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Oral Absorption, Intestinal Metabolism and Human Oral Bioavailability

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1. Introduction

Most of the drugs that are available in the marketplace are administered via the oral route, which is a convenient and cost effective route of administration (Lipinski 1995; Lipinski 2000; Lipinski et al. 2001; Lipinski 2004; Abrahamsson and Lennernas 2005). Thus, oral bioavailability is one of the key considerations for discovery and development of a new chemical entity (NCE). It is well recognized that poor oral bioavailability is one of the major causes of therapeutic variability, associated with the variable drug exposure (Beierle et al. 1999; Bardelmeijer et al. 2000; Katsura and Inui 2003). This is particularly important for drugs with narrow therapeutic window or potential for resistance development such as antibiotics and cytotoxic drugs (Bardelmeijer et al. 2000). Hellriegel *et al.* reported a significant inverse relationship between the oral bioavailability of drugs from several therapeutic classes and the coefficient of inter-individual variability in their oral bioavailability (Hellriegel et al. 1996).

Oral bioavailability is a product of fraction absorbed, fraction escaping gut-wall elimination, and fraction escaping hepatic elimination; and the factors that influence bioavailability can be divided into physiological, physicochemical, and biopharmaceutical factors. It has been well established that physicochemical properties determine oral absorption and drug metabolism. The "rule-of-five" devised by Lipinski and co-workers provided an important advance, with analysis of a large data set showing that compounds within certain physicochemical space tended to be more successful in clinical development than others. Using a dataset of 309 drugs, Varma *et al.* studied the interrelation of physicochemical properties and the individual parameters of oral absorption to define the physicochemical space for optimum human oral bioavailability (Varma et al. 2010). This analysis, which may provide a rational judgment on the physicochemical space to optimize oral bioavailability, will be discussed. Furthermore, the solubility and permeability as the fundamental properties of oral absorption will be discussed in-line with biopharmaceutics classification system. Uptake and efflux transporters are implicated as facilitating or limiting intestinal absorption. This book chapter will touch up on the latest findings on several chemistry approaches that has be directed to target the uptake transporters and circumvent the efflux transporters. Overall, this chapter will provides a better understanding of the interplay between gastrointestinal tract physiology/anatomy and drug physicochemical /biopharmaceutical factors in the absorption and metabolism mechanisms that affect oral

bioavailability humans; and enable a rational approach to design NCE with better absorption in humans.

2. Concepts and theoretical calculations of oral bioavailability

Bioavailability (F) is the extent to which an active moiety is absorbed from a pharmaceutical dosage form and becomes available in the systemic circulation (Thomas et al. 2006). Bioavailability is usually determined by calculating the respective plasma drug exposure assessed as the total area under the drug plasma concentration versus time curve (AUC) after oral and intravenous administration as:

$$\text{Absolute Bioavailability} = \frac{AUC_{oral}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{oral}} \quad (1)$$

In general, determinants of oral drug bioavailability include fraction of dose absorbed in the gastrointestinal tract (GIT) and fraction of dose that escapes elimination by the intestinal tract, liver, and lung. Thus, oral bioavailability can be defined mathematically by the following equation:

$$F = F_{abs} \cdot F_g \cdot F_h \quad (2)$$

Where F_{abs} is the fraction of the dose that is absorbed from the intestinal lumen to the intestinal enterocytes; F_g is the fraction of the dose that escapes pre-systemic intestinal first pass elimination; and F_h is the fraction of the dose that passes through the liver and escapes pre-systemic liver first-pass elimination. The fraction of the dose that escapes first-pass elimination across the intestine (F_g) and liver (F_h) can be estimated experimentally via the comparison of systemic exposures (AUC ratios) where the dosing routes are selected to isolate the contribution by a particular organ.

