Published in final edited form as:
Mol Pharm. 2009 ; 6(6): 1631–1643. doi:10.1021/mp900253n.

The Drug Transporter-Metabolism Alliance: Uncovering and Defining the Interplay

Leslie Z. Benet*
Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, University of California San Francisco, San Francisco, California 94143-0912

Abstract

Two decades ago the importance of transporter-enzyme interplay and its effects on drug bioavailability and hepatic disposition were first recognized. Here we review the history of uncovering and defining this interplay with a primary emphasis on studies from our laboratory. We review the early 1990s oral bioavailability studies that found that the highly lipophilic, poorly water soluble cyclosporine formulation on that market at that time did not have an absorption problem, but rather a gut metabolism problem. This led to studies of the interactive nature of CYP3A and P-glycoprotein in the intestine, and investigations of this interplay using cellular systems and isolated perfused rat organ studies. Studies investigating uptake transporter-enzyme interactions using cellular, perfused rat liver and intact rats are reviewed, followed by the human transporter-enzyme interaction studies. Work characterizing the rate limiting processes in the drug transporter-metabolism alliance is then addressed, ending with a review of areas of the interplay that require further studies and analysis.

Keywords

Transporters; metabolic enzymes; transporter-enzyme interplay; intestine; liver; Biopharmaceutics Drug Disposition System; BDDCS; atorvastatin; cyclosporine; digoxin; erythromycin; felodipine; glyburide; midazolam; rifampin; sirolimus; tacrolimus

Introduction

Less than 20 years ago when a drug exhibited poor oral bioavailability it was generally believed that this was due to either (a) physico-chemical properties, such as poor solubility in the GI fluids or poor permeability through the intestinal membrane, or alternatively due to (b) significant first-pass hepatic metabolism. Our laboratory in San Francisco was one of the first to hypothesize that for many drugs poor oral bioavailability could be due to interactive biochemical processes in the intestine and/or the liver rather, and often in addition to, the physico-chemical properties[1, 2]. Based on a series of early cellular, animal and human studies, we hypothesized that intestinal metabolic enzymes and efflux transporters, working coordinately as a protective mechanism, could be responsible for the poor bioavailability of a number of drugs. In this manuscript we briefly review the studies carried out in our laboratory to probe and understand the interactive nature of enzymes and transporters in the intestine and the liver.

*Address correspondence leslie.benet@ucsf.edu, University of California San Francisco, 533 Parnassus Ave., Room U-68, San Francisco, CA 94143-0912. Tel: 415-476 3853. Fax: 415-476-8887..

Human Oral Bioavailability Studies with Immunosuppressive Agents

In the early 1990s, our group carried out interaction studies in humans with cyclosporine, tacrolimus and sirolimus. At that time it was generally believed that the poor and variable absorption of these immunosuppressive agents was due to their poor solubility in the gastrointestinal fluids, resulting from the drugs' large molecular weight and high lipid/water partition coefficient. Our first study of the effects of a high fat meal on cyclosporine pharmacokinetics in healthy subjects[3] led us to believe that the unusual effects resulting from a high fat meal, i.e. no change in the absorption rate but a significant increase in the extent of absorption[4] and an increase in the clearance of cyclosporine,[5] could be explained by a lipid effect in the liver. However, biochemical and in vitro studies could not provide a rational explanation for the apparently anomalous results of our human clinical studies. In a 1992 study[6], we began to recognize that the accepted dogma relating cyclosporine's poor bioavailability to poor absorption might not be correct. In that work we noted that the increase in hepatic metabolism with concomitant rifampin administration could not explain the marked decrease in cyclosporine oral bioavailability. We proposed, based in part on the innovative studies of Watkins and coworkers[7,8], that gut metabolism of cyclosporine may account for the differences observed in the presence of rifampin, a known inducer of CYP3A in humans. A further study with cyclosporine and concomitant ketoconazole[9] supported this hypothesis but also gave clues that another biochemical modulator was involved. Our subsequent analysis of the oral and iv cyclosporine data following induction and inhibition led to a surprising conclusion, at that time[10]. That is, cyclosporine in the old commercially available Sandimmune® formulation did not have an absorption problem and that the drug on average was absorbed at least 86%, but rather cyclosporine underwent a marked first-pass gut extraction, which approximated 60% or more.

We realized that the outcomes of our cyclosporine studies could not be explained based only on changes in gut enzymes by drug inducers or inhibitors. We were the first to note and publish the striking overlap of substrate specificity and tissue distribution for CYP3A and P-glycoprotein[11]. We proposed that CYP3A and P-glycoprotein played complementary roles in drug absorption, distribution, metabolism and excretion by biotransformation and countertransport, particularly in the villi of the small intestine. Shortly following our publication of this coordinated protective mechanism[11], Schuetz et al.[12] demonstrated that modulators and substrates of P-glycoprotein and CYP3A coordinately upregulated these proteins in human colon carcinoma cells and that P-glycoprotein was a major determinant of rifampin-inducible expression of CYP3A in mice and humans[13]. Similar studies to those described above for cyclosporine were reported for tacrolimus and sirolimus[14–16].

Interactive Nature of CYP3A and P-glycoprotein in the Intestine

Figure 1 depicts our conception of the interaction between CYP3A and P-glycoprotein in the intestine. Three drug molecules are depicted. They are all the same drug and only differentiated by their outcome. Drug is absorbed by passive processes into the enterocyte where it may be metabolized by the enzyme. However, the drug is also subject to active efflux back into the intestine thereby allowing further access to the enzyme upon subsequent passive absorption. The open circle molecule enters the enterocyte, is not metabolized by CYP3A or effluxed back into the lumen by P-glycoprotein. It then proceeds in the hepatic portal vein to the liver. The solid circle molecule is absorbed into the enterocyte and is metabolized to the open square product upon its first encounter with the enzyme. The open square metabolite either passes into the hepatic portal blood or back into the gut lumen. However, the shaded diamond molecule is absorbed; it is not metabolized by the enzyme; it is effluxed back into the gut lumen by P-glycoprotein and this cycling occurs twice again, where upon the fourth entry into the enterocyte the shaded diamond molecule is metabolized. The cartoon in Fig. 1 depicts the

transporter as controlling the access of the drug to the enzyme, giving the enzyme multiple opportunities to prevent the intact xenobiotic from entering the bloodstream. Thus, we hypothesized that the enzyme and the transporter are working in a coordinated manner as a protective process to keep foreign substances out of the body. Note that if no P-glycoprotein were present, or if it were to be inhibited, the enzyme would only have one opportunity to metabolize the drug as it diffuses through the enterocyte. However, when the P-glycoprotein is present, or to an even greater extent when P-glycoprotein is induced, the enzyme has multiple opportunities to metabolize the drug.

We realized, based on Fig. 1, that intestinal metabolism of a drug could be changed as a function of P-glycoprotein activity without either inhibiting or inducing CYP3A enzymes. That is, if efflux by P-glycoprotein is inhibited, the drug molecule passes through the intestine in a single pass and intestinal metabolism will decrease since there is less access of the drug to the enzymes. We attempted to test this hypothesis by carrying out studies with a substance that had negligible effects on CYP3A but which we believe could affect P-glycoprotein. We undertook a study in healthy volunteers in which cyclosporine was dosed as the oral Sandimmune® formulation, with and without water-soluble vitamin E (TPGS, tocopherol polyethylene glycol 1000 succinate).[17] We demonstrated that this commercially available additive had no effect on CYP3A metabolism[17], but that it was an inhibitor of P-glycoprotein[18]. In 10 healthy volunteers, oral cyclosporine area under the curve (AUC) was increased 58±24% when the Sandimmune® formulation was given concomitantly with TPGS[17]. At that time we believed the bioavailability increase was due to P-glycoprotein inhibition. However, as will be discussed subsequently, there may be another explanation.

In the late 1990s, a series of studies, mostly in animals, demonstrated that inhibition or induction of intestinal CYP3A and/or P-glycoprotein had marked effects on the bioavailability of a number of drugs as summarized by Zhang and Benet[19]. These included studies of inhibitors and P-glycoprotein knockout animals on the bioavailability of paclitaxel,[20,21] inhibitors and inducers affecting HIV-1 protease inhibitors[22–24] and an investigational cysteine protease inhibitor[25], as well as studies with digoxin, which in rodents is the substrate for CYP3A, but not in humans[26,27].

