4.5 g/l glucose were prepared by Mediatech (Herndon, VA) and obtained from Fisher Scientific (Pittsburgh, PA). The di-OH vit D₃ stock solutions were prepared at 0.1 mg/ml in ethanol and stored at −70°C. Indinavir, [14C]indinavir, and indinavir metabolite standards were prepared at Merck and Co. (West Point, PA, and Rahway, NJ).

Cell Culture. Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD) and were used at passage 21 to 29. The cells were routinely maintained in Dulbecco’s modified Eagle’s medium supplemented with glutamine, nonessential amino acids, penicillin-streptomycin, and 5% FCS at 37°C in a humidified 5% CO₂/95% air environment. The di-OH vit D₃ induction of CYP3A4 in mixed Caco-2 cell cultures was performed as described by Schmiedlin-Ren et al. (1997). For comparisons between di-OH vit D₃-treated and untreated Caco-2 cell cultures, Caco-2 cells were plated at 2 × 10⁶ cells/cm² onto culture flasks or 24-mm-diameter polycarbonate filters (Costar Transwell, 0.2 µm pore size; Corning Glassworks, Corning, NY) coated with 2 µg/cm² EHS cell attachment matrix (extracellular matrix from EHS cells). After 5 days in culture, di-OH vit D₃ treatment was initiated by adding medium containing 0.1 µg/ml di-OH vit D₃. The di-OH vit D₃ was added to the medium immediately before feeding the cells, and the culture medium was changed every 2 to 3 days. The di-OH vit D₃ treatment was continued for 2 to 3 weeks before the cells were used for transport and metabolism studies.

Kinetics of M6 Formation. The di-OH vit D₃-treated and untreated Caco-2 cells grown in flasks were dissociated with trypsin-EDTA, resuspended in Dulbecco’s modified Eagle’s medium with 5% FCS, and washed by centrifugation (500g for 5 min), followed by washing two more times in HEPES-buffered HBSS, pH 7.4. The final cell pellets were resuspended at 5 million cells/ml in HEPES-buffered HBSS, and 0.5 ml/well was added in a 24-well tissue culture plate. Indinavir (10 mM stock solution in water) was then added to the cell suspension at final concentrations of 0.2 to 50 µM, and the mixtures were incubated in a tissue culture incubator at 37°C. After 4 h, viability of the cells was confirmed by trypan blue exclusion, 0.4 ml of the cell suspensions were transferred to glass tubes, and two volumes of acetonitrile were added to terminate metabolism and precipitate the cellular protein. An internal standard structurally related to indinavir was added to the mixture, precipitated protein was removed by centrifugation, and the samples were dried and reconstituted in 10% acetonitrile in water.

Transport and Metabolism of Indinavir. Before transport studies, culture medium was removed, and the filter-grown Caco-2 cells were preequilibrated in HEPES-buffered HBSS, pH 7.4. The HBSS was then replaced with 2 ml of fresh HBSS on the receiver side and 2 ml of HBSS containing indinavir on the donor side. In cases in which Pgp was inhibited with CsA, both the receiver and donor solutions also contained 5 µM CsA.

Initial time course and metabolism profiles were determined with donor solutions containing 5 to 10 µM [14C]indinavir at 0.1 to 0.2 µCi/ml. Samples (20 µl) from the donor and receiver compartments were taken at the indicated times, and the drug transport was quantified by scintillation counting. For evaluation of metabolism and drug transport, the total drug in the receiver and donor solutions was collected at 2 and 4 h, and drug remaining in the cells was extracted by the addition of 1 ml of ethanol to the apical side of the filters. The solutions were dried and reconstituted in 250 µl of 10% acetonitrile (concentrated 8-fold), and 100-µl samples were analyzed by radiochromatography (Chiba et al., 1996). Transport and metabolism studies using unlabeled drug were performed at the indicated concentrations in accordance with the procedure for labeled indinavir with the exception that the samples did not have to be concentrated due to the high sensitivity of the liquid chromatography/mass spectrometry (LC/MS) assay. The conversion of 50 µM testosterone to 6β-OH testosterone during 2-h incubations was measured on separate filters in parallel with the transport and metabolism assays to assess the level of CYP3A activity in the filters and was quantified according to Chiba et al. (1997).

