4
Drug Absorption Principles

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4.1 Drug Absorption and Bioavailability

Pharmacokinetics describes drug absorption, distribution, metabolism, and excretion processes. Absorption is the rate and extent at which drugs reach the systemic circulation from the site of administration. Distribution of a drug includes all the processes that are involved from the time when the drug reaches the circulation to the time when it (or a metabolite of the drug) leaves the body. Metabolism involves all the biochemical processes that result in a chemical change to the drug compound including both the metabolism in the gut wall, the liver, and blood circulation. Excretion is the process in which the drug is eliminated from the systemic circulation into bile, urine, feces, sweat, and air (Allen, 1982). The reader is referred to authoritative texts in this area for a detailed review.

Bioavailability means the rate and extent to which the API or active moiety is absorbed from a drug product and becomes available at the site of action (Atkinson, 2001; Chiou, 2001; Toutain and Bousquet-Melou, 2004). Drug absorption plays an important role in bioavailability (F) determination since the drug absorption contributes importantly to the time and extent that drug targets exposure to therapeutic drugs *in vivo*. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by the measurements intended to reflect the rate and extent to which active ingredient or active moiety becomes available at the site of action. Bioavailability can be mathematically represented by the equation: $F = F_a \times F_g \times F_h$, in which F_a is the fraction of drug absorbed, F_g is the fraction that escapes metabolism in the gastrointestinal tract, and F_h is the fraction that escapes first pass hepatic metabolism (Kwan, 1997; Sun *et al.*, 2004). Based on the above equation, one of the main factors governing the bioavailability of a compound is the fraction of the drug absorbed.

Oral drug absorption process occurs mainly in small intestinal regions, which includes passive transcellular diffusion, carrier-mediated transport processes, paracellular transport, and endocytosis. In general, lipophilic compounds are usually absorbed by passive diffusion through the intestinal epithelium. Many hydrophilic compounds are absorbed through a carrier-mediated process, while some small hydrophilic compounds may be transported through the paracellular junction. Under physiological conditions, the fastest absorption process may

dominate the absorption for a particular compound (Sun *et al.*, 2004; Cao *et al.*, 2005).

Absorption of a compound is governed by many processes. Two fundamental parameters govern drug absorption: drug solubility and gastrointestinal permeability (Amidon *et al.*, 1995). If both drug solubility and permeability are enhanced, there will be a great increase in the rate and extent of oral absorption. Therefore, the oral bioavailability of a drug is largely a function of its solubility characteristics in gastrointestinal fluids, absorption into the systemic circulation, and metabolic stability.

4.2 Types of Intestinal Membrane Transport

Intestinal membrane transport include paracellular and transcellular transport (Fig. 4.1). Transcellular transport can be further divided into passive diffusion, endocytosis, and carrier-mediated transport. Paracellular transport refers to the passage of solute without passage through the epithelium cells (Higuchi and Ho, 1988; Narawane and Lee, 1994; Ungell *et al.*, 1998; Oh *et al.*, 1999).

4.2.1 Passive Diffusion

Hydrophobic molecules can pass through the lipid bilayers by random molecular motions. The direction of mass transfer of molecules or substances by passive diffusion depends on the concentration gradient on the two sides of the membrane. Lipophilic compounds are generally absorbed by passive diffusion through the

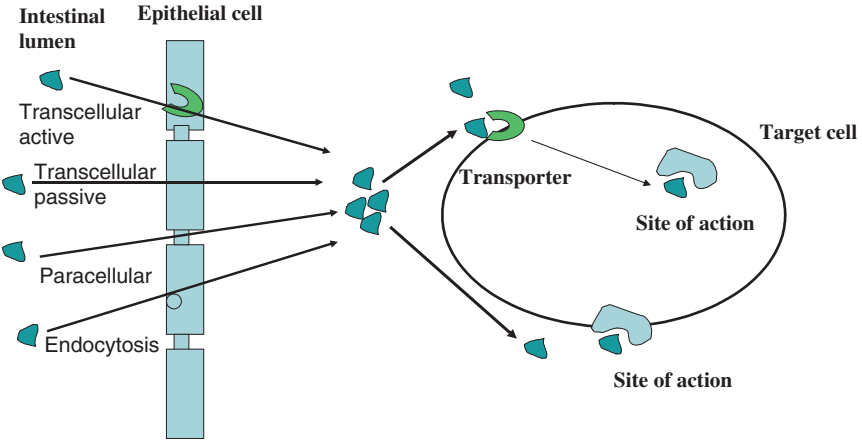

FIGURE 4.1. Drug transport and site of action (*See* Color Plate I)

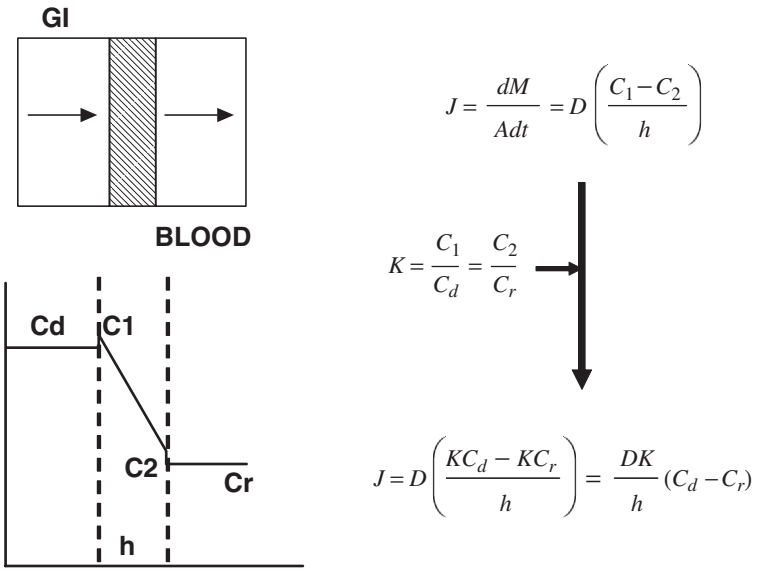

FIGURE 4.2. Fick's first law of diffusion

intestinal epithelium. The passive diffusion of the molecules is governed by Fick's first law (Lennernas, 1998; Yoon and Burgess, 1998; Chidambaram and Burgess, 2000).

Fick's first law of diffusion (Fig. 4.2)

$$J = \frac{\mathrm{d}M}{A\,\mathrm{d}t} = \frac{D(C_1 - C_2)}{h}, \tag{4.1}$$

where J is the flux (amount of material flowing through a unit cross section); M, the drug mass (g, mol); A, the surface area (cm^2); t, the time (s); D, the diffusion coefficient (diffusivity, $\mathrm{cm}^2\ \mathrm{s}^{-1}$) ; C_1, the drug concentration at membrane wall in intestinal lumen ($\mathrm{mol\,l}^{-1}$); C_2, the drug concentration at membrane wall in blood side ($\mathrm{mol\,l}^{-1}$); and h is the membrane thickness (cm).

The assumptions made by this model are the following: (1) steady state flux. The transfer of drugs reaches to steady state very fast and (2) the steady state follows sink conditions: both sides of the membrane are well stirred and homogenous.

Define partition coefficient K as $K = C_1/C_\mathrm{d} = C_2/C_\mathrm{r}$ (C_d as drug concentration in the gastric intestinal (GI) lumen, and C_r is the drug concentration in the blood), we can get (4.2)

$$J = D\left(\frac{KC_\mathrm{d} - KC_\mathrm{r}}{h}\right) = \frac{DK}{h}(C_\mathrm{d} - C_\mathrm{r}). \tag{4.2}$$

If $C_\mathrm{d} \gg C_\mathrm{r}$, then

$$J = \frac{DK}{h}C_\mathrm{d}. \tag{4.3}$$

Define permeability coefficient P as $P = DK/h$ (unit cm s^{-1}), then

$$J = PC.$$

Consider the absorptive surface area, we can get the final (4.4)

$$dM/dt = PA(C_d - C_r). \tag{4.4}$$

4.2.2 *Carrier-Mediated Transport*

Intestinal epithelial cell membranes are highly polarized. Apical membrane faces the external lumen with many microvilli to increase the membrane surface area. Many membrane transporters are located in this side facilitating absorption for most nutrients and many drugs, while basolateral membrane is toward blood (Rouge *et al.*, 1996; Shin *et al.*, 2003; Anderle *et al.*, 2004) (Fig. 4.3).

