Chapter 8
Analysis of Intestinal Transporters

Ikumi Tamai and Takeo Nakanishi

Abstract Intestinal transporters are involved in both influx (absorption) and efflux (exsorption) of various drugs and thereby affect the bioavailability of those drugs. Intestinal tissues are heterogeneous, exhibiting regional variations in physiology and transporter expression, as well as having highly variable intestinal luminal contents. Furthermore, intestinal absorption may proceed via plural mechanisms, such as simple diffusion, carrier-mediated transport, and paracellular transport, in parallel. Accordingly, it is not necessarily easy to identify the mechanism(s) involved in absorption of particular drugs. However, by employing combinations of several experimental methods, some transporters involved in drug absorption and exsorption have been found. P-glycoprotein and BCRP are key efflux transporters that serve to limit absorption of various drugs. As for influx transporters, the picture has not yet been fully clarified, but PEPT1 and OATP have been demonstrated to contribute to drug absorption, and they are expected to be available as target molecules for improving the absorption of orally administered drugs. This chapter focuses on the current understanding of intestinal drug transporters, especially the less-studied absorptive transporters, as well as methods to analyze intestinal absorption and transport processes.

Abbreviations

BBB	Blood–brain barrier
BCRP	Breast cancer resistance protein
HEK	Human embryo kidney

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MDR Multidrug resistance
OATP Organic anion-transporting polypeptide
PEPT Peptide transporter
Reb Rebamipide

8.1 Intestinal Transporters and Drug Absorption

Intestinal absorption is a key issue in the development of new drugs, and compounds that are pharmacologically active in vitro are sometimes dropped if they show poor oral bioavailability. Bioavailability is affected by multiple factors, including solubility, intestinal permeability, and intestinal and hepatic metabolism. Among these factors, intestinal permeability is influenced by the distribution and activity of influx and efflux transporters (Fig. 8.1). Intestinal influx transporters expressed at the apical membrane of enterocytes are physiologically essential for the absorption of nutrients such as amino acids, oligopeptides, bile acids, water-soluble vitamins, nucleosides, hexose, and other nutrients. On the other hand, efflux transporters such as P-glycoprotein (encoded by *MDR1*/*ABCB1*) and BCRP (*ABCG2*) function as an absorption barrier, thereby protecting organisms from xenobiotic (toxic) compounds. Most drugs are potentially recognized as xenobiotics, i.e., they are recognized as substrates of efflux transporters, but not influx transporters. However, some orally administered drugs are actively absorbed from the intestinal lumen, probably because they are misrecognized by influx transporter(s) due to their structural similarity to endogenous substrates; in other words, some transporter(s) show a rather

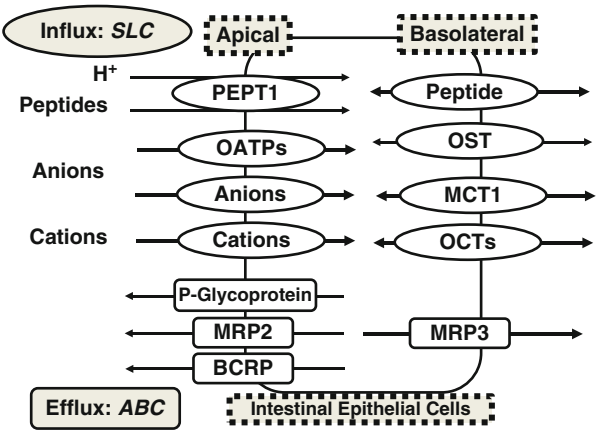

Fig. 8.1 Intestinal transporters affecting drug absorption. *Ovals* and *squares* indicate SLC and ABC transporters, respectively. Apical membrane transporters have been well studied, whereas information on basolateral transporters involved in drug transport is limited

broad substrate specificity. Influx transporters for nutrients expressed at the apical membrane usually translocate their substrates by utilizing a concentration gradient of sodium ions, protons, or other ions. So far, peptide transporter 1 (PEPT1) and organic anion-transporting polypeptides (OATPs) (especially OATP2B1) have been demonstrated to participate in drug transport in the absorptive direction. Accordingly, it may be feasible to utilize these transporters for oral delivery of certain drugs. But, drugs that are substrates of these transporters are sometimes also substrates of efflux transporters. In that case, their intestinal disposition could be influenced by both influx and efflux transporters, resulting in complex absorption characteristics. In this chapter, we will review and summarize (a) the current understanding of the role of transporter molecules in intestinal absorption (PEPT1, OATP2B1) and exsorption (P-glycoprotein and BCRP), (b) methods to analyze intestinal absorption and transport and problems associated with transporter studies, and (c) the application of influx transporters to oral delivery of drugs.

8.2 Oligopeptide Transporter PEPT1

8.2.1 Current Understanding of PEPT1-Mediated Intestinal Absorption

The oligopeptide transporter PEPT1 (*SLC15A1*) is the most extensively studied absorptive transporter. It is a member of the proton-dependent oligopeptide transporter (POT) family, which consists of four members: PEPT1, PEPT2 (*SLC15A2*), PHT2 (also termed PTR3/*SLC15A3*), and PHT1 (PTR4/*SLC15A4*). Among them, PEPT1 is expressed mainly at the apical membrane of intestinal epithelial cells and to a lesser extent in renal tubular epithelial cells and mediates intestinal absorption and/or renal reabsorption of di- and tripeptides as native substrates. PEPT2 expression is confined to the apical membrane of renal epithelial cells, and PEPT2 plays a key role in reabsorption of these oligopeptides from urine.