F_g can be estimated (Eq. 3) for a compound when doses are given orally and via a cannulated hepatic portal vein (h.p.v.) with the fraction absorbed (F_{abs}) either assumed to be complete or is known.

$$F_{abs} \cdot F_g = \frac{AUC_{oral}}{AUC_{h.p.v.}} \times \frac{Dose_{h.p.v}}{Dose_{oral}} \quad (3)$$

Similarly, F_h can be estimated for a compound when doses are given via a cannulated hepatic portal vein and intravenously (Eq. 4).

$$F_h = \frac{AUC_{h.p.v.}}{AUC_{i.v.}} \times \frac{Dose_{i.v}}{Dose_{h.p.v.}} \quad (4)$$

The details on scientific background and factors that influence F_h are outside the scope of this book chapter; interested readers are encouraged to refer to our recent reviews in these areas (Thomas et al. 2006; Hurst et al. 2007; Varma et al. 2010) and other chapters in this book that focus on metabolism and related topics such as induction and inhibition of drug metabolism, pharmacogenetics and metabolism: past, present and future, and effect of pharmaceutical excipients on drug metabolism.

3. Mechanism of oral absorption

Following oral dosing, drug molecules can cross the luminal membrane through various mechanisms that involve passive diffusion or active transport. Passive diffusion is comprised of two pathways: the paracellular pathway, in which drug diffuses through the aqueous pores at the tight junctions between the intestinal enterocytes; and the transcellular (lipophilic) pathway, which requires drug diffusion across the lipid cell membrane of the enterocyte. The active transport pathway is mediated by transporters and is divided into active drug influx and efflux. It is important to note that the relevance of each route is determined by the compound's physicochemical properties and its potential affinity for various transport proteins (Thomas et al. 2006; Hurst et al. 2007; Varma et al. 2010; Varma et al. 2010).

3.1 Passive diffusion

In paracellular diffusion, drug molecules are absorbed by diffusion and convective volume flow through the water-filled intercellular space (Lennernas 1995). In general, drugs that are absorbed through this pathway are small molecules (e.g., molecular weight [MW] < 250 g/mol) and hydrophilic in nature (Log P < 0). Because the junctional complex has a net negative charge, positively charged molecules pass through more readily, whereas negatively charged molecules are repelled (Karlsson et al. 1999). Furthermore, the paracellular pathway offers a limited window for absorption since it accounts for < 0.01% of the total surface area of intestinal membrane. In addition, the tight junctions between cells become tighter traveling from the jejunum towards the colon.

The transcellular pathway is the major route of absorption for most of drug molecules. In general, the rate of passive transcellular permeability is mainly determined by the rate of transport across the apical cell membrane, which is controlled by the physicochemical properties of the absorbed compound. Unlike the paracellular pathway, compounds that are absorbed through the transcellular pathway are unionised, with lipophilicity of Log P > 0 and MW > 300 g/mole. In addition, the hydrogen-bonding capacity determined by the number of hydrogen bond donors and hydrogen bond acceptors is < 10 and 5, respectively (Lipinski 1995; Lipinski 2000; Avdeef 2001).

3.2 Active transport

Enterocytes express several transporters, belonging to the adenosine triphosphate (ATP) binding cassette (ABC) superfamily and the solute carrier (SLC) superfamilies, on the apical and basolateral membranes for the influx or efflux of endogenous substances and xenobiotics (Table 1). Although a variety of transporters are expressed in the enterocytes, only a few are known to play a key role in the intestinal absorption of drugs. ABC transporters utilize ATP to drive the transport and are called primary active transporter. However, SLC transporters majorly use the ion gradients (H+, Na+ and Ca++ gradients) created across the membrane by primary active carriers (Na+/K+-ATPase, Na+/H+-ATPase) (Tsuji and Tamai 1996). ABC transporters expressed in the intestine include P-glycoprotein (P-gp; *ABCB1*), breast cancer resistance protein (BCRP; *ABCG2*), multidrug resistance proteins (MRP1-6; *ABCC1-6*). P-gp, BCRP, MRP2 and MRP4 are localized on brush-border (apical) membrane while certain MRPs are expressed on the basolateral

membrane of the enterocytes. These efflux transporters functionally limit the enterocytic levels of their substrates by reducing uptake and facilitating efflux. SLC transporters suggested as relevant at the intestinal apical surface of epithelial cells include, peptide transporter (PepT1; *SLC15A1*), organic anion polypeptide transporters (OATP1A2, *SLCO1A2;* OATP2B1,*SLCO2B1*), monocarboxylate transporters (MCT1; *SLC16A1*), sodium-multivitamin transport (SMVT; *SLC5A6*) and organic cation/zwitterion transporters (OCTN1, *SLC22A4;* OCTN2, *SLC22A5*). Several other SLC transporters including organic anion or cation transporters (OATs or OCTs; *SLC22*) have also been identified in the intestine, but seem to be of less importance in oral drug absorption (Englund et al. 2006; Seithel et al. 2006).

