CHAPTER 13

BIOPHARMACEUTICAL MODELING FOR MISCELLANEOUS CASES

> "The ability to simplify means to eliminate the unnecessary so that the necessary may speak."
>
> —Hans Hofmann

A specific physiological condition of the GI tract can be changed by a coadministered drug. For example, a proton pump inhibitor (PPI) increases the stomach pH. These cases are clinically important. In addition, owing to its specific effect, the clinical data of such cases can be used to validate a specific feature of biopharmaceutical modeling. Some GI physiology of animals are different from that of humans. Biopharmaceutical modeling can be used to estimate and elucidate the behavior of a drug under such situations.

13.1 STOMACH pH EFFECT ON SOLUBILITY AND DISSOLUTION RATE

The pH shift in the stomach can affect Fa% of dissociable drugs with low solubility. The stomach pH can be altered by a coadministered drug, such as PPIs, H2 blockers, and acid neutralizers. In the elder Japanese, the number of hypoacid patients are significant (40% at >50 years old) [1]. Therefore, biopharmaceutical modeling for these cases would be of significant importance to drug development. The effect of gastric pH on Fa% of several drugs is summarized in Table 12.4.

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13.1.1 Free Bases

In the case of free bases with low solubility, when the stomach pH is increased to a neutral pH, the oral absorption can be significantly reduced. Biopharmaceutical modeling for such cases is discussed in Section 8.6. To avoid variable oral absorption in patients, it is preferable to design an API and/or a formulation that gives high Fa% even with high stomach pH. For example, if Fa% with high stomach pH is 80%, the maximum variation caused by the stomach pH would be 20%. Fa% with high stomach pH can be easily estimated using Fa_{SS} equation (Section 8.5).

13.1.2 Free Acids and Undissociable Drugs

On the other hand, the effect of the stomach pH for free acids and undissociable drugs has not been clinically observed (to the best of the author's knowledge). No literature information about the effect of H_2 blocker and PPI on the oral absorption of NSAIDS (as free acids) is available. As the effect of the stomach pH can be negligible, Fa_{SS} equation can be used to estimate Fa% (Section 8.5).

13.1.3 Salts

The effect of gastric pH on Fa% of several salts is summarized in Table 12.4. Even when dosed as a salt form API, many bases with low solubility precipitate out as a free base in the stomach when the stomach pH is elevated by an acid-reducing agent, resulting in reduced Fa%.

In the case of a salt of an acid drug, the salt form might convert to a free form when in contact with the low pH fluid in the stomach. This conversion would not occur when the stomach pH is high. For example, the oral absorption of raltegravir potassium significantly increased when dosed with a PPI [2].

As discussed in Section 8.7, biopharmaceutical modeling for a salt is currently difficult.

13.1.4 Chemical and Enzymatic Degradation in the Stomach and Intestine

In the case of acid-degradable drugs such as triazolam [3], an increase in the stomach pH can increase Fa%. Other marketed drugs that undergo acid hydrolysis are erythromycin [4], omeprazole, lansoprazole, pantoprazole [5], etc. Usually, an acid-catalyzed degradation follows first-order kinetics (k_{deg}). Degradation % in the stomach can be estimated as

$$\text{Degraded}\,\% = \frac{k_{\text{deg}}}{k_{\text{deg}} + K_{t,\text{stomach}}} \tag{13.1}$$

where k_{deg} is the degradation rate constant.

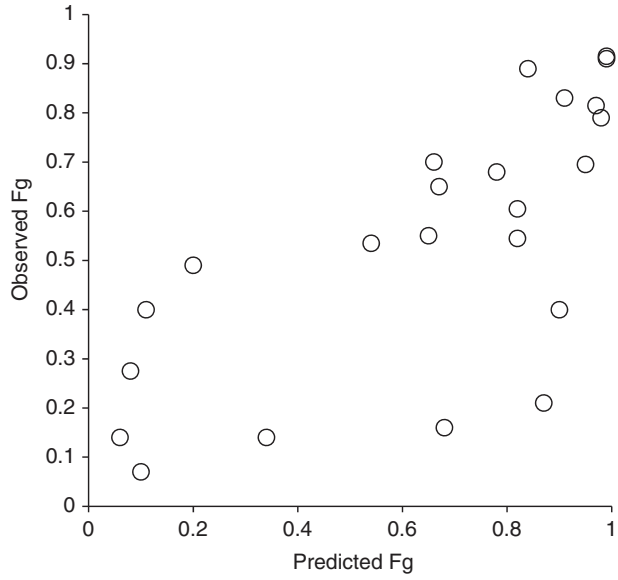

Figure 13.1 Fg prediction using *in vitro* metabolic clearance with the Q_{gut} method. In the original publication [6], PS_{perm} was calculated based on the linear log P_{app}–log P_{eff} relationship. Log P_{app} was obtained in either Caco-2 or MDCK-MDR1 cells.

13.2 INTESTINAL FIRST-PASS METABOLISM

Prediction of the intestinal first-pass metabolism has been extensively investigated, mainly for CYP3A4 metabolism, and also for glucuronic and sulfonic conjugations. Figure 13.1 shows the prediction of Fg using the Q_{gut} model [6]. As discussed in Section 4.10, it is difficult to accurately predict Fg from *in vitro* data (Figs. 4.29 and 13.1).

Fg can be calculated from F, Fa, and Fh as $Fg = F/(Fa \cdot Fh)$. However, Fh estimation from CL_h and Q_h ($Fh = 1 - CL_h/Q_h$) could have some errors [7]. The use of intestinal CYP3A4-specific inhibitor, such as grape fruit juice, might be useful to evaluate clinical Fg for CYP3A4 [8].

Substantial sulfate conjugation has been reported for terbutaline and fenoterol ($Fg < 0.3$) [9–11]. These drugs are hydrophilic drugs, and the mechanism to access the enzymes in the enterocyte is not well known.[1] Glucuronization significantly reduces the bioavailability of some drugs that have planner phenolic groups such as raloxifen (BA 2%) [12, 13].

[1] As the surface area of the basolateral membrane is threefold larger than that of the apical membrane and the basolateral pH is ca. 1 unit higher than the apical pH, the passive membrane clearance of a base is ca. 30-fold higher in the basolateral membrane. Owing to the subepithelial diffusion resistance, removal of a drug from the basolateral membrane would not be infinitely fast. Therefore, it might be possible that a base drug that permeated through the paracellular pathway may diffuse into the cytosol. This point requires further investigation.

To predict the extent of DDI via intestinal first-pass metabolism, metabolic and escaping clearances have to be quantitatively predicted for both the victim and inhibitor drugs (Section 4.10). Even though a fully mechanistic simulation model is available, the estimation method for escaping clearance has not been thoroughly validated.

13.3 TRANSIT TIME EFFECT

13.3.1 Gastric Emptying Time

Gastric emptying pattern can be changed by the pharmacological effect of a drug. Propantheline increases gastric emptying, resulting in faster T_{max} of paracetamol, whereas metoclopramide had the opposite effect (Fig. 13.2) [14].

Alprazolam has the muscle relaxant effect and reduces the gastric motility, resulting in the double peaks in its plasma concentration profiles after oral administration in rats [15, 16]. However, the double peak phenomena was not observed in humans.

Avitriptan clearly showed a double peak in C_p–time profile conjugated with the stomach emptying simultaneously measured by Gamma scintigraphy in humans [17].

In dogs, celiprolol showed double peak C_p–time profile, which is associated with MMC [18]. Interestingly, faster gastric emptying resulted in the increased bioavailability. The most likely explanation was that an increase in intestinal concentration (caused by faster gastric emptying) saturated the P-gp efflux. However, in rats, the double peak was observed even after duodenum administration [19], suggesting that another reason might also be involved.

Ranitidine and cimetidine also change the gastric motility [20]. The double peak phenomena were observed in humans [21].

13.3.2 Intestinal Transit Time

Cisapride reduces the intestinal transit time of a drug. When dosed with cisapride, the bioavailabilities of drugs with low permeability were reduced, for example, sotalol [22], cimetidine [23], ranitidine [24], and digoxin [25] (decreased by 30%, 18%, 26% and 12%, respectively). Figure 13.3 shows the effect of cisapride on the C_p–time profile of sotalol [22]. T_{max} of sotalol was shortened from 2.8 to 1.2 h, and the AUC was decreased by 30%.

13.4 OTHER CHEMICAL AND PHYSICAL DRUG–DRUG INTERACTIONS

13.4.1 Metal Ions

Tetracyclines, quinolones, and bisphosphonates make complexes with metal ions in the GI tract. Antacids ($Mg(OH)_2$, etc.) and milk (contains Ca^{2+}) should not

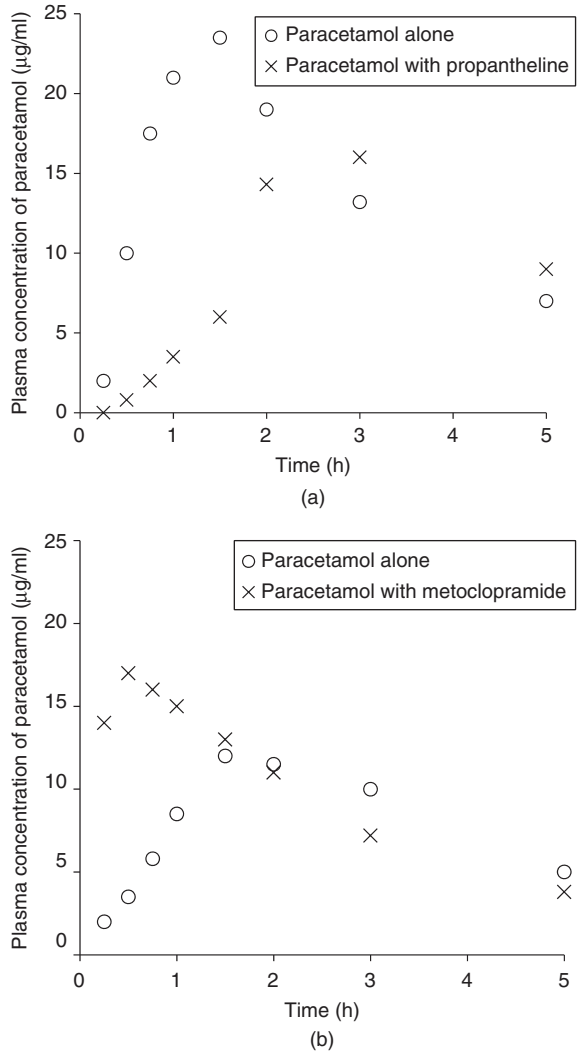

Figure 13.2 The effect of (a) propantheline and (b) metoclopramide on the C_p–time profile of acetaminophen. *Source:* Adapted from Reference 14 with permission.

be taken simultaneously [26–28]. Biopharmaceutical modeling for this kind of DDI has not been reported.