Investigating the CYP3A-P-glycoprotein Interactions Using Cellular Systems

The Caco2 (colon carcinoma) cell line is frequently used to model the human intestinal absorption of drugs[28]. Although Caco2 cells express the P-glycoprotein efflux transporter, they lack CYP3A. We characterized the expression of CYP3A4 and efflux transporters, P-glycoprotein as well as MRP1 and MRP2, in CYP3A4-transfected Caco2 cells after an induction protocol[29]. The role of P-glycoprotein in modulating the extent of intestinal drug metabolism was examined in these CYP3A4-transfected Caco2 cells grown as monolayers. The metabolism and transport of several model CYP3A4 and P-glycoprotein compounds, as listed in Table 1, were determined when dosed on the apical side of the cells (mimicking human intestinal absorption) alone or together with the P-glycoprotein inhibitor GG918 (200nM) or the dual CYP3A4 and P-glycoprotein inhibitor cyclosporine (10μ). We hypothesized based on Figure 1 as described above, that for compounds that were substrates of both CYP3A4 and P-glycoprotein, inhibition of intestinal P-glycoprotein would not only increase absorption by blocking efflux but also decrease total metabolism resulting in a significantly enhanced intestinal bioavailability.

The drugs tested included: K77[30], an investigational cysteine protease inhibitor currently being developed to treat Chagas' disease; sirolimus[31], an immunosuppressive; midazolam[31], an anesthetic agent and felodipine[30], a Ca^{2+}-channel blocker. Each of these drugs was a known substrate for CYP3A4 having a K_m for metabolite formation approximately equal to the dose

administered (shown in brackets, Table 1). K77 was found to be the best P-glycoprotein substrate tested, exhibiting a 9-fold greater basolateral (B)asolateral to (A)pical flux compared with its A to B flux. Sirolimus transport exhibited only a 2.5-fold efflux ratio across the cells and is considered a weaker P-glycoprotein substrate. Midazolam and felodipine are not substrates for P-glycoprotein in the Caco2 cell system, as indicated by their efflux ratios of 1, and acted as negative controls for these studies. Negative controls were necessary to ensure that the specific P-glycoprotein inhibitor GG918 was not affecting the cells in any way except via inhibition of P-glycoprotein. All drugs tested were significantly metabolized while traversing the cells as indicated by the extraction ratios ranging from 25% to 60% (Table 1, drug alone). As expected, incubation with cyclosporine resulted in decreased extraction ratios for all compounds, as cyclosporine is a known, potent CYP3A4 inhibitor.

Incubation of felodipine and midazolam with GG918 (a P-glycoprotein inhibitor that does not inhibit CYP3A4) did not change the transport profiles or the extraction ratios of either of these two drugs. This was expected as these compounds were negative controls for the P-glycoprotein function, and the effects of GG918 should be negligible. K77 and sirolimus transport profiles and extraction ratios, however, were significantly decreased by GG918. Consistent with complete inhibition of P-glycoprotein, efflux transport of both K77 and sirolimus was abolished. By inhibiting P-glycoprotein, the extraction ratio for K77 went from 33 to 14% (a 58% decrease), indicating that when the transporter was inactivated, there was decreased exposure of K77 to CYP3A4. The extraction ratio for sirolimus was decreased 25% in the presence of GG918 (from 60 to 45%) consistent with its moderate interaction with P-glycoprotein, when compared with K77. Therefore, selective inhibition of the transporter yielded significant effects on the extent of metabolism by CYP3A4 for the two drugs that were substrates of CYP3A4 and P-glycoprotein. These data support the role for P-glycoprotein in increasing the exposure of drugs to CYP3A4 in the intestine by allowing repeated cycling of drug via diffusion and active efflux as demonstrated in Figure 1.

Our cellular system allowed us to also measure the extraction ratio going from the B to A side of the CYP3A4-Caco-2 cell system. The extraction ratios of K77 and felodipine after both apical and basolateral dosing are shown in Figure 2. The extraction ratios for the apical dose were presented and discussed with respect to Table 1. From Figure 2 we see that the extraction ratio of K77 going from B to A was only 6%. That is, relative to the amount of drug that entered or crossed the cell, 5.5 times more was metabolized from an apical dose. In the presence of GG918 the apical extraction was decreased from 33% to 14% indicating that the extent of metabolism was lower when P-glycoprotein was not active. However, the opposite trend was observed from a basolateral dose where the extraction ratio increased from 6% to 12%, indicating that decreased P-glycoprotein clearance resulted in an increase in the extent of metabolism. Thus, when P-glycoprotein was inhibited the extraction ratio for K77 did not differ between apical and basolateral dosing, as only the enzyme elimination was involved, just as was seen for the non P-glycoprotein substrate felodipine, where extraction ratios (Fig. 2) were similar from both an apical and a basolateral dose (26% vs. 24%). These extraction ratios were unchanged with GG918 but significantly decreased in the presence of cyclosporine, indicating that felodipine served as a negative control for P-glycoprotein activity.

Investigating the CYP3A-P-glycoprotein Interaction Using Perfused Rat Organs

Following the cellular studies described above, studies using the rat single pass intestinal perfusion model (with mesenteric vein cannulation) were performed to determine whether a similar drug metabolism-efflux transporter alliance existed in vivo[32]. The extent of metabolism of two test compounds, K77, a dual CYP3A and P-glycoprotein substrate, and midazolam, a substrate demonstrating CYP3A metabolism but no P-glycoprotein effect, were compared

when each drug was perfused alone and in the presence of the P-glycoprotein inhibitor GG918. The appearance of K77 in the mesenteric blood was increased 2.5-fold in the presence of GG918 indicating that P-glycoprotein was inhibited. Midazolam permeability was unchanged by GG918, as expected since it is not a P-glycoprotein substrate. Estimates of the extent of metabolism were determined by calculating the fraction metabolized from the disappearance of drug from the gut lumen and the extraction ratio for the formation of known CYP3A metabolites. For K77, both the fraction metabolized (95±3% vs. 85±4% with GG918) and the extraction ratio (49±12% and 37±3%) were decreased when P-glycoprotein was inhibited, whereas both measures of the extent of metabolism for midazolam were unchanged with P-glycoprotein inhibition[32]. The data obtained from the single pass intestinal perfusion system were the first to demonstrate the specific interaction of P-glycoprotein with CYP3A in this isolated organ. These in vivo data support the proposed interplay between P-glycoprotein and CYP3A as P-glycoprotein, when active, appears to enhance the extent of metabolism of the dual CYP3A and P-glycoprotein substrate as was seen in the cellular systems.

The role of P-glycoprotein and CYP3A in modulating hepatobiliary drug disposition was examined ex situ using the isolated perfused rat liver (IPRL) system. In contrast to in vitro models like microsomes, hepatocytes and liver slices, the IPRL preserves hepatic architecture, cell polarity and bile flow. Yet the system avoids the influences from in vivo non-hepatic routes and hormonal effects. Wu and Benet[33] examined the transport and metabolism of tacrolimus (1.2μM), an immunosuppressive that is a substrate for both P-glycoprotein and CYP3A, alone and in the presence of transporter and enzyme inhibitors. Troleandomycin (20μM), a specific CYP3A inhibitor, GG918 (1μM) an inhibitor of P-glycoprotein but not CYP3A, and cyclosporine (10μM), a dual CYP3A and P-glycoprotein inhibitor, were added 5 minutes before the addition of tacrolimus. Tacrolimus and its major desmethyl metabolites were measured by LC/MS/MS[33]. Figure 3a depicts the perfusate concentration-time profiles for tacrolimus without (control) and in the presence of the three different P-glycoprotein-CYP3A inhibitors over 30 min. The rat liver rapidly eliminates tacrolimus as the control curve decreases by 2 decades over the 30 min perfusion period. Concomitant troleandomycin increased tacrolimus area under the curve (AUC) 2.3-fold ($p < 0.05$) as would be expected for this CYP3A inhibitor in the liver. As seen in Figure 3A, GG918 decreased the AUC by 23% ($p < 0.05$) presumably by increasing the residence time of tacrolimus in the hepatocytes, as was observed for the cellular studies in Figure 2 where the extraction ratio was increased upon basolateral dosing in the presence of GG918. Cyclosporine, which inhibits both P-glycoprotein and CYP3A, demonstrated a profile between that of troleandomycin- and GG918-treated livers, revealing a composite of the two opposite directions of the interaction.

IPRL perfusions of felodipine, a CYP3A but not a P-glycoprotein substrate, are depicted in Figure 3B. The AUC over the 30 min perfusion in the presence of troleandomycin was increased 40% ($p < 0.05$) versus control (Figure 3B). Since felodipine is not a P-glycoprotein substrate, the curves for control and GG918 are superimposable. Similarly the curves for concomitant cyclosporine and concomitant troleandomycin with tacrolimus yield the same increase in area since we had chosen the inhibitory concentrations of troleandomycin and cyclosporine to yield equipotent CYP3A inhibition. We recognized from the control felodipine study that the difference between cyclosporine and troleandomycin on tacrolimus in Figure 3A results from the combined effects of inhibiting CYP3A metabolism but increasing access to the enzyme by inhibiting the efflux transporter P-glycoprotein. Overall, for tacrolimus the inhibition of the enzyme outweighs the inhibition of the efflux transporter but still demonstrates a difference between the troleandomycin and cyclosporine inhibition effects.

