Functional interactions between P-glycoprotein and CYP3A in drug metabolism

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The interaction between drug-metabolising enzymes and active transporters is an emerging concept in pharmacokinetics. In the gut mucosa, P-glycoprotein and cytochrome P450 (CYP)3A functionally interact in three ways: i) drugs are repeatedly taken up and pumped out of the enterocytes by P-glycoprotein, thus increasing the probability of drugs being metabolised; ii) P-glycoprotein keeps intracellular drug concentrations within the linear range of the metabolising capacity of CYP3A; and iii) P-glycoprotein transports drug metabolites formed in the mucosa back into the gut lumen. In comparison with the gut mucosa, in hepatocytes the spatial sequence of CYP3A and P-glycoprotein is reversed, resulting in different effects when the activity of one or both are changed. CYP3A and P-glycoprotein are both regulated by nuclear receptors such as the pregnane X receptor (PXR). There is significant genetic variability of CYP3A, P-glycoprotein and PXR and their expression and activity is dependent on coadministered drugs, herbs, food, age, hormonal status and disease. Future pharmacogenomic and pharmacokinetic studies will have to take all three components into account to allow for valid conclusions.

Keywords: cytochrome P450, drug–drug interactions, drug metabolism, drug transport, oral bioavailability, P-glycoprotein, pharmacokinetic variability

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1. Introduction

In a key review article, Wacher et al. [1] suggested the functional interaction between cytochrome P450 (CYP)3A and P-glycoprotein and coregulation based on their location in intestinal mucosa cells and hepatocytes, an almost complete overlap in substrates, inhibitors and inducers and adjacent location of their genes.

The CYP3A subfamily is the predominant CYP family in the human liver. In humans, the CYP3A subfamily includes CYP3A4, -3A5, -3A7 [2] and -3A43 [3]. CYP3A4 represents the predominant CYP in microsomal tissue of adults and is involved in the metabolism of > 50% of all currently prescribed drugs [4]. In the liver, CYP3A5 constitutes < 8% of the total CYP3A content and is found in only 10 – 20% of adult livers in the Caucasian population [5-7]. In vitro studies have shown an almost complete substrate overlap with CYP3A4, with CYP3A5 having equal or less activity [8]. CYP3A7 is the primary fetal CYP3A enzyme and diminishes during infancy [9]. In a study investigating the expression of CYP3A4 transcription in the livers of 63 Caucasians, CYP3A5 and -3A7 contributes an average of 2% and CYP3A43 an average of 0.3% transcripts to the CYP3A mRNA pool [10]. This study also found that CYP3A5 and -3A7, but not CYP3A4, were expressed in the adrenal gland and only CYP3A5 was expressed in the kidney.

P-glycoprotein is a member of the ATP-binding cassette (ABC) transporter family. The ABC transporter family is currently the largest known family of
Functional interactions between P-glycoprotein and CYP3A in drug metabolism

Table 1. The effect of P-glycoprotein inhibition on the extraction ratios of drugs across CYP3A4-overexpressing Caco-2 cell monolayers.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Substrate for Efflux ratio</th>
<th>Extraction ratio % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP3A</td>
<td>P-gp</td>
</tr>
<tr>
<td>K77 (10 µM)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sirolimus (1 µM)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Midazolam (3 µM)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Felodipine (10 µM)</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

The table was modified from Benet et al. [19] with permission from Bentham Science Publishers Ltd, and is based on data from references [21] and [22]. Intracellular amounts (pmol) are shown in brackets. All values are means ± SD (n = 3). K77 is an investigational cysteine protease inhibitor currently under development for the treatment of Chagas’ disease. Sirolimus is an immunosuppressive agent, midazolam is a benzodiazepine and felodipine is a Ca²⁺-channel blocker. Cyclosporin is an immunosuppressant and is a known CYP3A and P-gp substrate and inhibitor. In contrast, GG-918 is a specific P-gp inhibitor that was demonstrated not to affect CYP3A activity [21]. All four drugs have an apparent \( K_{iC} \) in human microsomes for metabolite formation close to the concentrations tested.

The extraction ratio (ER) was calculated using the following equation:

\[
ER = \frac{\Sigma \text{metabolites}_{(\text{apical, basolateral, intracellular})}}{\Sigma \text{metabolites}_{(\text{basolateral, intracellular})} + \Sigma \text{metabolites}_{(\text{apical, basolateral, intracellular})}}
\]

Because intracellular drug concentrations may change when drug transporters are modulated, the parent drug levels have been included in the denominator of the equation [19].

B to A: ratio apical to basolateral; B to A: ratio basolateral to apical, GG-918: N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxa-4-acridine carbocamine; K77: K11777, N-methyl-piperazine-Phe-homoPhe-vinylsulfone-phenyl; P-gp: P-glycoprotein.

transmembrane proteins. Forty-eight genes have been assigned to this family, which, based on sequence homologies and domain organisation, has been categorised into seven distinct subfamilies [101]. P-glycoprotein is a 170-kDa transmembrane glycoprotein and is encoded by the human multiple drug resistance protein (MDR)-1 (ABCB1) gene. It consists of two homologous halves. Each comprises six transmembrane domains and a cytoplasmic nucleotide binding domain [11,12]. P-glycoprotein is expressed at high levels on the apical surfaces of epithelial cells in the biliary membrane of the liver, small intestine and colon mucosa cells, the proximal tubule of the kidney, the adrenal gland and the pancreatic duct [13]. It also is a key component of the blood–brain barrier [14].