13.4.2 Cationic Resins

Resin-based drugs, such as chorestylamine and sevelamer, can bind to various drugs and reduce oral absorption of the drug [29, 30]. Biopharmaceutical modeling for this kind of DDI has not been reported.

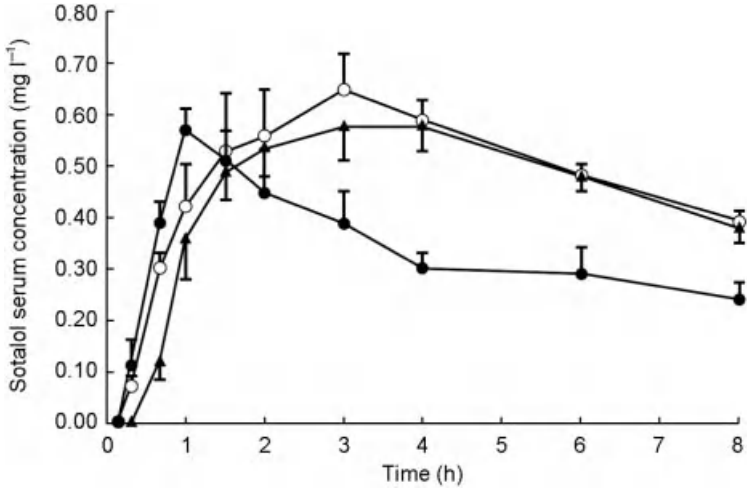

Figure 13.3 Mean (±S.E.) sotalol serum concentrations of seven healthy subjects after the administration of 80 mg sotalol as an oral solution (open circles), as an oral solution also containing 20 mg cisapride (closed circles), and as a sublingual tablet (triangles). *Source:* Adapted from Reference 22 with permission.

13.5 SPECIES DIFFERENCE

13.5.1 Permeability

Figure 13.4 shows the relationship of Fa% among human, rat, dog, and monkeys [31–33]. All of the drugs plotted are drugs with high solubility. This data suggests that the intestinal epithelial membrane permeability is comparable among human, rats, and monkeys, but not dogs. In dogs, Fa% of paracellular pathway permeants was higher than that in humans, whereas that of transcellular permeants were comparable. This is due to the larger paracellular pore size in dogs. Therefore, for permeability-limited absorption drugs (BCS III), rats and monkeys would be the appropriate species to predict human Fa%. The dog data should be carefully interpreted, especially for base or neutral drugs with MW < 500.

The good correlation of Fa% in rats and humans (Fig. 13.4) looks as if it contradicts to the experimental data that P_{eff} in rats is 6- to 15-fold lower than that in humans (Table 8.1) [34]. However, as the radius of the rat intestine is smaller than that of the human intestine, the permeation rate and Fa% in rats and humans becomes similar.

$$k_{\text{perm}} = \frac{2\text{DF}}{R_{\text{GI}}} P_{\text{eff}} \left(= \frac{\text{SA}_{\text{GI}}}{V_{\text{GI}}} P_{\text{eff}} \right) \tag{13.2}$$

The difference in P_{eff} and the similarity of Fa% between rats and humans are well captured by the GUT framework as it considers the difference in plicate and villi structure (Figs. 8.9 and 13.5) [35]. For dogs, the GUT framework also well

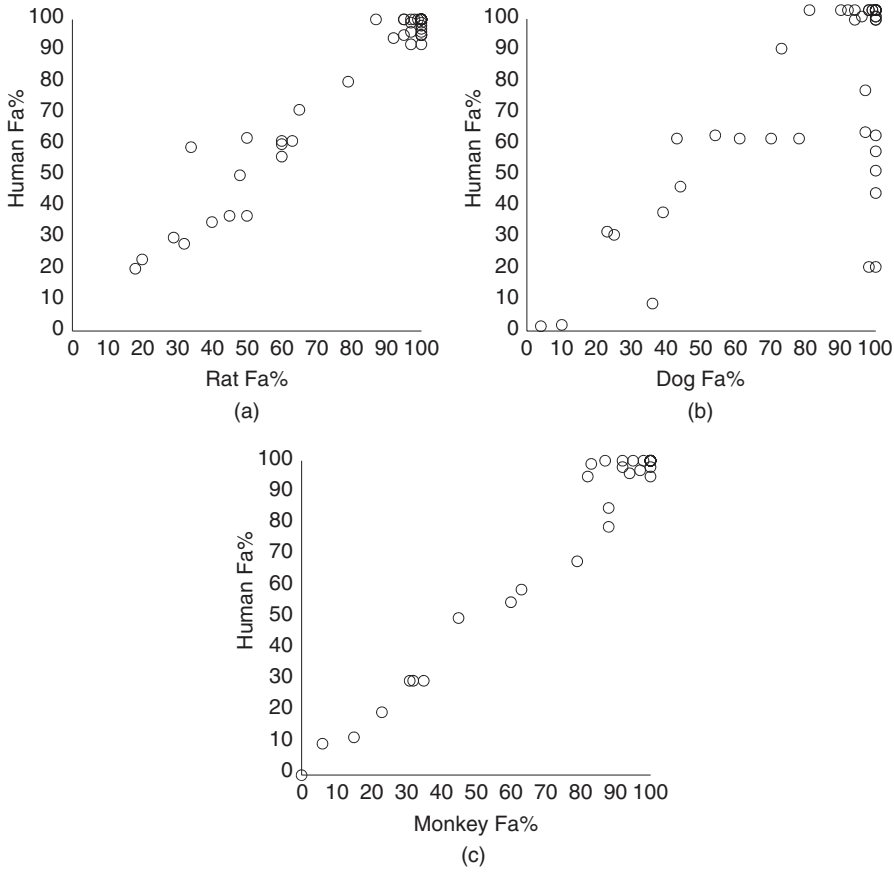

Figure 13.4 Comparison of Fa% in humans with that in (a) rats, (b) dogs, and (c) monkeys. *Source:* Replotted from References 31–33.

captures the difference of Fa% for the paracellular permeants and the similarity of Fa% for the others. The paracellular pathway model is necessary to simulate the species difference for dogs.[2]

13.5.2 Solubility/Dissolution

Species differences in solubility/dissolution perspectives have not been well characterised. As the bile concentration in rats is fivefold higher than that in fasted state humans because of continuous secretion of bile (rats lack the gallbladder), Fa% of drugs with low solubility would be overestimated. This is in good

[2]In one commercial software (as of 2011), the dog P_{eff} value of any drug is assumed to be threefold larger than the human P_{eff} values regardless of the permeation pathway of a drug. However, this assumption is not valid for the transcellular- and UWL-controlled cases. For these cases, the dog P_{eff} should be threefold lower than the human P_{eff}.

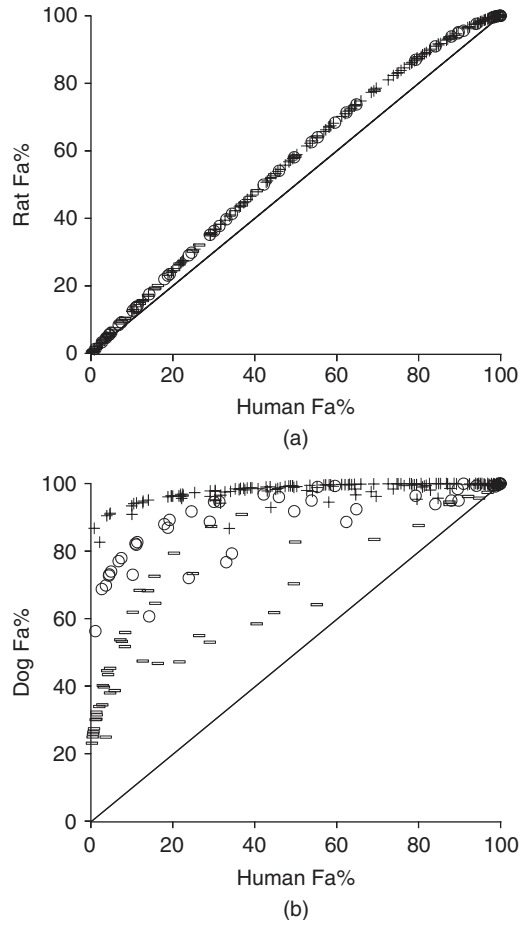

Figure 13.5 Theoretical prediction of Fa% correlation between rats, dogs, and humans by the GUT framework.

agreement with real-life experiences in drug research that as the animal species becomes larger, Fa% of drugs with low solubility becomes lower.

From a practical perspective, dogs are most frequently used as an animal species for the investigation of the performance of drug formulation. However, dogs have a little higher bile concentration, higher agitation strength, and shorter intestinal transit time compared to humans. The stomach pH of dogs should be controlled to mimic that of humans (Sections 6.3.2.1 and 7.10.3). Dogs are also appropriate for investigating the food effect [36] (Section 7.10.3).

13.5.3 First-Pass Metabolism

It is well known that there are significant species differences in the intestinal and liver first-pass metabolism.