These isolated perfused rat organ studies[32,33] together with the cellular system studies of Cummins et al.[29–31] led to our understanding of the special relationship between the metabolic enzyme and the efflux transporter in the intestine and the liver as depicted in Figure 4[34]. In the

intestine where the efflux transporter is encountered prior to the metabolic enzyme, drugs diffusing into the enterocytes will be effluxed back into the lumen by P-glycoprotein and have another opportunity to diffuse into the enterocytes thereby allowing more metabolites to be formed and less parent traversing the membrane. In contrast in the liver where the drug enters from the basolateral side and encounters the metabolic enzyme prior to efflux into the biliary canaliculi, drugs diffusing into the hepatocytes will be pumped out by P-glycoprotein but not diffuse back in because it would be against a concentration gradient, thus less metabolites are formed and more parent traverses the membrane.

Investigating the Uptake Transporter-Enzyme Interaction Using Cellular and Animal Studies

The focus of our laboratory over the past 15 years has been to uncover and define drug transporter-metabolism interplay. Our focus initially was on the efflux transporter P-glycoprotein, as described above. However, similar efflux transporter-enzyme interplays will occur in the intestine and the liver with the other important drug efflux transporters, BCRP and MRP2. As depicted in Figure 4, drugs enter the hepatocytes in the liver via the basolateral membrane. Thus, we began to investigate the hepatic uptake-enzyme interplay. The hepatic uptake transporters are members of the solute carrier (Slc) family and are generally categorized into three sub families: the organic anion-transporting peptides (OATP in humans/Oatp in rodents), the organic anion transporters (OAT/Oat) and the organic cation transporters (OCT/Oct). When we began our work Oatp1 (gene symbol: *Slc21a1*) Oatp2 (*Slc21a5*), Oatp4 (*Slc21a10*), Oat2 (*Slc21a7*), Oat3 (*Slc21a8*) and Oat1 (*Slc21a1*) had been demonstrated to be expressed in the rat liver and localized in the basolateral membrane[35, 36] Of all the hepatic uptake transporters the Oatp family has been the most extensively studied. Whereas OATs transport mainly low molecular weight compounds, OATPs mediate the uptake of much larger, poorly soluble and structurally diverse substrates such as digoxin[36, 37], erythromycin[38], atorvastatin[39], and glyburide[40]. These four drugs are also substrates for hepatic CYP enzymes (digoxin in rats but not in humans) as well as being substrates for hepatic efflux transporters. To demonstrate the effect of inhibiting the uptake transporter on hepatic metabolism we chose rifampin, which had been previously shown to inhibit Oatp2[36], as our model inhibitor. We demonstrated in rat hepatocytes that inhibition of Oatp2 would markedly decrease digoxin uptake and its metabolism[41]. We then studied the disposition of digoxin in the IPRL and the influence of the Oatp inhibitor rifampin and the P-glycoprotein inhibitor quinidine[42]. Digoxin and its primary metabolite were measured in perfusate and liver by LC/MS. The IPRL AUC from 0 to 60 min was determined. The AUC ± S.D. of digoxin was increased from control (3880±210nM•min) by rifampin (5,200±240nM•min; $p<0.01$) and decreased by quinidine (3,220±340nM•min; $p<0.05$). We concluded that rifampin limited the hepatic entrance of digoxin and reduced hepatic exposure of digoxin to CYP3A by inhibiting the basolateral Oatp2 uptake transporter, whereas quinidine increased the hepatic exposure of digoxin to CYP3A by inhibiting the canalicular P-glycoprotein transport. These data emphasize the importance of uptake and efflux transporters on hepatic drug metabolism[42].

The effects of hepatic uptake and efflux transporters on erythromycin disposition and metabolism were examined by comparing results from rat hepatic microsomes, freshly isolated hepatocytes and in vivo studies[43]. Uptake studies carried out in freshly isolated rat hepatocytes showed that erythromycin and its metabolite (N-desmethyl-erythromycin) are both substrates of Oatp1a4 and Oatp1b2. Whereas rifampin and GG918 exerted minimal effects on metabolism in microsomes, rifampin (2.5μM) and GG918 (0.5μM) significantly decreased and increased erythromycin metabolism in hepatocytes, respectively, as expected based on the digoxin results reported above. The results of the iv bolus dosing studies are given in Table 2. Note that single dose coadministration of rifampin significantly decreased total blood clearance ($p<0.05$), which resulted from decreasing biliary elimination more than 50% ($p<0.001$), having no effect

on renal clearance, while the remaining clearance (CL_{other}, primarily metabolic) also decreased ($p=0.05$). These results would be expected if rifampin inhibited hepatic uptake. With GG918 inhibition both biliary and renal clearance were markedly decreased ($p<0.001$), but unexpectedly we also saw CL_{other} decrease, which is opposite of the results found for digoxin as described above. We will return to this anomalous result in the discussion of studies in humans.

Atorvastatin is primarily metabolized by CYP3A in the liver to two active hydroxyl metabolites, and the sequential transport system governed by hepatic uptake and efflux transporters is important for the drug disposition and metabolism. We initially assessed the interaction of atorvastatin with Oatp and Mrp2 in vitro and ex situ using the IPRL. Rifampin was chosen as the inhibitor for Oatp. Its inhibitory effects on Mrp2 and metabolism were also tested using MRP2-overexpressing cells and rat microsomes, respectively[39]. Our results indicated that inhibition of Oatp-mediated uptake seemed to be the major determinant for the interaction between atorvastatin and rifampin. Metabolites of atorvastatin were subject to Oatp-mediated uptake as well, suggesting that they undergo a similar disposition pathway as the parent drug[39].

Following our cellular and IPRL studies[39] atorvastatin was administered orally (10mg/kg) and intravenously (2mg/kg) to rats in the absence and presence of a single intravenous dose of rifampin (20mg/kg)[44]. Rifampin markedly increased the plasma concentration of atorvastatin and its metabolites when atorvastatin was administered orally. The AUC for atorvastatin also increased significantly after iv dosing of atorvastatin with rifampin, but the extent was much less than that observed for oral atorvastatin dosing. Significant increases in plasma levels were observed for both metabolites as well. The seven-fold higher AUC ratio of metabolites to parent drug following oral versus intravenous atorvastatin dosing suggested that atorvastatin undergoes extensive gut metabolism[44]. Both hepatic and intestinal metabolism contribute to the low oral bioavailability of atorvastatin in rats. In the presence of rifampin the liver metabolic extraction was significantly reduced, most likely because of rifampin's inhibition of Oatp-mediated uptake, which leads to reduced hepatic amounts of parent drug for subsequent metabolism. Gut extraction was also significantly reduced, but we were unable to elucidate the mechanism of this effect because iv rifampin caused gut changes in availability. These studies reinforced our hypothesis that hepatic uptake is a major contributor to the elimination of atorvastatin and its metabolites in vivo.

Uptake Transporter-Enzyme Interaction Using Human Studies

As described earlier, our human studies with immunosuppressive agents and inhibitors and inducers of enzymes and transporters[6,9,10,14–17] were the impetus for our work investigating transporter-enzyme interplay in the intestine and the liver in humans. Predicting drug pharmacokinetics and pharmacodynamics in the individual patient is a worthy goal, and since more than 50% of metabolized drugs are substrates for CYP3A4 the erythromycin breath test (EBT) is a commonly used non-invasive phenotype probe for this enzyme[45]. However, marked variability has been noted when using the EBT to predict metabolism of other CYP3A4 substrates and the resulting drug-drug interactions[46]. We previously hypothesized that the lack of correlation may be due in good part to the effects of the erythromycin uptake and efflux transporters, which alter the quantity of erythromycin available for metabolism[47]. The effects of hepatic uptake and efflux transporters on erythromycin disposition and metabolism in cellular and animal studies were described earlier in this review[43].

Erythromycin metabolism in the liver initially requires uptake into the cells, which is mediated by OATPs[48]; after injection, [^{14}C-*N*-methyl] erythromycin in partially broken down by CYP3A4 to its *N*-demethylated metabolite and ^{14}C-formaldehyde. The radio labeled

formaldehyde is then predominantly converted to ^{14}C-formate and then finally to ^{14}C-CO_2, which is exhaled through the lungs. The majority of the unmetabolized parent drug and the *N*-demethylated metabolites are excreted by the efflux transporter P-glycoprotein. An increase in EBT ^{14}C-CO_2 excretion is considered to be a marker for CYP3A4 induction, whereas a decrease in ^{14}C-CO_2 excretion is considered to be a marker for CYP3A4 inhibition. But the ability of EBT to predict the metabolism of other CYP3A4 substrates may be compromised if these substrates inhibit uptake or efflux transporters, and could cause misleading interpretation of CYP3A enzyme activity. Kurnik et al.[49] reported that concomitant administration of the potent investigational P-glycoprotein inhibitor tariquidar significantly increased EBT values without changing CYP3A4 activity. Frassetto et al.[50] demonstrated that single iv dose rifampin administration decreased EBT values. Thus, concomitant administration of an efflux inhibitor, tariquidar, in the liver prevented erythromycin from being eliminated from the liver and increased metabolism as depicted in Figure 4, while inhibition of the uptake transporter by rifampin prevented erythromycin from entering the liver and caused decreased metabolism. These studies demonstrate that the effects of transporters on EBT will compromise the ability of EBT to be a predictor of the clearance of other CYP3A4 drugs in patients.