Transporter protein	*Gene*	Orientation	Drug Substrates
P-gp/MDR1	*ABCB1*	Apical efflux	Actinomycin D, cerivastatin, colchicine, cyclosporine A, daunorubicin, digoxin, docetaxel, doxorubicin, erythromycin, etoposide, fexofenadine, imatinib, indinavir, irinotecan, ivermectin, lapatinib, loperamide, losartan, nelfinavir, oseltamivir, paclitaxel, quinidine, ritonavir, saquinavir, sparfloxacin, tamoxifen, terfenadine, topotecan, verapamil, vinblastine, vincristine.
BCRP	*ABCG2*	Apical efflux	Abacavir, ciprofloxacin, dantrolene, dipyridamole, enrofloxacin, erlotinib, etoposide, furosemide, gefitinib, genistein, glyburide, grepafloxacin, hydrochlorothiazide, imatinib, irinotecan, lamivudine, lapatinib, methotrexate, mitoxantrone, prazosin, rosuvastatin, tamoxifen, triamterene, zidovudine.
MRP1	*ABCC1*	Basolateral efflux	Daunorubicin, doxorubicin, epirubicin, grepafloxacin, methotrexate, vincristine.
MRP2	*ABCC2*	Apical efflux	Indinavir, methotrexate, ritonavir, saquinavir, vinblastine.
MRP3	*ABCC3*	Basolateral efflux	Etoposide, methotrexate.
MRP4	*ABCC4*	Apical efflux	Ceftizoxime, topotecan.
PepT1	*SLC15A1*	Apical uptake	Ampicillin, bestatin, captoril, cephalexin, enalapril, fosinopril, oseltamivir, valciclovir.
OATP 1A2	*SLCO1A2*	Apical uptake	Fexofenadine, levofloxacin, methotrexate, ouabain, rosuvastatin, saquinavir.

Transporter protein	*Gene*	Orientation	Drug Substrates
OATP 2B1	*SLCO2B1*	Apical uptake	Atorvastatin, bosentan, fluvastatin, glyburide, pitavastatin, pravastatin, montelukast, rosuvastatin.
MCT1	*SLC16A1*	Apical uptake	Arbaclofen placarbil, carindacillin, gabapentin enacarbil, ketoprofen, naproxen, phenethicillin, propicillin.
SMVT	*SLC5A6*	Apical uptake	Gabapentin enacarbil
OCTN1	*SLC22A4*	Apical uptake	Quinidine, verapamil.
OCTN2	*SLC22A5*	Apical uptake	Cephaloridine, imatinib, ipratropium, tiotropium, quinidine, verapamil.
CNT1	*SLC28A1*	Apical uptake	Cytarabine, gemcitabine, zidovudine.
CNT2	*SLC28A2*	Apical uptake	Clofarabine, fluorouridine, ribavirin.
ENT1	*SLC29A1*	Basolateral efflux	Cladribine, clofarabine, cytarabine, gemcitabine, ribavirin.
ENT2	*SLC29A2*	Basolateral efflux	Clofarabine, gemcitabine, zidovudine.

Table 1. Major human intestinal efflux and uptake transorters involved in drug transport. Gene, transport orientation and the clinical drug substrates of each transporter is included (Polli et al. 2001; Mahar Doan et al. 2002; Murakami and Takano 2008; Giacomini et al. 2010; Klaassen and Aleksunes 2010).

