JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 429–433

Received December 5, 2002
Accepted December 31, 2002
DOI 10.1211/002235702973
ISSN 0022-3573

Commentary

Intestinal drug absorption and bioavailability: beyond involvement of single transport function

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Intestinal permeability (P_{eff}) is one of the key biopharmaceutic parameters that determine the in-vivo rate and extent of intestinal drug absorption in man and animals (Amidon et al 1995; Lennernäs 1998). The physicochemical properties of a drug and the complex physiological and biochemical conditions of the gastrointestinal tract together will determine in-vivo intestinal P_{eff} (Lennernäs 1998; Winiwarter et al 1999). The intestinal P_{eff} is often a value based on multiple parallel transport processes such as passive transcellular diffusion, carrier-mediated absorption and carrier-mediated efflux (Figure 1). The relation between various absorption mechanisms may differ along the intestinal tract. During the past decade a significant research effort has been made to investigate the effect of cellular carrier efflux on the pharmacokinetics of drugs and their metabolites (Suzuki & Sugiyama 2000; Borst & Elfebrink 2002). Most of our mechanistic understanding of intestinal efflux has been obtained from studies in cell cultures and knockout animals (Hunter et al 1993; Kim et al 1998; Fromm et al 2000; van Tellingen 2001; Fricker & Miller 2002). Unfortunately, comparisons with the complex in-vivo situation are rare (Lindahl et al 1996; Sandström et al 1998, 1999; Chiou et al 2001; Yu et al 2002; Wacher et al 2002; Tannergren et al 2003). The in-vivo evaluation of the role of drug transporters in pharmacokinetics suffers from the lack of good in-vivo probe molecules and specific transport inhibitors. There is also a need to use other in-vivo/in-situ approaches besides plasma concentration measurement since this may not give enough resolution for mechanistic assessment (Tucker et al 2001). Intestinal perfusion techniques (such as Loc-I-Gut), for instance, may enable significantly more direct in-vivo investigation of biliary secretion and intestinal absorption and efflux, without the influence of gastrointestinal motility, pH changes and metabolism (Lennernäs et al 1992; Lennernäs 1998; Tannergren et al 2003). This commentary will briefly discuss the clinical significance of the carrier-mediated efflux on intestinal absorption and first-pass gut wall metabolism of drugs.

The human intestinal epithelium expresses efflux proteins that may limit the absorption of xenobiotics and secrete intracellularly formed metabolite(s), including conjugates, back into the intestinal lumen. The multidrug-resistant transporter gene MDR1 (human genome (HUGO) nomenclature: adenosine triphosphate (ATP)-binding cassette transporter gene ABCB1), which codes for P-glycoprotein (P-gp), is the most extensively studied transporter gene. However, there are other multidrug transporters involved, such as the multidrug-resistant protein family (MRP1–MRP9 or ABCC1–ABCC9) and breast-cancer-resistant protein (BCRP1 or ABCG2). These ABC transporters are also expressed in numerous tissues such as the liver, kidney, testes, placenta and blood–brain barrier and are expected to affect the disposition of drugs and their metabolites (Suzuki & Sugiyama 2000; Borst & Elfebrink 2002).

Several reports based on in-vitro investigations in various cell models have suggested that carrier-mediated intestinal efflux may be a major reason for incomplete absorption and variable bioavailability of drugs, as well being a target for drug–drug and specific food–drug interactions (Kim et al 1998; Spahn-Langguth and Langguth 2001; Cummins et al 2002). It has also been suggested that apical efflux and intracellular metabolism may work in concert to increase the intestinal first-pass effect (e.g., in $F = fa\,(1–E_G)\,(1–E_H)$ where fa is the fraction dose absorbed, E_G is the extraction in the gut wall and E_H is the extraction in the liver) (Zhang & Benet 2001). However, in-vivo evidence of the clinical significance of carrier-mediated intestinal efflux as a general detoxification process affecting the absorption/bioavailability of a large number of drugs has also been challenged (Sandstrom et al 1998; Chiou et al 2001; Yu et al 2002).

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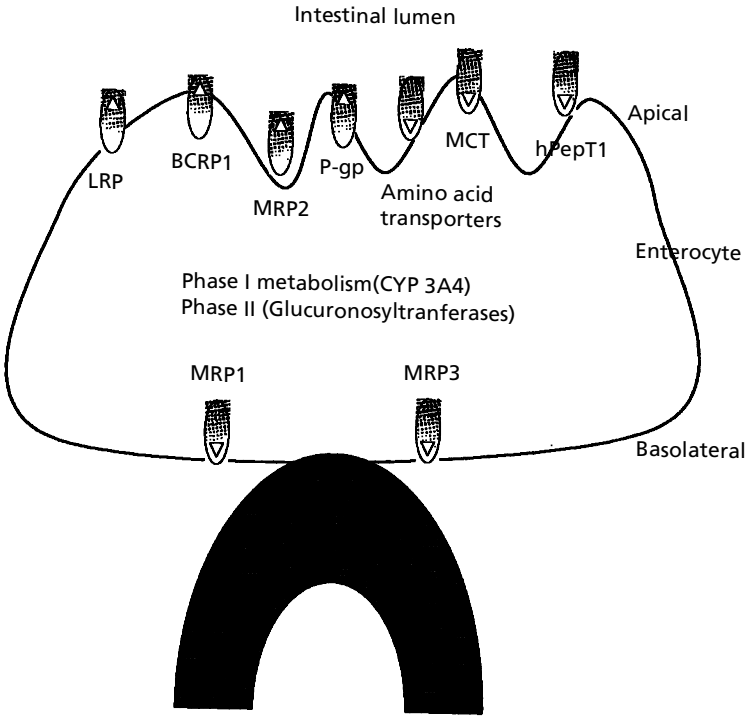