Frassetto et al.[50] also showed that concomitant administration of lansoprazole, dosed as a 30mg iv infusion over 30 minutes yielded a significant increase (p=0.018) in the EBT in 8 healthy women. In that study, we dosed lansoprazole as an approved drug P-glycoprotein inhibitor and in women it yielded similar results to that found by Kurnik et al.[49] with the experimental P-glycoprotein inhibitor tariquidar. However, Frassetto et al.[50] found no significant change in the EBT in 8 healthy men, where 4 showed increased EBT values and 4 showed decreased EBT values. In contrast, the Kurnik study was carried out only in men. Thus, some uncertainty concerning erythromycin disposition and transporter effects may still exist, as we noted earlier for the anomalous results in the IPRL[43], where digoxin metabolism was decreased with P-glycoprotein inhibition.

Lau et al.[51] investigated the inhibitory effect of a single rifampin dose on atorvastatin kinetics in 11 healthy volunteers in a randomized, crossover study. Each subject received two 40 mg doses of atorvastatin separated by one week and on one of those occasions, a 30 min infusion of 600 mg rifampin was administered prior to dosing. Plasma concentrations of atorvastatin and its two active hydroxyl metabolites were measured for up to 24 hours after dosing. As depicted in Figure 5, rifampin significantly increased the total AUC of atorvastatin acid by 6.8 ±2.4-fold and that of 2-hydroxy-atorvastatin acid and 4-hydroxy-atorvastatin acid by 6.8±2.5-fold and 3.9±2.4-fold, respectively. The AUC values of the lactone forms of the parent drug and its two active metabolites were also significantly increased, but to a lower extent. The data confirm that OATP1B transporters represent a major hepatic uptake system for atorvastatin and its active metabolites. A discussion of rate limiting steps in the drug transporter metabolism alliance, as exemplified by the atorvastatin studies described here, will be undertaken in the next section of this review.

We have chosen to use rifampin as our model inhibitor of hepatic OATP uptake. We chose rifampin since it is a potent inhibitor of the OATP transporters; it can be dosed in humans via the intravenous route; and we have shown that a single intravenous dose of rifampin has minimal effects on CYP3A4 and other transporters[51]. However, historically, rifampin is well known in the scientific community as an inducer of enzymes and transporters and it is frequently used as the model inductive compound in drug interaction studies for drugs seeking approval from the regulatory agencies. We previously demonstrated in cellular and animal models that this dual uptake inhibitor potential, together with inducing effects, can lead to misinterpretation of the mechanisms involved[52]. Most recently we investigated the effects of single iv doses and multiple oral doses of rifampin on glyburide exposure and blood glucose levels in 9 healthy volunteers[53]. A single iv dose of rifampin significantly increased AUC of

glyburide and its primary metabolite. Blood glucose levels were significantly lower than those observed after dosing with glyburide alone. Multiple doses of rifampin induced an increase in liver enzyme levels, leading to a marked decrease in glyburide exposure and blood glucose levels. When iv rifampin was administered after multiple oral doses of rifampin, the inhibition of hepatic uptake transporters masked the induction effect and the AUCs for glyburide and its hydroxyl metabolite were similar to those seen under control conditions. This study demonstrated how measurement of the levels of both the parent drug and its primary metabolite are useful in unmasking simultaneous drug-drug induction and inhibition effects and in characterizing enzymatic vs. transporter mechanisms.

Characterizing Rate Limiting Processes in the Drug Transporter-Metabolism Alliance

In 2005, Wu and Benet[54] proposed a Biopharmaceutical Drug Disposition Classification System (BDDCS), a modification of the FDA's Biopharmaceutics Classification System (BCS) [55], as a tool for predicting the relevance of transporters and enzymes in drug disposition. The BCS categorizes drugs into four classes according to their solubility and permeability. The FDA uses the BCS system as a science-based approach to allow waiver of in vivo bioavailability and bioequivalence testing of immediate-release solid oral dosage forms for Class 1 high solubility, high permeability drugs, when such drug products also exhibit rapid dissolution[55]. A drug substance is considered “highly soluble” when the highest dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1–7.5 at 37°. A drug substance is considered to be “highly permeable” when the extent of absorption (parent drug plus metabolites) in humans is determined to be ≥90% of an administered dose. Wu and Benet[54] reviewed 130 compounds that had been classified in the literature into the four BCS categories. They recognized that the major route of elimination for the high permeability Class 1 and Class 2 drugs in humans was metabolism, while the major route of elimination for the poorly permeable Class 3 and Class 4 drugs in humans was renal and biliary excretion of unchanged drugs. Since, at least for drugs on the market, the extent of metabolism is better characterized than the extent of absorption, Wu and Benet[54] proposed that drugs be categorized in terms of extent of metabolism and solubility versus permeability and solubility. BDDCS predicts transporter effects in the gut and the liver as shown in Figure 6.

BDDCS predicts that for highly permeable, extensively metabolized, highly soluble Class 1 drugs, transporter effects in the gut and the liver will be minimal and the rate limiting steps of elimination will be related to enzymatic processes. For Class 2 drugs, transporter-enzyme interplay will be most important. As shown in Figure 6 and as described earlier, in the gut the interplay between efflux transporters and intestinal metabolic enzyme will be an important consideration. In the liver, hepatic uptake, hepatic efflux and enzymatic processes may all be important. For Class 3 drugs, uptake transporters effects will predominate but can be modulated by efflux transporters once the drug enters the cell. This prediction was confirmed very recently by Watanabe et al.[56] using isolated hepatocytes, kidney slices, integration plot analyses, and in vivo pharmacokinetic studies using 12 minimally metabolized drugs in rats. The authors conclude: “This study suggests that the uptake process is the determining factor for organ clearance of minimally metabolized drugs, and uptake assays using isolated hepatocytes in kidney slices are useful for evaluating the uptake clearance.” Although Class 3 and 4 drugs, such as erythromycin and furosemide, are predominantly eliminated unchanged in the bile and urine of humans, the relatively low fraction of metabolism that does occur will be predicted based on transporter-enzyme interplay as proposed for Class 2 compounds in BDDCS. For erythromycin this was demonstrated earlier in this review for both animal[43] and human[49,50] studies.

Recently Kusuhara and Sugiyama[57] addressed the rate limiting steps in transporter-mediated organ elimination. The extraction of drugs during a single pass through an organ of elimination is determined by the blood flow rate to the organ, the protein binding of the drug in the blood and the intrinsic ability of the organ to remove drugs from the blood circulation (CL_{int}). Thus the classical relationship would hold for BDDCS Class 1 drugs where transporter effects are minimal. When transporter-metabolism interplay occurs or when transporter-transporter interplay are involved in the organ clearance, Kusuhara and Sugiyama depict in Figure 7 the hepatic relationship and define the in vivo and in vitro techniques that can assist in predicting hepatobiliary drug elimination. When hepatic efflux transporter effects are relevant for Class 2, 3 and 4 drugs, then CL_{int} is the sum of the metabolic intrinsic clearance, $CL_{int,\,met}$, and the transporter related intrinsic efflux clearance into the bile, $PS_{int,\,bile}$, as depicted in Fig. 7. That is,

$$CL_{int} = CL_{int,met} + PS_{int,bile} \quad (1)$$

Now the intrinsic organ clearance ($CL_{int,\,org}$) is given by Eq. 2:

$$CL_{int,org} = [PS_{inf} / (PS_{eff} + CL_{int})] \cdot CL_{int} \quad (2)$$

where PS_{inf} and PS_{eff} represent the intrinsic clearance for cellular uptake (influx) and efflux into the systemic circulation, respectively.

At the boundary condition where $CL_{int} >> PS_{eff}$, i.e., where the great majority of drug taken up by the hepatocytes is metabolized or excreted into bile, then $CL_{int,\,org}$ can be approximated by PS_{inf} :

$$CL_{int,org} \cong PS_{inf} \quad (3)$$

This appears to be the case for the atorvastatin IPRL study[39], in vivo rat studies[44] and our human study[51]. Watanabe et al.[58] concur with this conclusion where using multiple indicator dilution perfusion studies, in vitro rat hepatocyte uptake studies and in vitro rat liver microsomes clearance measurements they determine that the overall in vivo intrinsic clearance measurements approximate the in vitro uptake clearance measurements. They further predict in vivo human hepatic uptake clearance and suggest that these values approximate overall human intrinsic clearance measurements for atorvastatin and three other statins: pravastatin, pitavastatin and fluvastatin. These are excellent ground breaking analyses, however, I suggest that further tests should be applied, incorporating efflux transporter and metabolism inhibition studies such as those carried out in our laboratory, as reviewed here. Drug interaction studies with efflux transporter inhibitors (quinidine with digoxin and GG918 with erythromycin) show significant clearance changes and would not be consistent with hepatic uptake being the rate limiting step.