4. Absorption kinetics

When an active uptake process is involved, the overall transport of a drug across the intestinal enterocytes can be defined by model incorporating saturable and nonsaturable components (Eq. 5).

$$J = \frac{J_{\max,\inf} \cdot C}{K_{m,\inf} + C} + K_D C \tag{5}$$

As outlined in the equation above, the total flux (J) of a compound across intestinal membrane is determined by four variables: J_{max}, which is the maximal uptake rate, K_m, which is the transporter substrate binding affinity, K_D, which is the kinetic constant for nonsaturable transport, and C, which is the luminal drug concentration. The impact of intestinal transporters on the overall absorption of drug across the intestine is determined by the percentage component of the active process ($J_{max}C/(K_m+C)$) to the total flux, J, of the drug molecule. In general hydrophilic drug have low K_D values and therefore there transport rates are mainly driven by the transporter activity, while for lipophilic drugs the passive component is usually high and the role of transporters is expected to be minimal.

5. Physiological, physicochemical and biopharmaceutical factors that impact oral drug absorption

The factors that alter the rate and extent of oral drug absorption can be divided into three main categories: physiological, physicochemical and biopharmaceutical factors (Sabnis 1999; Horter and Dressman 2001; Kramer and Wunderli-Allenspach 2001; Zhou 2003; Pouton 2006).

5.1 Physiological factors that impact oral drug absorption

5.1.1 Gastro-intestine anatomy and physiology

In humans, the GIT consists mainly of the stomach, small intestine (the duodenum, jejunum, and ileum) and large intestine (cecum, colon and rectum). The total length of the human GIT is 8.35 m and the relative size of the human small intestine, which is considered the primary site of drug absorption, to the total length of the GI tract is 81%. As for the large intestine, its relative size in humans is 19%. It may also be pointed out that the cecum, which is the major site of microbial digestion, forms only 5% of the length of the human large intestine (DeSesso and Jacobson 2001).

The surface area is attributed to the fact that the human small intestine has three anatomical modifications that significantly increase the surface area of the human small intestine (Shargel and Yu 1999). The human small intestine has grossly observable folds of mucosa (plicae circularis or folds of kerckring) that increase the surface area threefold. From the plicae circularis, microscopic finger-like pieces of tissue called villi project, which increase the surface area by 10-fold for humans. Each villus is covered in microvilli, which increase the surface area by 20-fold. Unlike the small intestine, the large intestine surface area does not have villi and is divided into geographical areas by transverse furrows. In addition, the large intestine enterocytes differ slightly from that of the small intestine and its microvilli are less packed (Kararli 1995). Overall, this significantly contributes to the smaller surface area of the large intestine in humans and is consistent with the fact that small intestine is the major site of drug absorption in humans.

5.1.2 Unstirred water layer

Adjacent to the intestinal membrane is an unstirred water layer, which is a potential barrier for the absorption of various drug molecules across the intestinal membrane (Winne 1976; Hayton 1980). The thickness of this layer in humans is only 25 μm (Strocchi et al. 1996). Chiou *et al.* quantitatively studied the impact of the unstirred water layer adjacent to the intestinal membrane on the rate and extent of absorption of passively absorbed drugs with different membrane absorption half-lives (10 – 300 min) in humans (Chiou 1994). Results of this analysis suggested that the presence of the unstirred water layer is generally expected to have a relatively mild or insignificant effect on the rate of absorption and an insignificant effect on the extent of absorption (Kimura and Higaki 2002).

5.1.3 Gastrointestinal transit times

The absorption rate of a drug molecule is generally a function of drug absorption through the GIT, which is determined by the residence time and absorption in each GIT segment

(Kimura and Higaki 2002). In general, gastric transit time impacts the systemic exposure of rapidly dissolved and well absorbed drugs. However, intestinal transit time influences the absorption of drugs with limited mucosal permeability, carrier mediated uptake, drugs subject to intestinal degradation, or products whose dissolution is the rate limiting step for systemic absorption (Martinez and Amidon 2002). In contrast to gastric transit time, intestinal transit time is independent of the feeding conditions and the physical composition of the intestinal contents (Garanero et al. 2005) . The human intestinal transit time is ~3 – 4 h (DeSesso and Jacobson 2001; Kimura and Higaki 2002). Several studies suggested that in human small intestine, there is a gradient of velocity where the small intestinal transit in the proximal intestine was faster compared with the distal intestine. The transit time in human large intestine can vary in the range of 8 – 72 h (DeSesso and Jacobson 2001).