During the last 10 years, a large body of literature has confirmed Wacher and colleagues’ hypotheses [1]. As discussed in the following review, the results of those studies are likely to revolutionise our mechanistic understanding of pharmacokinetics, drug–drug interactions, and intersubject variability for the pharmacokinetics, drug–drug interactions, and intersubject variability for drug metabolism [15].

Please note that in this review the term ‘drug’ refers specifically to drugs that are CYP3A and P-glycoprotein substrates. A most recent list of such drugs can be found in reference [16].

2. Functional interactions between CYP3A and P-glycoprotein in the small intestine

P-glycoprotein and CYP3A functionally interact in three ways [17]. First, drugs are repeatedly taken up and pumped out of the enterocytes by P-glycoprotein. Repeated exposure to CYP3A enzymes increases probability of drugs being metabolised. Second, P-glycoprotein keeps intracellular drug concentrations within the linear range of the metabolising capacity of CYP3A enzymes. Third, P-glycoprotein transports drug metabolites formed in the mucosa back into the gut lumen.

2.1 Effect of P-glycoprotein on intestinal metabolism

In vitro and in vivo studies using specific CYP3A and/or P-glycoprotein substrates and inhibitors demonstrated that specific inhibition of P-glycoprotein decreases the metabolic extraction ratio in the small intestine. Those results have been reviewed in detail by Benet et al. [18-20].

The effect of P-glycoprotein on intestinal drug metabolism was studied in vitro using CYP3A4-transfected Caco-2 cells grown as monolayers [21,22]. The transport and metabolism of two P-glycoprotein and CYP3A substrates, K77 and sirolimus, and two CYP3A4-only substrates, midazolam and felodipine, were studied (Table 1). Drugs were given to the apical side of the cell culture system to simulate human intestinal absorption. K77 was found to be the best P-glycoprotein substrate.
tested and exhibited a ninefold greater basolateral-to-apical than apical-to-basolateral flux [19]. The sirolimus efflux ratio was only 2.5, indicating that sirolimus is a weaker P-glycoprotein substrate than K77. Midazolam and felodipine had efflux ratios of 1 and, thus, were confirmed not to be P-glycoprotein substrates. Both compounds served as negative controls. When added to the Caco-2 cells alone, all compounds tested were significantly metabolised in the cells (Table 1). As expected, addition of the CYP3A inhibitor cyclosporin significantly decreased the extraction ratios of all four drugs. However, whereas the extraction ratios of the CYP3A-only substrates felodipine and midazolam were reduced by 46 – 60%, the reduction of the extraction ratios of the CYP3A and P-glycoprotein substrates K77 and sirolimus were greater and found to be 74 – 83%. Those results already suggested a contribution of P-glycoprotein because cyclosporin is also a P-glycoprotein inhibitor. The involvement of P-glycoprotein was confirmed when K77 and sirolimus were added to the Caco-2 cell cultures in combination with the specific P-glycoprotein inhibitor GG-918. Specific P-glycoprotein inhibition resulted in a significant reduction of extraction ratios without a change in CYP3A activity. K77, the better P-glycoprotein substrate, was more affected (58% decrease) than sirolimus (25% decrease). Interestingly, a change in intracellular concentration (Table 1) does not explain the results [19]. When P-glycoprotein is inhibited, concentrations of K77 and sirolimus increase and more substrate is available to the CYP3A enzymes. Thus, an increase in the extraction ratio would be expected. However, when P-glycoprotein was inhibited, the opposite effect was found. It can be speculated that the observed reduction in metabolism is at least in part due to saturation of CYP3A enzymes, resulting in more nonmetabolised drug passing through the cell and inhibition of P-glycoprotein-mediated recycling of those drugs that usually result in repeated presentation of drug molecules to the CYP3A enzymes. These results indicate that inhibition of intestinal P-glycoprotein may not only increase absorption by blocking efflux transport, but also decrease total metabolism, resulting in significantly enhanced intestinal bioavailability [19].

Analysis of the impact of P-glycoprotein on the intestinal extraction ratio of verapamil, a P-glycoprotein and CYP3A substrate, using a four compartment model supported a nonlinear relationship between the extent of drug metabolism and drug transport [23]. As one of the reasons for this nonlinearity, saturable drug metabolism in the intestine was discussed.

In vitro, those mechanisms were confirmed using the rat single-pass intestinal perfusion model with mesenteric vein cannulation [24]. Again, K77, a CYP3A and P-glycoprotein substrate, and midazolam, a CYP3A but not a P-glycoprotein substrate, were compared in the absence and presence of the specific P-glycoprotein inhibitor GG-918. In the presence of GG-918, for K77 both the fraction metabolised (95 ± 3% versus 85 ± 4% with GG-918) and the extraction ratio (49 ± 12% and 37 ± 3%) were decreased. In contrast, the permeability and metabolism of midazolam remained unaffected. This study confirmed that also in vivo P-glycoprotein enhances the extent of intestinal metabolism of drugs that are substrates of CYP3A and P-glycoprotein [24].