There are several reports on regional differences in the expression of PEPT1 along the intestine. In humans, relatively high mRNA expression of PEPT1 is maintained from duodenum through ileum, while the expression is much lower, though still significant, in colon (Meier et al. 2007). Another study showed that PEPT1 mRNA and protein expression decreases in the order of duodenum > jejunum > ileum; PEPT1 is also expressed in the stomach in some individuals, but not in colon (Terada et al. 2005). In rats, Pept1 mRNA expression was shown to be higher in the lower small intestine in the fed state, while its expression in the upper small intestine was increased in the starved state, becoming comparable to that in the lower small intestine (Naruhashi et al. 2002). Accordingly, PEPT1 is essentially expressed throughout the small intestine, though with some regional differences, and its expression level is affected by food and other factors, showing considerable inter- and intraindividual variability.

Although PEPT1 and PEPT2 accept di- and tripeptides as endogenous substrates, they also accept several peptide-mimetic drugs as substrates and contribute to their intestinal absorption and renal reabsorption (Matthew 1991; Daniel and Kottra 2004). PEPT1 is thought to exhibit broader selectivity for drugs than PEPT2 (Tsuji et al. 1987; Tamai et al. 1988; Terada et al. 2000). The structural requirements of di- and tripeptide substrates include the presence of one or two peptide bonds, an amino terminal, and a carboxyl terminal. Oligopeptides meeting these requirements are high-affinity substrates of PEPT1. However, several drugs that are transported by PEPT1 do not meet these criteria, so PEPT1 seems to possess a broader substrate selectivity. As for endogenous substrates of PEPT1, some 400 dipeptides and 8,000 tripeptides may be formed from ingested proteins, which may contain 20 different amino acids, though some of these peptides may have negligible affinity for PEPT1. This is in marked contrast to the amino acid transporters, which show high selectivity for substrate amino acids according to size and charge. In other words, the substrate selectivity of PEPT1 is strict in terms of molecular size, but not as regards the amino acid residues that constitute the di- and tripeptides. Accordingly, it seems likely that PEPT1 could mediate the intestinal absorption of various peptide-mimetic drugs.

The driving force for PEPT1-mediated transport is an inwardly directed proton gradient. Microclimate pH in the close vicinity of intestinal epithelial cells is maintained at a weakly acidic level by sodium/proton exchange, so that the environment is proton-rich. An interesting point is that the optimal pH of transport by PEPT1 is variable among substrates. Electrically neutral peptides containing an amino moiety and a carboxyl moiety show optimal transport at about pH 6. However, transport of acidic peptides (with a predominance of anionic moieties) is greater at lower pH, while transport of basic peptides (with a predominance of cationic moieties) is greater at neutral pH (Wenzel et al. 1996; Steel et al. 1997). Figure 8.2 shows the pH dependence of transport of peptides and peptide mimetics in Caco-2 cells. The transport activity for glycylsarcosine (a neutral peptide) was highest at pH of 5.5 or 6, while that for carnosine (β-alanylhistidine, a cationic peptide) was highest at neutral pH. In the case of acidic beta-lactam antibiotics, cefixime and FK089, higher transport activity was observed at lower pH, while neutral cefadroxil exhibited optimal transport at pH 6. Although the mechanism that determines the optimal pH of PEPT1-mediated transport is not clear, the ionization state of substrates clearly influences the apparent transport.

8.2.2 Potential Contribution of PEPT1 to Drug Absorption

As described above, PEPT1 exhibits relatively broad substrate selectivity and likely contributes to intestinal absorption of clinically important substrate drugs, though other transporters may also be involved (Tsuji and Tamai 1996; Brandsch et al. 2008). Interaction of PEPT1 with drugs was first established by characterizing PEPT1-mediated transport of orally active beta-lactam antibiotics, such as

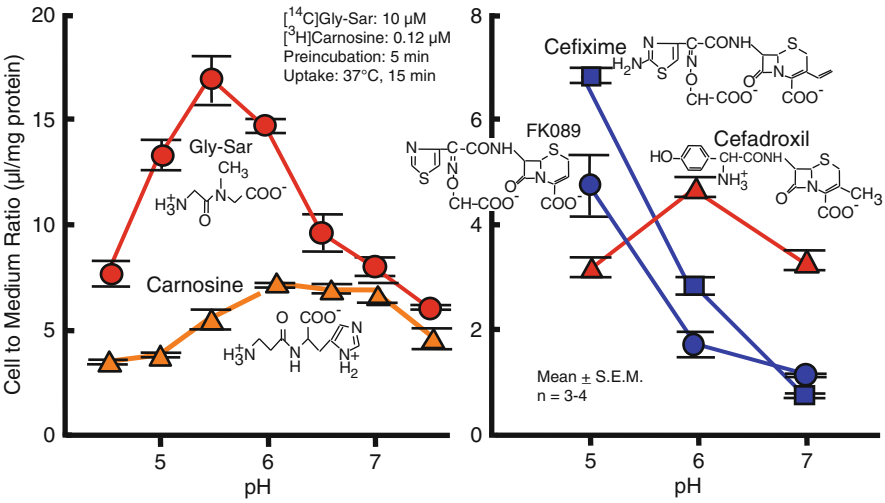

Fig. 8.2 Effect of pH on PEPT1-mediated transport of peptides and peptide mimetics. Uptake of test compounds by Caco-2 cells at various pH values is shown. Gly-Sar (glycylsarcosine) and cefadroxil are neutral, carnosine (beta-alanylhistidine) is cationic because of the histidine residue, and cefixime and FK089 are anionic. Neutral compounds showed optimal pH for transport of around pH 6, while anionic compounds show an increase of permeability with decrease of pH. Cationic carnosine shows an optimal pH value close to neutral. Optimal pH for transport by PEPT1 is substrate dependent

