The barrier function of CYP3A4 and P-glycoprotein in the small bowel

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Abstract

CYP3A4 present in small bowel enterocytes can catalyze substantial metabolism of some orally administered drugs and, thus, exerts a first-pass effect. Recent data indicate that the P-glycoprotein (the MDR 1 gene product) in the enterocyte brush border also limits the bioavailability of many of the same drugs that interact with CYP3A. It has been proposed that P-glycoprotein and CYP3A4 may be functionally linked because (a) the two proteins are co-localized within the digestive tract and within enterocytes, (b) they share many of the same substrates and (c) they are co-inducible in response to at least some xenobiotics. There are several potential mechanisms whereby the functions of P-glycoprotein and CYP3A4 could be complimentary. First, Pgp may limit absorption in the proximal small bowel, shifting it to more distal, less catalytically efficient segments that contain lower amounts of CYP3A4. Second, Pgp may function to prolong the duration of absorption. This might increase the duration of exposure of drug to and, hence, the extent of metabolism by enterocyte CYP3A4. Finally, Pgp may preferentially remove from the enterocyte primary drug metabolites that are themselves substrates for CYP3A4. This would limit product inhibition and facilitate primary metabolism catalyzed by CYP3A4. Characterization of the roles of CYP3A4 and Pgp in limiting oral drug availability may be aided by recent success in the development of human intestinal cell lines that stably express both CYP3A4 and Pgp.

1. Introduction

1.1. Intestinal cytochrome P450 3A4

There is now overwhelming evidence that some orally administered drugs that are substrates for cytochrome P450 3A4 (CYP3A4) can undergo significant first-pass metabolism within small intestinal epithelial cells (enterocytes). These data are reviewed elsewhere in this volume and will not be discussed extensively here. CYP3A4 appears to be the most abundant cytochrome P450 present in human small bowel [1,2]; it is found only in the mature enterocytes lining the villus and is not present in crypt cells (Fig. 1). CYP3A4 present in human enterocytes appears to be functionally and structurally identical to CYP3A4 present in human liver. The complete coding region of intestinal CYP3A4 cDNA has been sequenced in our laboratory and determined to be identical to its liver counterpart (unpublished observations). In addition, the pattern of metabolites produced from several CYP3A4 substrates, and the kinetic properties of the reactions, appear to be essentially identical in human small bowel and liver [3–6]. It therefore appears that catalytic properties of CYP3A4 determined in human liver microsomes, or in recombinant systems, can generally be extrapolated to the small bowel.

The related enzyme, CYP3A5, is also detectable in enterocytes in about 70% of adults, and is not coregulated with CYP3A4 [7–10]. Its structure, function and contribution to first-pass metabolism has not been established. However, CYP3A5 appears to be a minor enterocyte enzyme in most individuals [7].

1.2. Intestinal P-glycoprotein

The potential role of P-glycoprotein (Pgp, the MDR-1 gene product) in determining oral availability of some drugs has only recently been appreciated. Pgp is a versatile xenobiotic pump that was first discovered in cancer cells (reviewed in [11]). Pgp functions to make certain cancer cells resistant to several chemotherapeutic agents by pumping them