Depending on the direction and category of transported solutes, drug carrier to mediate transport can also be classified into uniporter, symporter, and antiporter. Uniporter is the carrier-mediated transport with single solute; symporter facilitates the transport of two solutes with same direction, while antiporter facilitates the transport of two solutes with opposite directions. Based on the concentration gradient of the solutes and energy involved in the process, drug carrier can be classified into facilitated diffusion and active transport.

4.2.2.1 Facilitated Diffusion

Carrier proteins are involved in facilitated diffusion. This process does not need energy. Similar to passive diffusion, transport direction of facilitated diffusion depends on the solutes concentration gradient (from higher concentration to lower

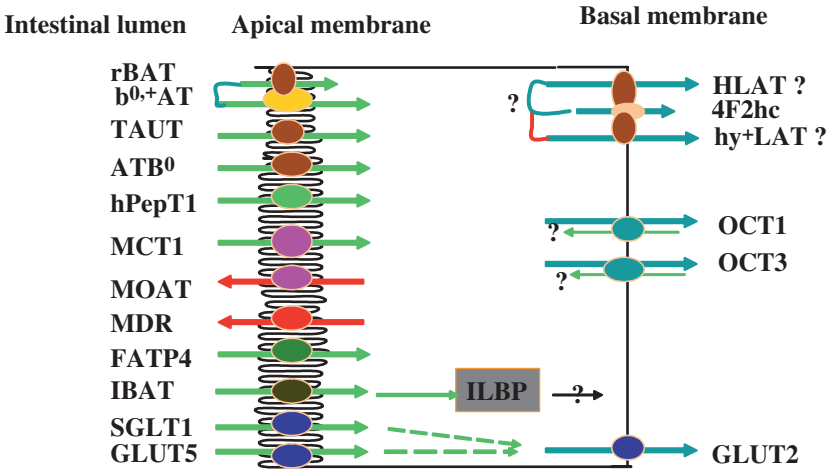

FIGURE 4.3. Apical and basolateral transporters coupling for absorption (*See* Color Plate II)

concentration) (Cainelli *et al.*, 1974; Feher, 1983). However, facilitated diffusion has higher transport rate than what would be expected from passive diffusion alone.

4.2.2.2 Active Transport

Active transport is the primary mode by which molecules are transported against electrical and/or chemical concentration gradients. The process involves a membrane bound protein molecule that binds reversibly to the solute molecule at a specific site. The complex then undergoes a change in conformation that translocates the solute to the other side of the membrane. Factors that can affect this transport include energy, temperature, and stereospecificity of the molecule. Similar to enzyme kinetics, active transport also exhibits saturable kinetics and can be inhibited by similar structural analogs.

$$J = \frac{J_{\max} C}{K_{\mathrm{m}} + C}, \tag{4.5}$$

where J is the drug flux (mg s^{-1}); $J_{\max}$, the maximum drug flux; C, the drug concentration (mg ml^{-1}); and K_{m}, the drug affinity to carrier (mg ml^{-1}).

At low concentration, $C \ll K_{\mathrm{m}}$, first order absorption prevails

$$J = \frac{J_{\max}}{K_{\mathrm{m}}} C. \tag{4.6}$$

At high concentration, $C \gg K_{\mathrm{m}}$, zero-order absorption prevails

$$J = J_{\max}. \tag{4.7}$$

In contrast to passive diffusion, drugs with active transport absorption mechanism may have a concentration dependent and/or dose-dependent absorption (Fig. 4.4). Drug flux can be competitively inhibited by other substrates.

4.2.3 Paracellular Transport

Paracellular transport refers to transport solutes in between cells, without passage through the epithelial cells themselves. It is now well recognized that the intercellular junctions between epithelial cells of capillaries are "leaky," allowing paracellular transport of small molecules (Daugherty and Mrsny, 1999; Trischitta *et al.*, 2001). Paracellular transport is passive transport, follows drug concentration gradients, and does not require energy.

4.2.4 Endocytosis

Endocytosis is a process in which a substance or compound gains entry into a cell without passing through the lipid cell membrane. Based on the mechanisms and molecules involved, this process can be subdivided into different types: pinocytosis, phagocytosis, and receptor-mediated endocytosis. In each case, endocytosis

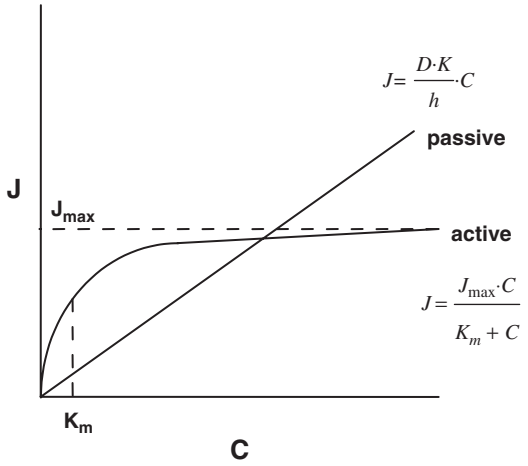

FIGURE 4.4. Active transport shows nonlinear pharmacokinetics

results in the formation of an intracellular vesicle by the invagination of the plasma membrane and membrane fusion. Drug molecules can be transported into the cells by this process (Hansen *et al.*, 2005; Liang *et al.*, 2006).

4.2.5 Which Absorption Path Dominates Drug Absorption?

Although different mechanisms of oral drug absorption have been shown in small intestinal regions, under physiological conditions, several routes may contribute to drug absorption at the same time. Usually, the fastest route dominates the absorption of a particular compound (Burton *et al.*, 2002; Cao *et al.*, 2005). In general, passive diffusion is the main mechanism for absorption of many lipophilic compounds, while the carrier-mediated process governs the absorption of transporter substrates. In some cases, paracellular junction is the route for the absorption of some small hydrophilic compounds with molecular weight less than 300.

4.3 Three Primary Factors Influence Drug Absorption

Permeability, solubility, and dissolution are the three primary factors that influence drug absorption (Narawane and Lee, 1994; Lennernas, 1998; Zhou *et al.*, 2005). Permeability reflects the physiological properties of membrane to the solutes. Fraction of drug absorbed is determined by the drug permeability through intestinal wall. Solubility is one of the physicochemical properties of drug molecules to affect drug absorption. The drug molecules have to be dissolved in solution in order for absorption to occur in the intestinal tract. Dissolution is the dosage form variable to determine the rate and extent of drug dissolved in solution.

4.3.1 Membrane Permeability

4.3.1.1 Effective Permeability

Passive permeability (P) of molecules across a membrane can be expressed as

$$P_{\text{passive}} = \frac{J}{C} = \frac{DK}{h}, \tag{4.8}$$

where K is the partition coefficient, D is the diffusion coefficient, and h is the thickness of the cell membrane. The diffusion coefficient (D) depends on the molecular weight or size of a molecule. K is a measure of the solubility of the substance in lipid. Therefore, the passive permeability is related to membrane and drug properties. For a specific drug, the passive membrane permeability should be a constant P_{m} and independent to drug concentration.

The permeability for active absorption can be presented by

$$P_{\text{active}} = \frac{J}{C} = \frac{J_{\max} C}{K_{\text{m}} + C} \frac{1}{C} = \frac{J_{\max}}{K_{\text{m}} + C}, \tag{4.9}$$

where J is the drug flux, $J_{\max}$ is the maximum drug flux, C is the drug concentration, and K_{m} is the drug affinity to the carrier. Obviously, active permeability is dependent on drug concentration.