In Table 2 both the uptake inhibitor rifampin and the efflux inhibitor GG918 increase erythromycin AUC significantly. The decrease in $CL_{biliary}$ and CL_{other} (most likely metabolism) with no change in CL_{renal} is consistent with inhibition of hepatic uptake. We would expect the decrease in $CL_{biliary}$ (53%) and CL_{other} (40%) to be approximately equivalent, which they are considering the variability observed. However, we also see significant decreases in these two clearance measurements with concomitant GG918, which should not be observed if Eq. 3 holds. Here we see that $CL_{biliary}$ decreases 69%, which is very comparable to the decrease in CL_{renal} (71%), suggesting that P-glycoprotein inhibition in the liver and kidney is equivalent. However, CL_{other} now only decreases 37%, obviously not consistent with biliary

and renal changes. These data suggest that other processes may be at work, which are not yet fully defined. For example, rifampin could also be inhibiting basolateral efflux, but GG918 is not.

Our IPRL tacrolimus studies[33] (Fig. 3A) and our digoxin IPRL studies[42] exhibit increased clearance when hepatic efflux is inhibited, which we justified when discussing Fig. 4. So, obviously for the in vivo studies with erythromycin and GG918 (Table 2), the full organ clearance Eq. 2 must be considered. However, we suspect that other interactive processes may be at work that have not yet been adequately defined. For example, the $CL_{int,\ met}$ portion of Eq. 1 is assumed to be independent of transport processes in the derivation of Kusuhara and Sugiyama[57] and in all other treatments of intrinsic metabolic clearance. This is emphasized in the first derivation of intrinsic clearance as proposed by Rowland et al.[59], where the partition coefficient between unbound concentration in the measured systemic circulation and that in the eliminating organ is assumed to be constant (usually = 1). However, inhibition, induction or activation of transporters could potentially change this ratio so that, in essence, what we now call intrinsic metabolic clearance is not intrinsic, but rather varies with transporter changes. Our studies with atorvastatin in humans[51] not only showed inhibition by rifampin of atorvastatin uptake into the liver, but also caused a change of the ratio of the atorvastatin lactone to the atorvastatin acid, thereby changing the amount of substrate available to the enzymes in the liver since as we have shown earlier, metabolism by CYP3A4 of atorvastatin occurs via the lactone, not the acid[60]. The present analysis follows our recently published reviews of the role of transporters in the pharmacokinetics of orally administered drugs[61] and the effects of drug transporters on volume of distribution[62].

Conclusions

Transporter-enzyme interplay and its importance in drug disposition were recognized in the early 1990s. Since then a number of investigators have elucidated the importance of this process via cellular, isolated organ, whole animal and human studies. Here we have reviewed primarily the studies carried out in our laboratory over the past two decades to uncover and define the importance of this phenomenon with respect to the intestine and the liver characterizing inhibition and induction of apical and basolateral transporters and how drug metabolism can change independent of any change in the metabolic enzymes. Finally, we have pointed out some areas that require further definition in understanding and predicting the effects of transporter-enzyme interplay on drug disposition.

Acknowledgments

This work was supported in part by NIH Grants GM075900 and GM061390, and by an unrestricted grant from Amgen.

References

(1). Wacher VJ, Salphati L, Benet LZ. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv. Drug Del. Rev 1996;20:99–112.

(2). Benet LZ, Wu C-Y, Hebert MF, Wacher VJ. Intestinal drug metabolism and antitransport processes: a potential paradigm shift in oral drug delivery. J. Control. Rel 1996;39:139–143.

(3). Gupta SK, Manfro RC, Tomlanovich SJ, Gambertoglio JG, Garovoy MR, Benet LZ. Effect of food on the pharmacokinetics of cyclosporine in healthy subjects following oral and intravenous administration. J. Clin. Pharmacol 1990;30:643–653. [PubMed: 2391396]

(4). Gupta SK, Benet LZ. Absorption kinetics of cyclosporine in healthy volunteers. Biopharm. Drug Dispos 1989;10:591–596. [PubMed: 2611359]

(5). Gupta SK, Benet LZ. High fat meals increase the clearance of cyclosporine. Pharm. Res 1990;7:46–48. [PubMed: 2300535]

(6). Hebert MF, Roberts JP, Prueksaritanont T, Benet LZ. Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. Clin. Pharmacol. Ther 1992;52:453–457. [PubMed: 1424418]

(7). Kolars JC, Schmiedlin-Ren P, Scheutz JD, Fang C, Watkins PB. Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. J. Clin. Invest 1992;90:1871–1878. [PubMed: 1430211]

(8). Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, Merion RM, Watkins PB. CYP3A gene expression in human gut epithelium. Pharmacogenomics 1994;4:247–259.

(9). Gomez DY, Wacher VJ, Tomlanovich SJ, Hebert MF, Benet LZ. The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine. Clin. Pharmacol. Ther 1995;58:15–19. [PubMed: 7628178]

(10). Wu C-Y, Benet LZ, Hebert MF, Gupta SK, Rowland M, Gomez DY, Wacher VJ. Differentiation of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporine. Clin. Pharmacol. Ther 1995;58:492–497. [PubMed: 7586942]

(11). Wacher VJ, Wu C-Y, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-Glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol. Carcinog 1995;13:129–134. [PubMed: 7619215]

(12). Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinatedly upregulate these proteins in human colon carcinoma cells. Mol. Pharmacol 1996;49:311–318. [PubMed: 8632764]

(13). Schuetz EG, Schinkel AH, Relling MV, Schuetz JD. P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and humans. Proc. Natl. Acad. Sci. USA 1996;93:4001–4005. [PubMed: 8633005]

(14). Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, Roberts JP, Hebert MF. Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. Clin. Pharmacol. Ther 1997;62:41–49. [PubMed: 9246018]

(15). Hebert MF, Fisher RM, Marsh CL, Dressler D, Berkersky I. Effects of rifampin on tacrolimus pharmacokinetics in healthy volunteers. J. Clin. Pharmacol 1999;39:91–96. [PubMed: 9987705]

(16). Floren LC, Christians U, Zimmerman JJ, Neefe L, Schorer R, Rushworth D, Harper D, Renz J, Benet LZ. Sirolimun oral bioavailability increases ten-fold with concomitant ketoconazole. Clin. Pharmacol. Ther 1999;65:159.

(17). Chang T, Benet LZ, Hebert MF. Effect of water-soluble vitamin E on cyclosporine pharmacokinetics in healthy volunteers. Clin. Pharmacol. Ther 1996;59:297–303. [PubMed: 8653992]

(18). Dintaman JM, Silverman JA. Inhibition of P-glycoprotein by D-α-tocopheryl polyethylene glycol 100 succinate (TPGS). Pharm. Res 1999;16:1550–1556. [PubMed: 10554096]

(19). Zhang Y, Benet LZ. The gut as a barrier to drug absorption. Clin. Pharmacokinet 2001;40:159–168. [PubMed: 11327196]

(20). van Asperen J, van Tellingen O, Sparreboom A, Schinkel AH, Borst P, Nooijen WJ, Beijnen JH. Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. Br. J. Cancer 1997;76:1181–1183. [PubMed: 9365166]

(21). Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, Borst P, Nooijen WJ, Beijnen JH, van Tellingen O. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc. Natl. Acad. Sci. USA 1997;94:2031–2035. [PubMed: 9050899]

(22). Yamaji H, Matsumura Y, Yoshikawa y. Takada K. Pharmacokinetic interactions between HIV protease inhibitors in rats. Biopharm. Drug Dispos 1999;20:241–247. [PubMed: 10594868]

(23). Lin JH, Chiba M, Chen IW, Nishime JA, deLuna FA, Yamazaki M, Lin YJ. Effect of dexamethasone on the intestinal first-pass metabolism of indinavir in rats: evidence of cytochrome P-4503A and P-glycoprotein induction. Drug Metab. Dispos 1999;27:1187–1193. [PubMed: 10497146]

(24). Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Romen DM, Wilkinson GR. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J. Clin. Invest 1998;101:289–294. [PubMed: 9435299]

(25). Zhang Y, Hsieh Y, Isumi T, Lin ET, Benet LZ. Effects of ketoconazole on the intestinal metabolism, transport and oral bioavailability of K02, a novel vinylsulfone peptidomimetic cysteine protease

inhibitor and a P450 3A, P-glycoprotein dual substrate, in male sprague-dawley rats. J. Pharmacol. Exp. Ther 1998;287:246–252. [PubMed: 9765344]

(26). Salphati L, Benet LZ. Effects of ketoconazole on digoxin absorption and disposition in rat. Pharmacology 1998;56:308–313. [PubMed: 9654217]