2.2 Interaction between intestinal efflux transporters and drug metabolites

When the intestinal metabolism of the immunosuppressants tacrolimus and sirolimus (both substrates of CYP3A and P-glycoprotein) was studied in pig duodenal mucosa mounted in an Ussing chamber, it was found that both drugs were metabolised in the small intestine and that, surprisingly, the metabolites were almost exclusively found on the luminal side [25,26]. Because the parent drugs were added to the luminal chamber at a concentration of 10 µM and were present in the luminal chamber at 100-fold higher concentrations than the metabolites, those results could only be explained by active efflux transport of the metabolites against a concentration gradient, a higher affinity of the metabolites to P-glycoprotein than the parent drugs and/or that the CYP3A-mediated changes resulted in change of affinity to an efflux transporter other than P-glycoprotein. A latter study in Caco-2 cells confirmed that sirolimus metabolites are transported from the mucosa cell back through the apical (luminal) membrane [22]. The metabolites were confirmed to be P-glycoprotein substrates, but the study also found evidence that other transporters are involved [22]. The hypothesis that metabolism changes the affinity to other transporters than those playing a role for the parent was further confirmed in an in vitro study assessing the functional interaction between transport and metabolism by comparing the transport of losartan and its active metabolite EXP-3174 (EXP) across cell monolayers [27]. Epithelial layers of Caco-2 cells as well as ABCB1-overexpressing Madin–Darby canine kidney (MDCK) cells, in comparison with the wild type, were used to characterise the transcellular transport of losartan and EXP. Losartan was found to be a P-glycoprotein substrate with significantly higher basolateral-to-apical than apical-to-basolateral flux with a ratio of 31 ± 1 in ABCB1-overexpressing MDCK cells and a ratio of 4 ± 1 in Caco-2 cells. The metabolite was only transported in Caco-2 cells with a basolateral-to-apical/apical-to-basolateral ratio of 5 ± 1, while lacking active transport in the ABCB1-overexpressing MDCK cells. Those results suggest a functional interaction between CYP3A-dependent metabolism and intestinal efflux in a way, such that drugs are converted to metabolites that are substrates for transporters different from those that are involved in the intestinal transport of the parent (Figure 1) [27]. It can be hypothesised that inhibitors or inducers of proteins, which are involved in the active transport of the metabolite, may have an impact on the pharmacokinetics of the parent drug, although the parent itself might not be a substrate of such transporters.
Functional interactions between P-glycoprotein and CYP3A in drug metabolism

2.3 Local differences in intestinal CYP3A and P-glycoprotein expression and effect on oral bioavailability

The small intestine plays a significant role in drug metabolism, drug interactions and oral bioavailability of drugs that are CYP3A and/or P-glycoprotein substrates. Oral bioavailability is also a significant contributor to overall pharmacokinetic variability of such drugs. As discussed above, in the small intestine drug efflux pumps and CYP enzymes, most importantly P-glycoprotein and CYP3A, form a cooperative barrier against the absorption of xenobiotics (Figures 1 and 2). The following ranking of transporter mRNAs in the duodenum was found [28]: MRP3 > MRP1 > MRPI > MRP2 > MRP5 > MRP4 > MRPI (ABCC3 >> ABCB1 >> ABCB2 >> ABCC5 >> ABCC4 >> ABCC1). In the ileum transporter mRNA concentrations were as follows: ABCB1 >> ABCC3 >> ABCC1 ≈ ABCC5 ≈ ABCC4 ≈ ABCC2. Mouly and Paine [29] determined P-glycoprotein expression in mucosa scrapings of every other 30-cm segment of the intestines of four unrelated donors. P-glycoprotein expression increased from proximal to distal. Within-donor variation of P-glycoprotein expression was, depending on the region compared, 1.5- to 3-fold [29]. In healthy volunteers, Fricker et al. [30] showed that the absorption of the CYP3A and P-glycoprotein substrate cyclosporin was dependent on the location of absorption and followed the rank order stomach > jejunum/ileum > colon. The decrease in absorption was inversely correlated to the expression of ABCB1 mRNA (stomach < jejunum < colon) [30]. CYP activity is a function of the distance along the intestine from the duodenum to the ileum [31,32]. CYP3A concentration and activity increases slightly from the duodenum to the jejunum and then decreases towards the ileum [32,33].

Because drug transporters and CYPs are differently expressed in different parts of the human intestine the importance of their interactions and their effect on the pharmacokinetics of a specific drug will depend on the site of absorption from the gut. Again, it is important to consider...
that drugs and/or their metabolites may be substrates for different drug-metabolising enzymes and transporters.

3. Different functional interactions between CYP3A and P-glycoprotein in the liver and small intestine and impact on drug–drug interactions

In a perfused rat liver model, Wu and Benet showed that specific inhibition of P-glycoprotein significantly decreased the concentration of tacrolimus, a CYP3A and P-glycoprotein substrate, in the perfusate, a surrogate for systemic exposure [34]. However, when felodipine, a CYP3A but not a P-glycoprotein substrate, was studied in the same model, there was no difference in felodipine perfusate concentrations in the absence and presence of the specific P-glycoprotein inhibitor. This and other studies indicated that, whereas inhibition of P-glycoprotein in the small intestine reduces CYP3A-dependent metabolism [21,22,24], it increases hepatic metabolism [34-36]. There are two major differences between intestinal mucosa cells and hepatocytes that can explain those findings. The abundance of CYP3A enzymes in hepatocytes is significantly higher than in mucosa cells. An increase of substrate concentrations in the hepatocytes leads to more CYP3A-mediated metabolism. However, due to the lower metabolic capacity, an increase of substrate concentrations in the mucosa cells may saturate CYP3A relatively fast and then drug can pass the mucosa cells nonmetabolised (Figure 1). The other difference is that drug enters the mucosal cell through the apical membrane where P-glycoprotein is located. The drug may come into contact with P-glycoprotein first and, thus, P-glycoprotein regulates access to CYP3A enzymes (Figure 2). In comparison with the intestinal mucosa, drugs entering the hepatocyte encounter CYP3A and P-glycoprotein in the reverse order [16,19]. Drugs pass through the basolateral membrane and may come into contact with CYP3A before they reach P-glycoprotein located in the apical (biliary) membrane (Figure 2).