5.1.4 The GIT pH

The extent of ionization plays a pivotal role in determining the drug dissolution rate and passive permeability across the GIT. Therefore, it becomes clear that the pH at the absorption site is a critical factor in facilitating or inhibiting the dissolution and absorption of various ionizable drug molecules (DeSesso and Jacobson 2001). It should be stressed that the pH of the luminal content (chyme) is altered by the luminal secretions. The pH of chyme is acidic and can be as low as 2.3. When the chyme arrives in the duodenum, it is quickly neutralized by the secretion of the pancreatic bicarbonate and bile. The pH values of chyme become progressively more alkaline in the distal portion of the small intestine in humans. However, the pH of chyme in the large intestine is generally more acidic than the pH observed in the small intestine in humans, possibly due to the fermentation mediated by the microbial flora (Kararli 1995).

5.1.5 Bile fluid

Bile is produced by hepatocytes and drained through the many bile ducts that penetrate the liver (DeSesso and Jacobson 2001). During this process, the epithelial cells add a watery solution that is rich in bicarbonates which increases the alkalinity of the solution. In humans, the bile is stored and concentrated up to five times its original potency in the gall bladder. It is to be noted that the human gall bladder secrets bile at a rate of 2 – 22 ml/kg/day. In humans, bile acts as a detergent to emulsify fats by increasing the surface area to help enzyme action, and thus aid in their absorption in the small intestine. In addition to bicarbonate solution, bile is composed of bile salts, such as the salts of taurocholic acid and deoxycholic acid, which are combined with phospholipids to break down fat globules in the process of emulsification by associating their hydrophobic side with lipids and the hydrophilic side with water. Emulsified droplets are then organized into many micelles which increases their absorption. Because bile increases the absorption of fats, it also plays a pivotal role in the absorption of the fat-soluble vitamins and steroids (Hanano 1993; Kirilenko and Gregoriadis 1993).

5.1.6 Bacterial microflora

In humans, bacterial microflora exists in most of the GIT and become an important component of the luminal content. However, there is no bacterial microflora in the stomach and upper

small intestine. This is mainly attributed to the low pH of the human gastric content. However, a large number of bacterial microflora populates the human's distal small and large intestines (Cummings and Macfarlane 1997). These bacterial microflora play a role in the metabolism of various chemicals and xenobiotics through hydrolysis, dehydroxylation, deamidation, decarboxylation and reduction of azide groups (Lichtenstein 1990; Cummings and Macfarlane 1997; Blaut et al. 2003). Among these reactions, hydrolysis of the glucuronide conjugates is the most important metabolic reaction that is mediated by the glucronidase enzyme and produced by the bacterial microflora found in the GIT of humans.

5.1.7 Lymphatic absorption

The intestinal lymphatic route plays a key role in the absorption of drugs that are highly lipophilic. It has many advantages, such as increase in the oral bioavailability of highly lipophilic drugs by avoiding hepatic first pass effect, direct targeting of lymphoid tissue, indirect targeting of specific sites associated with low-density lipoprotein receptors, and alteration in the rate of oral drug input to the systemic circulation thereby providing opportunity for controlled release drug formulation (Cheema et al. 1987; Trevaskis et al. 2005; Trevaskis et al. 2006; Trevaskis et al. 2006).

5.1.8 Intestinal drug transporters

P-glycoprotein (P-gp)

P-gp (MDR1; *ABCB1*), an ATP-dependent transmembrane efflux pump belonging to ABC superfamily, shows affinity to a wide variety of structurally unrelated compounds (Juliano and Ling 1976). It is expressed as a 1280 amino acid long (MW ~170 kDa) single chain glycoprotein with two homologous portions of equal length, each containing six transmembrane (TM) domains and two ATP binding regions separated by a flexible linker polypeptide region (Schinkel et al. 1993; Ambudkar et al. 1999).