(27). Song S, Suzuki H, Kawai R, Sugiyama Y. Effect of PSC 833, a P-glycoprotein modulator, on the disposition of vincristine and digoxin in rats. Drug Metab. Dispos 1999;27:689–694. [PubMed: 10348798]

(28). Artursson P, Borchardt RT. Intestinal drug abosorption and metabolism in cell cultures: Caco-2 and beyond. Pharm. Res 1997;14:1655–1658. [PubMed: 9453050]

(29). Cummins CL, Mangravite LM, Benet LZ. Characterizing the expression of CYP3A4 and efflux transporters (P-gp, MRP1, and MRP2) in CYP3A4-transfected Caco-2 Cells after induction with sodium butyrate and the phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Pharm. Res 2001;18:1102–1109. [PubMed: 11587480]

(30). Cummins CL, Jacobsen W, Benet LZ. Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. J. Pharmacol. Exp. Ther 2002;300:1036–1045. [PubMed: 11861813]

(31). Cummins CL, Jacobsen W, Christians U, Benet LZ. CYP3A4-transfected Caco-2 cells as a tool for understanding biochemical absorption barriers: studies with sirolimus and midazolam. J. Pharmacol. Exp. Ther 2004;308:143–155. [PubMed: 14569063]

(32). Cummins CL, Salphati L, Reid MJ, Benet LZ. In vivo modulation of intestinal CYP3A metabolism by P-glycoprotein: studies using the rat single-pass intestinal perfusion model. J. Pharmacol. Exp. Ther 2003;305:306–314. [PubMed: 12649383]

(33). Wu C-Y, Benet LZ. Disposition of tacrolimus in isolated perfused rat liver: influence of troleandomycin, cyclosporine, and GG918. Drug Metab. Dispos 2003;31:1292–1295. [PubMed: 14570757]

(34). Benet LZ, Cummins CL, Wu C-Y. Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. Int. J. Pharm 2004;277:3–9. [PubMed: 15158963]

(35). Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S, Giacomini KM. Cloning and functional expression of a human liver organic cation transporter. Mol. Pharmacol 1997;51:913–921. [PubMed: 9187257]

(36). Shitara Y, Sugiyama D, Kusuhara H, Kato Y, Abe T, Meier PJ, Itoh T, Sugiyama Y. Comparative inhibitory effects of different compounds on rat oatpl (slc21a1)- and Oatp2 (Slc21a5)-mediated transport. Pharm. Res 2002;19:147–153. [PubMed: 11883641]

(37). Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. Biochim. Biophys. Acta 2003;1609:1–18. 2003. [PubMed: 12507753]

(38). Sun H, Huang Y, Frassetto L, Benet LZ. Effects of uremic toxin on hepatic uptake and metabolism of erythromycin. Drug Metab. Dispos 2004;32:1239–1246. [PubMed: 15286055]

(39). Lau YY, Okochi H, Huang Y, Benet LZ. Multiple transporters affect the disposition of atorvastatin and its two active hydroxy metabolites: application of in vitro and ex situ systems. J. Pharmacol. Exp. Ther 2006;316:762–771. [PubMed: 16258024]

(40). Satoh H, Yamashita F, Tsujimoto M, Murakami H, Koyabu N, Ohtani H, Sawada Y. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. Drug Metab. Dispos 2005;33:518–523. [PubMed: 15640378]

(41). Lam JL, Benet LZ. Hepatic microsome studies are insufficient to characterize in vivo hepatic metabolic clearance and metabolic drug-drug interacations: studies of digoxin metabolism in primary rat hepatocytes versus microsomes. Drug Metab. Dispos 2004;32:1311–1316. [PubMed: 15483198]

(42). Lau YY, Wu C-Y, Okochi H, Benet LZ. Ex situ inhibition of hepatic uptake and efflux significantly changes metabolism: hepatic enzyme-transporter interplay. J. Pharmacol. Exp. Ther 2004;308:1040–1045. [PubMed: 14634033]

(43). Lam JL, Okochi H, Huang Y, Benet LZ. In vitro and in vivo correlation of hepatic transporter effects on erythromycin metabolism: characterizing the importance of transporter-enzyme interplay. Drug Metab. Dispos 2006;34:1336–1344. [PubMed: 16698890]

(44). Lau YY, Okochi H, Huang Y, Benet LZ. Pharmacokinetics of atorvastatin and its hydroxy metabolites in rats and the effects of concomitant rifampicin single doses: relevance of first-pass

effect from hepatic uptake transporters, and intestinal and hepatic metabolism. Drug Metab. Dispos 2006;34:1175–1181. [PubMed: 16624870]

(45). Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, Guzelian PS. Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450. J. Clin. Invest 1989;83:688–697. [PubMed: 2913056]

(46). Rivory LP. Erythromycin breath test. Clin. Pharmacol. Ther 2001;70:395–399. [PubMed: 11673756]

(47). Benet LZ. There are no useful CYP3A probes that quantitatively predict the in vivo kinetics of other CYP3A substrates and no expectation that one will be found. Mol. Interv 2005;5:79–83. [PubMed: 15821156]

(48). Baker AL, Kotake AN, Schoeller DA. Clinical utility of breath tests for the assessment of hepatic function. Semin. Liver Dis 1983;3:318–329. [PubMed: 6359427]

(49). Kurnik D, Wood AJJ, Wilkinson GR. The erythromycin breath test reflects P-glycoprotein function independently of cytochrome P450 3A activity. Clin. Pharmacol 2006;80:228–234.

(50). Frassetto LA, Poon S, Tsourounis C, Valera C, Benet LZ. Effects of uptake and efflux transporter inhibition on erythromycin breath test results. Clin. Pharmacol. Ther 2007;81:828–832. [PubMed: 17361125]

(51). Lau YY, Huang Y, Frassetto L, Benet LZ. Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. Clin. Pharmacol. Ther 2007;81:194–204. [PubMed: 17192770]

(52). Lam JL, Shugarts SB, Okochi H, Benet LZ. Elucidating the effect of final-day dosing of rifampin in induction studies on hepatic drug disposition and metabolism. J. Pharmacol. Exp. Ther 2006;319:864–870. [PubMed: 16905688]

(53). Zhang HX, Huang Y, Frassetto LA, Benet LZ. Elucidating rifampin's inducing and inhibiting effects on glyburide pharmacokinetics and blood glucose in healthy volunteers: unmasking the differential effects of enzyme induction and transporter inhibition for a drug and its primary metabolite. Clin. Pharmacol. Ther 2009;85:78–85. [PubMed: 18843263]

(54). Wu C-Y, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a Biopharmaceutics Drug Disposition Classification System. Pharm. Res 2005;22:11–23. [PubMed: 15771225]

(55). Food and Drug Administration. Guidance for Industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. Food and Drug Administation; Rockville, MD: 2000. Retrieved from www.fda.gov/cder/guidance/index.htm

(56). Watanabe T, Maeda K, Kondo T, Nakayama H, Horita S, Kusuhara H, Sugiyama Y. Prediction of the hepatic and renal clearance of transporter substrates in rats using in vitro uptake experiments. Drug Metab. Dispos 2009;37:1471–1479. [PubMed: 19364828]

(57). Kusuhara H, Sugiyama Y. In vitro-in vivo extrapolation of transporter-mediated clearance in the liver and kidney. Drug Metab. Pharmacokinet 2009;34:1336–1344.

(58). Watanabe T, Kusuhara H, Maeda K, Kanamaru H, Saito Y, Hu Z, Sugiyama Y. Investigation of the rate-determining process in the hepatic elimination of HMG-CoA reductase inhibitors in rats and humans. Drug Metab. Dispos. 2010 in press.

(59). Rowland M, Benet LZ, Graham GG. Clearance concepts in pharmacokinetics. J. Pharmacokinet. Biopharm 1973;1:123–135. [PubMed: 4764426]

(60). Jacobsen W, Kuhn B, Soldner A, Kirchner G, Sewing K-F, Kollman PA, Benet LZ, Christians U. Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin. Drug Metab. Dispos 2000;28:1369–1378. [PubMed: 11038166]

(61). Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. Pharm. Res 2009;26:2039–2054. [PubMed: 19568696]

(62). Grover A, Benet LZ. Effects of drug transporters on volume of distribution. AAPS J 2009;11:250–261. [PubMed: 19399628]

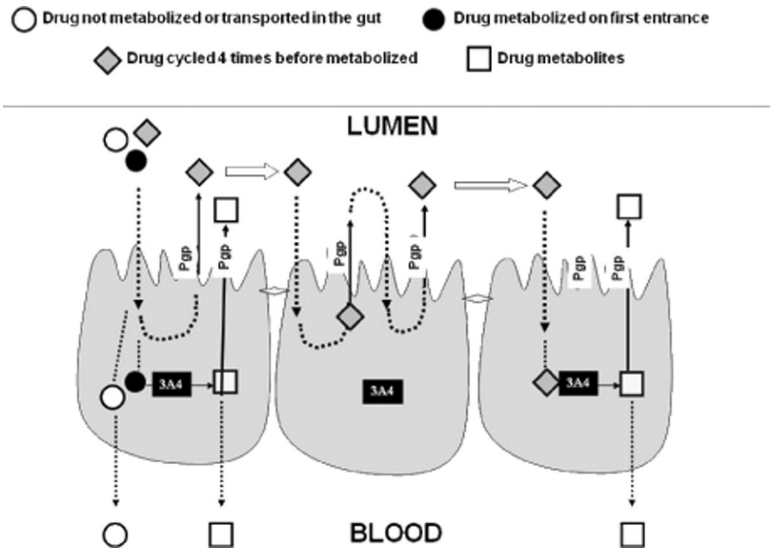

Figure 1.
Cartoon depicting CYP3A4 and P-glycoprotein interplay in the enterocytes.