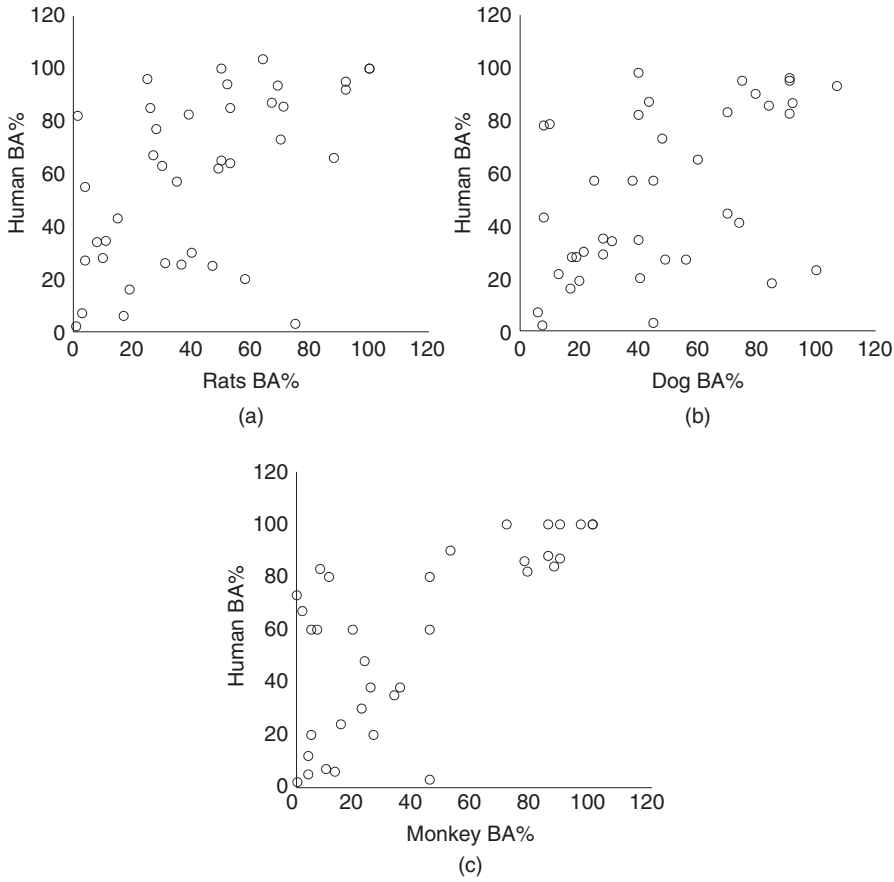

Figure 13.6 Comparison of bioavailability in humans with that in (a) rats, (b) dogs, and (c) monkeys. *Source:* Figures (a) and (b) replotted from References 32 and 43.

Figure 13.6 shows the comparison of bioavailability (F) in humans, with that in rats, dogs, and monkeys. All of the drugs plotted are drugs with high solubility. As Fa is rather consistent among rats, monkeys, and humans, Fg · Fh is suggested to have significant species differences. The oral bioavailability of CYP3A4 and UDT substrates, is markedly lower in monkeys than in humans [37], due to extensive first-pass metabolism in the monkey intestine [38–40]. On the other hand, pharmacokinetics after intravenous administration in humans is closer to that in monkeys than that in rats and dogs [41, 42].

Figure 13.7 shows the comparison of the biliary excretion percentages of drugs in humans with that in rats and dogs. Rats tend to overestimate biliary excretion in humans [44, 45]. Existence of MW cutoffs for biliary excretion has been reported. Minimum MW cutoffs for rats, guinea pigs, rabbits, dogs, and humans were reported to be 325, 400, 475, 400, and 500, respectively [44, 45].

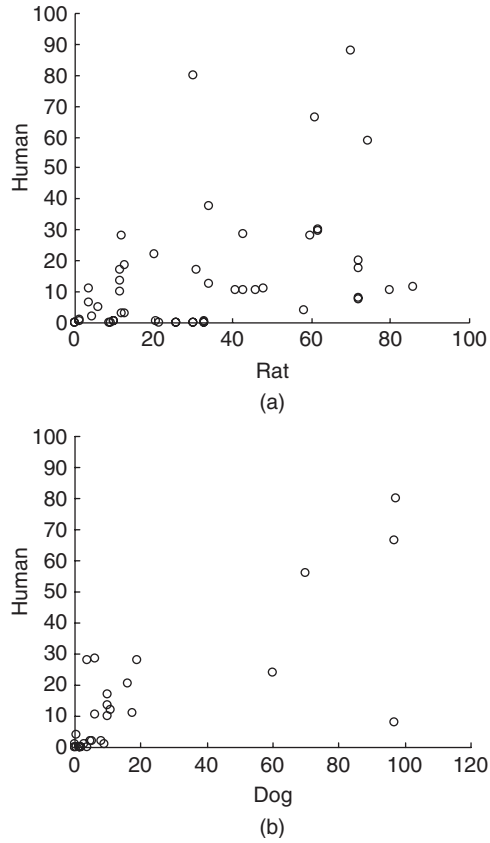

Figure 13.7 Biliary excretion in rats, dogs, and humans [44, 45].

These cutoffs were determined for organic anions and may not be applied for other types of drugs.

13.6 VALIDATION OF GI SITE-SPECIFIC ABSORPTION MODELS

Considering the significant physiological difference, the stomach, the small intestine, and the colon should be separately considered.

13.6.1 Stomach

The stomach has a much smaller surface area and shorter transit time compared to the small intestine. It is often suggested that its low pH is also a disadvantage for permeability of a basic drug (however, this would be arguable as the microclimate pH at the surface is higher [46]). Therefore, the permeability of a drug in the stomach is often neglected in biopharmaceutical modeling. However, the stomach

pH has a significant impact on the dissolution of dissociable drugs (Sections 8.6 and 13.1).

13.6.2 Colon

The colon also has a much smaller surface area compared to the small intestine. For epithelial-membrane-limited cases, by the shape and surface parameters for colon (PE, VE, and DF), colonic permeability can be appropriately expressed (Section 6.1.2).

For BCS III compounds, relative BA% from the colon is ca. 20% compared to that in the small intestine [47]. The preferable drug characteristics for colonic absorption are discussed in Section 11.8.5.2.

The general relationships between P_{oct}, P_{eff}, k_a, and Fa in the small intestine and colon predicted by the GUT framework are shown in Figure 13.8. Since the estimations of K_{bm} and $P_{trans,0}$ by P_{oct} were rough estimations, this figure should be taken as general trends. The difference of P_{eff} and k_{perm} in $\log P_{oct} > 1.5$ region is due to the difference of h_{UWL} and bile-micelle concentration. It was suggested that, compared to the small intestine, 0.5 log unit higher lipophilicity would be required to have a similar Fa in the colon. As the lipophilicity increased, the rate-limiting step changes from epithelial membrane permeation to UWL permeation, resulting in a superficial empirical relationship between the permeability ratio and $\log P_{oct}$.

13.6.3 Regional Difference in the Small Intestine: Fact or Myth?

13.6.3.1 Transporter. A site-specific membrane permeation of a drug in the small intestine ("absorption window") is often discussed in the literature, especially for transporter substrates. There are many reports investigating the GI position dependency of membrane permeability of drugs using *ex vivo* and *in situ* methods such as the Ussing chamber and intestinal perfusion methods (Table 13.1, Fig. 13.9). However, a significant difference (>2-fold) in permeability is rarely observed. As discussed in Section 6.4, the transporters such as P-gp, PEP-T1, and OATP are expressed more or less all along with the small intestine.

There is little *in vivo* and clinical evidence that shows more than twofold difference in permeability or Fa% caused by possible regional differences in the small intestine. From a C_p–time curve, it is perhaps nearly impossible to conclude the site-specific absorption of a drug in the small intestine. The bimodal peak in a C_p–time profile can be explained by an erratic gastric emptying and bile-micelle binding for many cases.

13.6.3.2 Bile-Micelle Binding and Bimodal Peak Phenomena. Pafenolol shows a double peak C_p–time profile in both humans and rats. Bile-micelle binding was suggested as the reason (Fig. 12.10) [56]. Owing to the site-specific reabsorption of bile acids, the unbound fraction increases at the end of the ileum. Especially, in the case of an efflux transporter substrate, a decrease in unbound

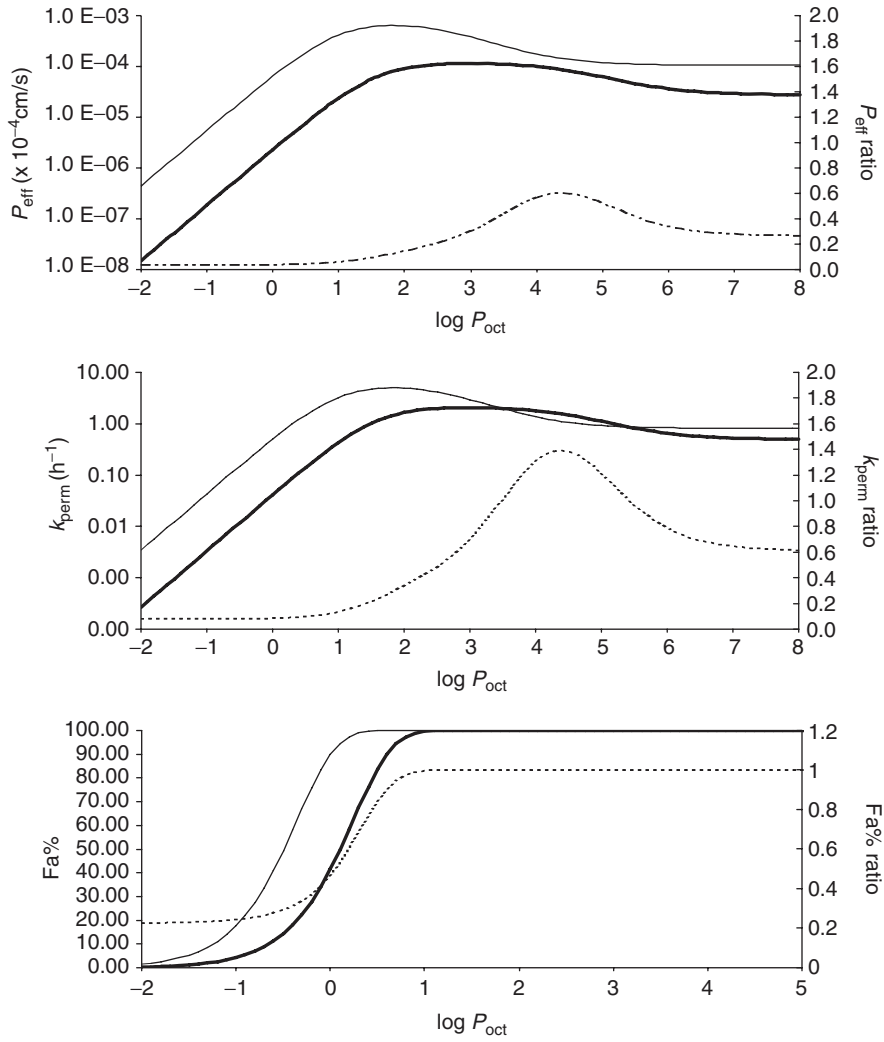

Figure 13.8 Comparison of the general relationship between P_{oct}, P_{eff}, k_a, and Fa in the small intestine and the colon. Solid line, small intestine; bold line, colon; dotted line, ratio of small intestine/colon.

drug concentration by bile-micelle binding can desaturate the efflux transporter in the proximal and middle small intestine.