cefaclor (neutral), cephalexin (neutral), cefadroxil (neutral), cefixime (anionic), and ceftibuten (anionic). Anticancer agent ubenimex and antihypotensive midodrine have dipeptide-like structures and are transported by PEPT1. Several angiotensin-converting enzyme inhibitors, such as captopril and enalapril, have been suggested to be substrates of PEPT1, though a more recent study suggested a negligible contribution of PEPT1 to the membrane permeability of those angiotensin-converting enzymes inhibitors (Knütter et al. 2008). Some prodrugs lacking peptide bond(s) in their structure are accepted as substrates of PEPT1. Antiviral drugs such as valacyclovir (valine ester of acyclovir) and valganciclovir (valine ester of ganciclovir) are transported by PEPT1 (Balimane et al. 1998; Sugawara et al. 2000). The amino acid delta-aminolevulinic acid is also a substrate of PEPT1 (Döring et al. 1998). Considering the broad substrate selectivity of PEPT1, other drugs in clinical use could also be substrates.

To date, there are no clinically available drugs that were previously designed to be recognized by PEPT1 in the expectation of higher intestinal membrane permeability. Although PEPT1 targeting seems to be an attractive strategy for oral drug delivery, it faces many challenges (Ezra et al. 2000; Eriksson et al. 2005; Steffansen et al. 2005). In the next section, studies performed by the present authors to improve intestinal drug absorption via PEPT1 are described.

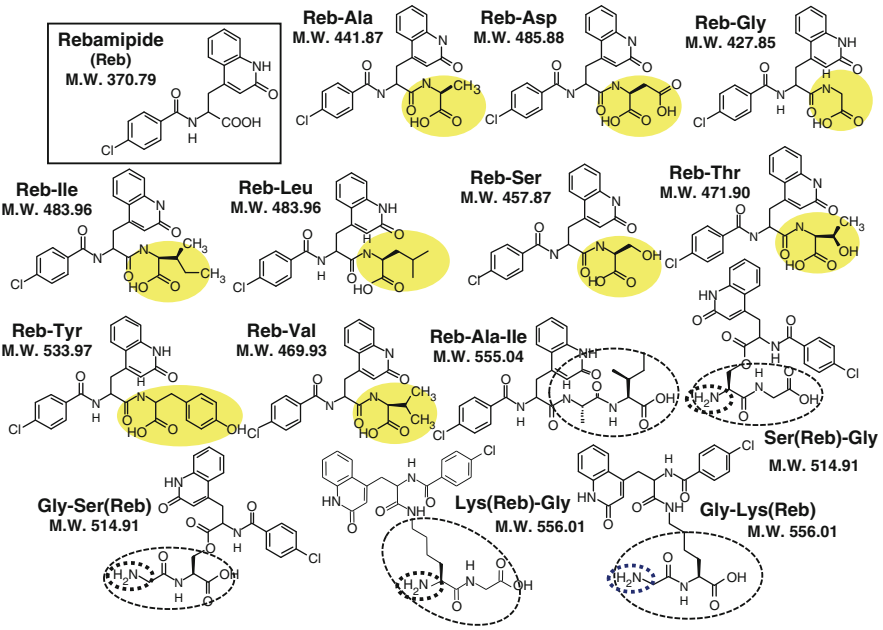

Fig. 8.3 Structures of synthesized amino acid and peptide derivatives of rebamipide. Derivatives shown by *dotted circles* have significant affinity for PEPT1. Four compounds (Ser(Reb)-Gly, Gly-Ser(Reb), Lys(Reb)-Gly, and Gly-Lys(Reb)) retain a free amino group as shown by the *dotted oval*. This figure is cited from Kikuchi et al. (2009) with modifications

8.2.3 Application of PEPT1 to Oral Drug Delivery

Strategies to utilize PEPT1 for oral drug delivery can be classified into two broad types: derivation of non-PEPT1 substrate drugs to peptide-mimetic compounds that can be recognized by PEPT1 and activation of PEPT1 function to increase PEPT1-mediated absorption of weakly recognized substrate drugs without structural modification of the compounds.

In the case of the first strategy, one option is to design compounds that mimic di- or tripeptides. Such compounds need not necessarily contain a peptide bond, as in the case of the antiviral agents described in the previous section. In this approach, the compounds are usually designed as prodrugs that can be transported by PEPT1 and subsequently cleaved to generate the active form after having been absorbed (Tamai et al. 1998).