Therefore, the total effective permeability is dependent on drug concentration for drugs that absorbed through both passive diffusion and active transport, and it can be expressed as follows

$$P_{\text{eff}} = P_{\text{passive}} + P_{\text{active}} = P_{\text{m}} + \frac{J_{\max}}{K_{\text{m}} + C}. \tag{4.10}$$

However, at very low concentration, $C \ll K_{\text{m}}$, drug permeability is independent to drug concentration

$$P_{\text{eff}} = P_{\text{m}} + \frac{J_{\max}}{K_{\text{m}}}. \tag{4.11}$$

At high concentration, $C \gg K_{\text{m}}$, drug permeability is dependent on drug concentration.

$$P_{\text{active}} = \frac{J}{C} = \frac{J_{\max} C}{K_{\text{m}} + C} \frac{1}{C} = \frac{J_{\max}}{C} \approx 0, \tag{4.12}$$

$$P_{\text{eff}} = P_{\text{m}}. \tag{4.13}$$

Therefore, the permeability vs. concentration plot can be generated as in Fig. 4.5.

4.3.1.2 Fraction of Drug Absorbed

Drug permeability through intestinal wall will determine the fraction of drug absorbed (F_{a}). F_{a} can be estimated by drug permeability through intestinal wall

$$F_{\text{a}} = 1 - \text{e}^{-2\text{An}}; \quad \text{An} = \frac{T_{\text{res}}}{T_{\text{abs}}}; \quad T_{\text{abs}} = \frac{R}{P_{\text{eff}}}, \tag{4.14}$$

where, T_{res} is the small intestine transit time ($\sim$3 h), T_{abs} is the absorptive time (h), R is the radius of small intestine (2 cm), and P_{eff} is the drug permeability through intestinal wall.

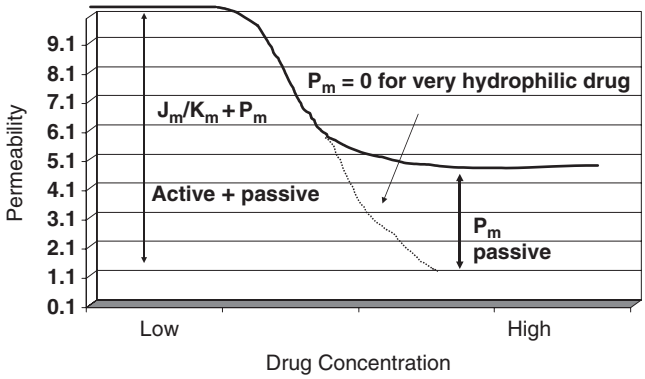

FIGURE 4.5. Active and passive permeability at low and high drug concentration

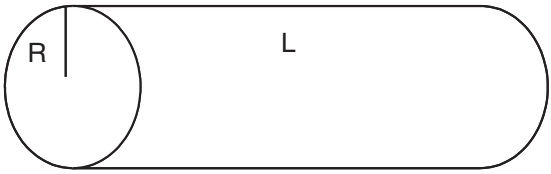

FIGURE 4.6. Model for intestinal absorption compartment

4.3.1.3 Permeability and Absorption Rate Constant

Absorption rate constant can be expressed as

$$K_a = P\frac{A}{V} = P\frac{2\pi RL}{\pi R^2 L} = \frac{2P}{R}, \tag{4.15}$$

where K_a is the absorption rate constant with unit $1\,s^{-1}$, P is the permeability (cm s^{-1}), A is the membrane surface area (cm^2), and V is the volume of absorption compartment (cm^3) (Fig. 4.6). However this equation tends to overestimate absorption by 12.5-fold, so $K_a = P/(2\pi R)$ may be more realistic.

4.3.2 Solubility

Solubility is the most important physicochemical property of drug molecules, which can affect the drug absorption. The drug molecules have to be dissolved in the solution for the absorption to occur in the intestinal tract. The solubility of a solute is the maximum quantity of solute that can dissolve in a certain quantity of solvent or quantity of solution at a specified temperature. The extent of ionization and oil/water partition coefficient K of the drug contribute to both drug solubility and membrane permeability. In general, low K indicates high solubility in water and high K indicates high solubility in lipid. However, the drug molecules with high lipid solubility usually possess high membrane permeability.

Ionization and pH play an important role in drug water solubility (Zhou *et al.*, 2005). Ionized form is usually more water soluble than unionized form, but unionized form is easier for absorption in the GI tract by passive diffusion than ionized form. For weakly basic drugs, more unionized form would be predominant in intestine at high pH (5–8), which favors absorption. For weakly acid drug, more ionized form would be predominant in intestine. Although in theory that ionized weak acid is not favorable for absorption in intestine, the larger surface area of intestine will compensate this weakness to produce complete absorption for many weakly acidic drug.

4.3.3 Dissolution of Solid Dosage Forms

If drugs are administered in solid dosage forms, they must be dissolved in the GI tract before absorption can take place. For drugs with low solubility and high dose, the dissolution will be slow, and the dissolution rate will be the rate-limiting step for absorption. Factors that affect dissolution will control the whole absorption process.

Noyes–Whitney equation can be used to describe the dissolution rate as following

$$\frac{\mathrm{d}m}{\mathrm{d}t} = A\frac{D}{h}(C_\mathrm{s} - C) \quad C = 0 \text{ at sink condition,} \tag{4.16}$$

where $\mathrm{d}m/\mathrm{d}t$ is the rate of solid dissolution, A is the solid surface area, D is the diffusion coefficient, h is the thickness of unstirred boundary layer, C_s is the drug aqueous solubility, and C is the concentration at h (Fig. 4.7).

For drugs with low solubility, formulation strategies such as micronization (increases A), ionization (increases C_s), solubilization (surfactants), and

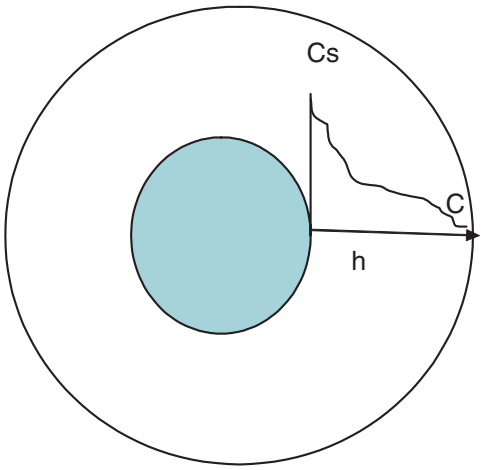

FIGURE 4.7. Model for dissolution of solid drugs

disintegrants can be used to enhance dissolution and fraction of drug absorbed (Anderson and Pitman, 1980; Frenning and Stromme, 2003; Schreiner *et al.*, 2005; Jinno *et al.*, 2006).

4.4 Secondary Factors Influencing Drug Absorption

4.4.1 Biological Factors of Gastrio Intestinal Tract

GI tract plays important roles in secretion, digestion, and absorption. Many biological factors, such as gastric emptying, gastric and intestinal pH, GI content, GI motility, GI surface area, and blood flow (Fleisher *et al.*, 1990) can affect drug absorption.

4.4.1.1 Gastric Emptying Time

Gastric emptying time refers to the time needed for the stomach to empty the total initial stomach contents. During digestion, gastric emptying depends on the tone of proximal stomach and pylorus, which is under reflex and hormonal control. Generally, anything that slows down gastric emptying is likely to slow down the rate (not extent) of drug absorption, and thus affect onset of the therapeutic response. A lot of factors promote gastric emptying, such as hunger, lying on right side, noncaloric liquid intake, drugs (metoclopramide, prokinetic drugs), and some excipients. On the other hand, factors, such as meals (especially with fatty, bulky, and viscous food), lying on left side, and other drugs (tricyclic antidepressants, anticholinergics, and alcohol) retard gastric emptying. Gastric emptying of solution-type dosage forms and suspensions of fine drug particles is generally much faster and less variable than that of solid, nondisintegrating dosage forms and aggregated particles. For drugs with high solubility and high membrane permeability, gastric emptying rate will control the absorption rate and onset of the drugs. There will be a direct relation between gastric-emptying rate and maximal plasma concentration, and an inverse relation between gastric-emptying rate and the time required to achieve maximal plasma concentrations.

4.4.1.2 Surface Area

Surface area of different regions of GI influences drug absorption. Small intestine has largest effective surface area for drug absorption due to the presence of folds of mucosa, villi, and microvilli. For carrier-mediated drug absorption, small intestine is also the most important region for most drug transporters that are also expressed in this area. In contrast, stomach and large intestine have no villi, microvilli, or less transporter expression.