This leads to significant differences in drug–drug interactions with CYP3A and P-glycoprotein substrates in the small intestine and liver (Table 2) [16,19]. However, as indicated in Table 2, the situation may be more complex for specific drugs as additional transporters such as absorptive efflux transporters, and hepatic uptake transporters may play a significant role [16,35]. The clinical relevance of those differences in liver and intestine has not yet been confirmed in clinical trials and, due to the complexity of mechanisms involved and the differences of drugs in terms of their affinities to drug-metabolising enzymes and transporters, will be difficult, if not impossible to prove, by retrospectively analysing published drug–drug interaction studies.

4. Coregulation of CYP3A and P-glycoprotein

The nuclear receptor family constitutes a large family of related receptors that serve as targets for > 10% of all commonly prescribed drugs [37]. Important members of the family are the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). The human orthologue of PXR is also called steroid and xenobiotics receptor (SXR) and is encoded by NR1I2. PXR is a promiscuous receptor that is activated by a wide variety of xenobiotics and endogenous compounds such as progesterone, phytoestrogens, dexamethasone, plant products such as hyperforin (the active compound of St John’s Wort), bile acids, insecticides, and drugs such as rifampin, peptide mimetic protease inhibitors and paclitaxel [38-40]. On binding of the activating compound, PXR forms a heterodimer with the retinoid X receptor. The heterodimer functions as a transcription factor and interacts with the cognate response element in the 5′ regulatory region of the CYP3A4, but not the CYP3A5, gene [41]. Meanwhile, it has been shown that in the human liver and intestine, the PXR as well as CAR can induce CYP3A4 and -3A5 expression via an everted repeat separated by a 6-base pair (ER6) motif, 100 base pairs upstream from the transcription start site [42].
Among others, the PXR regulates gene expression of the drug-metabolising enzymes CYP2A, -2B, -2C and -3A, various glutathione S-transferases, and uridine diphosphate glucuronosyltransferase (UGT) 1a as well as the drug transporters MDR1A and -1B, MRP3 and OATP2 in mice [41]. The CAR regulates the expression of the drug-metabolising enzymes CYP1A, -2A, -2B and -3A and various glutathione S-transferases as well as the drug transporters MRP1, -2 and -3 [41]. In mice, there were several overlaps, but also several genes that were differentially regulated by those two receptors [43]. Based on their data, Maglich et al. [43] hypothesised that PXR plays a more important role for the regulation of drug-metabolising enzymes and drug transporters in the small intestine and CAR a more important role for gene regulation of those proteins in the liver. This also means that the coordinated regulation of drug-metabolising enzymes and drug transporters may be organ specific [44].

Synold et al. [39] were the first to describe coregulation of drug metabolism and efflux via CYP3A and MDR1 in liver and intestine by the human receptor PXR/SXR. This data indicated that paclitaxel reduces its own oral bioavailability by activating the PXR and induces its own metabolism and biliary elimination. Interestingly, this study also showed that hydroxylated paclitaxel metabolites as well as the structurally similar doctaxel did not interact with the PXR [39]. The observation that PXR interactions are not necessarily associated with certain classes of drugs was confirmed by another study that showed that the HIV protease inhibitor ritonavir is a relatively strong activator of PXR, whereas saquinavir is a relatively weak activator, and nelfinavir and indinavir are unable to activate PXR [40]. Ritonavir binds and activates PXR with a half-maximal effect concentration (EC50) of 2 µM. This is well within the therapeutically relevant expression and activity of the enzyme [46,47]. The CYP3A5*3 allele results in premature termination of translation and a nonfunctional truncated protein. This allele is mainly responsible for differences in CYP3A5 distribution among ethnic groups.

ABCB1 is highly polymorphic and 49 single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms in the ABCB1 gene have been described [48,49]. Seven SNPs are located in introns and eleven change the amino acid sequence [50]. The distribution of allelic variants differs greatly among ethnic groups [46]; however, most allelic ABCB1 variants are silent. The exonic single nucleotide SNPs C3435T and G2677T/A have been shown to correlate with expression and/or function of P-glycoprotein [50,51]. Subjects who were homogenous for the 3435T allele in exon 26 showed an average of twofold lower P-glycoprotein expression in the small intestine than the ‘wild type’ [50]. In terms of CYP3A and ABCB1 polymorphisms, so far the best-studied group of drugs is the immunosuppressants, specifically the calcineurin inhibitors cyclosporin and tacrolimus [51]. Both are critical dose drugs. Prediction of adequate dosing can be expected to facilitate management of immunosuppressive drug regimens and may improve clinical long-term outcome. However, results have been inconsistent. [52] A link between the polymorphisms of CYP3A4 and -3A5 and ABCB1 genes and the daily dose to achieve adequate tacrolimus blood concentrations has been shown [53,54]. Another study confirmed the role of CYP3A4 and -3A5 polymorphisms in tacrolimus pharmacokinetics, but did not support the role of ABCB1 polymorphisms [55]. In the case of cyclosporin, a study in liver transplant patients showed that the ABCB1 exon 26 C3435T is a major determinant of the cyclosporin concentration/dose