Another option is to conjugate the candidate compound to a peptide structure that is recognized and transported by PEPT1. For example, rebamipide, an antiulcer drug that exhibits very low membrane permeability after oral administration, was molecularly modified by attaching amino acid or dipeptide moieties to it (Fig. 8.3), and the transport of these compounds was evaluated in vitro in PEPT1-expressing

cultured cells and Caco-2 cells (Kikuchi et al. 2009). The amino acid derivatives exhibited low or negligible affinity for PEPT1 and/or low solubility and were considered inadequate as prodrugs. However, several dipeptide derivatives of rebamipide (Reb), including Ser(Reb)-Gly, Gly-Ser(Reb), Lys(Reb)-Gly, and Gly-Lys(Reb), showed moderate affinity for PEPT1 in in vitro inhibition studies. These four peptide derivatives retain the intact amino and carboxyl terminals of the peptide moiety, since the hydroxyl group of serine or ε-amino group of lysine was used to carry the Reb moiety. Serine has a smaller molecular weight than lysine and may be more suitable for prodrugs. Further study on the transport of these peptide derivatives of rebamipide showed that Ser(Reb)-Gly and Gly-Ser(Reb) are PEPT1 substrates as well as PEPT1 inhibitors, whereas Lys(Reb)-Gly and Gly-Lys(Reb) are only inhibitors. Thus, it is possible to improve the membrane permeability by linking a suitable peptide moiety to a poorly permeable drug, which can then be carried across the intestinal membrane via PEPT1. There are several issues still to be solved, including adequate stability of the peptide derivatives in the intestinal lumen and efficient cleavage to generate the active compound after absorption, in order to obtain the pharmacological effect. However, it was established that PEPT1 can accept rebamipide–peptide derivatives with molecular weight larger than 500, since the molecular weight of rebamipide is 370 and that of the conjugated peptide part in this case is about 150. So, further modifications could be possible to improve the stability and affinity of the peptide moieties in order to optimize the delivery of various low-molecular-weight drugs.

The alternative strategy of activating/optimizing PEPT1 activity has also been proven effective (Nozawa et al. 2003). PEPT1 is an active transporter utilizing a proton gradient as the driving force, and so the transport is affected by luminal pH. As described above, the optimal pH for PEPT1-mediated transport can vary depending on the substrate. For example, the anionic beta-lactam antibiotic cefixime is a substrate of PEPT1, but its bioavailability is not high (about 30 % in human and rat). Since it is not metabolized and is excreted almost wholly in the unchanged form in urine, intestinal membrane permeability is considered to be the limiting factor, even though the compound is a substrate of PEPT1. In an in vitro experiment, higher permeability was observed at more acidic pH than at physiological pH (Fig. 8.2). At pH 5, cefixime exhibited comparable PEPT1-mediated permeability to cefadroxil, which is hydrophilic, but is absorbed almost completely. Accordingly, if the pH at the surface of the intestinal lumen can be modified to be more acidic, intestinal absorption of cefixime via PEPT1 may be considerably increased. When an acidic polymer, Eudragit L100-55, is added to a solution, the pH is maintained at an acidic level, depending on the concentration of the polymer, since the polymer releases protons. Therefore, if the polymer is coadministered with cefixime, it is expected that PEPT1-mediated intestinal absorption of cefixime would be enhanced due to the lowered intestinal luminal pH. Indeed, the strategy of administering cefixime as a 5 % Eudragit L100-55 solution was successful in increasing the bioavailability of cefixime from 27 to 62 % (Nozawa et al. 2003). In this experiment, the pH in the intestinal lumen was estimated to have been modified to about pH 5, based on the results of in vitro experiments. This strategy is unique, since it requires only an

appropriate formulation technology without any need for chemical modification of the active pharmaceutical ingredient, and so it should be readily applicable to other weak PEPT1 substrates.

8.3 Organic Anion-Transporting Polypeptide (OATP)

8.3.1 Current Understanding of OATP2B1-Mediated Intestinal Absorption

OATP2B1 (OATP-B, *SLCO2B1*) is expressed in various tissues, including the small intestine, liver, lung, and ovary (Tamai et al. 2000). This broad tissue expression profile is a distinctive feature of OATP2B1; in contrast, OATP1B1 and OATP1B3 are expressed exclusively in the liver (Abe et al. 1999; König et al. 2000). OATP2B1, OATP1B1, and OATP1B3 are commonly expressed at the sinusoidal membrane of hepatocytes (Kullak-Ublick et al. 2001). However, due to their specific and abundant expression in the liver and broad substrate selectivity, OATP1B1 and OATP1B3 are thought to be key transporters for hepatic uptake of drugs, as well as certain endogenous compounds, such as bile acids, bilirubin, and conjugated metabolites of steroid hormones (Mikkaichi et al. 2004; Kusuhara and Sugiyama 2009). OATP2B1 has been suggested to be involved in hepatic uptake of drugs in clinical use and may also play a significant role in drug disposition in other tissues, including intestine (Tamai 2012). The difference between the tissue expression profiles of these liver-specific OATPs (OATP1B1 and OATP1B3) and the more ubiquitous OATP2B1 could be partly explained by the difference in their regulatory transcription factors, HNF1alpha for liver-specific OATPs and general transcription factor Sp1 for OATP2B1 (Maeda et al. 2006).

Functionally, OATP2B1 is characterized by the pH dependence of its substrate transport. When OATP2B1 is expressed in HEK293 cells, uptake of estrone-3-sulfate by the cells is higher at acidic pH 5.0 than at pH 7.4. The increase is due to an increase of V_{max} (sevenfold), with only a slight increase of K_m (1.5-fold) at pH 5.0 compared with pH 7.4 (Kobayashi et al. 2003; Nozawa et al. 2004). Although the mechanism of the increase of transport activity at acidic pH remains unclear, FCCP, a protonophore, caused a significant decrease of uptake at acidic pH to 42 % of the control, with a smaller decrease at neutral pH (to 81 % of the control). In addition to estrone-3-sulfate, dehydroepiandrosterone sulfate, fexofenadine, and pravastatin were taken up to a greater extent at acidic pH (pH 5.0) than at neutral pH (pH 7.4) via OATP2B1 (Nozawa et al. 2004). Furthermore, pravastatin has been shown to exhibit proton-gradient-dependent transport in brush-border membrane vesicles prepared from rabbit small intestine, based on the observation of overshoot uptake in the presence of a proton gradient (Tamai et al. 1995; Shirasaka et al. 2011). Similar pH dependence was reported in OATP2B1-transfected cells and Caco-2

cells (Kis et al. 2010). Accordingly, these studies strongly support the idea that proton-coupled cotransport or exchange transport with hydroxyl ion contributes to the pH dependence of OATP2B1 transport activity. Since the physiological microclimate pH in the intestinal lumen is weakly acidic, as mentioned above, it may be important to characterize OATP2B1-mediated transport of drugs at acidic pH, but not neutral pH, in order to understand the physiological and pharmacological relevance of OATP2B1.