Figure 1 The intestinal permeability (P_{eff}) of drugs in-vivo is the total transport parameter that be will be determined by different transport mechanisms acting in both directions. A few of the most important transport proteins that may be involved in the intestinal transport of drugs and their metabolites across intestinal epithelial membrane barriers in man are displayed. P-gp, P-glycoprotein; BCRP1, breast-cancer-resistant protein; LRP, lung-cancer-resistant protein; MRP1–3, multidrug resistant protein family; hPepT1, oligopeptide carrier for di- and tripeptides; MCT, $H^{(+)}$-monocarboxylic acid co-transporter. CYP 3A4 is the important intracellular oxidation CYP P450 enzyme to which approximately 50–60% of all clinically used drugs are substrates. The Phase II metabolism will produce hydrophilic conjugates that often are excreted by various membrane efflux carrier proteins.

In reality, few human studies have found clinical significance in the intestinal efflux mechanisms for bioavailability and drug–drug interactions (Gramatte et al 1996; Gramatte & Oertel 1999; Greiner et al 1999; Boyd et al 2000; Hoffmeyer et al 2000; Schwarz et al 2000; Sakaeda et al 2001; Tucker et al 2001). Many drugs that were initially suggested to undergo significant efflux in-vitro were later shown to be completely absorbed in-vivo (Wacher et al 2002; Yu et al 2002; Petri & Lennernäs 2003). This apparent discrepancy between in-vitro and in-vivo results may be due to several factors: a large difference between conditions for absorption in-vivo and in-vitro (e.g. expression and function of various proteins, pH and blood flow); the concentration of an investigated drug used in-vitro having been significantly lower than that used in-vivo; a larger contribution of passive transcellular diffusion to in-vivo P_{eff}; a poorly defined pharmacological and physiological function of some efflux proteins along the intestine; difficulties in separating transport and metabolic processes in pharmacokinetic models; and a correlation between in-vitro efflux activity and in-vivo absorption not having been established, most likely owing to the low number of in-vivo studies performed, with more direct approach(es) than standard plasma pharmacokinetic investigations (Lennernäs et al 1992).

Drugs which do not undergo metabolism but which have a high specificity for a transporter are needed, together with direct in-vivo methods, for accurate investigations of the clinical significance of transporters in pharmacokinetics (Tucker et al 2001). For instance, digoxin, talinolol and fexofenadine have been suggested as specific probe compounds for assessing the phenotype for P-gp significance (Gramatte et al 1996; Greiner et al 1999; Hamman et al 2001). A good example of the clinical significance of ABC transporters was studied by Greiner et al (1999), who observed that the plasma pharmacokinetics of orally administered digoxin were significantly lower during rifampin treatment. This finding was attributed to an increased expression of intestinal P-gp since rifampin acts upon the human pregnane X receptor (hPXR). It has also been shown that a polymorphism in exon 26 (C34 35T) can result in a decreased level of intestinal expression of P-gp, along with an increased oral bioavailability of digoxin (Hoffmeyer et al 2000; Sakaeda et al 2001). Atorvastatin, verapamil and quinidine have likewise been shown to affect the pharmacokinetics of digoxin in man (Angelin et al 1987; Hedman et al 1990, 1991; Boyd et al 2000). In these reports the drug–drug interactions have been suggested to be due to inhibition of a transporter (such as P-gp) in either the intestine or the liver. The truth may be that both organs are involved, as well as an influx carrier being involved in the liver, where the organic anion transport polypeptide

(OATP) located at the sinusoidal membrane may play a role. In general, drug metabolism adds a further complexity in clinical studies aiming to elucidate the role of transporters. Inhibition of intestinal efflux (especially of P-gp) has been reported to be the major cause behind an increased bioavailability when certain drugs were co-administered (Floren et al 1997; Watkins 1997; Kim et al 1998). However, in many of these reports the most likely main mechanism was inhibition of CYP 3A4. The role of the cellular efflux in-vivo at the intestinal level remains unclear (van Zuylen et al 2000). The reason for this over-interpretation of the role of enterocyte efflux activity on intestinal drug absorption may be a high passive membrane diffusion of the parent drug and the overlapping specificities of the substrates and inhibitors to both CYP 3A4 and P-gp (Sandström et al 1998; Wacher et al 1995). Additional factors may be saturation of the efflux carrier due to high drug concentration in the intestinal lumen (Sandström et al 1998; Yu et al 2002).