Weitschies reported that talinolol showed double peak phenomena, whereas coadministered acetaminophen did not [57], suggesting that the bimodal peak was not caused by the irregular gastric emptying. It is interesting that when a food was taken after 1 h administration of talinolol, the double peak phenomena disappeared. One possible explanation would be that the two peaks collapsed as the intestinal transit time of the chymes already residing in the small intestine

TABLE 13.1 Regional Difference in Permeability

	Upper Intestine	Lower Intestine	Colon
Rat			
Ussing chambers (P_{app} (10^{-6} cm/s)) [48]			
Almokalant	11.4	16.23	30.18
Antipyrine	39.74	43.07	92.74
Atenolol	5.95	5.08	1.7
Creatinine	7.74	7.63	2.54
dDAVP	2.37	1.12	0.88
D-Glucose	56.75	51.93	2.8
Erythritol	8.25	5.7	2.02
Foscarnet	5.05	3.33	2.37
Gemfibrozil	59.12	69.65	100.26
Inogatran	3.33	3.95	1.32
L-Leucine	71.32	19.64	8.88
Mannitol	5.88	3.68	1.32
Metoprolol	34.91	78.07	85.09
Omeprazole	29.6	40.7	69.3
Phenytoin	24.04	46.13	66.1
Propranolol	28.95	41.3	87.9
Raffinose	4.37	2.63	2.48
Salicylic acid	19.74	19.51	17.6
Terbutaline	3.4	2.9	1.2
SPIP (P_{eff} (10^{-4} cm/s)) [49]			
Antipyrine	1.6	1.3	0.75
Atenolol	0.06	0.01	0.02
Fluvastatin	1.6	1.3	0.99
Metoprolol	0.33	0.53	0.09
Naproxen	2.1	2.1	2.5
Others			
Fexofenadine[a]	1	0.5	—
Bepotastine[b]	83	27	6
Rabbit			
SPIP permeation clearance (ml/min/cm) [50]			
Gancyclovir	0.44	0.63	0.47
Lobucavir	1.1	1.5	0.46
Human			
Talinolol[c]	1	0.52	—
Gabapentin[d]	29.6	15.2	7.9

[a] Perfusion/serum AUC ratio [51].
[b] % absorbed at 30 min in closed loop at 2 mM [52].
[c] Ratio of AUC in human intestinal perfusion study (upper intestine = 1) [53].
[d] AUC after regional dose in humans (μg h/ml) [54].

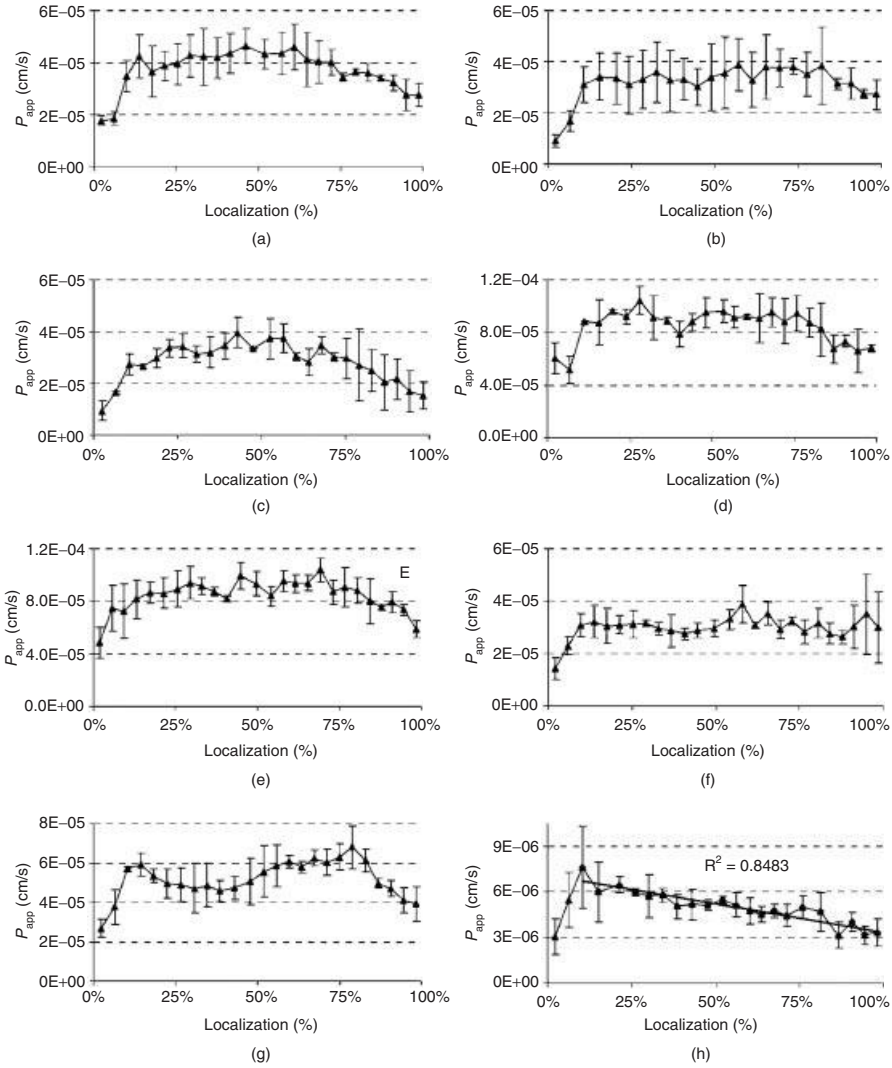

Figure 13.9 P_{app} (cm/s) along the rat small intestine. (a) mannitol, neutral; (b) lucifer yellow, anionic; (c) ranitidine, cationic: passive paracellular diffusion; (d) Testosterone; (e) antipyrine: passive transcellular diffusion; (f) L-Dopa; (g) glycylsarcosine: influx transporters LAT2 and PepT1, respectively; and (h) Digoxin: efflux transporter (P-glycoprotein). Each figure shows the P_{app} values (cm/s) plotted against the localization of each everted sac as percentage of the total length of the small intestine. The points are the means ± S.D ($n = 3$). Source: Adapted from Reference 55 with permission.

became significantly shortened by the house keeping wave (clean up the intestine before the next food comes) [58]. Ranitidine showed double peak phenomena in rats with intact bile duct, but not with bile duct-cannulated rats [59].

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CHAPTER 14

INTESTINAL TRANSPORTERS

> "How wonderful we have met with a paradox. Now we have some hope of making progress."
>
> —Neils Bohr

Biopharmaceutical modeling of carrier-mediated CM) transport processes is still under extensive investigation. At present, an apparent K_m value is used for kinetic analysis in many cases. However, as discussed in Section 4.8, the effect of UWL and differences of apical and cytosolic concentrations have to be taken into account when calculating the intrinsic K_m value, which is required for mechanistic modeling. In addition, the expression levels of transporters in the human intestine have not been quantified with sufficient accuracy. CM transports have been mainly investigated *in vitro*, and the results have been well summarized in many excellent review articles [1]. However, knowledge about the role of CM transport in *in vivo* oral absorption is limited. Even though many drugs have been identified as substrates for transporters *in vitro*, nonlinearity in AUC has been rarely reported, probably because the K_m values are often very high (>1 mM) [2] or contribution of CM transport is insignificant *in vivo* [3, 4].[1] This point is further discussed in Section 14.4. In this chapter, the literature information about

[1] A cellular or vehicle uptake assay does not reflect the paracellular permeation, resulting in underestimation of the contribution of passive permeation.

Biopharmaceutics Modeling and Simulations: Theory, Practice, Methods, and Applications, First Edition. Kiyohiko Sugano.

in vivo CM transports is mainly reviewed as the starting point for the development of biopharmaceutical modeling.

14.1 APICAL INFLUX TRANSPORTERS

Theoretically, as the dissolved drug concentration in the GI tract exceeds the K_m value, the main permeation route could change from CM transport to passive transport (Fig. 7.31). To appropriately simulate this change, not only the K_m and V_{max} values but also the contribution of passive permeation should be appropriately estimated. In the literature, the following equation is sometimes used to analyze the apparent dose–Fa% relationship [5]:

$$\mathrm{Fa} = \frac{X_{abs}}{\mathrm{Dose}} = \frac{X_{abs,max,CM}}{K_{dose} + \mathrm{Dose}} + \mathrm{Fa}_{passive} \tag{14.1}$$

where X_{abs} is the amount of the absorbed drug and K_{dose} is the half of the maximum absorbable dose ($X_{abs,max,CM}$) via CM transport.

14.1.1 Case Example 1: Antibiotics

Several antibiotics permeate the intestinal epithelial membrane via PEP-T1. It was demonstrated that Fa% of PEP-T1 substrates in humans can be predicted from the CM uptake clearance in Caco-2 [6] (Fig. 14.1).

Amoxicillin shows the subproportionality in dose–AUC profile (Fig. 14.2) [5, 7–9]. The dose–AUC data suggested that *in vivo* K_{dose} value at 2500-mg dose would be [ca. 55 mM (20 mg/ml) in the intestinal fluid] in the clinical dose range. After i.v. administration, the dose–AUC profile was linear. Several *in vitro* assays suggested that PEP-T1 plays a dominant role in cellular uptake of amoxicillin. The K_m value measured by the rat SPIP study was found to be 0.058 mM [10]. In the same report, significant contribution of passive transport was also noted (ca. 50%). In another report, no transmembrane permeation was observed in MDCK-PEP-T1 cells [11]. The solubility curve of amoxicillin is U shaped, with a minimum at pH 5, 5.5 mg/ml at 37°C [12], which is lower than the apparent K_m value in the clinical situation.

Cefatrizine also shows a dose-subproportional AUC increase between 500 and 1000 mg [8 and 17 mM (3.8 and 6.9 mg/ml) in the human small intestine[2]][13]. The K_m for the rat PEP-T1 was 0.6 mM [14]. The solubility of cefatrizine is 4.6 mg/ml.

The rate of oral absorption of cefadroxil was reduced by ca. 50% as the dose was increased from 5 to 30 mg/kg [7.4 to 44.5 mM, K_i (hPEP-T1, GlySar) = 7.2 mM, K_m (rPEP-T1) = 5.9 mM] [2, 14]. Coadministration of cephalexin [45 mg/kg (70 mM), K_i (hPEP-T1, GlySar) = 14.4 mM] decreased the oral

[2]Based on V_{GI} = 130 ml. In this section, the intestinal concentration of a drug is calculated in the same manner.