4.4.1.3 GI Transit Time

GI transit time or mean resident time (MRT) can also influence oral drug absorption. Increase in the GI residence time (or decrease of motility) leads to enhanced

drug absorption potential. Stomach MRT is about 1.3 h while the small intestine MRT is around 3 h. The longer MRT in small intestine will contribute to a higher drug absorption potential.

4.4.1.4 Intestinal Motility

Intestinal motility is another factor that influences oral drug absorption. Intestinal movement includes propulsion and mixing. Propulsive movement determines the intestinal transit time and is important for slow release dosage forms, enteric-coated drug that is only released in intestine, slowly dissolving drugs, and carrier-mediated absorption. Mixing movement increases dissolution rate where the drug molecule contacts with endothelial surface area for absorption.

4.4.1.5 Components, Volume, and Properties of Gastrointestinal Fluids

Components, volume, and properties of gastrointestinal fluids especially GI pH will change the drug's ionization, solubility, dissolution rate, and therefore affect drug absorption. The rate of dissolution from a dosage form, particularly tablets and capsules, is dependent on pH. Acidic drugs dissolve most readily in alkaline media and will have a greater dissolution in the intestinal fluids than in gastric fluids. Basic drugs will dissolve most readily in acidic solution, and thus the dissolution will be greater in gastric fluids than in intestinal fluids. GI pH depends on general health of the individual, disease conditions, age, type of food, and drug therapy. Antichlolinergic drugs and H_2-blockers increase gastric pH and significantly decrease bioavailability of some weakly basic drugs with pH-dependent solubility.

4.4.1.6 Food

Food influences drug absorption in different ways. High fat food may stimulate bile salt secretion, increases drug solubility and dissolution, and increases bioavailability for certain drugs with low solubility. High protein may increase gastric pH, thus decrease dissolution of weak basic drugs and bioavailability. High calorie food decreases gastric emptying rate, delays the rate of absorption, and delays the onset of therapeutic drugs. At the same time, food components may compete for drug absorption that is mediated by transporters. For instance, grapefruit juice inhibits efflux pump (P-gp) and increases bioavailability of P-gp substrates. In addition, food components may form complex with drugs (complexation) and decrease drug absorption and bioavailability as seen in the example that tetracycline forms a complex with calcium in milk to hinder its absorption.

4.4.1.7 Blood Flow

Blood flow in the GI tract also plays an important role in drug absorption. GI tract is highly vascularized and receives 28% of the cardiac output. The higher blood flow promotes the higher drug absorption, especially for those active-absorption mediated and highly permeable drugs.

4.4.1.8 Age

Age can also influence the drug absorption. Newborns, for example, have less acidic gastric fluids, smaller gut fluid volume, slower gastric emptying rate, less intestinal surface area and blood flow, and thus have relatively lower drug absorption.

4.4.2 Dosage Factors Influencing Absorption

Dosage form factors include excipients and dosage forms, which may affect drug absorption (Rouge *et al.*, 1996; Badawy Sherif *et al.*, 2006). The disintegrants can enhance the dissolution rate of the drugs and increase absorption. Surfactants such as Tween-80 may increase drug solubility of poorly soluble drugs, and increase drug absorption through enhancement of the drug permeability. The coating of enteric-coated tablets such as cellulose acetate can only be dissolved in the intestine at high pH (> 5), which protects the drug from degradation in gastric condition and against drug stimulation of gastric mucosa. In such cases, the controlled release dosage form will have a completely different absorption profile as compared with immediate release dosage forms.

4.5 Evaluation of Oral Drug Absorption in Human

4.5.1 Drug Absorption Assessment Using In Vivo Data

4.5.1.1 Estimation of Fraction of Drug Absorbed Using Experimental Intestinal Permeability *In Vivo*

An *in vivo* method has been successfully established to measure human intestinal permeability by *in situ* intestinal perfusion (Lennernas *et al.*, 1997; Sun *et al.*, 2002, 2002; Cao *et al.*, 2006). A perfusion tube, as illustrated in Fig. 4.8, is placed in the human jejunum to allow drug passage through a 10-cm intestinal segment. The drug concentration is measured at the inlet and outlet of the perfusion tube. The drug permeability is then calculated with the following equation

$$P_{\text{eff, human}} = Q(1 - C_{\text{out}}/C_{\text{in}})/2\pi RL, \tag{4.17}$$

where $P_{\text{eff, human}}$ is drug permeability in the human intestine, Q is the perfusion flow rate (2 min ml^{-1}), C_{in} is inlet drug concentration of the perfusion tube, C_{out} is outlet drug concentration of the perfusion tube, R is human small intestine radius (2 cm), and L is the 10-cm perfusion segment. When the permeability is plotted against the fraction of drug absorbed, the relationship can be established (4.18) (Fig. 4.9) (Amidon *et al.*, 1988, 1995; Oh *et al.*, 1993)

$$F_{\text{a}} = 1 - \exp(-2\text{An}) = 1 - \exp(-2P_{\text{eff, human}}T_{\text{res}}/R), \tag{4.18}$$

where F_{a} is the fraction of drug absorbed, $P_{\text{eff, human}}$ is drug permeability in human intestine, T_{res} is transit time in human small intestine (3 h), R is the radius of human small intestine (2 cm).

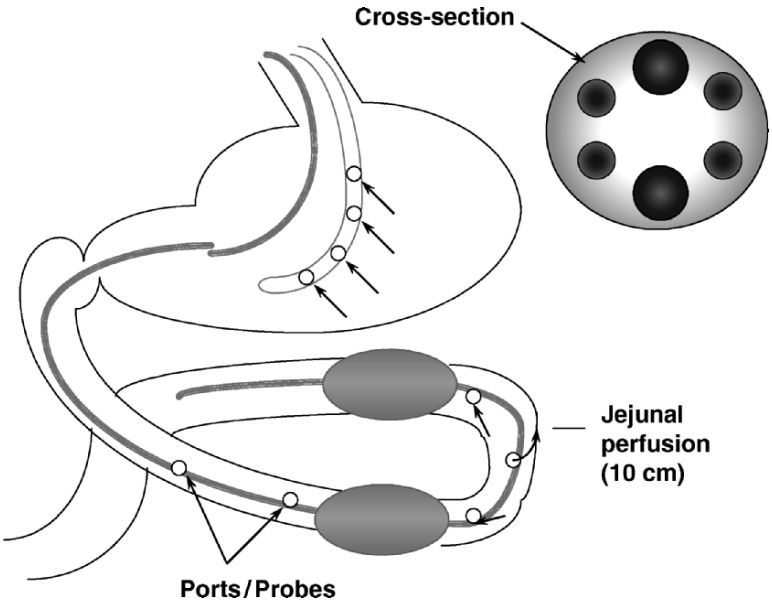

FIGURE 4.8. Perfusion tube for *in situ* human intestinal permeability measurement

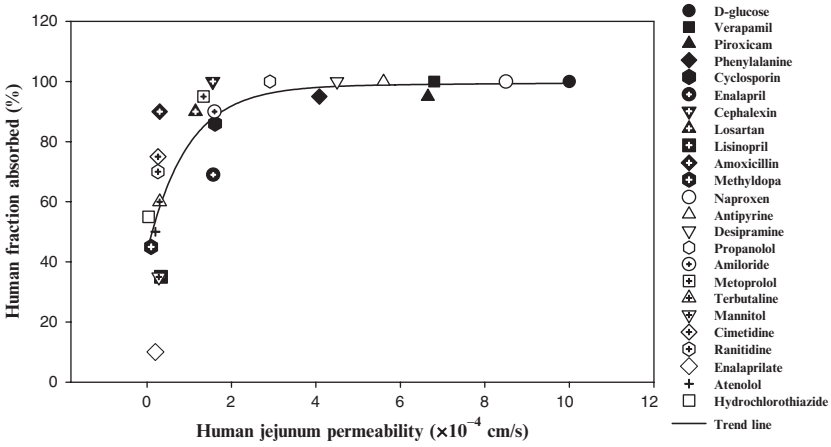

FIGURE 4.9. Prediction of the fraction of drug absorbed using human jejunum permeability. Drugs are labeled with different symbols. *Closed symbols* are drugs absorbed through carrier-mediated process, while *open symbols* are drugs absorbed through passive diffusion (Sun *et al.*, 2002)