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**Table 2. Differential predicted extraction of drugs that are substrates of both CYP3A and P-glycoprotein after inhibition of CYP3A and/or active transporters in intestine or liver (according to [16,19]).**

<table>
<thead>
<tr>
<th></th>
<th>Intestine</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibit P-gp</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Inhibit CYP3A</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Inhibit P-gp + CYP3A</td>
<td>↓↑</td>
<td>↓↑↔</td>
</tr>
</tbody>
</table>

See also Figures 1 and 2. In both liver and intestine, inhibition of CYP will decrease extraction in those organs. However, possibly due to the reverse sequential locations of P-gp and CYP (Figure 2) in the intestinal mucosa and intestine, inhibition of P-gp in the intestinal mucosa will reduce gut drug metabolism, whereas in the liver inhibition of P-gp was found to increase metabolism. Inhibition of intestinal efflux and metabolism resulted in a more potent inhibition of gut extraction, whereas in the liver the effect was found to go either way, depending on the affinity of substrate and inhibitor to CYP and P-gp. It must be noted that the concepts summarised in this table were based on data from cell and perfused organ models. The relevance for pharmacokinetics and drug–drug interactions in humans still needs to be confirmed. In addition, this table is limited to the CYP3A/P-gp interaction. As soon as a drug is also the substrate of another efflux and/or uptake transporter, and other drug-metabolising enzymes, the transporter interaction will be more complex.

CYP: Cytochrome P450; P-gp: P-glycoprotein.

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5. **Sources of variability**

5.1 **Pharmacogenetics and -genomics**

More than 30 CYP3A4 allelic variants have been identified [101]. Today, there is no consensus as to whether there is a direct correlation between function and CYP3A4 polymorphisms. CYP3A4 alleles may have little clinical importance. The reasons are the relatively low allele frequencies and the limited alterations in enzyme expression and catalytic function [45,46].

The CYP3A5*1 allele is associated with high CYP3A4 expression and activity, whereas CYP3A5*3 is associated with irrelevant expression and activity of the enzyme [46,47]. The CYP3A5*3 allele results in premature termination of translation and a nonfunctional truncated protein. This allele is mainly responsible for differences in CYP3A4 distribution among ethnic groups.

ABCB1 is highly polymorphic and 49 single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms in the ABCB1 gene have been described [48,49]. Seven SNPs are located in introns and eleven change the amino acid sequence [50]. The distribution of allelic variants differs greatly among ethnic groups [46]; however, most allelic ABCB1 variants are silent. The exonic single nucleotide SNPs C3435T and G2677T/A have been shown to correlate with expression and/or function of P-glycoprotein [50,51]. Subjects who were homogenous for the 3435T allele in exon 26 showed an average of twofold lower P-glycoprotein expression in the small intestine than the ‘wild type’ [50]. In terms of CYP3A and ABCB1 polymorphisms, so far the best-studied group of drugs is the immunosuppressants, specifically the calcineurin inhibitors cyclosporin and tacrolimus [51]. Both are critical dose drugs. Prediction of adequate dosing can be expected to facilitate management of immunosuppressive drug regimens and may improve clinical long-term outcome. However, results have been inconsistent. [52] A link between the polymorphisms of CYP3A4 and -3A5 and ABCB1 genes and the daily dose to achieve adequate tacrolimus blood concentrations has been shown [53,54]. Another study confirmed the role of CYP3A4 and -3A5 polymorphisms in tacrolimus pharmacokinetics, but did not support the role of ABCB1 polymorphisms [55]. In the case of cyclosporin, a study in liver transplant patients showed that the ABCB1 exon 26 C3435T is a major determinant of the cyclosporin concentration/dose...
Table 3. Contribution of CYP3A and P-gp in the interpatient variation of the oral bioavailability of drugs that are CYP3A and P-gp substrates.

<table>
<thead>
<tr>
<th></th>
<th>Liver CYP3A</th>
<th>Intestinal P-gp</th>
<th>Intestinal CYP3A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent oral clearance</td>
<td>56%</td>
<td>17%</td>
<td>-</td>
</tr>
<tr>
<td>Cmax</td>
<td>32%</td>
<td>30%</td>
<td>-</td>
</tr>
</tbody>
</table>

Data were taken from reference [65]. The pharmacokinetics of the CYP3A and P-gp substrate cyclosporin was assessed in 25 kidney transplant patients and was correlated to CYP3A activity in the liver and CYP3A and ABCB1 expression in the small intestine. Forward multiple regression analysis revealed that liver CYP3A and intestinal P-gp contributed to inter-patient variability of apparent oral clearance and the maximal cyclosporin concentration in blood (Cmax), a surrogate marker for the rate of absorption. Although intestinal expression of CYP3A varied 10-fold among the subjects, it did not contribute to the inter-individual pharmacokinetic parameters evaluated. This data suggested that P-gp-mediated efflux, but not CYP3A metabolism is a rate-limiting step in cyclosporin absorption.