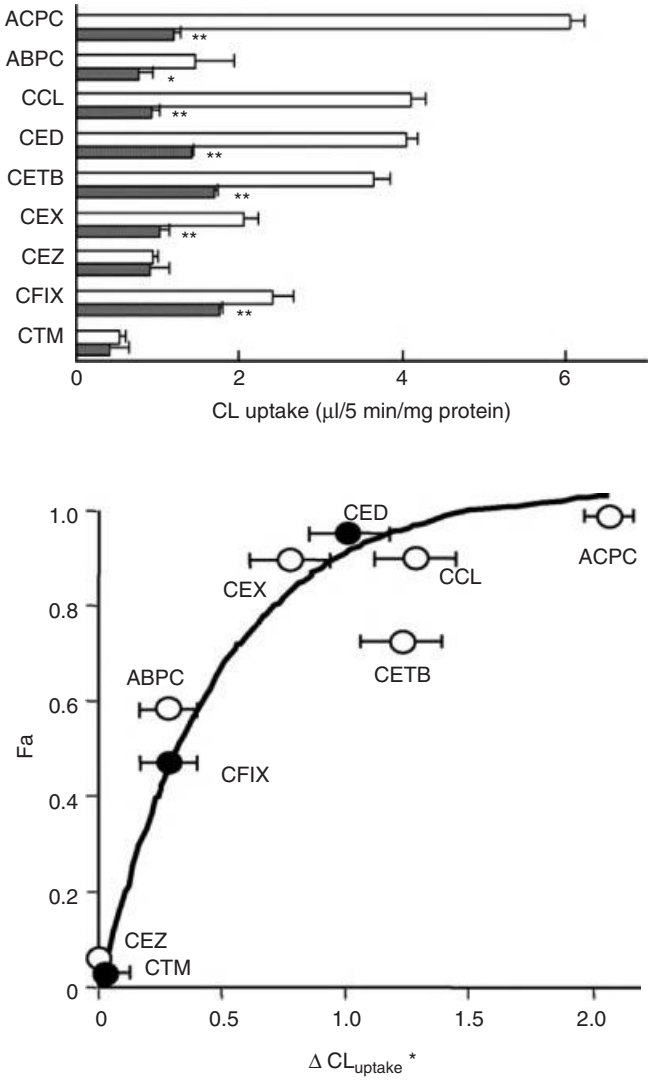

Figure 14.1 Prediction of clinical Fa% from Caco-2 uptake clearance ($^{*}P < 0.05$, $^{**}P < 0.01$). *Source*: Adapted from Reference 6 with permission.

absorption rate of cefadroxil by 50% [15]. As the amount of urinary excretion remained the same, it was suggested that Fa% was not changed as cefadroxil was completely absorbed even at a high dose or in the presence of cephalexin. Therefore, the contribution of PEP-T1 to the oral absorption rate of cefadroxil would likely to be ca. 50%, but the other process(es) is fast enough to give a sufficient oral absorption. However, in the other two reports, dose-subproportionality in the absorption rate was not observed in the 250- to 1500-mg dose range [16, 17].

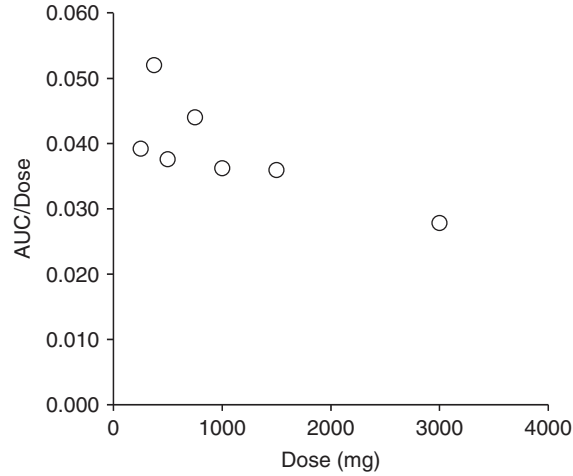

Figure 14.2 Dose–normalized AUC of amoxicillin [5, 7–9].

14.1.2 Case Example 2: Valacyclovir

Valacyclovir is a prodrug of acyclovir and has been suggested to be absorbed via PEP-T1 [18, 19]. The K_m value for human PEP-T1 is 1.6 mM [20]. Valacyclovir is rapidly converted to acyclovir in the body. The dose-normalized AUC (as of acyclovir) after dosing valacyclovir is plotted in Figure 14.3. In this dose range

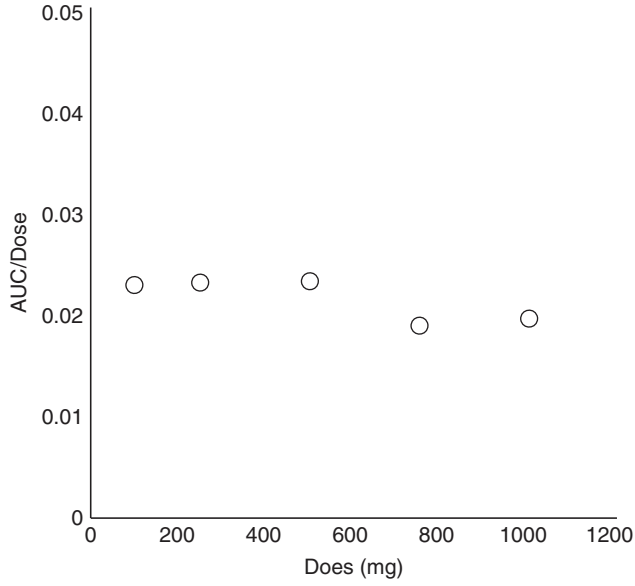

Figure 14.3 Dose–normalized AUC of acyclovir after dosing valacyclovir [21].

[100–1000 mg (2.1–21 mM)] little reduction of AUC/dose was observed [21]. In addition, moderate reduction of total urinary excretion (ca. 40%) was observed in this dose range after single-dose administration (but not after multiple-dose administration). The therapeutic dose is 500–1000 mg. The acyclovir AUC after dosing valacyclovir (500 mg) did not correlate with the PEP-T1 expression level [22] and was affected only a little by coadministration of PEP-T1 inhibitor [cephalexin 500 mg (11 mM) [6, 23]. From the molecular weight of valacyclovir free base (=324) and pK_a (=7.47), Fa% via the paracellular pathway is predicted to be 39%. From the urinary excretion, Fa% was estimated to be from 50% (at high dose) to 80% (at low dose). Therefore, the contribution of PEP-T1 would likely to be ca. 50% or less. The increase of oral absorption by the prodrug approach may be partly explained by the increase in solubility, as acyclovir shows solubility-permeability-limited absorption at >400-mg dose range (Section 8.5).

14.1.3 Case Example: Gabapentin

Gabapentin is a substrate of LAT-1. The dose dependency of Fa% of gabapentin in humans is shown in Figure 14.4). Fa% is 74% at <400-mg dose and decreases to 36% at higher doses. Fa% of gabapentin in humans was appropriately predicted from rat P_{eff} data [24]. From the dose–Fa% relationship, it can be suggested that passive transport contributes ca. 50% of total permeability at <400-mg dose. In *in situ* and *ex vivo* studies in rats (perfusion and chamber methods, respectively), permeability of gabapentin is about twofold higher than that of mannitol (a paracellular marker) at 0.01 mM concentration [25]. In the presence of an inhibitor, the permeability of gabapentin was reduced to a value similar to that of mannitol. Considering the similarity of MW (171 vs 180) and charge (neutral), data

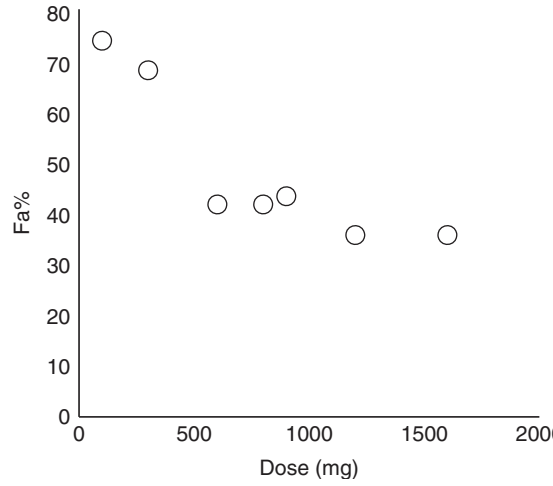

Figure 14.4 Fraction of dose absorbed as a function of oral dose of gabapentin to humans [24].

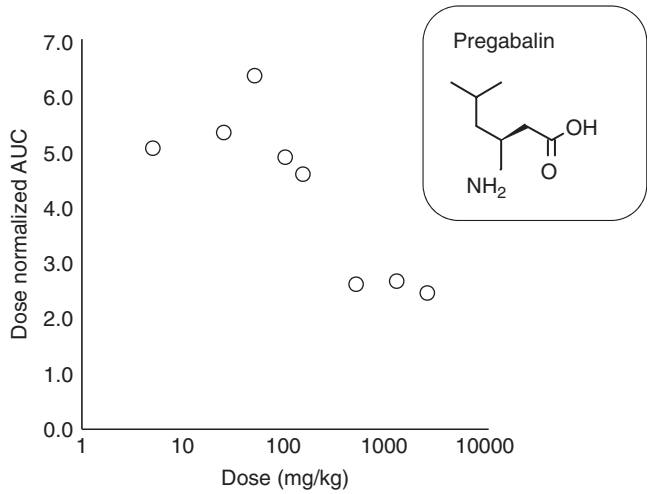

Figure 14.5 Dose-normalized AUC of pregabalin in rats [26]

from rats suggest that paracellular permeation explains about half of the total permeability. From the molecular weight of gabapentin and pK_a [3.68 (acid) and 10.70 (base)], Fa% via the paracellular pathway is predicted to be 70%. This is in good agreement with the clinical data of gabapentin. The contribution of CM and passive transport is likely to be ca. 50:50 to the net permeability of gabapentin at <400-mg dose.

Pregabalin has a chemical structure similar to gabapentin; however, the contribution of CM transport to its oral absorption was suggested to be more significant than that for gabapentin [26]. However, the oral PK is linear in the 50–300 mg range in humans. The oral absorption in rats was nonlinear in the 50 ∼ 500 mg/kg range (Fig. 14.5) [27].