$$\mathrm{An} = P_{\mathrm{eff,\,human}} \times T_{\mathrm{res}}/R.$$

However, when *in situ* intestinal perfusion is performed, low drug concentrations are used for permeability measurements. In this case, the drug concentration is always below its solubility limit. Since the fraction of drug absorbed is a function

of its solubility and permeability, (4.18) is not suitable for predicting the fraction of drug absorbed when high drug concentration above *in vivo* solubility limit is used in the experiment. This model has been further modified to overcome this problem by utilizing different calculation methods according to the drug's solubility (Yu *et al.*, 1996; Yu and Amidon, 1999)

$$F_a = 1 - \exp(-2\mathrm{An}), \quad \text{when} \quad C_{in} < S, \quad C_{out} < S, \tag{4.19}$$

$$F_a = 2\mathrm{An}/D_0, \quad \text{when} \quad C_{in} > S, \quad C_{out} > S, \tag{4.20}$$

$$F_a = 1 - 1/[D_0 \exp(-2\mathrm{An} + D_0 - 1)], \quad \text{when} \quad C_{in} > S, \quad C_{out} < S, \tag{4.21}$$

where F_a is the fraction of drug absorbed, $\mathrm{An} = P_{\mathrm{eff,\,human}} \times T_{res}/R$, C_{in} is inlet drug concentration of the perfusion tube, C_{out} is outlet drug concentration of the perfusion tube, $P_{\mathrm{eff,\,human}}$ is drug intestinal permeability in human, T_{res} is transit time in human small intestine (3 h), D_0 is dose number [D_0 = (dose/volume)/S], and S is drug solubility. The challenge for this method is that drug intestinal permeability has to be obtained *in vivo* in human, which is very difficult and not available during early stages of drug discovery and development. Meanwhile the relationship between C_{out} (or C_{in}) and solubility is also difficult to determine *in vivo*.

4.5.1.2 Estimation of Maximum Absorbable Dose Using *In Vivo* Absorption Rate Constant and Drug Solubility

Another method has been proposed to estimate maximum absorbable dose (MAD) based on the *in vivo* absorption rate constant (Curatolo, 1987) with the following equation

$$\mathrm{MAD} = SK_aVT, \tag{4.22}$$

where S is drug solubility, K_a is absorption rate constant, V is intake water volume (250 ml), and T is transit time in small intestine (3 h). For instance, MAD could be estimated using different K_a values (Table 4.1). However, K_a has to be obtained from *in vivo* pharmacokinetic studies in animals or humans, which are usually not available during early stages of drug discovery and development. Alternatively K_a can be estimated by *in vivo* drug permeability if it is available by (4.23)

$$K_a = P_{\mathrm{eff,\,human}}(A/V) = P_{\mathrm{eff,\,human}}(2\pi RL/\pi R^2 L) = P_{\mathrm{eff,\,human}}(2/R), \tag{4.23}$$

where A is the surface area, V is the volume, R is the radius, and L is the length of small intestine.

However, it is also difficult to estimate the appropriate volume for the calculation in this method. Although standard water intake is 250 ml, the daily gastric secretion volume is 2,000 ml; intestine secretion volume is in the range of 1,500–2,000 ml; and bile and pancreatic secretion is 500–1,500 ml (Dressman *et al.*, 1998).

TABLE 4.1. Estimation of maximum absorbable dose (MAD) using absorption rate constant (K_a) in human with following equation: $MAD = SK_aVT$

K_a (min^{-1})	Solubility (mg ml^{-1})	MAD (mg)
0.001	0.001	0.045
0.001	0.01	0.45
0.001	0.1	4.5
0.001	1	45
0.01	0.001	0.45
0.01	0.01	4.5
0.01	0.1	45
0.01	1	450
0.1	0.001	4.5
0.1	0.01	45
0.1	0.1	450
0.1	1	4,500

4.5.1.3 Estimation of MAD from Drug *In Vivo* Permeability in Humans and Drug Solubility

At the steady state of *in situ* human intestinal perfusion, drug flux J is a function of permeability, drug concentration, and absorption surface area (Amidon *et al.*, 1988, 1995; Oh *et al.*, 1993),

$$J = dm/dt = P_{\text{eff, human}} S\, dA. \tag{4.24}$$

Then,

$$\text{MAD} = P_{\text{eff, human}} SAT = P_{\text{eff, human}} S2\pi RLT, \tag{4.25}$$

where J is drug flux, $P_{\text{eff, human}}$ is drug permeability in human intestine, S is drug solubility, A is absorption surface area, T is transit time in small intestine (3 h), R is the radius of small intestine (2 cm), and L is the length of small intestine (6 m). It is worth noting that the small intestine surface area for drug absorption should include surface area of villi and microvilli, but the surface area calculated in (4.24) is only the intestinal tube surface area without such consideration. However, since the permeability obtained in the *in situ* perfusion is calculated by (4.17), where the surface area also does not include villi and microvilli, the error is cancelled in the MAD calculation in (4.25), and it does not affect the MAD estimation if human intestinal permeability is used. The examples for estimation of MAD using permeability with (4.25), or using calculated K_a from human permeability with (4.22) and (4.23) are summarized in Table 4.2.

In comparison of the examples in Tables 4.1 and 4.2, it seems that MAD might be underestimated using the absorption rate constant in (4.22) due to the assumption of 250 ml of volume in the calculation. MAD might be overestimated using permeability in (4.9) due to the assumption that the drug is absorbed at the maximum concentration (at its solubility) in the whole small intestinal region (6 m)

TABLE 4.2. Estimation of MAD using drug intestinal permeability in human with following equation: $\mathrm{MAD} = P_{\mathrm{eff,\,human}} S 2\pi RLT$, $\mathrm{MAD} = P_{\mathrm{eff,\,human}} SA_{\mathrm{eff}} T$, or with calculated absorption rate constant (K_{a}) with following equations: $K_{\mathrm{a}} = P_{\mathrm{eff,\,human}}(2/R)$ and $\mathrm{MAD} = SK_{\mathrm{a}}VT$

$P_{\mathrm{eff,\,human}}$ ($\times 10^{-4}$ cm s^{-1})	Solubility (mg ml^{-1})	MAD (mg) calculated from $P_{\mathrm{eff,\,human}}$	MAD (mg) calculated from effective absorption surface area	Calculated K_{a} from $P_{\mathrm{eff,\,human}}$ (min^{-1})	MAD (mg) from calculated K_{a}
0.1	0.001	0.813	0.086	0.0006	0.027
0.1	0.01	8.13	0.864	0.0006	0.27
0.1	0.1	81.3	8.64	0.0006	2.7
0.1	1	813	86.4	0.0006	27
1	0.001	8.13	0.864	0.006	0.27
1	0.01	81.3	8.64	0.006	2.7
1	0.1	813	86.4	0.006	27
1	1	8,138	864	0.006	270
10	0.001	81.3	8.64	0.06	2.7
10	0.01	813	86.4	0.06	27
10	0.1	8,138	864	0.06	270
10	1	81,388	8,640	0.06	2,700

with maximum surface area over the entire 3 h absorption period, while in reality only partial small intestine is used at a given time. Therefore, the effective absorption surface area of 800 cm^2 is proposed to calculate MAD (Curatolo, 1987). The examples for estimation of MAD using this effective surface area are also summarized in Table 4.2. MAD using the effective absorption surface area seems more appropriate. If the MAD based on permeability and solubility is below the required clinical dose, formulation development, and delivery would be very challenging.

4.5.2 Drug Absorption Assessment Using In Vitro Data

When MAD is estimated with *in vivo* data, either the *in vivo* absorption rate constant, or the drug *in vivo* intestinal permeability is required for the calculation. However, during early stages of drug discovery and development, *in vivo* data are usually unavailable. The challenge is to optimize the process for selecting compounds to evaluate *in vivo* human studies based on *in vitro* data. Fortunately, drug permeability in Caco-2 cells and drug solubility are routinely screened in the pharmaceutical industry. These data can be utilized to predict fraction of drug absorbed and MAD in humans to identify the best candidates for further clinical development.