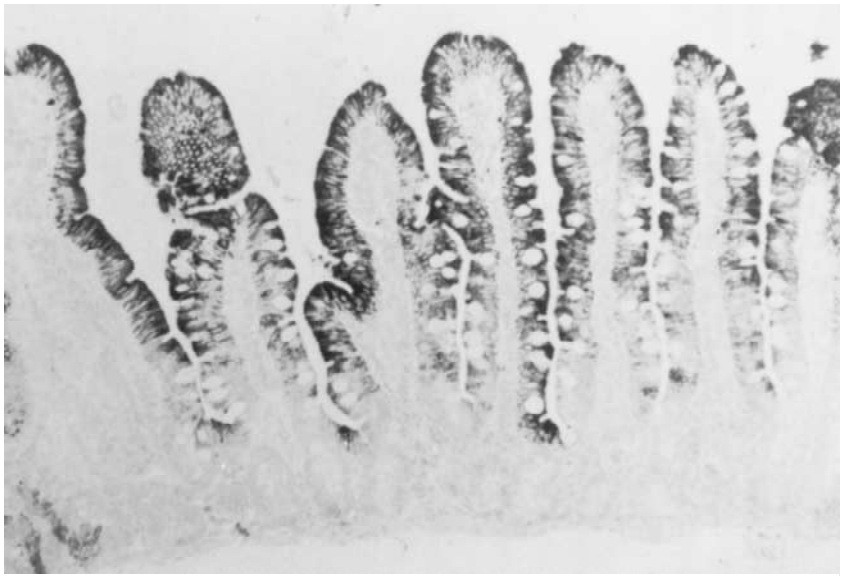

Fig. 1. Localization of CYP3A in human small bowel mucosa. Full thickness specimens of proximal jejunum were obtained from a human organ donor, formalin fixed, and reacted with a polyclonal antibody that recognizes CYP3A4. The biopsy is oriented with lumen above and the serosa below. CYP3A4 (dark staining) is detected only in mature epithelial cells (enterocytes) lining the villi and is not present in the crypt cells, or in other cell types present in the mucosa. Within enterocytes, the highest concentration of CYP3A4 appears to be near the absorptive surface, just below the brush border.

out of the cells, maintaining intracellular concentrations at sublethal levels (MDR stands for multiple drug resistance). Pgp is expressed in a variety of normal (non-cancerous) human tissues, including liver, brain, adrenal gland, kidney and intestinal tract epithelia [12]. In the small bowel, Pgp is present on the apical membrane of the mature epithelial cells and is not detectable in the crypt cells (Fig. 2). Pgp is oriented in the apical membrane to pump xenobiotics from inside the cells back into the lumen of the intestine (i.e., a ''countertransport'' function) [13]. In humans (as opposed to rodents), there is only one MDR1 gene, and it is generally assumed that intestinal Pgp is functionally identical to Pgp present in other epithelial cells and in cancer cells. Pgp appears to have approximately the same molecular weight in human intestinal cells as in other cell types (as judged by mobility on polyacrylamide gels) [14–16]. However, the complete coding region of human intestinal Pgp cDNA has not been sequenced to the author's knowledge. Kinetic data regarding chemical inhibition of enterocyte Pgp vs. Pgp in other cell types is sparse and somewhat conflicting [16–23]. It can therefore not be assumed at present that characteristics of Pgp transport determined in cells other than enterocytes will be directly applicable to oral drug absorption.

2. Transport function of intestinal Pgp

2.1. Effect on drug absorption

The demonstration of Pgp in enterocytes provides a mechanism to account for prior observations concerning intestinal (non-biliary) excretion for certain xenobiotics. For example, erythromycin [24], digoxin [24] and some β-blockers [25] and antibiotics [26–29] undergo active secretion from blood into the small bowel intestinal lumen, and appear to be substrates for Pgp [5,21,30]. Direct evidence that Pgp inhibits the absorption of orally administered drugs comes from several sources. First, several investigators have used isolated loops of rat jejunum to study the absorption of compounds in the absence and presence of inhibitors of Pgp. One study [31]

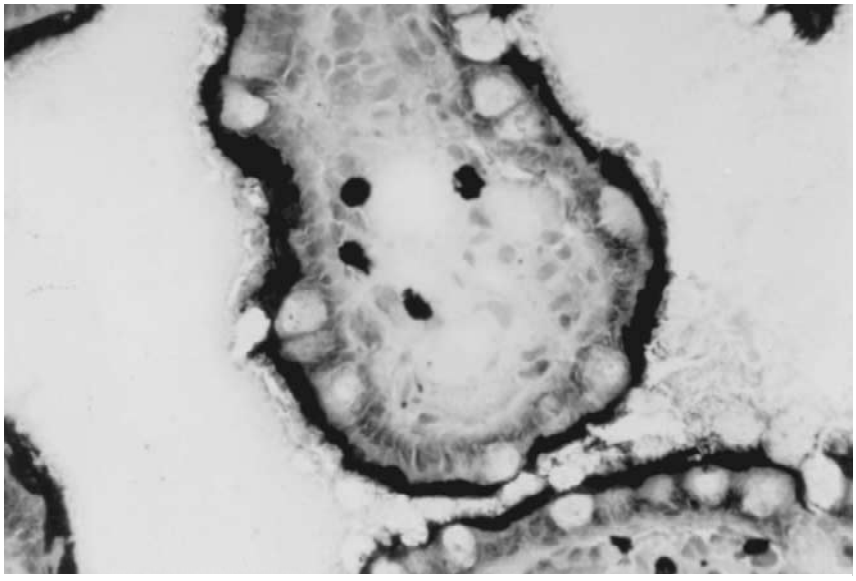

Fig. 2. Localization of P-glycoprotein in a human small bowel villus tip. The above tissue was frozen without formalin fixation (accounting for the poorer histology compared with Fig. 1) and reacted with an antibody that recognizes P-glycoprotein. Pgp (the black staining) is detected only in the apical brush border membrane of the enterocytes. Pgp is therefore in close proximity to CYP3A4 (Fig. 1) at the apex of the enterocyte. Reproduced with permission from the authors of reference [12].