The broad substrate selectivity and some conflicting data regarding the effect of pH on OATP2B1 activity might be explained by the presence of multiple binding sites with differential substrate/inhibitor affinity and pH sensitivity (Satoh et al. 2005; Shirasaka et al. 2012). Other transporters may also have more than one substrate binding site. Accordingly, further studies are required to clarify the structural requirements for substrates of OATP2B1 and to establish optimum conditions for the application of OATP2B1 for oral drug delivery.

8.3.2 Pharmacogenomics of OATP2B1

Table 8.1 shows non-synonymous mutations found in the *SLCO2B1* gene. Among these genetic variants, *SLCO2B1*3*, which contains the mutation c.1457C>T (causing the amino acid change Ser486Phe), resulted in a decrease of transport activity for estrone-3-sulfate in HEK293 cells expressing the variant compared with that of wild-type *SLCO2B1*1*, after correction for expressed protein amount (Nozawa et al. 2002). This change was explained by a decrease of V_{max} to 43 % of that of the wild-type enzyme, with a negligible change of affinity, i.e., K_m 2.97 μM (*1) and 2.31 μM (*3). Individuals carrying the *SLCO2B1*3* allele showed decreased intestinal absorption of fexofenadine, in accordance with the difference of in vitro activity (Imanaga et al. 2011). Plasma concentration of celiprolol was similarly affected by the genotype *SLCO2B1*3* (Ieiri et al. 2012). Wild-type homozygotes of CC showed the highest plasma concentration, followed by heterozygotes CT and mutant homozygotes TT at the therapeutic dose of 100 mg. These results indicate involvement of OATP2B1 in celiprolol absorption. Interestingly, such an effect of genetic mutation was not detected at microdose levels (37.5 μg). The dose-dependent effect of the genotype of *SLCO2B1* gene was explained in terms of the contribution of P-glycoprotein to exsorption of celiprolol. At therapeutic doses, P-glycoprotein is saturated and has no apparent effect on celiprolol efflux, leaving OATP2B1 as the predominant determinant of intestinal absorption of celiprolol, while at microdose levels the effect of P-glycoprotein is predominant.

The allele frequency of *SLCO2B1*3* (c.1457C>T) was 30.9 % in Japanese. This frequency is relatively high, and individuals with genotype(s) associated with impaired transport activity may show decreased efficacy of substrate drugs. There is an ethnic difference in this SNP, because its frequency is low in Finns (2.8 %). On the other hand, OATP2B1*3 has higher activity for the transport of rosuvastatin

Table 8.1 Major single-nucleotide polymorphisms of the *OATP2B1* gene

rs number	Exon	Nucleotide variation	Amino acid variation	Allelic frequency		
				Japanese	Europeans	Africans
rs56837383	3	c.43C>T	P15S			
rs148248368	4	c.343C>T	P115S			
rs35199625	6	c.601G>A	V201M		2.1 (Laitinen and Niemi 2011)	
rs12422149	8	c.935G>A	R312Q		13.6 (Laitinen and Niemi 2011) 8.2 (Mougey et al. 2009)	13 (Mougey et al. 2009)
rs1621378	10	c.1175C>T	T392I	0 (Nozawa et al. 2002)		
rs111782322	10	c.1240G>A	G414S			
rs2306168	11	c.1457C>T	S486F	31 (Nozawa et al. 2002)	2.8 (Laitinen and Niemi 2011)	
rs140407559	11	c.1526G>A	R509H			
rs143480565	12	c.1624G>A	V542M			
rs145875125	13	c.1638C>A	N546K			
rs149242910	12	c.1642G>A	V548M			
rs149765874	15	c.2071G>A	V691I			

than wild-type OATP2B1, when expressed in HeLa cells (Ho et al. 2006). Accordingly, the effect of genetic polymorphisms on transport activity might be variable among substrates. Further studies on the mechanisms of alteration of apparent activity caused by mutation seem necessary. Another variant, c.1175C>T, which causes the amino acid substitution of Thr at codon 392 with Ile (*SLCO2B1*2*), resulted in a slight decrease in uptake of estrone-3-sulfate in HEK293 cells compared with the wild-type (Nozawa et al. 2002). Other variants of OATP2B1, such as c.43C>T (Pro15Ser), c.601G>A (Val201Met), and the three-amino-acid deletion (26–28, Gln-Asn-Thr), were preliminarily reported to have lowered transport activity for rosuvastatin (Ho et al. 2006). The variant c.935G>A, which causes a nonsynonymous mutation of OATP2B1 (Arg312Gln), has also been reported, though its effect on activity is poorly understood. The relationship between the *SLCO2B1* genotype and the pharmacological effect of the leukotriene receptor antagonist montelukast was examined in patients with asthma (Mougey et al. 2009). Compared to the wild-type allele 935G, individuals with 935A heterozygously exhibited a weaker response to treatment and a lower plasma concentration of montelukast. Based on these pharmacogenomic studies, it is clear that OATP2B1 contributes to drug absorption in vivo in humans.