14.2 EFFLUX TRANSPORTERS

14.2.1 Effect of P-gp

Prediction of the effect of P-gp on *in vivo* oral absorption of drugs from *in vitro* data has not been investigated for humans, partly because there is little quantitative evaluation of data of the P-gp effect in humans. A specific inhibitor for P-gp would be of great help to progress in this area.

By using knockout (KO) mice, the predictability of the Caco-2 cells for the P-gp effect was investigated. The bioavailability ratio of KO and wild-type mice was found to correlate with the absorption quotient obtained by the Caco-2 cell assay (Fig. 14.6) [28].

The oral absorption of maraviroc was suggested to be reduced by P-gp (Fig. 14.7; Fig. 12.12). The exposure after oral administration was found to be dose-suprapropertional, whereas it was dose-linear after i.v. administration

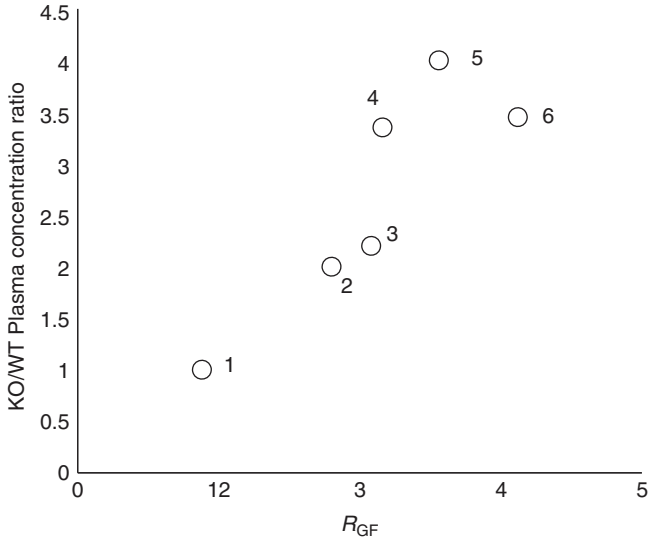

Figure 14.6 Correlation of R_{GF} ($P_{app,inhibitor}/P_{app,no\ inhibitor}$ in Caco-2) with oral plasma concentration in P-gp KO and wild-type mice after i.v. correction for effects of P-gp on drug clearance. Data points are 1, verapamil; 2, digoxin; 3, paclitaxel; 4, S09788; 5, saquinavir; and 6, tacrolimus. *Source:* Replotted from Reference 28.

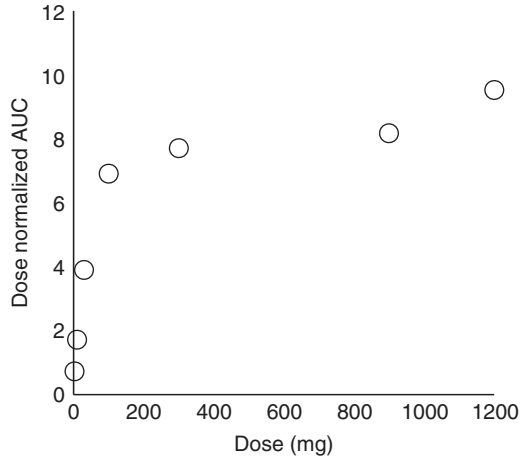

Figure 14.7 Dose–AUC dependency of maraviroc.

[29, 30]. The K_m value for P-gp is 37 μM [31]. Maraviroc is mainly metabolized by CYP3A4 ($K_m = 21$ μM) [31]. The intrinsic hepatic clearance is 44 ml/min/kg, suggesting that Fg is 0.91 (Section 4.10.2). The unbound drug concentration in the portal vein is estimated to be less than 1 μM ($=\text{dose} \times k_{abs} \times \text{Fg} \times f_{u,p}/Q_h$). Therefore, the dose supralinearity was suggested to be due to the P-gp efflux in the oral absorption process, rather than first-pass metabolisms and systemic

clearance processes. Considering that the cytosolic unbound drug concentration would be one-tenth of that on the apical side (see next section), the dose strength that saturates P-gp efflux can be estimated as 25 mg from the *in vitro* K_m value (assuming $V_{GI} = 130$ ml). This value is in good agreement with the clinical observation.

14.2.2 Drug–Drug Interaction (DDI) via P-gp

Drug–drug interaction (DDI) via P-gp has been extensively discussed in the literature [32]. As an empirical rule, the following criteria has been proposed to determine the possible DDI via intestinal efflux transporters [33].

$$\frac{[I]_2}{\mathrm{IC50}} \geq 10$$
$$[I]_2 = \frac{\mathrm{Dose}}{250\ \mathrm{ml}} \tag{14.2}$$

Theoretically, owing to the concentration gradient across the epithelial membrane, the concentration of a drug in the cytosol is lower than that in the intestinal fluid (Section 4.9). The following criteria would be useful to diagnose the DDI and saturation of efflux transport [34]:

$$\text{Undissociable } C_{\mathrm{dissolv}} f_{\mathrm{mono}} \times 1/3 > K_m, K_i$$
$$\text{Base } (\mathrm{p}K_a > 7.5) C_{\mathrm{dissolv}} f_{\mathrm{mono}} \times 1/10 > K_m, K_i$$
$$\text{Acid } (\mathrm{p}K_a < 5.5) C_{\mathrm{dissolv}} f_{\mathrm{mono}} \times 1/2 > K_m, K_i$$

where $C_{\mathrm{dissolv}} f_{\mathrm{mono}} = \mathrm{Dose} f_{\mathrm{mono}}/V_{GI}$ (Do $<$ 1) or $C_{\mathrm{dissolv}} f_{\mathrm{mono}} = S_{\mathrm{dissolv}} f_{\mathrm{mono}}$ ($= S_{\mathrm{blank}}$) (Do $>$ 1) [35]. It should be noted that here K_m and K_i are the intrinsic values (Section 4.9.5). Owing to the asymmetry of apical and basolateral membranes and difference of apical and cytosolic pH, when the permeation of the drug is epithelial membrane limited, the unbound drug concentration in the cytosol is roughly 1/2, 1/3, and 1/10 of the apical unbound concentration for acid, undissociable, and base drugs, respectively. For the UWL-limited case, the cytosolic concentration becomes smaller further. These theoretical results are in good agreement with the empirical rule of $[I]_2/\mathrm{IC50} > 10$ as the rule is obtained mainly based on the DDI with base drugs.

The K_m values for P-gp are usually less than 100 μM for many drugs. For the MW = 400 and $K_m = 100$ μM case, the doses for >50% saturation would be at ca. 20, 50, and 3 mg for undissociable, base, and acid drugs, respectively.

Figure 14.8 shows the relationship between $[I]_2/\mathrm{IC50}$ and the digoxin AUC and C_{max} ratio with/without inhibitor [36]. Even though the DDI via an intestinal transporter has been extensively discussed in the literature, clinical evidences seem to be sparse. Compared to the DDI via intestinal CYP3A4, the DDI via intestinal P-gp was reported to be milder, usually less than two- to three-fold change in AUC [35, 36]. From the theoretical perspective, as discussed in

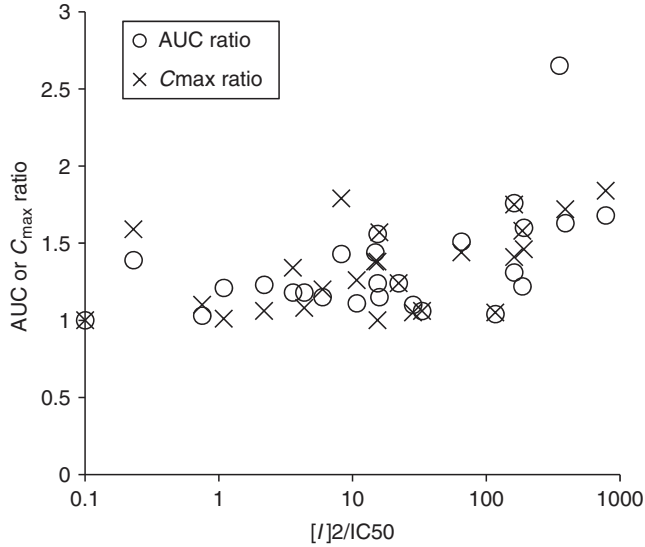

Figure 14.8 Relationship between $[I]_2/IC_{50}$ of various inhibitors and clinical AUC and C_{max} ratios of digoxin (with/without an inhibitor) [36].

Section 4.9, the maximum difference in Fa% with and without P-gp inhibition (ca. sevenfold) would be observed for moderately lipophilic basic drugs (Fig. 4.22).

Digoxin has most often been used as a probe compound for P-gp in clinical studies. However, the appropriateness of digoxin as a probe has been questioned [37, 38]. The effect of P-gp on Fa of a target drug in humans is difficult to quantify, as there is no appropriate specific inhibitor for intestinal P-gp. P-gp not only affects the intestinal absorption but also affects the biliary and renal eliminations.

Induction of P-gp also causes DDI. After repeated dose of rifampin, the AUC of digoxin after oral administration was significantly reduced, whereas after i.v. administration, it was unchanged (Fig. 14.9) [39]. The AUC of talinolol was also reduced by rifampin [40], but it is not conclusive whether this is via intestinal P-gp or change in drug disposition [41].

14.3 DUAL SUBSTRATES

14.3.1 Talinolol

Talinolol is a substrate of both P-gp and OATP in the small intestine. As the metabolism of talinolol is negligible, it is a suitable substrate for transport studies. Figure 14.10 shows the dose-normalized AUC change of talinolol in humans [42]. This dose dependency was suggested to be due to the saturation of P-gp.