showed that the absorption of etoposide was significantly enhanced in the presence of an antibody directed to Pgp (applied to the luminal surface). Other studies have tested the ability of chemical inhibitors of Pgp to influence the absorption of xenobiotics in isolated loops of rat jejunum. In their recent review, Tsuji and Tamai [32] presented a figure (reproduced in Fig. 3) that summarized data indicating that certain drugs have slower rates of absorption across rat intestinal mucosa than would be predicted by their lipophilicity alone. Many of these drugs are now known to be substrates for Pgp, and most were shown to have more rapid absorption when administered with the Pgp inhibitor cyclosporin A (Fig. 3). It is interesting that the presence of cyclosporin A did not result in a shift back to the absorption rate predicted by lipophilicity alone in most cases (Fig. 3). It is possible that the investigators did not use sufficiently high concentrations of cyclosporin A to achieve complete inhibition of Pgp function (the concentrations used were not given). Alternatively, it may be that Pgp is one of multiple countertransport proteins responsible for the relatively poor absorption of these compounds.

2.2. Studies with Caco-2 cells

The human colon cancer cell line, Caco-2, has also been used to study the potential role of Pgp in influencing the oral absorption of drugs. Caco-2 cells form confluent and polarized monolayers when maintained in culture and differentiate towards the mature small bowel enterocyte phenotype. A variety of devices are commercially available to facilitate the establishment of polarized monolayers of Caco-2 cells with separate apical (corresponding to the lumen of the intestinal tract) and basal (corresponding to the serosal side) compartments. Drugs are then added to one side of the monolayer (usually the apical side to mimic absorption) and the rates of transport or diffusion to or from either media compartment is measured. Functional Pgp is expressed along the apical surface of Caco-2 cell monolayers and has been shown to mediate efflux from the basolateral to apical media compartments of some drugs in this model [17,18,20–22,25,33]. Recent data support the idea that countertransport proteins other than Pgp are also present in Caco-2 cells, and may be involved in the transport of xenobiotics that are

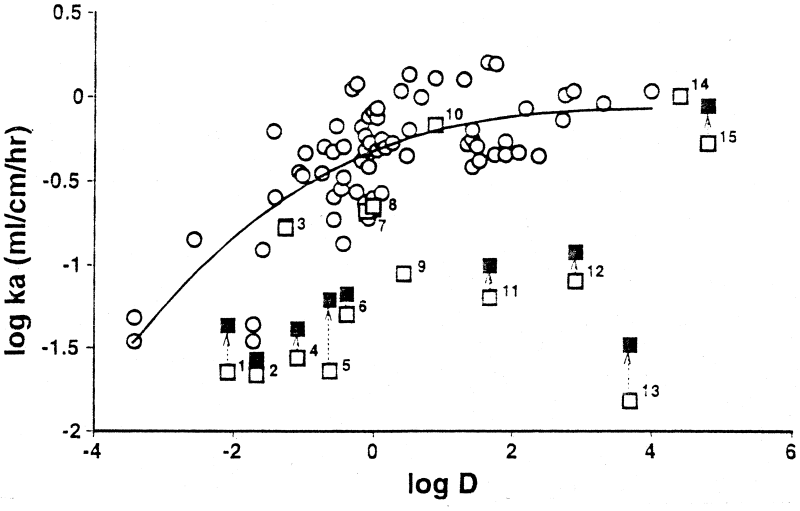

Fig. 3. Relationship between lipid solubility and the rate of absorption from the lumen of rat small bowel for a variety of different compounds. The results shown with the squares represent the relationship between intestinal absorption clearance (ka) observed from the in situ jejunal loop in the presence (solid) and absence (hollow) of cyclosporin A and octanol-buffer (pH 7.0) partition coefficients (log *D*) that were determined [32]. 1, atenolol; 2, nadolol; 3, acetamide; 4, celiprolol; 5, acebutolol; 6, doxorubicin; 7, timolol; 8, sulfathiazole; 9, quinidine; 10, sulfamethoxazole; 11, digoxin; 12, cyclosporin A; 13, vinblastine; 14, β-estradiol and 15, verapamil. Reproduced with permission from the authors [32].

also transported by Pgp [21,34]. A countertransport protein termed MRP (multidrug resistance-associated protein) has recently been reported to be present in Caco-2 cells [35].