LC/MS Analysis of Indinavir and Its Metabolites. Indinavir and its metabolites were separated on a 5-µm Betasil C-18 reversed phase column (50 × 3 mm) (Keystone analytical) using a linear gradient from 30 to 80% solvent B over the first 2 min, holding at 80% B for 0.5 min, and returning to 30% B over 1 min, in which solvent A is 5 mM ammonium acetate, pH 4.5, and solvent B is 70% acetonitrile. Indinavir and its metabolites were detected by LC/MS with a Scieix API 150 using APCI at capillary temperature of 425. Ions were monitored at m/z of 523, 529, 614, 630, and 613 for M6, M5, indinavir, addition of oxygen to indinavir, and the internal standard, respectively.

Results

Expression of CYP3A4 and Indinavir Metabolism in Caco-2 Cells. We applied the Caco-2 cell model to study the influence of Pgp efflux on the CYP3A4-mediated metabolism

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**Note:** The content above is a sample of the text that would typically appear in a scientific manuscript, focusing on studies involving drug transport and metabolism, particularly focusing on the role of Pgp and CYP3A4 in the context of drug metabolism and the use of Caco-2 cells as a model system. The text is designed to illustrate the type of information and formatting that might be expected in a scientific publication.
of indinavir. When grown under conventional culture conditions, Caco-2 cells express many of the barrier properties of the intestinal epithelium, including expression of the drug efflux pump Pgp, but fail to express significant CYP3A4 activity. CYP3A activity as determined by 6β-OH-testosterone production was low in the Caco-2 filters grown in the absence of di-OH vit D$_3$ and was below the limits of detection in most cases (Table 1). To induce expression of CYP3A4, Caco-2 cells were grown on the extracellular matrix from EHS cells (EHS matrix) in the presence of di-OH vit D$_3$. Caco-2 cell filters treated with di-OH vit D$_3$ for 2 weeks showed a dramatic increase in 6β-OH-testosterone production that was inhibited by the CYP3A4 inhibitor 1 μM ketoconazole. Over different passages of cells (passages 21–28), the level of CYP3A activity in Caco-2 cells treated for 2 to 3 weeks varied as much as 4-fold with an average value of 17.4 ± 11.3 pmol/min 6β-OH-testosterone produced per 4.7-cm$^2$ filter on incubation with 50 μM testosterone.

Metabolism of indinavir by CYP3A4 has previously been characterized (Balani et al., 1996; Chiba et al., 1996; Lin et al., 1996). M6, the product of N-depyridomethylation, is the predominant metabolite produced, with hydroxylation of M6 (M5), pyridine N-oxidation, and hydroxylation of the phenylmethyl (M4b) and the indan moieties (M2, M3, and M5) occurring at lower levels. Chromatograms of incubation mixtures from di-OH vit D$_3$-treated and untreated cells revealed unique peaks in the di-OH vit D$_3$-treated cells with m/z corresponding to M6, M5, and two peaks consistent with hydroxylation of indinavir (Fig. 1). Radiochromatographic analysis of 14C-labeled indinavir after incubation with di-OH vit D$_3$-treated Caco-2 cells showed that M6 was the most abundant metabolite formed. The amount of M6 formed in different preparations of Caco-2 cells correlated well with the level of CYP3A activity in the cells as indicated by 6β-OH-testosterone formation ($r^2 = 0.89$), and no significant M6 formation was detected when indinavir was incubated with untreated Caco-2 cells.

Formation of M6 as a function of indinavir concentration was evaluated with suspensions of untreated and di-OH vit D$_3$-treated Caco-2 cells. No M6 formation was observed with untreated Caco-2 cells. In di-OH vit D$_3$-treated Caco-2 cells, M6 formation was saturable with high affinity ($K_m = 4–6$ μM). This value is slightly higher than the $K_m$ observed with human intestinal (2.5 μM) and liver (1.3 μM) microsomes (Chiba et al., 1997). The higher $K_m$ value in the cell suspensions most likely reflects an intracellular drug concentration that is lower than that in the extracellular buffer due to Pgp efflux of indinavir.