The jejunal permeability and the intestinal and hepatic extraction of fluvastatin, a drug completely metabolised by CYP 2C9, have been investigated in man by using the Loc-I-Gut technique (Lennernäs et al 1992; Lindahl et al 1996). The human in-vivo P_{eff} ($2.4 \pm 1.4 \times 10^{-4}$ cm s^{-1}) was high, despite a significant in-vitro intestinal efflux mediated by MRP2 (efflux ratio > 5 at a clinically relevant concentration in Caco-2 cells) (Lindahl et al 1996; unpublished data). This was most likely due to the significant contribution of passive intestinal permeability to the overall absorption of fluvastatin, which is in accordance with its lipophilic properties (acid pK_a 4.3, log $D_{6.5}$ 2.0, PSA 81 $Å^2$, HBD 3) (Lindahl et al 1996; Winiwarter et al 1998). A similar pattern has been shown for other drugs investigated with the Loc-I-Gut perfusion technique. For instance, the jejunal P_{eff} for ciclosporin, a well-known CYP 3A4 and P-gp substrate, has been found to be high despite the drug being subjected to significant efflux in in-vitro cell models (Fricker et al 1996; Lown et al 1997; Sun et al 2002). This may be due to a high contribution of passive diffusion as well as to the fact that these intestinally located efflux proteins may be easily saturated owing to high drug concentrations adjacent to the intestinal membrane.

Highly passive permeable drugs are often lipophilic and will often also undergo significant drug metabolism (van der Waterbeemd et al 2001). The main enzyme in the small intestine is CYP 3A4 and it has been suggested to work in concert with intestinal efflux proteins (such as P-gp) to increase the intestinal first-pass extraction (via apical recycling) (Wacher et al 1995; Zhang & Benet 2001). Verapamil has been shown to be mainly transported by passive diffusion and to be only minimally effluxed by P-gp (Sandström et al 1998). However, verapamil is still subjected to significant first-pass gut-wall extraction (about 50%) in the human small intestine (Fromm et al 1996; Sandström et al 1998; von Richter et al 2001). This suggests that the apical recycling hypothesis is not applicable as a major explanation for the high degree of intestinal metabolism of verapamil.

As for fexofenadine, it has been shown to be a substrate for P-gp in Caco-2 and L-MDR1 cells, and its disposition is altered in knockout mice lacking the gene for mdr1a (Cvetkovic et al 1999; Soldner et al 1999). The jejunal in-vivo P_{eff} of the drug has been reported to be low ($0.1–0.2 \times 10^{-4}$ cm s^{-1}) and variable (Tannergren et al 2003). Fexofenadine has also been suggested to be a good in-vivo probe since its pharmacokinetics have been suggested to be determined by P-gp and not by its metabolism rate. Concomitant oral administration of fexofenadine and ketoconazole has been shown to lead to an increase in the maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve (AUC), which is consistent with inhibition of P-gp-mediated transport, since fexofenadine is not subjected to any metabolism and has been reported based on in-vitro methods to be a P-gp substrate in-vitro (Cvetkovic et al 1999; Davit et al 1999; Soldner et al 1999; Simpson and Jarvis 2000). Hence, the expected acute effect of ketoconazole when administered in combination with fexofenadine in the human jejunal perfusion model would be an increase in jejunal P_{eff} and the plasma AUC of fexofenadine; however, no acute effect on the jejunal P_{eff} of fexofenadine has been reported (Tannergren et al 2003). This suggests that P-gp does not limit the intestinal transport of fexofenadine in-vivo, as also observed by Drescher et al (2002), who recently showed that polymorphism in the MDR1 gene did not explain the high variability in the plasma concentrations of fexofenadine. They also concluded that mechanisms other than P-gp are likely to affect the pharmacokinetics of fexofenadine. For istance, intestinal transport of fexofenadine may be mediated by several transport proteins that, along with passive diffusion, work simultaneously in the absorptive and secretory direction. It has also been shown in-vitro that fexofenadine is a substrate for members of the organic anion-transporting polypeptide (OATP) family (Cvetkovic et al 1999). Preliminary in-vivo perfusion data from our group suggest that liver extraction (influx and efflux carriers), and not intestinal P_{eff}, is affected by verapamil (unpublished data). Therefore, the current transport drug–drug interaction for fexofenadine may be located in the intestine or the liver (or both) and will certainly involve other transporters besides P-gp.

Our understanding of the expression of transporters and their in-vivo activity and clinical significance in different human tissues is at an early stage. There is also a need for more direct in-vivo data to validate in-vitro methods of studying drug transport with regard to both quantitative and qualitative aspects in biopharmaceutics and pharmacokinetics.

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