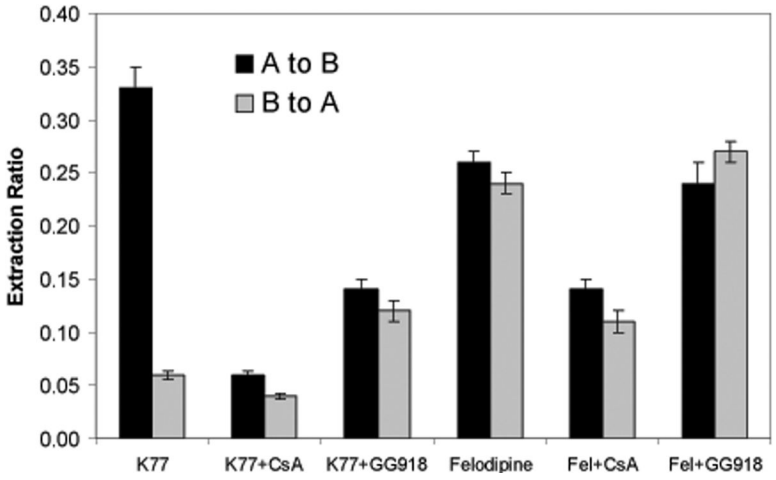

Figure 2.

Extraction ratios of K77 and felodipine after an apical or basolateral dose.[30] The effect of inhibiting P-glycoprotein on the extraction ratio is unmasked by incubating the substrates with GG918, whereas the dual effect of inhibiting CYP3A4 and P-glycoprotein on the extraction ratio is elucidated by inhibiting with cyclosporine (CsA). Data are presented as the mean ± S.D. (*n*=3).

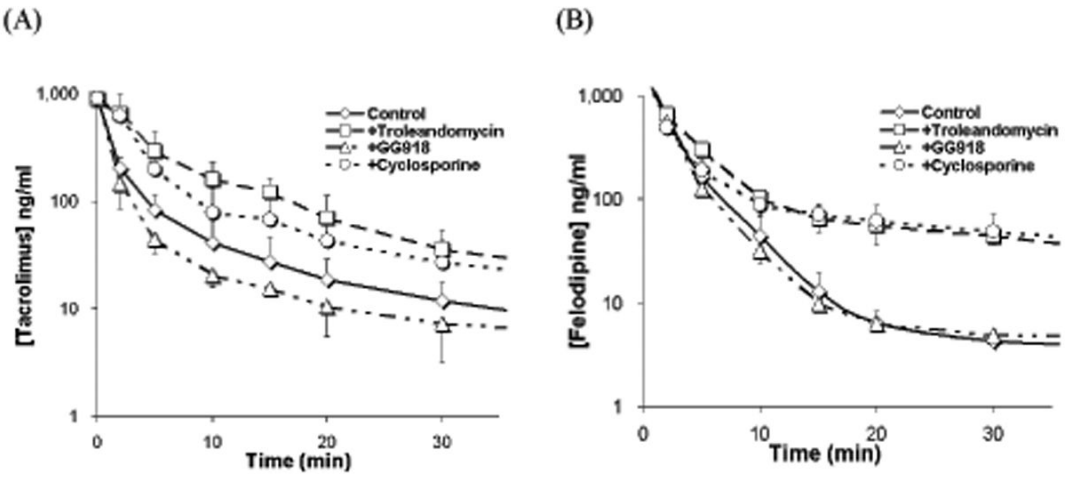

Figure 3.

Perfusate concentration-time profile for tacrolimus (A) and felodipine (B) without (control) and in the presencne of three different P-glycoprotein/CYP3A inhibitors over 30 min[33].

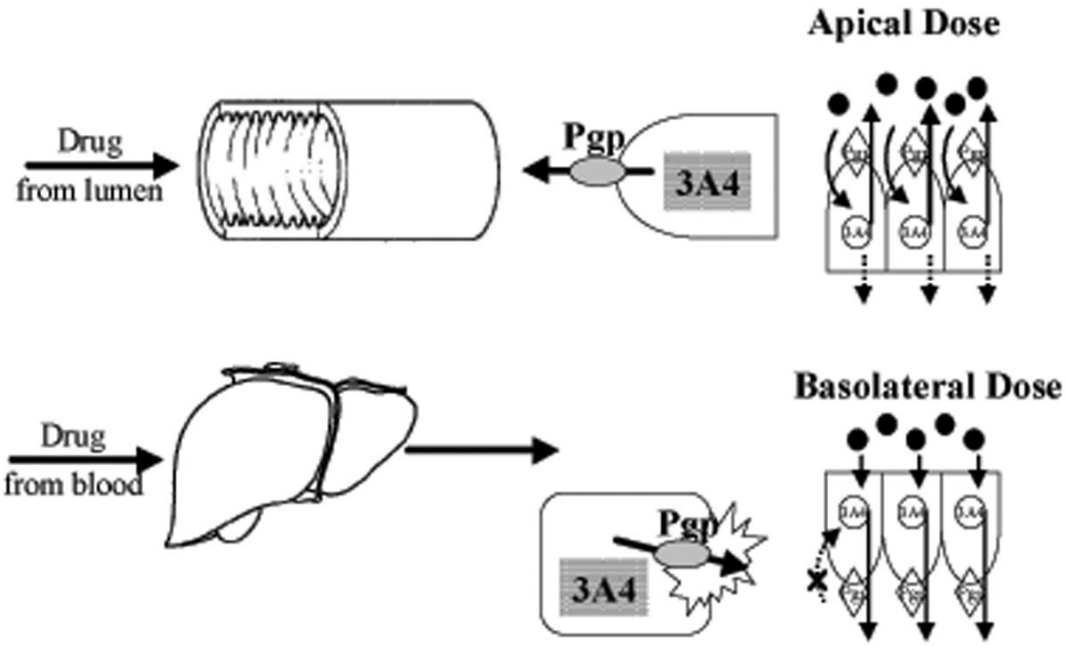

Figure 4.
The special relationship between the enzyme and efflux transporter in the intestine and the liver[34].