8.3.3 Drug–Fruit Juice Interaction at OATP2B1

OATP transporters may be involved in drug–fruit juice interaction during intestinal absorption. Previously known pharmacokinetic interactions with fruit juices were explained mainly in terms of the inhibitory effects of ingredients of fruit juice on intestinal drug-metabolizing enzymes and efflux transporters (e.g., P-glycoprotein), resulting in increased plasma concentration of the affected drugs (Bailey 2010). However, in 2002, it was reported that fruit juices such as grapefruit, orange, and apple juices reduced the plasma concentration of fexofenadine after oral administration (Bailey et al. 2007). Since the observed effect could not be explained by previously reported drug–juice interactions at drug-metabolizing enzymes and/or exsorptive transporters, interaction may also occur at intestinal absorptive transporters. It was hypothesized that OATP transporters, especially OATP1A2, were involved in this interaction based on the results of in vitro transport studies. In another study, a species difference between rat and human in the effect of fruit juice on the plasma concentration of talinolol after oral administration was observed, i.e., the plasma concentration of talinolol was increased and decreased in rat and human, respectively, upon ingestion with grapefruit juice (Spahn-Langguth and Langguth 2001; Schwarz et al. 2005). Because talinolol is not metabolized and is a substrate of P-glycoprotein, a decrease in its plasma concentration in human cannot be explained by interaction at P-glycoprotein, though the increase of plasma concentration in rat could be explained by inhibition of P-glycoprotein. We found that talinolol is a substrate of human OATP and rat intestinal Oatps, and naringin in grapefruit juice inhibits both human and rat OATPs/Oatps at a concentration that is achievable following ingestion of grapefruit juice (Shirasaka et al. 2009, 2010). Further, rat but not human P-glycoprotein was inhibited by a juice ingredient at the same concentration as in juice. Accordingly, the effect of grapefruit juice on talinolol absorption exhibited species difference due to the difference in the affinity of grapefruit juice ingredient(s) for P-glycoprotein between human and rat, whereas the juice inhibited intestinal OATPs similarly in human and rat. Fexofenadine absorption was reduced by ingestion with grapefruit juice or apple juice due to inhibition of OATP1A2 (Bailey et al. 2007) or OATP2B1 (Imanaga et al. 2011). Furthermore, reduction of intestinal absorption of statins (Shirasaka et al. 2011), montelukast (Mougey et al. 2009), and aliskiren (Tapaninen et al. 2010) by grapefruit juice was explained in terms of inhibition of OATP2B1 and/or OATP1A2. Other fruit juices and beverages also affect drug absorption by interacting with OATPs. Absorption of fexofenadine was reduced by orange juice and apple juice (Dresser et al. 2002; Imanaga et al. 2011). Green tea catechins including epicatechin gallate and epigallocatechin gallate inhibited OATP2B1 and OATP1A2 (Roth et al. 2011). Accordingly, OATPs are likely involved in a variety of drug–beverage interactions during the intestinal absorption process. The results of these studies on drug–juice interaction in humans, as well as in vitro studies with transporter-expressing cells, represent strong evidence that these transporters contribute to drug absorption.

8.3.4 Potential Contribution of OATP1A2 to Drug Absorption

OATP1A2 protein is expressed at the apical membrane of human enterocytes, like OATP2B1 (Glaeser et al. 2007), and accepts as substrates various drugs that are mostly also substrates of OATP2B1. Accordingly, it is not easy to distinguish the contributions of OATP1A2 and OATP2B1 to the absorption of common substrate drugs. However, several reports show that OATP1A2 is expressed at a much lower level than OATP2B1 in human intestine or even at a negligible level, and *SLCO2B1* was reported to be more abundant in enterocytes (Tamai et al. 2000; Meier et al. 2007). In addition, there is convincing evidence that the in vivo effects of genetic polymorphisms of OATP2B1 parallel the in vitro transport activity of the mutated OATP2B1, which is again consistent with a significant contribution of OATP2B1 to drug absorption. Thus, although OATP1A2 may also contribute to drug absorption, this remains to be established. It seems likely that marked interindividual variability in expression level and/or changes in expression level of OATP1A2 in response to various factors may account for the conflicting observations regarding its expression in intestinal tissues.

8.4 Intestinal Efflux Transporters

8.4.1 P-Glycoprotein-Mediated Exsorption as an Absorption Barrier

It is well understood that efflux transporters such as P-glycoprotein and breast cancer resistance protein (BCRP) affect drug absorption by transporting drugs into the intestinal lumen. As shown in Fig. 8.4, there appears to be a hyperbolic relationship between intestinal permeability and lipophilicity of drugs, though there is an upper limit of the permeability (Terao et al. 1996). However, several drugs exhibited lower permeability than would be expected from this correlation, and many of these drugs were substrates of P-glycoprotein. When cyclosporin A was added as a P-glycoprotein inhibitor, an increase in permeability was observed for many of these drugs. These results suggest that P-glycoprotein is a major component of the absorption barrier. Indeed, studies with P-glycoprotein-expressing cells, Caco-2 cells, and intestinal tissues have indicated that P-glycoprotein-mediated intestinal exsorptive transport is a molecular mechanism of poor intestinal absorption of drugs (Fromm 2003; Lin and Yamazaki 2003). However, some P-glycoprotein substrate drugs show good intestinal absorption in clinical use. One of the reasons for such apparently inconsistent observations, despite active exsorption mediated by P-glycoprotein, might be because the intestinal luminal concentration in the clinical setting is high enough to saturate P-glycoprotein-mediated transport. Another possible reason is regional difference in the expression of P-glycoprotein, with higher expression at the lower part of the small intestine. Several P-glycoprotein