CYP: Cytchrome P450; P-gp: P-glycoprotein.

ratio [56], whereas a study in kidney graft patients only found a weak correlation between cyclosporin dose requirements and ABCB1 SNPs [57]. In renal transplant patients, ABCB1 SNPs were not associated with sirolimus concentration/dose ratios [58]. Population-dependent distribution of CYP3A4, CYP3A5 and ABCB1 SNPs is most likely to be responsible for the ethnic differences in cyclosporin, tacrolimus and possibly also sirolimus pharmacokinetics [49,59-61].

The CYP3A5*1 allele is associated with high intestinal and hepatic expression and activities of CYP3A5 and with low concentration/dose ratios. More than 60% of African-Americans, compared with < 10% of the Caucasian population, possess this allele [49]. The situation for P-glycoprotein is similar. Only 24% of Caucasians, but 68% of African-Americans are carriers of the CC genotype of the ABCB1 C3435T genetic variation that is associated with low immunosuppressant exposure [61].

The expression of CYP3A4, CYP3A5, CYP3A43 and PXR mRNA were found closely correlated in 46 Caucasian human livers [7]. Thus, variability in expression of CYP3A and possibly also ABCB1 may be explained by the allelic variations in the PXR, one of their transcriptional regulators [46,62]. A total of 38 SNPs were identified, including 6 SNPs in the coding region [63]. The frequency of PXR*2 in African-Americans was 20% and PXR*2 was not found in Caucasians. However, this variant did not affect CYP3A expression. It is yet unknown as to whether the PXR SNPs are of clinical relevance.

In summary, studies evaluating the effect of CYP3A and ABCB1 polymorphisms on pharmacokinetics show that ABCB1 seems to be a better predictor than CYP3A [50,64]. This is not surprising as it was shown in a clinical trial that P-glycoprotein in the small intestine, but not CYP3A, significantly contributes to interpatient pharmacokinetics of cyclosporin (Table 3) [65]. This can probably be explained by the fact that cyclosporin after oral administration undergoes significant intestinal metabolism and that P-glycoprotein-mediated efflux controls cyclosporin concentrations in the mucosa cells and thus access of cyclosporin to intestinal CYP3A. In contrast to cyclosporin, CYP3A5 polymorphism was a better predictor of tacrolimus pharmacokinetics than ABCB1 [50]. Interestingly, it was found that the results of CYP3A4 and CYP3A5 genotyping did not sufficiently reflect the interindividual variability of midazolam, a CYP3A, but not a P-glycoprotein, substrate [66].

Those studies clearly demonstrate that predictive clinical values of CYP3A and ABCB1 polymorphism will depend on whether a drug is a good P-glycoprotein and/or CYP3A substrate (Table 1). It seems reasonable to expect that the genetic polymorphism of the transporter or drug-metabolising enzyme that is first in the elimination pathway will control access of a drug to the subsequent metabolism/transport sites and will have the most significant effect on overall pharmacokinetics. Thus, the route of administration will have a significant impact (Table 2). P-glycoprotein/CYP3A interactions will also be affected by the presence of drugs potentially interacting with PXR, which itself exhibits significant polymorphism [62]. This is a significant problem in specific patient populations such as transplant patients who receive a multitude of drugs that are CYP3A and P-glycoprotein substrates and that interact with the PXR.

5.2 Age
Fetal activity of CYP3A is 30 – 75% that of adults [67]. CYP3A activity increases throughout infancy and commonly exceeds adult activity by the age of 1 year. It continues at this level during childhood and CYP3A activities decrease gradually to adult levels by the end of puberty. A study in 90 healthy volunteers aged 0 – 86 years showed that P-glycoprotein activity in peripheral blood lymphocytes was highest in cord blood and progressively declined with age [68]. In human intestinal and liver tissues from individuals of different age ranging from neonatal to 85 years, a close correlation between PXR mRNA and the mRNAs of CYP3A4 and ABCB1 was shown [69]. PXR mRNA in the liver and intestine reached maximum levels in young adults (15 – 38 years of age) and decreased to half the maximal levels in older individuals (67 – 85 years) [69].

5.3 Gender
There is evidence in the literature that women have a higher clearance for drugs that are CYP3A and P-glycoprotein substrates than men after intravenous administration [70]. Those...
differences seem to be influenced by the menstrual cycle. Drugs that are only CYP3A, but not P-glycoprotein, substrates, did not exhibit gender-dependent differences in pharmacokinetics. Overall, those findings suggested a significant involvement of P-glycoprotein [70]. However, analysis of 94 well-characterised surgical liver samples revealed twofold higher CYP3A concentrations in women and no difference in P-glycoprotein expression [71]. In terms of CYP3A, similar differences were found for the small intestine when tacrolimus and cyclosporin metabolism rates were assessed in microsomes isolated from human duodenal mucosa [72,73]. However, a recent study in 46 healthy men and 45 healthy women did not find a gender difference for CYP3A4, CYP3A5 and P-glycoprotein expression in the proximal small intestine [74]. When in this study pre- and postmenopausal women were compared, CYP3A4 was 20% lower in postmenopausal women. Expression of the PXR was correlated with CYP3A expression, but did not show gender differences [71]. There was also no gender difference in ABCB1 expression in this study.