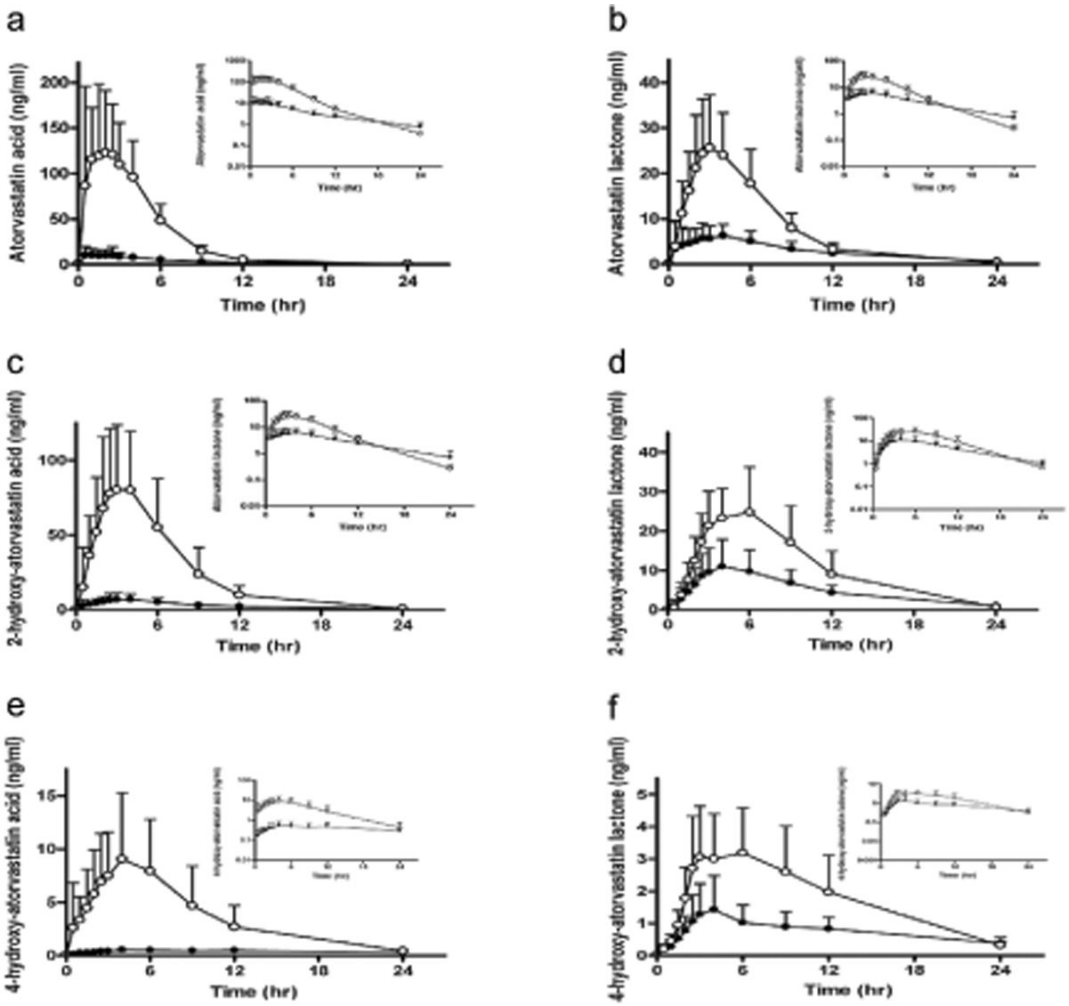

Figure 5.

Mean plasma concentrations (± S.D.) of (A) atorvastatin acid; (B) atorvastatin lactone; (C) 2-hydroxy-atorvastatin acid; (D) 2-hydroxy-atorvastatin lactone; (E) 4-hydroxy-atorvastatin acid, and (F) 4-hydroxy-atorvastatin lactone in 11 healthy volunteers after a single oral dose of 40 mg atorvastatin with and without 600 mg rifampin given as an intravenous 30-min infusion. The insets depicts the same data on a semilogarithmic scale. Solid circles indicate the atorvastatin-alone control group; open circles indicate the rifampin-treatment group.

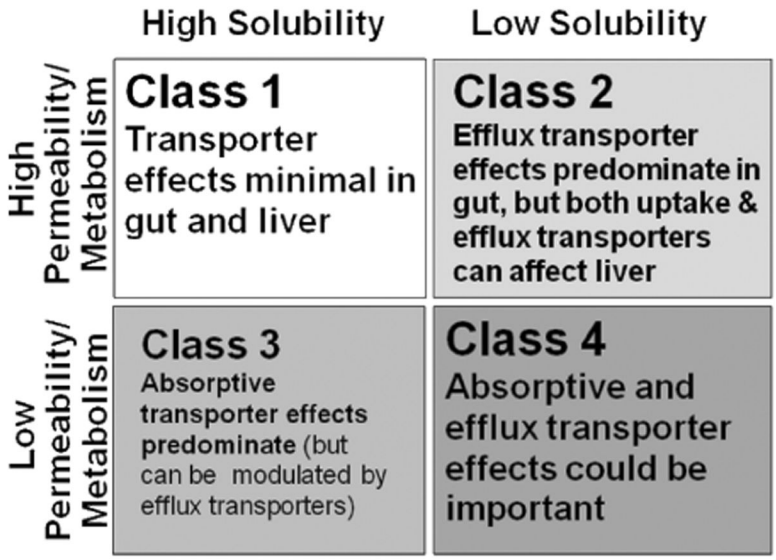

Figure 6.
Transporter effects predicted by BDDCS in the gut and the liver.

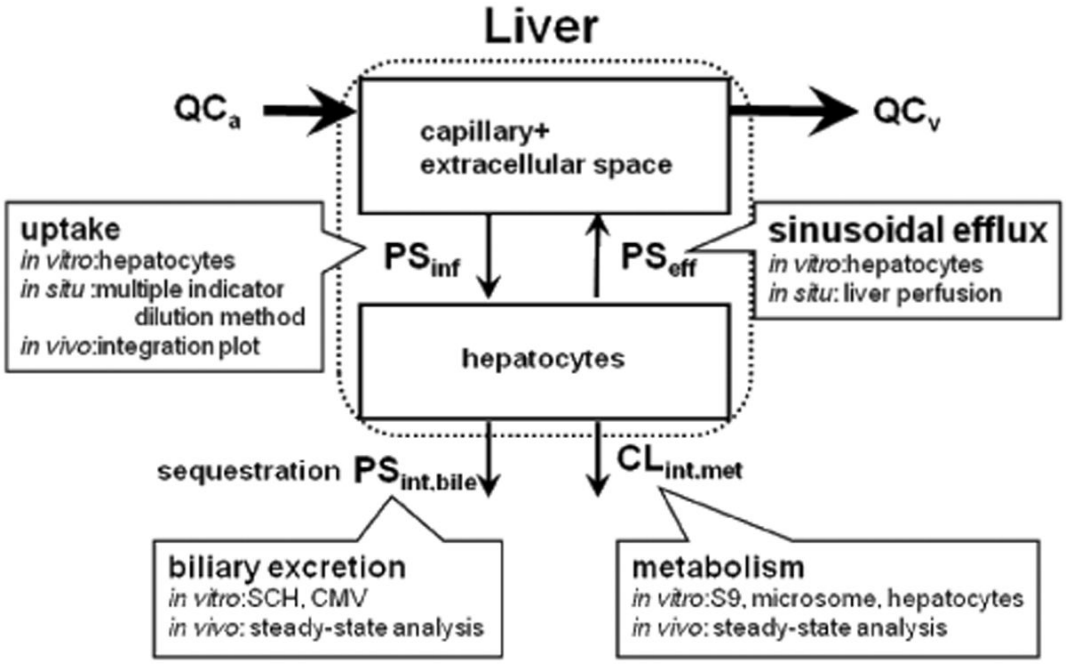

Figure 7.

In vivo and in vitro techniques to determine the scaling factors for the hepatobiliary transport of drugs. Q: hepatic blood flow rate, Ca: drug concentration in the arterial blood, Cv: drug concentration in the hepatic venous blood, PS_{inf}: intrinsic uptake clearance, PS_{eff}: intrinsic clearance for sinusoidal efflux, PSint, bile: intrinsic clearance for the biliary excretion, $CL_{int,\,met}$: intrinsic clearance for metabolism.

Table 1

Extraction ratios and characteristics of CYP3A and P-glycoprotein substrates tested across CYP3A4-Caco-2 cells[30,31]

Drug	Substrate for		Efflux ratio	Extraction ratio % (S.D.)		
	3A4[a]	P-gp[b]	B to A/A to B	Drug alone	Drug + cyclosporine	Drug + GG918
K77[c] [10μM]	Yes	Yes	9	33(3)	5.7(0.3)	14(1)
Sirolimus [lμM]	Yes	Yes	2.5	60(5)	15(1)	45(1)
Midazolam [3μM]	Yes	No	1	25(2)	10(1)	23(2)
Felodipine [l0μM]	Yes	No	1	26(1)	14(1)	24(2)

[a] 3A4, CYP3A4.

[b] P-gp, P-glycoprotein.

[c] K77, K11777: *N*-methyl-piperazine-Phe-homoPhe-vinylsulfone-phenyl.

TABLE 2

Pharmacokinetic parameters of erythromycin in rats after i.v. dosing with and without rifampin or GG918 co-administration[43]. Data are depicted as the mean ± S.D, n = 6.

PK Parameters	Rifampin group		GG918 group	
	Erythromycin Only	With Rifampin	Erythromycin only	With GG918
$t_{1/2}$ (min)	151 ± 27	219 ± 47*	124 ± 33	250 ± 77*
$AUC_{0\text{-inf}}$ (μg/ml · min)	224 ± 55	348 ± 73**	240 ± 29	537 ± 221**
CL_{blood} (ml/min/kg)	47.2 ± 12.5	29.8 ± 6.1*	42.1 ± 5.7	21.7 ± 9.0**
$MRT_{0\text{-inf}}$(min)	147 ± 27	237 ± 66*	109 ± 28	242 ± 135*
V_{ss}(l/kg)	6.30 ± 1.12	6.80 ± 1.11	4.51 ± 0.98	3.94 ± 1.28
$CL_{biliary}$ (ml/min/kg)	15.5 ± 2.9	7.23 ± 1.46***	11.2 ± 2.0	3.50 ± 1.16***
CL_{other} (ml/min/kg)	25.4 ± 9.0	15.3 ± 6.7†	26.9 ± 5.8	17.0 ± 8.5*
CL_{renal} (ml/min/kg)	6.26 ± 2.18	7.21 ± 2.44	4.02 ± 0.93	1.18 ± 0.61***
$CL_{renal,\ met}$ (ml/min/kg)	0.644 ± 0.127	0.691 ± 0.090	0.691 ± 0.192	0.421 ± 0.183*

† $p = 0.05$

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$, significantly different from erythromycin only.