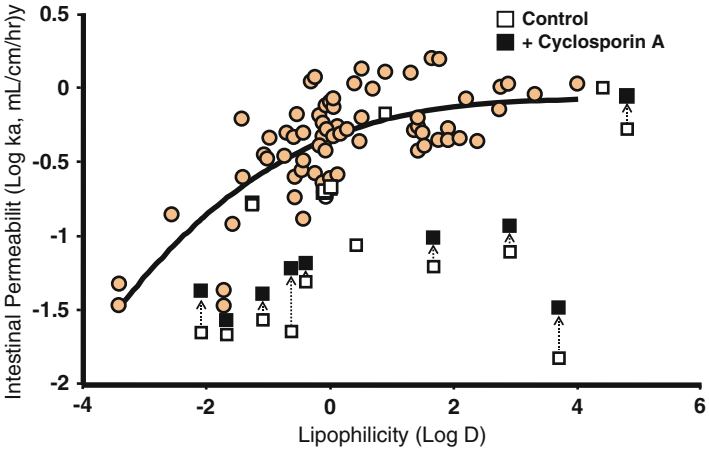

Fig. 8.4 Relationship between intestinal permeability and lipophilicity of various drugs. *Open and closed squares* show the absorption rate constants in the absence and presence of cyclosporine A as a P-glycoprotein inhibitor. The absorption rate constant is increased in the presence of cyclosporine A, showing that P-glycoprotein limits drug absorption to some extent. This figure is cited from Terao et al. (1996)

substrate drug in clinical use, such as quinidine, bepotastine, and azasetron, exhibit high intestinal permeability. As suggested above, this can be explained in terms of the high concentration of these drugs in the intestinal lumen after oral administration and/or higher absorptive permeability at the upper part of the small intestine, thereby avoiding exsorption mediated by P-glycoprotein due to its low expression in this region. However, these drugs are often not delivered into the brain across the blood–brain barrier (BBB) due to the efficient efflux transport via P-glycoprotein, since the plasma concentration may not be high enough to saturate P-glycoprotein at the BBB. If the pharmacological target of a drug is peripheral tissue, it is a good strategy to have high affinity for P-glycoprotein, since high intestinal permeability and low blood–brain barrier permeability can be expected due to the saturation of P-glycoprotein in gut but not in brain. Accordingly, care is needed in estimating the effect of efflux transporters on drug absorption, since it is not rare that drugs at clinically used doses are present at a high enough concentration in the intestinal lumen to saturate P-glycoprotein there. This is just one of the difficulties involved in predicting in vivo effects of transporters on drug absorption from in vitro studies.

8.4.2 BCRP-Mediated Exsorption as an Absorption Barrier

BCRP was named after its function in breast cancer. Ironically, it is frequently not expressed in breast tumor tissues, but it is expressed in several normal tissues, such as the intestine, liver, and placenta, which are involved in determining drug

pharmacokinetics. There is a species difference in the expression level in kidney, with high expression in murine kidney and low expression in human kidney, but common expression in intestinal tissues of both species (Maliepaard et al. 2001; Tanaka et al. 2004; Borght et al. 2006; Huls et al. 2007). It is known that BCRP is a characteristic marker of certain stem cell-like and progenitor cell populations in normal tissues; these cells show little or no staining with Hoechst33342, because of active BCRP-mediated efflux of the dye (Zhou et al. 2001). This reflects the physiological role of BCRP in protecting organisms from xenobiotics and toxins (Ross and Nakanishi 2010). More recently, a pharmacogenomic study of BCRP revealed that reduced activity of BCRP function due to genetic polymorphisms is associated with increased risk of hyperuricemia/gout (Matsuo et al. 2009). Since uric acid is a uremic toxin, BCRP may be one of the transporters that limits uric acid concentration in serum (Hosomi et al. 2012). BCRP is expressed in small intestine at relatively high levels, and since it serves as an efflux transporter for a wide variety of drugs, particularly chemotherapeutics, it is likely to function as an absorption barrier to drugs (Polgar et al. 2008; Nakanishi and Ross 2012). Sulfasalazine is used to treat ulcerative colitis and its target is the colon. Sulfasalazine is metabolized to sulfapyridine and 5-aminosalicylate by colonic bacteria, and the formed 5-aminosalicylate is the active agent. Accordingly, it is important that sulfasalazine is delivered to the colon without absorption in the small intestine. Since sulfasalazine is a substrate of BCRP, intestinal absorption of the drug is indeed low. However, individuals with a genetic variant of BCRP (c.421C>A in *ABCG2* gene) exhibited higher absorption of sulfasalazine due to decreased BCRP-mediated exsorptive transport in small intestine (Yamasaki et al. 2008). In the case of sulfasalazine, low, BCRP-limited absorption at the small intestine is clinically advantageous, but for most of substrate drugs, intestinal BCRP may reduce their bioavailability.

8.5 Evaluation of Absorption Mechanisms

As described above, absorptive (influx) and exsorptive (efflux) intestinal transporters may significantly influence the absorption of orally administered drugs. Although there is considerable evidence that this is the case for efflux transporters such as P-glycoprotein and BCRP, the contributions of influx transporters to drug absorption generally remain to be clarified. The reason for this is because multiple pathways exist for drug absorption, including simple diffusion, paracellular transport and carrier-mediated transport. Absorption via these mechanisms proceeds in parallel, and consequently elucidation of the relative contribution of each mechanism is not easy.