5.4 Herbs and food
The importance of food–drug interactions and interactions with herbal medicines has increasingly been recognised during the last years [75,76]. CYP3A-mediated metabolism and P-glycoprotein transport seem especially vulnerable [76,77]. Grapefruit juice and St John’s Wort are the best-studied food and herb drug interactions. The interaction between drugs and grapefruit juice is mainly driven by depletion of CYP3A enzymes in the small intestine [78], but effects of grapefruit juice components on active transporters in the intestine have also been described [79]. The active compound of St John’s Wort, hyperforin, induces CYP3A and P-glycoprotein via the PXR, resulting in reduced drug exposure and efficacy [50]. However, the relative contribution of CYP3A and P-glycoprotein induction to the overall effect of St John’s Wort remains unknown [79]. Recently, it has also been hypothesised that high-fat meals affect pharmacokinetics by inhibiting influx and efflux transporters including P-glycoprotein [16].
5.5 Disease

The activity of CYP3A is reduced by high intracellular free radical concentrations, infectious diseases, inflammation and immune reactions [80,81]. The activity of ABC transporters is affected in similar ways [82]. In mice, it was found that pro-inflammatory cytokines suppress the hepatic expression of ABCB1, ABCCC2, ABCCC3, oatp2, ntcp and bsep in addition to CYP3A enzymes [83]. The results of this study suggested involvement of the PXR. The overall effect of inflammation is increased exposure to drugs and/or their metabolites due to reduced first-pass effect and inhibited hepatic elimination. A good example is a clinical trial including 51 bone marrow transplant patient treated with cyclosporin [84]. During immunoreactions such as rejection of liver transplants and graft-versus-host disease, hepatic efflux transporters seem to be more affected than CYP3A-dependent metabolism. In this study, liver graft-versus-host disease led to accumulation of cyclosporin metabolites and a shift of the blood metabolism pattern from first-generation metabolites (metabolites that are changed only at one site) to second- and higher-generation metabolites (metabolites modified at two or more sites) that are the results of further metabolism of first-generation metabolites (Figure 3) [85]. Retrospectively, those results can be explained by CYP3A/efflux transporter interactions as shown in Table 2. When biliary efflux from the hepatocytes is inhibited, it is reasonable to assume that cyclosporin and first-generation metabolites will reach higher intracellular concentrations and the intracellular residence time increases. This leads to more extensive metabolism. Instead of being eliminated into bile, metabolites show up in relatively high concentrations in the blood. The effect of inflammation on CYP3A and P-glycoprotein may also explain the observation that hepatitis C positive liver transplant patients require a lower tacrolimus dose than hepatitis C negative patients [86].

6. Impact on drug development

Recently, Wu and Benet [16] suggested a modification of the Biopharmaceutics Classification System (BCS), the Biopharmaceutics Drug Disposition Classification System, to also allow for predicting overall drug disposition. At present, the BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate-release solid oral dosage forms: solubility, permeability and dissolution.

The proposed modification is based on the observation that class 1 (high solubility and high permeability) and 2 (low solubility and high permeability) drugs are primarily eliminated via drug metabolism, whereas class 3 (high solubility and low permeability) and 4 (low solubility and low permeability) drugs are primarily eliminated unchanged [16]. Review of the literature showed that most class 2 compounds are also substrates for active drug transporters and it is reasonable to expect that drug-metabolising enzyme/transporter interactions will play a role in their pharmacokinetics [16].

Changing the BCS permeability component to a route of elimination component will allow for prediction of routes of drug elimination and of when transporter/drug-metabolising enzyme interactions will result in clinically significant effects such as low and variable oral bioavailability and drug–drug interactions [16]. In addition, this modified classification may also be relevant for pharmacogenetic predictions. As discussed above, the impact of polymorphisms depends on whether a drug is a good substrate of drug transporters and/or drug-metabolising enzymes. According to Wu and Benet’s [16] observations, elimination of class 1 drugs are driven mainly by drug metabolism. Thus, it can be speculated that for class 1 drugs CYP polymorphisms have a significant impact on their pharmacokinetics. Class 2 drugs, however, have a significant active transporter component affecting their pharmacokinetics. Again, it can be speculated that pharmacokinetics of those drugs may be more impacted by polymorphisms of transporter genes, as those regulate the substrate concentrations at the CYP enzymes.

Because most of today’s new molecular entities are large-molecular-mass lipophilic compounds that fall into BCS class 2, a Biopharmaceutics Drug Disposition Classification System that takes drug-metabolising enzyme/active drug transporter interactions into account may have significant impact on drug development and may affect regulatory requirements [16].

7. Expert opinion

It was our goal to review emerging concepts of the interaction between CYP3A and P-glycoprotein and to give a critical status report. It is important to note that further experimental work will be required to confirm those concepts, some of which, as discussed above, must still be considered speculation. Although intriguing, their clinical relevance in humans still needs further assessment. Most of the key work has been done in cell and animal models that allow for testing specific hypotheses and for specific targeting of the CYP3A/P-glycoprotein interactions. In addition, clinical studies have been using specific probes to evaluate some of the mechanisms in humans. The situation with drugs that are substrates of a multitude of drug-metabolising enzymes and active transporters and for which transport and metabolism in several organs are involved in their pharmacokinetics is a lot more complex and it may not be possible to extrapolate in vitro studies solely assessing the CYP3A/P-glycoprotein interaction to the situation in patients. As mentioned below, this situation is further complicated by the fact that as of yet the transporter field is developing rapidly and it is reasonable to assume that only a part of the transporters relevant for clinical pharmacokinetics are currently known. This review focuses on CYP3A/P-glycoprotein interaction as it is the best studied and most of the aforementioned concepts were developed based on this
enzyme/transporter pair. There are other similar interactions between other drug-metabolising enzymes and transporter. At present, the clinically most important example is the interaction between the immunosuppressants mycophenolic acid and cyclosporin, which is based on the disruption of the cooperation between UGT and MRP-2 (ABCC2) in the liver via inhibition of MRP-2 by cyclosporin [87]. Many other relevant drug-metabolising enzyme/active transporter interactions have not yet been studied or discovered. Three components play a key role in the absorption and elimination of BCS class 2 drugs: drug-metabolising enzymes, drug transporters and the nuclear xenobiotic receptors. All three, alone or in combination, can be the target of drug–drug interactions. Again, it must be taken into account that also our knowledge about nuclear receptors, their networks and their role in drug pharmacokinetics and drug–drug interactions is probably still in its infancy.