Immunohistochemical analysis using monoclonal antibody provided evidence for localization of P-gp in a wide range of tissues, particularly in columnar epithelial cells of the lower GIT, capillary endothelial cells of brain and testis, canalicular surface of hepatocytes and apical surface of proximal tubule in kidney (Thiebut et al. 1987). Due to selective distribution at the port of drug entry and exit, P-gp has been speculated to play a major physiological role in the absorption, distribution and excretion of xenobiotics and endogenous substrates. Overall, P-gp functions as a biochemical barrier for entry of drugs across intestine and brain, as well as a vacuum cleaner to expel drugs from the intestine, liver, kidney, etc. A number of clinically important drugs are P-gp substrates (Table 1), which are as diverse as anthracyclines (doxorubicin, daunorubicin), alkaloids (reserpine, vincristine, vinblastine), specific peptides (valinomycin, cyclosporine), steroid hormones (aldosterone, hydrocortisone) and local anesthetics (dibucaine) (Polli et al. 2001; Mahar Doan et al. 2002; Varma et al. 2003; Takano et al. 2006). P-gp substrates, digoxin and talinolol, show pharmacokinetic changes in human upon coadministration with P-gp inhibitors (Gramatte et al. 1996; Fenner et al. 2009). Greiner *et al.*, studied the effect of rifampicin pretreatment on the oral pharmacokinetics of digoxin and suggested that rifampicn induced duodenal P-gp expression and thus significantly reduced AUC of digoxin (Greiner et al. 1999). Similarly, rifampicn decreased talinolol oral exposure, which is consistent with ~4 fold increase in duodenal P-gp expression (Westphal et al. 2000).

P-gp affinity screening using various *in vitro* culture models is now an integral part of drug discovery due to wide substrate specificity and clinical relevance in drug disposition and associated drug-drug interactions (DDIs) (Varma et al. 2004a). Tailoring of molecules to reduce substrate specificity to P-gp may help in improving the oral bioavailability of drugs. Seelig and coworkers suggested that the partitioning into the lipid membrane is the rate-limiting step for the interaction of a substrate with P-gp and that dissociation of the P-gp-substrate complex is determined by the number and strength of the hydrogen bonds formed between the substrate and the transporter (Seelig and Landwojtowicz 2000). Several studies have related the binding affinity (K_m) of P-gp for substrates and modulators to their lipid-water partition coefficient (Log P). Evidence suggests that a drug with high Log P will accumulate to a high concentration within the cytoplasmic membrane and favors binding to P-gp with low K_m value, while a drug with low partitioning will have a lower membrane concentration and a high K_m value. Three-dimensional structures of a large number of drugs revealed that the minimal common binding element consisting of two or three hydrogen bond acceptor (HBA) groups in a specific spatial distance. Since the TM sequences of P-gp are rich in hydrogen bond donor (HBD) groups, it is hypothesized that P-gp recognizes the HBA groups of the substrates through hydrogen bond formation in the lipid membrane environment (Seelig 1998; Seelig 1998). Didziapetris *et al.* studied 220 substrates and 1000 non-substrates and proposed the 'rule of four', which states that compounds with the HBA ≥ 8, a molecular weight (MW) > 400 g/mol and most acidic pK_a < 4 are likely to be P-gp substrates, while compounds with HBA ≤ 4, MW < 400 g/mol and most basic pK_a > 8 are not substrates to P-gp (Didziapetris et al. 2003). Although many such models describe the physicochemical attributes of P-gp interaction and are shown to have high predictability, existence of multiple binding sites and other complicating factors has prevented the development of a definitive SAR (Stouch and Gudmundsson 2002).

Breast Cancer Resistance Protein (BCRP)

BCRP (ABCP/MXR; *ABCG2*), a member of the ABC family of transporters, is considered a half-transporter with six TM domains and one ATP-binding domain at the amino terminus and is believed to homodimerize in order to function (Staud and Pavek 2005). It is composed of 655 amino acids with a MW of 72 kDa (Graf et al. 2003). An atomic model of BCRP was predicted by homology modeling based on the crystal structure of the bacterial multidrug exporter Sav1866, which suggested that BCRP had multiple drug binding sites (Hazai and Bikadi 2008; Muenster et al. 2008). BCRP expression can be traced to placenta, kidney, liver, testis, brain, mammary tissue, and intestine (Doyle and Ross 2003). Unlike P-gp, the expression of BCRP along the length of the small intestine does not vary significantly (Bruyere et al. 2010). Additionally, the mRNA level of BCRP is notably higher than other efflux transporters such as P-gp and MRP2 in the human intestine (Taipalensuu et al. 2001). Since BCRP is highly expressed on the apical membrane of enterocytes and effluxing substrates back into the lumen, it has been noted to play an important role as a detoxification efflux transporter and limiting drug absorption in the GIT (Zaher et al. 2006).