Several methods are available to evaluate intestinal transport and absorption (Table 8.2). For in vitro studies, isolated brush-border membrane vesicles, intestinal tissue-derived cultured cells such as Caco-2 cells, transporter-gene-transfected cultured cells, isolated enterocytes, and isolated intestinal tissues are widely used. These in vitro methods are useful for obtaining a mechanistic understanding of

Table 8.2 Analysis methods of intestinal transporters and absorption

Methods	Characteristics
In vitro	Good for mechanistic analysis
• Membrane vesicles:	Very artificial; easy to control experimental conditions
Brush-border and basolateral membranes from intestinal epithelial and cultured cells	Suitable for evaluation of driving force, affinity, inhibitors
	Limited applicability due to adsorption of some drugs on membrane
• Isolated epithelial cells	Cells are viable and intracellular conditions are well maintained
	No polarity
• Intestinal epithelial cell-derived cultured cells	
Caco-2	Maintained polarity; both influx and efflux can be measured
LS180	Expressional regulation can be evaluated
• Isolated intestinal tissue	Uptake and permeation can be measured in real tissue
Everted intestine	Maintaining viability is difficult
• Transporter-gene-transfected cultured cells	Responsible transporter molecule can be determined
HEK293, MDCK, LLCPK1, HeLa	Once established, easy to study with good reproducibility
	Influx and efflux can be evaluated (MDCK, LLCPK1)
Xenopus oocytes	Usually shows low background activity
	Handling is cumbersome compared with cultured cells
In situ	Intermediate between in vitro and in vivo; good for confirmation of in vitro hypotheses and as a bridge to in vivo study
• Intestinal perfusion	Intestinal permeability can be measured with blood circulation (viable)
	Low throughput
• Intestinal closed loop	Absorption mechanism can be estimated
	Luminal condition can be partly (but not completely) controlled
In vivo	Oral availability and absorption mechanism can be evaluated
• Gene knockout animal	Transporter effect on absorption can be assessed
	Limited availability
	Species difference between murine and human
• Clinical human study	Human absorption is directly evaluated
Effect of genetic polymorphism	Identification of responsible transporter is possible
Effect of interaction with drug and food (beverage)	Responsible transporter can be estimated

membrane transport, including identification of transporter molecules and their functional roles, driving forces for the transport, inhibitor selectivity, and affinity of particular drugs for the transporters. However, these methods do not necessarily throw light on the in vivo contribution of each mechanism. As in situ methods, intestinal perfusion and the intestinal closed loop method are useful for evaluation of apparent permeability and for rough estimating the roles of various transporter molecules and can form a bridge between in vitro and in vivo analyses. Although it is possible to estimate the involvement of certain mechanisms in drug absorption by means of in vitro and in situ intestinal transport studies, precise evaluation of the contributions of transporters to drug absorption in animals may require the use of transporter-gene knockout animals. At present, mice in which *Mdr1a*/*1b* (Schinkel et al. 1994, 1997), *Bcrp1* (Jonker et al. 2002; Krishnamurthy et al. 2004), *Pept1* (Hu et al. 2008), and some *Oatps* (Lu et al. 2008; Zaher et al. 2008; van de Steeg et al. 2010; Gong et al. 2011) have been knocked out are available. However, species difference is significant, especially in the case of Oatps, and that the correspondence between some human OATPs and murine Oatps remains controversial (Nakakariya et al. 2008). As for clinical studies in humans, transporters involved in drug absorption can be analyzed by evaluating the effects of genetic polymorphisms in the transporter gene on apparent functional activity. Another approach is to observe the alteration in pharmacokinetics of substrate drugs due to drug–drug and/or drug–food (or beverage) interactions. Indeed, findings on the modification of drug absorption by fruit juices and the effects of genetic polymorphisms that alter transporter function have already been described in this chapter. It is important to remember that, although transporter function can be analyzed relatively easily by means of the methods summarized in Table 8.2, it may still be difficult to fully understand the overall outcome of intestinal absorption processes in the complex in vivo environment, where the many variables may include transporter polymorphism, site-specific expression of transporters in the intestinal tract, microenvironmental changes of physiological pH or ion concentrations, variations in intestinal motility and contents, and modification of transporter function by dietary components.

8.6 Conclusion

Analysis of the mechanisms of intestinal drug absorption is extremely challenging, but there is now overwhelming evidence that PEPT1 and OATPs as influx transporters and P-glycoprotein and BCRP as efflux transporters play significant roles in the intestinal absorption of various drugs in clinical use. PEPT1 has broad substrate specificity and its expression is limited to small intestine, so it can potentially be utilized to enhance the absorbed fraction of appropriately designed prodrugs of soluble and poorly absorbable drugs. OATPs appear to be involved in absorption of many clinically used drugs, although the extent of their contribution varies from case to case. OATPs are often sites of drug–drug or drug–food (or beverage) interactions that influence the intestinal absorption of their substrate drugs.

Since P-glycoprotein and BCRP are exsorptive transporters, it is important that drugs should not be substrates of these transporters if they are to be efficiently absorbed. These four transporters seem to be the key players to interpreting alterations in intestinal absorption of drugs due to drug–drug or drug–food interactions. They are likely to be the main focus of future work to improve the bioavailability of new drugs, either by appropriate molecular modification of drug candidates or by modulation of the transporter function.

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