2.3. In vitro–in vivo correlations

The most direct data supporting a role for intestinal Pgp in limiting the oral availability of drugs in man has been provided recently from our research group [36]. We have developed techniques that allow quantitation of the enterocyte content of Pgp in patients and in normal healthy volunteers [7]. A fiberoptic endoscope is passed through the subject's mouth into the first portion of the small bowel (the duodenum) and several small (<5 mg wet weight) ''pinch'' biopsies of duodenal mucosa are obtained. The subject's throat is sprayed with an anesthetic and the subject receives light sedation prior to the procedure, which lasts for about 15 min. The mucosal biopsies obtained are then homogenized and the whole homogenate is subjected to immunoblot analysis. The blots are simultaneously developed with antibodies to Pgp, CYP3A4 and villin [7,14]. Villin is a constitutive enterocyte-specific protein whose level of expression appears to be relatively constant across individuals [7]. Enterocyte levels of Pgp and CYP 3A4 can therefore be estimated by normalizing the signals on the immunoblots for biopsy content of villin. This is necessary because the number of intact enterocytes can vary substantially among biopsies from the same individual [7]. For example, a deep biopsy will have much fewer enterocytes than a shallow biopsy. Without correction for biopsy villin content, biopsies obtained from the same patient can have a several-fold variation in the content of CYP3A4 or Pgp.

Using this technique, we have shown that the enterocyte concentrations of CYP3A4 and Pgp vary by up to 10-fold among kidney transplant recipients [14], and vary somewhat less among normal healthy adults not taking medication [37]. This technique has also allowed us to begin to define the roles of Pgp (and CYP3A4) in determining the oral availability of drugs. In a recent study of 20 stable renal transplant recipients [14], we found that variation in intestinal expression of Pgp and variation in liver expression of CYP3A4 (measured with the erythromycin breath test [38]) accounted for 73% of the observed inter-patient variation in oral clearance of cyclosporin A. Patients with higher enterocyte concentrations of Pgp tended to have lower oral clearances (Cl/F) of cyclosporin A. Variation in enterocyte concentrations of Pgp accounted for 17% of the variation in oral clearance in this patient population, and the contribution of Pgp was highly significant ($p<0.001$). Interestingly, a 10-fold inter-patient variation in enterocyte content of CYP3A4 had no detectable effect on the oral clearance of cyclosporin A in these studies.

3. Mechanism of intestinal Pgp action

3.1. Effect on cyclosporine bioavailability

The simplest explanation for the observed in vivo findings with cyclosporin A is that Pgp functions to prevent the complete absorption of the drug and that the vast majority of metabolism of cyclosporin occurs in the liver. Although this may be the case, several studies have suggested that the intestine can be a very substantial site for metabolism of orally administered cyclosporin A [6,39–41]. An alternate possibility is that Pgp may not prevent complete absorption of cyclosporin A, but may control the extent of intestinal metabolism of the drug. Although this is largely speculation, there are some data that support this theory.

First, CYP3A4 and Pgp appear to be functionally integrated, as there is great overlap between substrates for CYP3A4 and Pgp [42–44]. For example, cyclosporin A is a well characterized substrate for both CYP3A4 [45] and P-glycoprotein [46]. Indeed, to the author's knowledge, there has not yet been a single compound identified that is transported by Pgp that has been shown not to be a substrate for CYP3A4. Likewise, the author is unaware of a substrate of CYP3A4 that has been conclusively shown not to be transported by Pgp. It should be noted, however, that one recent study has suggested that the CYP3A4 substrate, midazolam, may not be transported by Pgp [34], and relatively few drugs have been tested in this regard.

Second, CYP3A4 and Pgp are co-localized in the small bowel (being present in villus enterocytes and not in crypt cells (Fig. 1A–B). Moreover, data obtained in rat and man suggest that CYP3A en-

zymes are localized primarily at the apex of the enterocytes, just below the brush border containing Pgp [10,47]. Although CYP3A4 is presumably bound within the endoplasmic reticulum (ER), much of the ER containing the enzyme appears to be closely associated with the apical plasma membrane.