It is common belief that one of the reasons for drug metabolism is making lipophilic drugs more water soluble, which, therefore, facilitates their elimination. There is a good possibility that drug metabolism also transforms drugs into better substrates for related transporters to facilitate their elimination [17]; this hypothesis requires further experimental confirmation.

Significant polymorphisms of PXR (NR1I2) have been reported. However, their clinical relevance is still unknown. One reason is the complexity of the network of genes regulated by nuclear receptors. In addition, studies have focused on using CYP3A expression as a surrogate marker for metabolic activity. As discussed above, this may not be correct depending on the drug and to which extent its pharmacokinetics is affected by active transporters. A complete picture requires the evaluation of drug-metabolising enzymes, uptake and efflux transporters and may depend on the nature of a drug as well as individual genetics. A complete understanding of PXR (NR1I2) polymorphisms will again require a complete understanding of the interaction between drug-metabolising enzymes, uptake and efflux transporters. The same applies for CYP3A and ABC-transporter gene polymorphisms. In light of these complex interactions, the general clinical applicability of diagnostic devices such as a microarray chip designed to routinely identify CYP polymorphisms can only be expected to be predictable for drugs for which uptake and efflux transporters have no significant influence on their pharmacokinetics.

Recent studies have acknowledged the interaction of CYP enzymes, ABC transporters and the PXR and have included measurement of all three in their clinical studies [50,60,83]. Current regulatory guidance, however, is still mostly focused on drug-metabolising enzymes [16,88,103]. Those regulatory guidelines for in vitro drug interaction testing are based solely on assays using isolated microsomes or CYP450 enzymes. Based on these experiments, they allow for the conclusion that lack of an in vitro drug–drug interaction is reassuring and can generally eliminate the need for further clinical evaluation [88,103]. As demonstrated in an in vitro study [36] and as discussed in detail by Wu and Benet [16], this can be misleading for drugs that are substrates for both intestinal enzymes and intestinal efflux transporters. In addition, inhibition of hepatic and renal uptake transporters can significantly increase systemic concentrations of drugs [16]. Such regulatory guidelines will require critical reevaluation.

Based on the fully sequenced human genome, it can be estimated that the number of human transporters and related proteins is in the thousands [104]. However, the number of active transporter proteins currently known is < 500. Thus, even if a drug-metabolising enzyme–drug transporter interaction is well-established, it is possible that a drug may also be the substrate for yet unknown transporters. It is reasonable to expect that in the future, as more transporters are identified, more clinically relevant drug-metabolising enzyme/drug transporter interactions will be described.

In conclusion, the field of drug-metabolising transporter/active transporter interactions in combination with pharmacogenetics and genomics is fast developing and the complexity of those interactions are still only partially understood. In vivo, drug-metabolising enzyme/transporter interactions depend on and can be influenced by:

- the affinity of a drug (and its metabolites) to CYP3A and P-glycoprotein
- the affinity of a drug (and its metabolites) to other drug-metabolising enzymes and other transporters such as uptake transporters
- the interaction of drug-metabolising enzymes and transporters with nuclear receptors
- polymorphisms of drug-metabolising enzymes, drug transporters and nuclear receptors
- the tissue distribution of a drug (and its metabolites) and expression patterns of drug-metabolising enzymes, drug transporters and nuclear receptors in tissues critical for pharmacokinetics
- age, gender, disease, drug–drug, herb–drug and food–drug interactions

Many of the concepts discussed above represent only a first attempt to assess and explain interaction mechanisms. Future studies will have to show as to whether those represent generally applicable concepts or will remain limited to the drug-metabolising enzymes, transporters and tissues based on which they have originally been established.

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This study provided experimental evidence for the hypothesis that drug metabolism changes the affinity to efflux transporters.

The results suggested that specific inhibition of P-glycoprotein has the opposite effect on the metabolism of dual P-glycoprotein/CYP3A substrates than in the gut mucosa due to the reverse order of CYP3A and P-glycoprotein in the path of a drug through the hepatocyte than through an intestinal mucosal cell.

The results suggested that hepatic drug metabolism is modulated by uptake and influx transporters and that the exclusive use of microsomal systems as supported by current regulatory guidelines may not be sufficient to predict or exclude clinically relevant drug–drug interactions.

The first publication to describe co-regulation of drug-metabolizing enzymes (CYP3A and CYP2C8) and drug transporters (MDR1 and MRPI) via the human nuclear receptor SXR.


of cytochrome P4503A5 and P-glycoprotein correlate with dose requirement. 


Functional interactions between P-glycoprotein and CYP3A in drug metabolism


Websites


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