**Pgp Activity in Caco-2 Cells.** Directional transport of indinavir (Fig. 2A) in di-OH vit D$_3$-treated Caco-2 cells showed 5- to 20-fold higher transport in the basolateral-to-apical direction than in the apical-to-basolateral direction. In the presence of the potent Pgp inhibitor CsA (5 μM), basolateral-to-apical transport of indinavir decreased and apical-to-basolateral transport increased to the point where no significant directional transport was observed. Comparison of untreated Caco-2 cells and Caco-2 cells treated with di-OH vit D$_3$ showed no significant difference between directional transport of indinavir, indicating that di-OH vit D$_3$ treatment neither induced nor suppressed Pgp activity (Fig. 2B).

**Metabolism and Transport of Indinavir in di-OH vit D$_3$-Treated Caco-2 Cells.** Directional transport studies were performed to evaluate the influence of Pgp efflux on metabolism of indinavir. Indinavir transport was determined after dosing to the apical or the basolateral side in the presence or absence of the Pgp inhibitor CsA. M6 retained in the cells and released into the apical and basolateral media were quantified by LC/MS. In the absence of CsA, M6 was almost exclusively secreted into the apical compartment (Fig. 3). Inhibition of Pgp by CsA resulted in M6 being released into both the basolateral and the apical compartments and some M6 being retained in the cells, suggesting that M6 is actively effluxed by Pgp. Similarly, M5 and OH-indinavir also showed selective efflux into the apical compartment (Fig. 4). In contrast to the apical secretion of indinavir metabolites, 6β-OH-testosterone did not show apical secretion (Table 1), suggesting that Pgp efflux cannot be generalized to all CYP3A-generated metabolites.

The total amount of M6 formed during the 4-h incubation was not significantly affected by the transport direction and showed a slight increase when Pgp was inhibited by CsA (Fig. 5A). Transport of indinavir in these filters was 5-fold higher in the basolateral-to-apical direction than the apical-to-basolateral direction. The basolateral-to-apical transport was reduced by the addition of CsA, whereas the apical-to-basolateral transport was enhanced by CsA (Fig. 5B). Normalization of the amount of metabolite formed to the amount of drug transport (Fig. 5C) shows that Pgp efflux results in more metabolite being formed per mole of drug transported across the cell monolayer for transport in the apical-to-basolateral direction than in the basolateral-to-apical direction. When Pgp efflux was inhibited by CsA, the amount of M6 formed normalized to drug transport was intermediate between the values observed for transport in both directions in the absence of CsA. Although CsA is also a CYP3A4 substrate, parallel incubations showed that CsA had only a small effect (20% inhibition) on testosterone metabolism by di-OH vit D$_3$-treated Caco-2 cells. Because the testosterone concentration (50 μM) used approximates the $K_m$ value for microsomal metabolism by CYP3A4 (Draper et al., 1998) whereas indinavir is used at 2.5 to 4 times its microsomal $K_m$ value, the inhibition observed represents an upper limit for inhibition of indinavir metabolism. Nevertheless, correction for this inhibition still shows increased metabolite formation relative to the amount of drug transport due to Pgp efflux. In separate sets of experiments, correction for inhibition of testosterone metabolism yielded values for M6 formation normalized to indinavir transport in the presence of CsA was 50 to 75% of the values obtained for apical-to-basolateral transport without inhibition of Pgp. Thus, the results indicate that countercurrent transport by Pgp increased the amount of metabolite formed relative to the amount of drug transported across the monolayer. By extrapolation, this suggests that