Finally, Pgp and CYP3A4 appear to be coordinately regulated in some instances [48]. It has been pointed out that CYP3A4 and Pgp genes are located close to one another on the same chromosome, and that tumors expressing Pgp tend to also express CYP3A4 [43]. In addition, at least some xenobiotics have been shown to be inducers of both CYP3A4 and Pgp in human enterocytes in vivo [37] or in a human intestinal cell line [48]. However, there does not appear to be a correlation between the relative levels of expression of CYP3A4 and Pgp in a given individual. That is, a person can have relatively high enterocyte CYP3A4 expression while having relatively low enterocyte Pgp expression (and vice versa) [14]. One confounding issue is that there does not appear to be a correlation between enterocyte levels of CYP3A4 mRNA and CYP3A4 protein [7], indicating that regulation of the enzyme is not at the level of transcription. This conclusion is also supported by the recent observation that grapefruit juice causes a marked fall in the enterocyte concentration of CYP3A4 protein while it does not influence the enterocyte concentration of CYP3A4 mRNA [49]. Although purely speculative, other dietary factors may also influence CYP3A4 protein levels within the enterocyte. Mechanisms underlying the regulation of Pgp expression in enterocytes have not been examined to date.

Fig. 4 illustrates several mechanisms whereby the functions of CYP3A4 and Pgp might be synergistic in limiting the oral availability of substrates. An intuitively attractive idea is that metabolites of compounds generated by CYP3A4 are better substrates for Pgp than are the parent compounds. There is some data with cyclosporin to support this hypothesis. We have performed studies in isolated loops of rat jejunum in vivo which demonstrated that primary metabolites generated by CYP3A4 were selectively pumped from the enterocyte back into the gut lumen [50]. Transport of cyclosporin A metabolites occurred in the presence of relatively high (presumably millimolar) concentrations of cyclosporin A in the intestinal lumen. Assuming that the primary

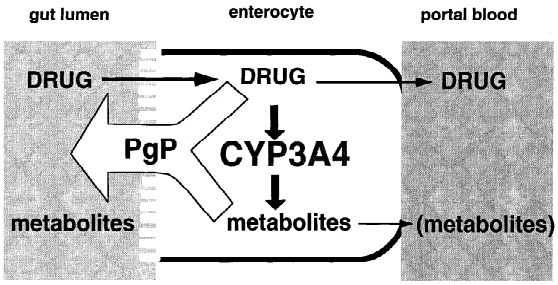

Fig. 4. Potential functional relationships between enterocyte P-glycoprotein and CYP3A4. In order to gain entry into the body (and into portal blood), most CYP3A4 and Pgp substrates must traverse small bowel epithelial cells (enterocytes) located near the tips of the intestinal villi. It appears that most drugs that are substrates for CYP3A4 are also transported by Pgp. In addition, metabolites generated by CYP3A4 may have higher affinity for Pgp than for the parent compounds (see discussion in text). The relative level of activity of Pgp may potentially influence the extent of oral availability of CYP3A4 substrates by (a) preventing parent drug diffusion across the apical brush border membrane (i.e., preventing absorption), (b) prolonging the duration of absorption (and hence increasing the extent of exposure of drug to enterocyte CYP3A4), or (c) by selectively removing metabolites from within the enterocyte (that are themselves substrates for CYP3A4), preventing product inhibition of primary drug metabolism.

metabolites of cyclosporin A generated in the enterocyte in vivo are substrates for Pgp (a reasonable but untested hypothesis), the affinity of the transporter for the metabolites would have to be significantly greater than for parent cyclosporin A. Similar observations were made by Gan et al. [51] in Caco-2 cell monolayers. They noted that the major primary metabolite of cyclosporin A produced within the cells was selectively transported across the apical cell membrane into the apical medium. This occurred in the presence of micromolar concentrations of cyclosporin A, again supporting the concept that metabolites of cyclosporin A generated by CYP3A4 are superior substrates for Pgp compared to the parent compound. Indeed, it is a very attractive idea to speculate that CYP3A4 is present in small bowel enterocytes to create better substrates for Pgp. Substrates that escape countertransport by Pgp and gain entry into the enterocyte would encounter CYP3A4 just beneath the brush border at the apex of the enterocyte [10,47]. Conversion of parent drug to more highly transportable (and hence less readily absorbed) metabolites at the cell apex would logical-

ly result in pumping of the metabolites into the intestinal lumen and elimination in stool.