The effect of naringin, a component of grapefruit juice, on the oral absorption of talinolol in rats is shown in Figure 14.11 [43]. Naringin has an IC_{50} of 12.7 μM for OATP (rat Oatp 1a5) and 604 μM for P-gp. Therefore it was suggested that

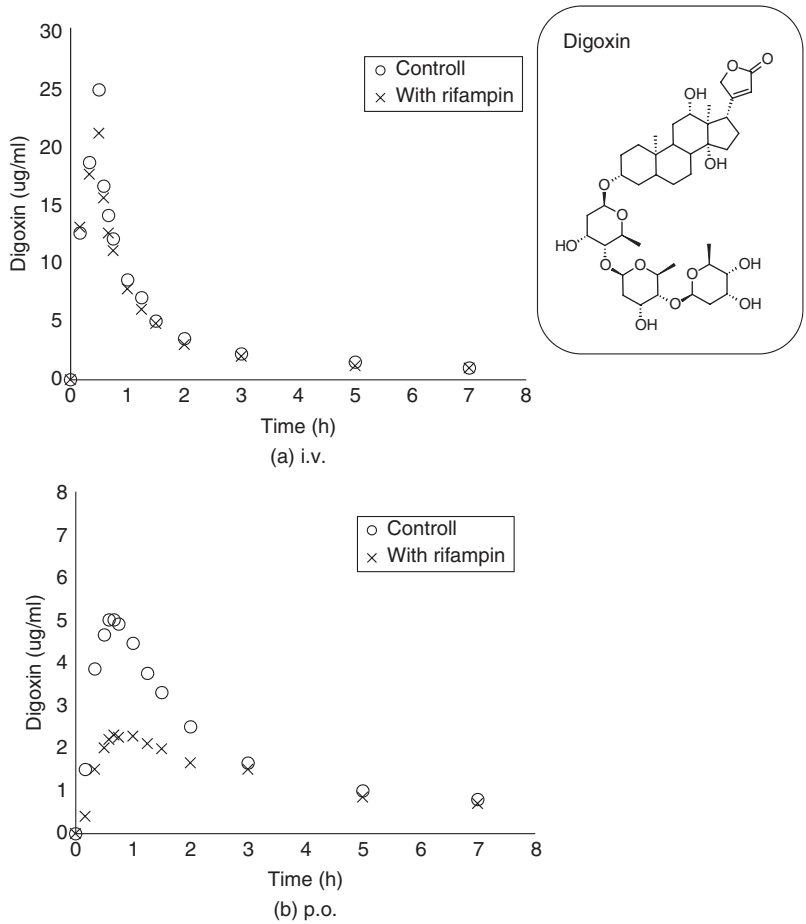

Figure 14.9 Mean ($n = 8$) plasma concentration (mean ± SD) time curves of intravenously (a) and orally (b) administered digoxin (1 mg) before and during coadministration of rifampin (600 mg). *Source:* Replotted from Reference 39.

at a low dose of naringin, only OATP was inhibited and AUC was decreased, whereas at a high dose, P-gp was inhibited and AUC was increased.

In a clinical study, 300 ml of grapefruit juice decreased the talinolol AUC and C_{max} values to 56% and 57% (Fig. 12.11), respectively, of those of water at 50-mg dose strength (1 mM) [44]. Talinolol T_{max} and $T_{1/2}$ were not affected. The 48-h cumulative excretion of talinolol into urine was reduced to 56% of that observed with water without alteration in renal clearance. The amounts of grapefruit juice constituents, naringin, dihydroxybergamottin, and bergamottin, were 712, 492, and 45 μM, respectively. K_m of talinolol for human OATP is 600–700 μM [45] and that for human P-gp is 74 μM [46].

The grapefruit juice effect in rats is different from that in humans. The K_m value of naringin is 604 μM for rat P-gp (Mdr1a), whereas it is >2000 μM

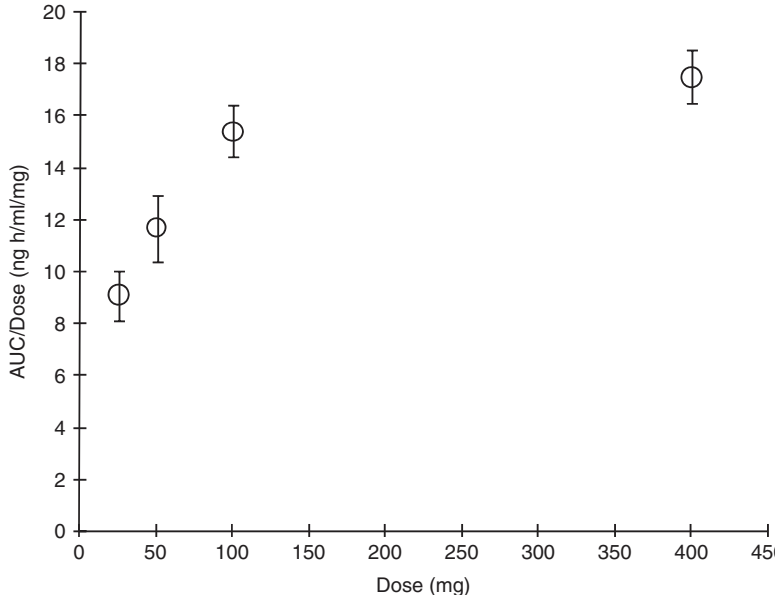

Figure 14.10 Dose-normalized AUC of talinolol [42].

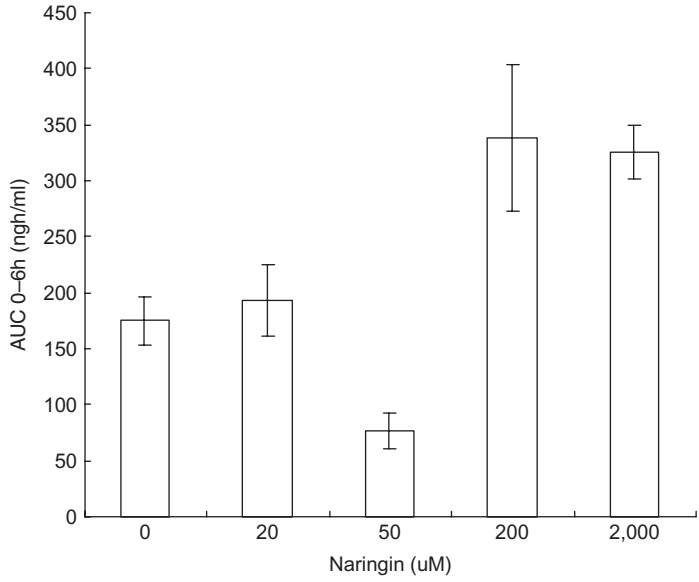

Figure 14.11 Effect of naringin on AUC of talinolol in rats [43]. Area under plasma concentration-time curve from 0 to 6 h is plotted against naringin concentration. Talinolol was administered as a racemic mixture at 10 mg/kg; 5 ml/kg. Data are represented as means ± SEM ($n = 4$).

for human P-gp (MDR1). Therefore, naringin in grapefruit juice of ca. 700 μM inhibited the rat P-gp but did not inhibit the human P-gp. In a clinical study, oral coadministration of the antibiotic erythromycin (2 g), a known P-gp inhibitor, significantly increased the talinolol serum AUC [47]. However, another P-gp substrate, verapamil, decreased the AUC of talinolol by 25% [48].

14.3.2 Fexofenadine

Fexofenadine is a well-established substrate for P-gp and OATP [49, 50]. The jejunum P_{eff} in humans is low ($0.1–0.2 \times 10^{-4}$ cm/s) and variable. According to the BCS, the drug is classified as a compound with low permeability (BCS class III) [51]. Fexofenadine has MW = 468 and is a zwitter ion. Therefore, the contribution of the paracellular pathway was estimated to be minimal.

The plasma exposure of fexofenadine was found to be linear over a wide clinical dose range of 40–800 mg (0.7–13 mM) [52]. In addition, fexofenadine had similar bioavailability (40% vs 28%) when given orally as a microdose (<100 μg) and at a higher dose of 120 mg [53]. P-gp inhibition by coadministration of verapamil and itraconazole was shown to increase AUC by threefold (so that BA% was increased to nearly 100%), whereas the elimination half-life was not changed [54, 55].

On the other hand, fruit juice (grapefruit, orange, and apple) significantly decreased the AUC of fexofenadine after oral administration (Fig. 12.13). Inhibition of OATP is suggested to be the mechanism behind these fruit juice effects. The estimated drug concentration at 120-mg dose is 1.8 mM. K_m for human OATP was 6 μM [50] and rat Oatp was 59 μM [56].

Fexofenadine displayed polarized transport in Caco-2 cells, with the $P_{app,BA}$ (apparent basolateral to apical permeability) being 28- to 85-fold higher than the $P_{app,AB}$ in the concentration range of 10–1000 μM [49]. $P_{app,BA}$ decreased with increasing concentration ($V_{max} = 5.21$ nmol/cm^2/s and apparent $K_m = 150$ μM). In addition, *in vitro* data obtained using Caco-2 cells suggest that the *in vitro* permeability was increased in the apical to basolateral direction by approximately two- to threefold in the presence of various P-gp inhibitors, such as verapamil, ketoconazole, and GF 120918 [49, 50, 57]. However, the $P_{app,AB}$ was independent of the concentration applied (10 μM–1000 μM range) (cf. drug concentration in the cytosol is lower than that in the apical side). This is in good agreement with the above-described dose-linear clinical PK.

Most reported clinical DDIs of fexofenadine, including those for verapamil and ketoconazole, partly resulting in increase in plasma levels (AUC), were explained by inhibition of intestinal efflux by P-gp [54]. However, it was recently found that there was no or a little acute effect of any of these two P-gp inhibitors, verapamil and ketoconazole, on the jejunum P_{eff} of fexofenadine in humans and rats [51, 56]. A recent *in vivo* perfusion study with simultaneous assessment of intestinal transport and plasma pharmacokinetics suggested that liver uptake of fexofenadine was mediated by OATP1B1 and/or OATP1B3, which could also be inhibited by verapamil and ketoconazole [58]. In addition, by using double

transfected cells expressing OATP1B1/multidrug-resistance-associated protein 2 (MRP2) and OATP1B3/MRP2, it was shown that OATP1B1 and OATP1B3 are involved in hepatic uptake of fexofenadine [59]. Therefore, this evidence suggested that the DDI could occur in the liver uptake process rather than in the intestinal membrane permeation process, which is also supported by a recent physiological-based pharmacokinetic model [60].

14.4 DIFFICULTIES IN SIMULATING CARRIER-MEDIATED TRANSPORT

As discussed in the previous sections, many drugs have been identified as substrates for a transporter(s). However, the contribution of a transporter to the net permeability of a drug *in vivo* is often difficult to estimate. Furthermore, it is difficult to predict the dose-nonlinearity *in vivo* from the *in vitro* K_m values. As examples of this fact, gabapentin, valacyclovir, cefadoxil, talinolol, and fexofenadine have already been discussed in Section 14.1.

14.4.1 Absorptive Transporters

14.4.1.1 Discrepancies Between In Vitro and In Vivo K_m Values. Simulation of dose dependency for CM transport is of great interest. However, as exemplified below, it is currently difficult to estimate the *in vivo* dose dependency from an *in vitro* K_m value.