### TABLE 1

Expression of CYP3A activity in di-OH vitamin D$_3$-treated Caco-2 cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Apical</th>
<th>Basolateral</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>6β-OH-Testosterone Formed/4 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di-OH vitamin D$_3$-treated cells</td>
<td>0.61 ± 0.08</td>
<td>0.57 ± 0.10</td>
<td>1.18 ± 0.18</td>
</tr>
<tr>
<td>Untreated cells</td>
<td>0.01 ± 0.02</td>
<td>0.01 ± 0.02</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>Di-OH vitamin D$_3$ + 1 μM</td>
<td>0.09 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>0.09 ± 0.22</td>
</tr>
</tbody>
</table>
efflux by Pgp would decrease the rate of absorption of indinavir, thus increasing the intestinal residence time, and increase the amount of indinavir metabolized by intestinal CYP3A4 relative to the amount of drug absorbed.

Concentration Dependence of M6 Formation during Indinavir Transport. Indinavir metabolism and drug transport were evaluated as a function of indinavir concentration in the donor compartment. Results for metabolite formation relative to drug transport in the apical-to-basolateral direction and the basolateral-to-apical direction are shown in Fig. 6. The kinetic parameters for M6 formation were determined from a nonlinear fit to Michaelis-Menton kinetics and confirmed by Scatchard analysis yielding a $K_m$ value of 8.1 μM and a $V_{max}$ value of $1.54 \times 10^{-4}$ nmol·min$^{-1}$·mg protein$^{-1}$ for Caco-2 cell filters at 0.16 mg cellular protein·cm$^{-2}$. The kinetics for Pgp efflux determined from the difference between apical-to-basolateral and basolateral-to-apical transport yielded a $K_m$ value of 140 μM and a $V_{max}$ value of $2.01 \times 10^{-1}$ nmol·min$^{-1}$·mg protein$^{-1}$, and the intrinsic permeability coefficient ($P_{app}$) reflecting the passive permeability of indinavir across Caco-2 cells was determined to be $3.87 \times 10^{-4}$ cm$^{-1}$·min$^{-1}$. Using these values, it is possible to simulate the effects of extracellular indinavir concentration ($C_o$) and transport direction on the ratio of metabolite to drug transport assuming that the kinetics of metabolism relative to the intracellular drug concentration is independent of Pgp activity as illustrated in Fig. 7. Under steady-state transport conditions, the rate of drug
entering the cell equals the rate of drug being removed from the cell interior via metabolism (\(V_{\text{met}}\)), active efflux (\(V_{\text{Pgp}}\)), and passive transport. Thus:

\[
P_{\text{ap}}C_o \text{ (or } P_{\text{bl}}C_o \text{ for basolateral to apical transport)} = P_{\text{ap}}C_i + P_{\text{bl}}C_i + V_{\text{Pgp}} + V_{\text{met}}
\]

where \(P_{\text{ap}}\) and \(P_{\text{bl}}\) are the passive permeability coefficients of the apical and basolateral membranes, respectively; \(C_o\) is the drug concentration in the donor compartment; and \(C_i\) is the drug concentration inside the cell. If we assume that the permeability coefficients for the apical and basolateral membranes are equal (\(P_{\text{ap}} = P_{\text{bl}} = P_{\text{ap, bl}}\)), then the intracellular concentration (\(C_i\)) can be estimated:

\[
C_i = \frac{(C_oP_{\text{ap}} - V_{\text{Pgp}} - V_{\text{met}})}{(2P_{\text{ap, bl}})}
\]

The permeability across the apical (\(P_{\text{ap}}\)) and basolateral membranes (\(P_{\text{bl}}\)) are related to the intrinsic permeability (\(P_{\text{ap, bl}}\)):

\[
\frac{1}{P_{\text{ap, bl}}} = \frac{1}{P_{\text{ap}}} + \frac{1}{P_{\text{bl}}}
\]