4.5.2.1 *In Vitro* Testing Conditions for Determining Drug Permeability in Caco-2 Cells and *In Vitro*/*In Vivo* Permeability Correlation

Many laboratories have established methods for measuring drug permeability in Caco-2 cells with different testing conditions (Chong *et al.*, 1996; Yee, 1997;

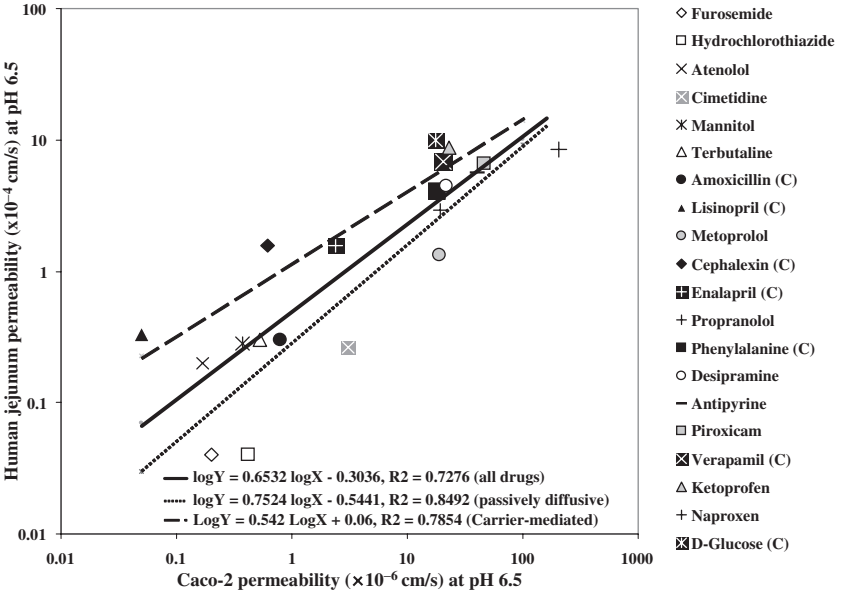

FIGURE 4.10. *In vitro/in vivo* permeability correlation of 20 drugs at pH 6.5. Correlation coefficient ($R^2 = 0.7276$) was calculated from the permeability of all 20 drugs. Correlation coefficient ($R^2 = 0.8492$) was calculated from the permeability of the following drugs: furosemide, hydrochlorothiazide, atenolol, cimetidine, mannitol, terbutaline, metoprolol, propranolol, desipramine, antipyrine, piroxicam, ketoprofen, and naproxen. Correlation coefficient ($R^2 = 0.7854$) was calculated from the permeability of the following drugs: cephalexin, enalapril, lisinopril, losartan, amoxicillin, phenylalanine, L-leucine, L-dopa, D-glucose, cyclosporin, and verapamil. Drugs are labeled with different symbols. *Black symbols* are drugs absorbed through carrier-mediated process, while *gray* and *open symbols* are drugs absorbed through passive diffusion (Sun *et al.*, 2002)

Pade and Stavchansky, 1998; Yamashita *et al.*, 2000). Some laboratories use buffer with pH 7.4 in both apical and basolateral sides of the Caco-2 cells, while others use pH 6.5 buffer at the apical side and pH 7.4 buffer at the basolateral side. When correlation analysis was performed between *in vitro* drug permeability in Caco-2 cells and *in vivo* drug permeability in humans, a better correlation was observed between human *in vivo* permeability and Caco-2 permeability measured at pH 6.5 than at pH 7.4 (Figs. 4.10 and 4.11). The correlation coefficient (R^2) of *in vitro* and *in vivo* permeability of 24 drugs assayed at pH 7.4 was 0.5126 in (4.26), while the *in vitro* and *in vivo* permeability correlation coefficient (R^2) of the 20 drugs determined at pH 6.5 was 0.7276 in (4.27) (Sun *et al.*, 2002).

$$\text{Log } P_{\text{eff, human}} = 0.4926 \,\text{Log } P_{\text{eff, Caco}-2} - 0.1454, \tag{4.26}$$

$$\text{Log } P_{\text{eff, human}} = 0.6532 \,\text{Log } P_{\text{eff, Caco}-2} - 0.3036. \tag{4.27}$$

However, if the drugs were absorbed through a carrier-mediated processes, such as cephalexin, enalapril, cyclosporin, amoxicillin, lisinopril, losartan, phenylalanine,

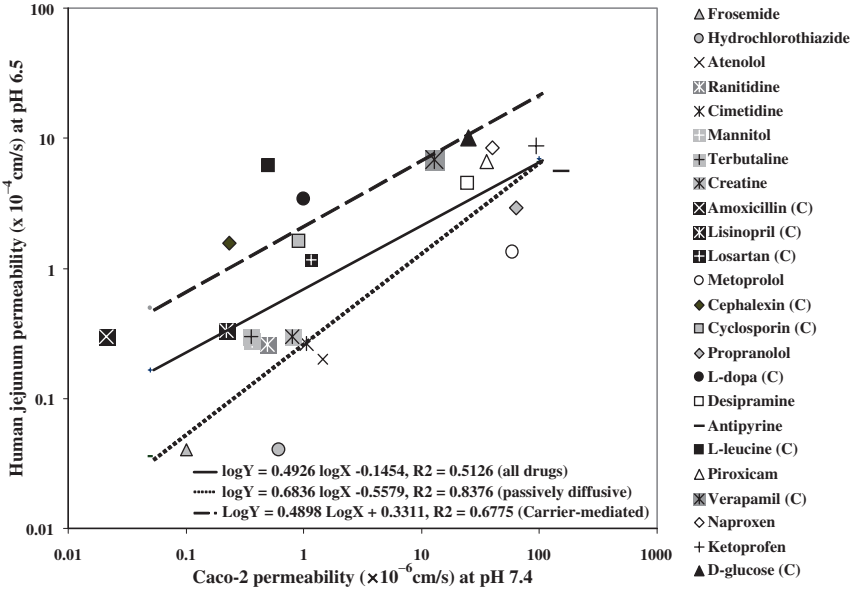

FIGURE 4.11. *In vitro*/*in vivo* permeability correlation of 24 drugs at pH 7.4. Correlation coefficient ($R^2 = 0.5126$) was calculated from the permeability of all 24 drugs. Correlation coefficient ($R^2 = 0.8376$) was calculated from the permeability of the following drugs: furosemide, hydrochlorothiazide, atenolol, ranitidine, cimetidine, mannitol, terbutaline, creatine, metoprolol, propranolol, desipramine, antipyrine, piroxicam, ketoprofen, and naproxen. Correlation coefficient ($R^2 = 0.6775$) was calculated from the permeability of the following drugs: cephalexin, enalapril, lisinopril, losartan, amoxicillin, phenylalanine, L-leucine, L-dopa, D-glucose, cyclosporin, and verapamil. Drugs are labeled with different symbols. *Black symbols* are drugs absorbed through carrier-mediated process, while *gray* and *open symbols* are drugs absorbed through passive diffusion (Sun *et al.*, 2002)

verapamil, L-dopa, D-glucose, and L-leucine were excluded, the *in vitro*/in vivo permeability correlation improves at both pHs, such that the permeability correlation coefficient (R^2) of 15 passively diffused drugs at pH 7.4 and 13 passively diffused drugs at pH 6.5 were 0.8376 in (4.28) and 0.8492 in (4.29), respectively.

$$\text{Log } P_{\text{eff, human}} = 0.6836 \,\text{Log } P_{\text{eff, Caco}-2} - 0.5579, \tag{4.28}$$

$$\text{Log } P_{\text{eff, human}} = 0.7524 \,\text{Log } P_{\text{eff, Caco}-2} - 0.5441. \tag{4.29}$$

4.5.2.2 Estimation of Fraction of Drug Absorbed In Humans Using *In Vitro* Drug Permeability in Caco-2 Cells

When *in vitro* drug permeability in Caco-2 cells is plotted against drug fraction absorbed in humans, a relationship could also be established as shown in Figs. 4.12 and 4.13 (Sun *et al.*, 2002). As these data clearly indicate, it might be difficult to predict the fraction of drug absorbed for the drugs with low Caco-2 permeability.