Considering the K_m and dose strength, cyclacillin (Fa% = 95%, hPEP-T1 K_m or K_i = 1.15 mM, hereafter the same), cephradine (94%, 8.3 mM), cefaclor (90%, 7.6 mM), cephalexin (90%, 2.9–7.5 mM), and ceftibuten (70%, 0.5–1.0 mM) [6] should be good examples to show a dose-subproportional oral absorption in humans. The AUC of cefaclor in humans was dose-linear between 250 mg (5.2 mM) and 500 mg (10.4 mM), but the C_{max} was slightly nonlinear (20% reduction) [61]. The AUC of ceftibuten in humans was dose-linear between 25 mg (0.43 mM) and 200 mg (3.4 mM), but the C_{max} was slightly nonlinear between 25 and 100 mg (26% reduction) [62]. The AUC and C_{max} of cephalexin in humans were dose-linear between 250 (5.5 mM) and 1000 mg (22 mM) [61]. On the other hand, in studies on rats, these compounds showed clear evidences for CM transports [10, 14, 63]. Cyclacillin (1500 mg) delayed the T_{max} of amoxicillin, but AUC was changed only slightly [64]. However, cyclacillin had only slight effect on the PK profiles of ampicillin and bacampicillin [64].

The oral absorption of celiprolol at 100-mg dose was significantly reduced by grapefruit juice [65]. In addition, its AUC was reduced by 50% in an OAPT2B1 gene mutant [65]. However, the K_m for humans was 21 μM [66] and the estimated intestinal concentration at 100 mg was 2.8 mM. Interestingly, when coadministered, the AUC of celiprolol correlated with that of atenolol [65]. In a mouse *ex vivo* study, the permeability of celiprolol was found to be similar to that of FITC-4000 (a paracellular marker) [66].

Pravastatin and oseltamivir were suggested to be predominantly absorbed by transporters (OATP and PEP-T1, respectively) from *in vitro* and rat data [67, 68]. The oral absorption of pravastatin in rats (100 mg/kg, 127 mM) was found to be decreased by more than 10-fold under the coexistence of naringin (1 mM, IC50 0.03 mM). The K_m of pravastatin for rat Oatp1a5 is 0.12 mM. The oral absorption of oseltamivir (30 mg/kg in rats, ca. 52 mM) was decreased by more than 10-fold under the coexistence of an inhibitor (125 mM GlySar), suggesting that PEP-T1 plays a predominant role in its oral absorption. *In vitro* (HeLa cell/PEP-T1), K_m was 8.59 mM. However, these substantial effects were not observed in humans [69, 70].

Ribavirin and riboflavin were also substrates for influx transporters [71, 72]. These compounds show dose-subproportional absorption in humans (600–2400 mg [71] and 5–30 mg [73]). The intestinal concentration estimated from the clinical dose is significantly higher than the K_m values obtained in *in vitro* experiments [5–10 μM (Na^+ nucleoside purine transporter) and <1 μM] [71, 72].

All in all, these extensive gaps between *in vitro* and *in vivo* observations suggest that further investigation is required to fully understand the role of transporters in oral absorption of drugs.

14.4.1.2 Contribution of Other Pathways. The contributions of PEP-T1 in *in vivo* oral absorption are reported to be ca. 60% for cefixime (by normal/KO mice comparison) [74], ca. 50% for cephalexin (from rat *in situ* permeability) [75], and 30–60% for GlySar (by normal/KO mice comparison) [76]. For ampicillin and ceftibuten, it was suggested that the paracellular component was significant [3, 4].

A similar aspect was also demonstrated for ACE inhibitors [77, 78], some of which are historically claimed to be substrates for PEP-T1. Of the 14 ACE inhibitors investigated, the majority displayed weak or no CM transport. Very low transports were observed *in vitro* only for four of the inhibitors, including enalapril, which has previously been cited as a drug that is transported by PEP-T1. Considering the low affinities, low transport activities, relatively moderate to high lipophilicity (log $D_{oct} > 0$ in 10 of 14 drugs), and daily dose (higher concentration would be achieved in the intestine at a clinical dose, compared to the concentration used in an *in vitro* study), it was suggested that it is highly unlikely that the peptide transporters dominantly controlled *in vivo* intestinal absorption of any of the ACE inhibitors.

14.4.2 Efflux Transporters

Even though many drugs have been diagnosed as substrates of P-gp in an *in vitro* model, its effect on *in vivo* oral absorption is minimal for many cases. This point has been clearly demonstrated by using P-gp KO animals (Table 14.1 and Table 14.2) [79–81].[3] Four reasons have been proposed to explain this

[3]This is in good contrast to the P-gp effect on the distribution of a drug in the brain.

TABLE 14.1 P-gp Knockout Data of Mice[a]

Drug	Plasma AUC or Concentration Ratio		BA Ratio	Brain to Plasma ratio
	i.v.	p.o.		
Amprenavir	NA	1.3	<1.3	21
Asimadoline	1.0	1.1	1.1	9.1
Benzo(a)pyrene	0.8	0.8	1.0	1.6
Cyclosporine A	1.1	0.6–0.9	0.5–0.8, 1.6	11–29
Digoxin	NA	2.4	2	4–28
Dihydroergocryptine	1.8	1.8	1.0	1.1
Erythromycin	1.5	3.4	2.3	1.2
Fexofenadine	1.0, 4.6	4.6, 6.5	1.0, 6.5	1.9
Fluconazole	1.2	1.2	1.0	0.9
Indinavir	0.7	2.0	2.9	3–10
Ivermectin	NA	1.9–3.7	<1.9–3.7	17–27
Loperamide	2.0	2.0	1.0	6.7
Nelfinavir	1.3	4.8	3.7	31
Paclitaxel	1.1–2	5.0–6.0	2.5–5.5, 3.18–6.71	7.9
Reserpine	1.2	1.2	1.0	2.4
Retinoic acid	1.0	1.1	1.1	1.0
Ritonavir	1.0	1.0	1.0	6.9
S 09788	NA	2.4	3.4	NA
Salinomycin	NA	NA	1.5	NA
Saquinavir	0.7–1.1	6.5	1.55, 5.9–9.3	2.2–6.8
Tacrolimus	2.3	8.2	3.5, 3.6	6
Topotecan	NA	2.3	<2.3	2.0
UK-224,671	1.1	>40	>36	NA
Verapamil	NA	1	1	8.3
Vinorelbine	NA	NA	1.5	NA
Rifampicin	NA	3.5	NA	NA
Talinolol	NA	2.9	NA	NA

Abbreviation: NA, not available.
[a] References 28, 84 and 85

discrepancy: (i) at a clinical dose, the concentration of a drug in the intestinal fluid is high enough to saturate P-gp [a low concentration (2–10 μM) is often used in an *in vitro* assay), (ii) the passive permeability of the drug is fast enough to overcome the effect of efflux transporter and to give 100% absorption, (iii) paracellular permeants are not prone to be affected by P-gp,[4] and (iv) no addition of plasma proteins in the basolateral side results in higher unbound

[4] As the surface area of the basolateral membrane is three-fold larger than that of the apical membrane and the basolateral pH is ca. 1 unit higher than the apical pH, the passive membrane clearance of a base is ca. 30-fold higher in the basolateral membrane. Owing to the subepithelial diffusion resistance, removal of a drug from the basolateral membrane would not be infinitely fast. Therefore, it might be possible that a base drug, which permeated through the paracellular pathway, may diffuse into the cytosol. This point requires further investigation.

TABLE 14.2 P-gp Knockout Data of Dogs[a]

Drug	Dose mg/kg	P-gp Expression	C_{max}	AUC
Fexofenadine	0.1	Wild type ($n = 5$)	53.9 ± 13.4 ng/ml	392 ± 77 ng h/ml
	0.1	P-gp null ($n = 6$)	90.7 ± 23.1 ng/ml	881 ± 249 ng h/ml
Quinidine	0.1	Wild type ($n = 5$)	16.5 ± 3.4 ng/ml	58.8 ± 12.8 ng h/ml
	0.1	P-gp null ($n = 6$)	20.0 ± 7.9 ng/ml	89.3 ± 21.8 ng h/ml
Loperamide	0.01	Wild type ($n = 5$)	80.8 ± 9.0 pg/ml	467 ± 85 pg h/ml
	0.01	P-gp null ($n = 6$)	101 ± 15 pg/ml	556 ± 91 pg h/ml
Drug	Dose (mg/kg)		BA%	
Quinidine	0.1	Wild type ($n = 3$)	41 ± 24	
	0.1	P-gp null ($n = 3$)	32 ± 13	
Loperamide	0.2	Wild type ($n = 2$)	67 (38–96)	
	0.2	P-gp null ($n = 3$)	46 ± 36	
Nelfinavir	2	Wild type ($n = 2$)	4.5 (4.4–4.6)	
	2	P-gp null ($n = 3$)	3.6 ± 2.6	
Cyclosporin	4	Wild type ($n = 2$)	40 (32–48)	
	4	P-gp null ($n = 3$)	44 ± 10	

[a] References 79 and 80.

fraction in an *in vitro* assay [82]. Considering the vacuum cleaner mechanism of P-gp, hydrophilic compounds would not be efficiently carried by P-gp (Section 4.9). Therefore, the effect of P-gp on the oral absorption of a drug can be significant only for drugs with low to moderate passive permeability. The clinical evidences suggested that this DDI via intestinal transporters is usually mild [83], compared to that via CYP3A4.

P-gp-CYP3A4 interplay has been under extensive investigation (Section 4.10.4). However, recent simulation study suggested that this interplay would occur only in limited conditions with K_m and passive permeability [86].

14.5 SUMMARY

In this chapter, the contributions of intestinal transporters to the oral absorption of drugs were reviewed focusing on the clinical data. In the literature, many drugs have been identified as substrates for transporters by using a highly sensitive *in vitro* assay (e.g., overexpression cells, use of low concentration, etc.). However, even though extensive literature survey was performed, it was difficult to find a case in which a CM transport contributed predominantly (>75%) to oral absorption of a drug in humans. There are several cases in which a drug diagnosed as a transporter substrate by an *in vitro* study is later questioned on contribution in *in vivo* pharmacokinetics. This situation illustrates the difficulties in predicting the contribution of CM transports from *in vitro* data. Biopharmaceutical modeling for CM transports is still challenging and requires further investigations in the future.

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