Assuming that \(P_{\text{ap}}\) and \(P_{\text{bl}}\) are equal, then \(P_{\text{ap, bl}} = 2 P_{\text{ap}} = 7.74 \times 10^{-4} \text{ cm}^{-1} \text{ min}^{-1}\). The concentration inside the cell (\(C_i\)) can then be determined as a function of extracellular indinavir concentration using the \(K_m\) (relative to extracellular indinavir) and \(V_{\text{max}}\) values to determine the rate of Pgp efflux (\(V_{\text{Pgp}}\)) and metabolism (\(V_{\text{met}}\)). Transport of drug into the receiver compartment is equal to \(P_{\text{bl}} C_i\) for transport in the apical-to-basolateral direction and \(P_{\text{ap}} C_i + V_{\text{Pgp}}\) for transport in the basolateral-to-apical direction. For the simulated curves in Fig. 6, the rate of metabolism as a function of \(C_i\) was derived using the \(V_{\text{max}}\) value determined for indinavir metabolism during the transport experiment and a \(K_m\) value (relative to intracellular drug concentration) of 2 \(\mu\)M (Chiba et al., 1997). The simulated curves for the ratio of metabolite formation to drug transport are indicated by the solid and broken lines in Fig. 6.

**Discussion**

It has been proposed that Pgp acts in synergy with CYP3A4 in the intestine to restrict oral absorption of drugs. According to this model, Pgp can enhance the extent of intestinal CYP3A4 metabolism by both 1) removal of CYP3A4-generated metabolites from inside the cells, thus preventing further interaction of metabolites with CYP3A4, and 2) prolonging the time required for absorption through repeated cycles of absorption and efflux, thus increasing the exposure of a drug to CYP3A4 before absorption into the systemic circulation (Gan et al., 1996; Watkins, 1997; Wacher et al.; 1998; Ito et al., 1999). However, no direct evidence to support this model has been obtained. This is in part due to the difficulty in separating the individual contributions of CYP3A4 and Pgp in vivo.
An in vitro model capable of emulating the transport properties of the intestinal epithelium that expresses both Pgp and CYP3A4 would provide a useful tool for assessment of the contribution of both activities. Although Caco-2 cells have been widely used as a model for intestinal absorption and Pgp transport, the expression of CYP3A4 activity is generally too low to study metabolite formation as a function of drug transport. Recently, Schmiedlin-Ren et al. (1997) demonstrated that the maintenance of Caco-2 cells in culture media containing di-OH vit D₃ induces expression of significant levels of CYP3A activity. The expression of CYP3A activity is primarily ascribed to increased CYP3A4 expression, although smaller amounts of CYP3A5 and 3A7 were also induced. Consistent with their results, we saw significant oxidation of testosterone to 6-β-OH-testosterone in di-OH vit D₃-treated Caco-2 cells, whereas this activity was barely detectable in untreated cells. Pgp activity in di-OH vit D₃-treated Caco-2 cells was comparable to activity observed in untreated cells. Thus, this system provides an intestinal model in which both CYP3A4 and Pgp activities are expressed and can be easily manipulated under controlled conditions to assess their roles and interactions in intestinal metabolism.

In the studies detailed here, we evaluated the interactions between intestinal CYP3A4 and Pgp using the human immunodeficiency virus protease inhibitor indinavir as a model substrate. Indinavir is a useful model compound because it is a substrate for both Pgp (Kim et al., 1998; Lee et al., 1998;
Oxidative metabolism of indinavir by CYP3A enzymes occurs with high affinity in liver microsomes and generates six metabolites. N-depyridomethylation (M6 formation) is the most prevalent route of metabolism, with N-oxide formation and oxidation of the phenylmethyl and indan moieties occurring in smaller amounts. The profile for indinavir metabolism in di-OH vit D3-treated Caco-2 cells was consistent with the pattern for CYP3A4-mediated oxidative metabolism, with M6 being the most prominent metabolite formed and secondary metabolism of M6 and oxidation of indinavir occurring at lower amounts.