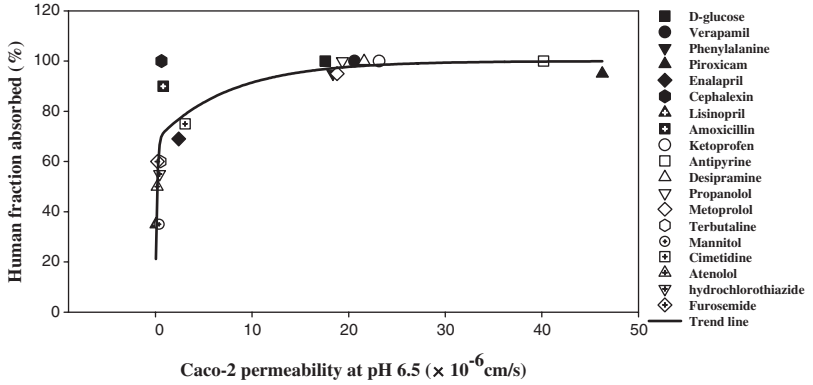

FIGURE 4.12. Prediction of the fraction of drug absorbed using Caco-2 permeability at pH 6.5. Drugs are labeled with different symbols. *Closed symbols* are drugs absorbed through carrier-mediated process, while *open symbols* are drugs absorbed through passive diffusion (Sun *et al.*, 2002)

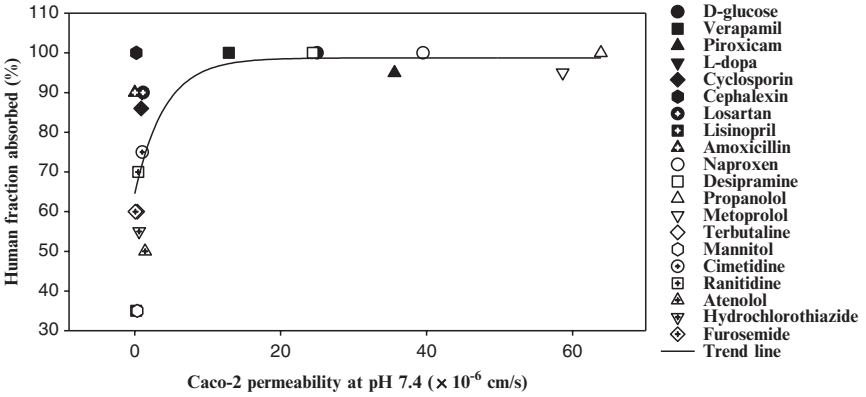

FIGURE 4.13. Prediction of the fraction of drug absorbed using Caco-2 permeability at pH 7.4. Drugs are labeled with different symbols. *Closed symbols* are drugs absorbed through carrier-mediated process, while *open symbols* are drugs absorbed through passive diffusion (Sun *et al.*, 2002)

More discrepancy was also observed for the drugs with carrier-mediated absorption routes especially when drug permeability in Caco-2 cells was obtained at pH 7.4 in the apical side.

4.5.2.3 Estimation of MAD in Human Based on *In Vitro* Data

Since an *in vitro* and *in vivo* drug permeability correlation has been established in (4.26), *in vivo* drug permeability in human could be easily estimated by *in vitro* drug permeability in Caco-2 cells. Although some of the transporter substrates showed high discrepancy from the *in vitro*/*in vivo* permeability correlation when Caco-2 permeability was obtained at pH 7.4, the overall correlation has shown

reasonable prediction when Caco-2 permeability was obtained at pH 6.5 (Sun *et al.*, 2002). Since MAD could be estimated using *in vivo* drug permeability in human with (4.25), the MAD could be estimated with *in vitro* drug permeability in Caco-2 cells in the following (4.30)

$$\mathrm{MAD} = P_{\mathrm{eff,\,human}} S A_{\mathrm{eff}} T = 10^{(0.6532\,\mathrm{Log}\,P_{\mathrm{eff,\,Caco}} - 0.3036)} S A_{\mathrm{eff}} T, \qquad (4.30)$$

where $P_{\mathrm{eff,\,human}}$ is the *in vivo* drug permeability in human, $P_{\mathrm{eff,\,Caco}}$ is the *in vitro* drug permeability in Caco-2 cells, S is the drug solubility, A_{eff} is the effective absorption surface area without considering villi and microvilli, and T is transit time in small intestine (3 h). As discussed earlier, the error associated when not considering surface area of villi and microvilli is cancelled in the MAD calculation using permeability in (4.25) and (4.30). In addition, the surface area of microvilli in Caco-2 cells is also irrelevant in calculation of MAD, since the human permeability is calculated with Caco-2 permeability by the correlation analysis.

Alternatively, the MAD can be estimated using (4.22) and (4.23), where K_{a} can be estimated with human permeability *in vivo* or Caco-2 permeability *in vitro* with the following (4.31)

$$K_{\mathrm{a}} = P_{\mathrm{eff,\,human}}(2/R) = (2/R)10^{(0.6532\,\mathrm{Log}\,P_{\mathrm{eff,\,Caco}} - 0.3036)}, \qquad (4.31)$$

where $P_{\mathrm{eff,\,human}}$ is drug *in vivo* permeability in human, $P_{\mathrm{eff,\,Caco}}$ is drug *in vitro* permeability in Caco-2 cells, and R is the radius of small intestine (2 cm). The examples of MAD estimation using *in vitro* drug permeability in Caco-2 cells are summarized in Table 4.3.

TABLE 4.3. Estimation of MAD using drug permeability in Caco-2 cells with following equation: $\mathrm{MAD} = 10^{(0.6532\,\mathrm{Log}\,P_{\mathrm{eff,\,caco}} - 0.3036)} S A_{\mathrm{eff}} T$, or with calculated absorption rate constant (K_{a}) with following equations: $K_{\mathrm{a}} = (2/R)10^{(0.6532\,\mathrm{Log}\,P_{\mathrm{eff,\,caco}} - 0.3036)}$ and $\mathrm{MAD} = S K_{\mathrm{a}} V T$

$P_{\mathrm{eff,\,Caco-2}}$ ($\times 10^{-6}$ cm s^{-1})	Solubility (mg ml^{-1})	MAD (mg) calculated from $P_{\mathrm{eff,\,Caco-2}}$	Calculated K_{a} from $P_{\mathrm{eff,\,Caco-2}}$ (min^{-1})	MAD (mg) from calculated K_{a}
0.1	0.001	0.0955	0.000663	0.0298
0.1	0.01	0.955	0.000663	0.298
0.1	0.1	9.545	0.000663	2.98
0.1	1	95.45	0.000663	29.8
1	0.001	0.429	0.002982	0.134
1	0.01	4.294	0.002982	1.34
1	0.1	42.94	0.002982	13.4
1	1	429.4	0.002982	134
10	0.001	1.932	0.01342	0.603
10	0.01	19.32	0.01342	6.03
10	0.1	193.2	0.01342	60.3
10	1	1,932	0.01342	603
100	0.001	8.696	0.060388	2.17
100	0.01	86.96	0.060388	27.17
100	0.1	869.6	0.060388	271.7
100	1	8,696	0.060388	2,717

4.5.3 Correlation of Oral Drug Bioavailability and Intestinal Permeability Between Rat and Human

Animal models are widely used to evaluate drug pharmacokinetics and drug absorption. However, the correlation of oral bioavailability (F) values of 48 drugs in rat and human has been studied and no correlation ($r^2 = 0.29$) was found due to low correlation of drug metabolism in rat and human (Cao *et al.*, 2006). Results of the F values comparison are shown in Fig. 4.14. In contrast, Chiou and Buehler observed low correlation in the bioavailability of 35 drugs between monkey and human with $r^2 = 0.502$ (Chiou and Buehler, 2002), which may be due to the closer physiological similarity between monkey and human. These data indicate that oral bioavailability in rat could not be used to predict oral drug bioavailability in human.