3.2. Interaction of Pgp with other CYP3A4 substrates/metabolites

There are several potential mechanisms whereby Pgp could influence the extent of metabolism of CYP3A4 substrates during absorption. Based on a variety of data outlined above, Pgp appears to influence the rate of absorption of several substrates. By prolonging absorption, the duration of exposure of the drug to the enzyme within the enterocyte is prolonged. If the enzyme is saturated during absorption, prolonging absorption should increase the extent of metabolism at the level of the intestine. For example, if the duration of absorption of cyclosporin A is largely determined by the level of P-glycoprotein, patients with relatively high enterocyte Pgp levels could have increased intestinal metabolism of cyclosporin A simply as a result of a prolonged absorption phase. Therefore, the effect of high enterocyte Pgp levels would be reduced oral availability of parent cyclosporin A, even though absorption could ultimately be complete. Gan et al. [51] noted that the rate of metabolism of cyclosporin A was greater when the drug was added to the apical (compared to the basolateral) side of Caco-2 cells, and proposed that this was due to an effect of Pgp in determining the ''residence time'' of the drug in the cells.

The second way in which the level of P-glycoprotein in the enterocyte could control the extent of metabolism of a substrate would be if, by reducing the rate of absorption, the result was to shift absorption from the proximal small bowel to more distal segments. The total mass of CYP3A4 per gram of mucosa falls significantly in distal areas of the small bowel [52]. Hence, moving absorption to more distal areas may result in less first-pass metabolism. This principle is supported by a study performed in rabbits using the CYP3A4 substrate, diltiazem [53]. Oral availability was significantly greater when diltiazem was infused into the distal small bowel than when it was administered to the proximal small bowel. This appeared to be the direct result of a reduction in metabolism during absorption. Similar results have been obtained in man with the CYP3A4 substrate, nefazodone [54]. It should be noted, however, that a similar human study with nifedipine (a well characterized CYP3A4 substrate) failed to show enhanced availability after distal small bowel administration [55]. A confounding factor is that the absorptive surface area per centimeter of bowel is considerably lower in the distal small bowel compared to the proximal small bowel, and the levels of Pgp may be increased [56]. These factors may function to reduce absorption of some CYP3A4 substrates in distal small bowel, off-setting the effect of reduced CYP3A4 levels.

The third way in which Pgp could influence first-pass metabolism might be by pumping primary metabolites out of the enterocyte, preventing secondary metabolism of substrates by CYP3A4. This could have the effect of accelerating the rate of primary metabolism if the affinity of the metabolites for Pgp transport was greater than that for the parent drug. For example, data outlined above suggests that primary metabolites of cyclosporin A are selectively transported out of enterocytes into the gut lumen and that the affinity for transport is greater for the metabolites than for parent cyclosporin A. Secondary metabolism of cyclosporin A is extensive, and CYP3A4 appears to be the principal enzyme involved in secondary metabolism [57]. Hence, patients with high Pgp expression might have relatively high rates of primary metabolism (and lower oral availability of parent cyclosporin A) but relatively low rates of secondary metabolism as a consequence of the rapid transport of metabolite out of the enterocyte. In this case, the level of Pgp would affect the extent of product inhibition and influence the oral availability of a drug, without necessarily changing the duration of the absorption phase, or the anatomical site of absorption.

4. Future directions

The potential interactions and synergy between CYP3A4 and P-glycoprotein remain largely speculative, mainly because methods available to study such interactions are limited at present. Mouse knock-out models are likely to provide some insight, but it should be remembered that rodents have two expressed Mdr1 genes and may not be a good model for the human situation. Relatively selective chemical inhibitors of Pgp or CYP3A4 that do not inhibit

both protein functions are not generally available. Native Caco-2 cells express adequate levels of functional Pgp, but do not appear to express CYP3A4 [34,58]. Recently, CYP3A4 has been genetically engineered into Caco-2 cells [59]. In addition, our group has reported culturing techniques that restore relatively high levels of functional CYP3A4 to these cells [34]. Such modified Caco-2 cells would appear to be the most promising model currently available to dissect the roles of CYP3A4 and Pgp, and to determine mechanisms of their potential synergy in limiting the oral availability of xenobiotics.

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