It has been proposed that one potential mechanism by which Pgp can enhance intestinal metabolism of drugs is by facilitating removal of the metabolites, thus preventing product inhibition due to competition for CYP3A4 (Watkins, 1997). The distribution of M6 formed during indinavir transport is consistent with this role. In the absence of a Pgp inhibitor, M6 was almost exclusively secreted into the apical compartment, and intracellular M6 was not detected. In the presence of the Pgp inhibitor CsA, both basolateral and intracellular M6 was detected, suggesting that the removal of M6 from the intracellular compartment is in part mediated by Pgp. This vectorial efflux of CYP3A-generated metabolites is consistent with findings previously reported for metabolites of CsA (Gan et al., 1996) and midazolam (Schmiedlin-Ren et al., 1997). However, Pgp-mediated efflux cannot be generalized to all CYP3A metabolites because 6β-OH testosterone did not show selective efflux into the apical compartment and its distribution was not affected by CsA. It is also worth noting that although the results are consistent with a role of Pgp in the removal of M6 from the intracellular compartment, accumulation of M6 within the cells when Pgp was inhibited did not affect the extent of indinavir metabolism in our study.

The second mechanism by which Pgp can increase the extent of metabolism by CYP3A4 is by decreasing the rate of absorption of a drug through repeated cycles of intracellular
uptake and efflux, thereby increasing the exposure of a drug to CYP3A4 before absorption in the systemic circulation. The results presented here clearly support this view. Although the total metabolite formed during the transport studies was not significantly affected by Pgp transport, resistance to transport conferred by Pgp efflux increased the amount of metabolite formed when normalized by the amount of parent drug transported across the monolayer. In extrapolation of these findings to in vivo absorption, Pgp efflux would have the effect of decreasing the rate of absorption, thus increasing the residence time resulting in increased metabolism. A model to explain these results is illustrated in Fig. 7. The net effect of Pgp efflux is to decrease the intracellular concentration of drug. Assuming the drug is transported across the basolateral membrane via passive diffusion, the observed drug transport across the epithelial layer will be a linear function of the intracellular drug concentration. Thus, a 50% decrease in the intracellular drug concentration due to Pgp efflux will result in a 50% decrease in the rate of drug transport. However, metabolism by CYP3A4 is not a linear function of the intracellular concentration, but it is saturable. Consequently, changes in intracellular concentration are not proportionally reflected in the rate of drug metabolism.

One limitation to this model is the assumption that the intracellular concentration of drug is uniform throughout the cells. If a drug is subject to extensive protein and membrane binding, it is possible that the distribution of drug within the cell may form a gradient from high concentration (the donor side) to low concentration (receiver side). Because cytochrome P-450s in enterocytes are localized toward the apical portion of the cell (Watkins, 1997), heterogeneity in the drug concentration within the cell could affect the relative influence of drug concentration on metabolism and transport. This could explain the small discrepancy between the results we obtained with indinavir and the results reported previously for CsA (Gan et al., 1996). Although both indinavir and CsA show a higher ratio of metabolite formation to drug transport for apical-to-basolateral transport than for basolateral-to-apical transport, the rate of CsA metabolism was also affected by the direction of transport. As opposed to the results we obtained showing essentially no effect of the direction of transport on the rate of M6 formation, Gan et al. (1996) found 2-fold higher metabolism of CsA when CsA was transported in the apical-to-basolateral direction than in the basolateral-to-apical direction. If we assume that cytochrome P-450s enzymes are localized in the apical portion of Caco-2 cells similar to their distribution in normal intestinal cells, then a gradient of CsA concentration could result in a lower concentration of CsA at the site of metabolism when the drug is administered to the basolateral side than when it is administered to the apical side. This could then be reflected as decreased metabolism observed for cyclosporin when administered to the basolateral compartment as opposed to the apical compartment.

In summary, the results presented here demonstrate the use of di-OH vit D₃-treated Caco-2 cells for study of the interactions between CYP3A4 and support a role of Pgp activity in enhancement of CYP3A4 intestinal metabolism.

References


Fig. 7. Model depicting the role of Ppp, CYP3A4, and passive membrane transport on drug absorption. The model depicts transport from the apical-to-basolateral direction under sink conditions. C₃ and C₄ represent the concentration of indinavir in the donor solution and inside the cell, and Pₐ and Pₜ correspond to the rate of passive drug transport across the apical and basolateral membrane, respectively. Vₙₐₑₙ represents the rate of indinavir metabolism.


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