Due to the structural similarities of intestinal membrane, drug absorption in animal models may be used to predict drug absorption in human. In order to depict the oral drug absorption process, *in situ* intestinal permeabilities of 17 drugs with different absorption mechanisms were evaluated in rat and human jejunum (Cao *et al.*, 2006). Since permeability is one of the primary factors governing absorption (Amidon *et al.*, 1995), studying the permeability correlation is useful when predicting human absorption from rat permeability. The tested drugs are absorbed by carrier-mediated processes as well as passive diffusion. For instance, valacyclovir, enalapril, and cephalexin are all absorbed through a peptide transporter (hPepT1). Leucine, phenylalanine, L-Dopa, and methyldopa are absorbed through amino acid transporters. Verapamil is a P-gp substrate. Cimetidine is an organic cation transporter substrate. Propranolol, atenolol, and furosemide are all absorbed through passive diffusion. The drug permeabilities in the rat jejunum were then correlated

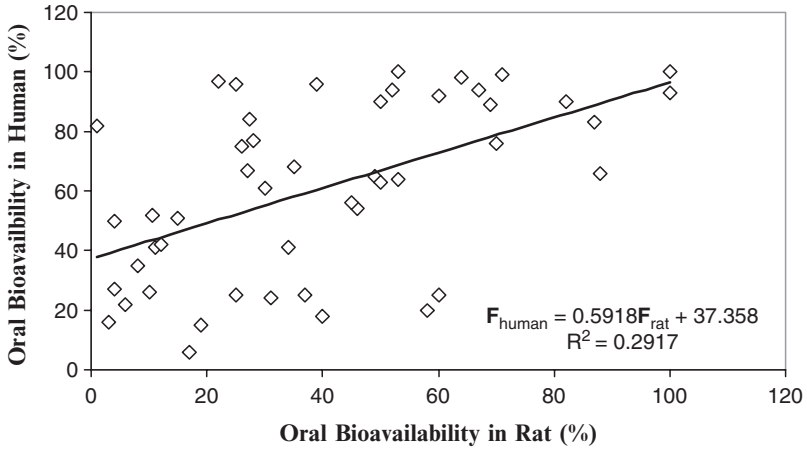

FIGURE 4.14. Correlation of oral bioavailability between rat and human. Total of 48 drugs were plotted. The equation describes the correlations for rat oral bioavailability (F_{rat}) and human oral bioavailability (F_{human}) (Cao *et al.*, 2006)

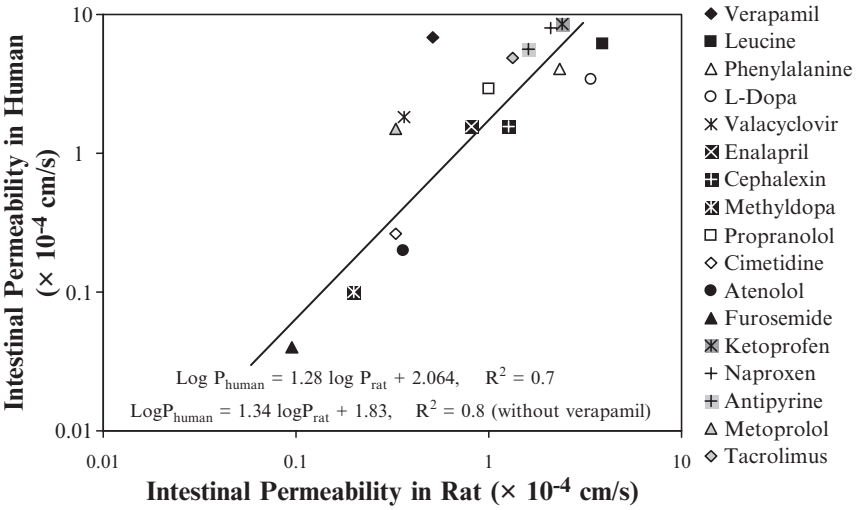

FIGURE 4.15. Correlation of drug permeability in rat jejunum and in human jejunum. Permeability coefficients (P_{eff}) were determined by *in situ* intestinal perfusion. The equations describe the correlations for rat permeability (P_{rat}) and human permeability (P_{human}) (Cao *et al.*, 2006)

with the drug permeabilities in the human jejunum (Fig. 4.15). It showed that drug permeability in the rat is generally five to ten-fold lower than the permeability in the human. However, both carrier-mediated and passively diffusing drugs showed a reasonable correlation ($r^2 = 0.7$). Interestingly, verapamil (a P-gp substrate) permeability in human deviates from the correlation curve. The permeability correlation between human and rat is highly increased ($r^2 = 0.8$) when verapamil is excluded in the analysis.

This study is in agreement with the other report, that the percentage of absorption of 98 drugs was correlated between rat and human with a correlation of $r^2 = 0.88$ (Zhao *et al.*, 2003). *In vivo* absorption in rats could be a useful method to predict the extent of absorption in humans. The permeability in rat for water soluble and poor water soluble compounds was used to predict the fraction of drug absorbed in humans (Watanabe *et al.*, 2004). In another study, a high correlation was found for a variety of compounds displaying various physicochemical and pharmacologic activities between the two species in the dose-independent absorption range (Chiou and Barve, 1998). However, a previous study reported that effective permeability estimates of passively absorbed solutes correlate highly in rat and human jejunum while carrier-mediated transport requires scaling between the models because the substrate specificity and/or transport maximum may differ (Fagerholm *et al.*, 1996). These discrepancies might be due to the different numbers of transporter substrates that are used in the correlation analysis. However, all of these studies indicate that reasonable permeability correlation between human and rat can be used to predict drug absorption in humans.

To understand the underlying mechanisms in the similarity in drug intestinal absorption between humans and rats, correlation analysis of the expression levels of transporters and metabolizing enzymes between rat and human intestine were further conducted (Cao *et al.*, 2006). Moderate correlations (with $r^2 > 0.56$) were found for the expression levels of transporters in the duodenum of human and rat. Although there is discrepancy observed in the expression of MDR1, MRP3, GLUT1, and GLUT3, other transporters (such as PepT1, SGLT-1, GLUT5, MRP2, NT2, and high affinity glutamate transporter) and the overall drug transporters expression share similar expression levels in both human and rat intestine with regional dependent expression patterns, which has high expression in the small intestine and low expression in the colon. These data provide the molecular mechanisms for the similarity and correlation of drug absorption (F_a) in the small intestine between rat and human. In contrast, the expression of metabolizing enzymes (CYP3A4/CYP3A9 and UDPG) showed 12- to 193-fold difference between human and rat intestine with distinct regional dependent expression patterns. No correlation was found for the expressions of metabolizing enzymes between rat and human intestine, which indicate the difference in drug metabolism in two different species and the challenges in predicting F_g and F from rat to human.

4.6 Summary

Drug absorption is a complicated process in which many physiological and physiochemical factors are involved. Understanding the principles of drug absorption benefits the designing of formulation strategies to enhance the bioavailability and *in vivo* drug activity. In summary, drug absorption mechanisms include passive diffusion and active transport. Permeability, solubility, and dissolution, GI physiological conditions, and dosage forms can influence the drug absorption rate. In general, if a drug has high water solubility and low membrane permeability (hydrophilic drugs), permeability usually limits absorption, unless it is carrier mediated or paracellular absorption dependent. Strategies which can enhance the drug permeability in dosage design could be used to increase this permeability controlled drug absorption. In contrast, if a drug has low solubility and high permeability (lipophilic drugs), solubility (and dissolution) usually limits absorption. Formulation strategies should be optimized in the dosage form to enhance the solubility (and dissolution) controlled drug absorption. If neither of the above two properties limits the absorption such as for drugs with high solubility and high permeability, then gastric emptying rate limits the drug absorption. Both *in vivo* and *in vitro* methods have been explored to assess drug absorption in human. Rat and human show similar drug absorption profiles and similar transporter expression patterns in the small intestine, while the two species exhibit distinct expression levels and patterns for metabolizing enzymes in the intestine. These data provide the molecular mechanisms for the similarity and correlation of drug absorption (F_a) in the small intestine between rat and human. Therefore,

rat can be used to predict oral drug absorption (F_a) in the small intestine of human, but not to predict drug metabolism (F_g and F_h) and oral bioavailability (F) in human.

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