BCRP exhibits broad substrate specificity and accepts diverse chemical space, as do other ABC transporters. Substrates to BCRP include (Table 1): chemotherapy agents (mitoxantrone, camptothecins, tyrosine kinase inhibitors), antivirals (zidovudine, lamivudine), HMG-CoA reductase inhibitors (statins), benzimidazoles, and antibiotics (ciprofloxacin, rifampicin) (Bailey et al. ; Merino et al. 2005; Huang et al. 2006; Takano et al.

2006; Ando et al. 2007; Dauchy et al. 2009; Ieiri et al. 2009). Some of the BCRP substrates are also effectively effluxed by P-gp. For example, etoposide, irinotecan and tamoxifen are substrates for both BCRP and P-gp (Table 1). In a clinical study, Kruijtzer *et al.* showed an increase in bioavailability of topotecan from 40% to 97% in the presence of GF120918, a potent inhibitor of BCRP and P-gp (Kruijtzer et al. 2002). Yamasaki *et al.* investigated the impact of genetic polymorphisms of ABCG2 (421c>A) and NAT2 on the pharmacokinetics of sulfasalazine, in 37 healthy volunteers and suggested sulfasalazine as a useful probe substrate for evaluating the role of BCRP in the intestinal disposition (Yamasaki et al. 2008). BCRP polymorphism significantly affects the pharmacokinetics of several HMG-CoA reductase inhibitors, including atrovastatin, rosuvastatin, fluvastatin and simvastatin lactone, but has no significant effect on pravastatin or simvastatin acid (Bailey et al. ; Huang et al. 2006; Ieiri et al. 2009; Keskitalo et al. 2009; Keskitalo et al. 2009). For example, rosuvastatin AUC was 100% and 144% greater in the c.421AA genotype population than in those with c.421CA and the c.421CC genotypes, respectively. Although, few clinical studies have been reported on the role of BCRP in the intestinal absorption, several studies using BCRP knock-out mice suggest significant impact (Merino et al. 2006; Seamon et al. 2006; Zaher et al. 2006; Yamagata et al. 2007).

Due to general selectivity, substrates of BCRP can be either negatively or positively charged, hydrophobic or hydrophilic, and unconjugated or conjugated. Several attempts were made to establish SAR for BCRP interaction, however, many analysis methods were based on the datasets of inhibitors (Saito et al. 2006; Matsson et al. 2007; Matsson et al. 2009; Nicolle et al. 2009). Yoshikawa *et al.* studied BCRP substrate specificity of 14 camptothecin (CPT) analogues, and noted that CPT analogues that showed ATP-dependent transport in BCRP-overexpressing membrane vesicles possess one –hydroxy or –amino group (Yoshikawa et al. 2004). Also CPT analogues showed a good correlation between polarity and BCRP-association, where highly polar compounds showed substrate specificity. It is likely that the presence of hydroxyl and amino functional groups facilitate hydrogen bonding with the amino acid residues at the binding site of BCRP. Furthermore, presence of a negative electrostatic potential area at position 10 for SN-38 and SN-398, but not in SN-22, suggests that CPT analogues with this feature are potential substrates for BCRP (Nakagawa et al. 2006). BCRP substrate specificity of a set of pyrrolobenzodiazepine (PBD) derivatives showed a good correlation with the electrostatic potential and aromaticity (Kaliszczak et al. 2010). PBDs with a greater number of HBA and the electronegativity and aromaticity of the C2 substitution show affinity to BCRP. Evidently, BCRP-mediated efflux could be circumvented by limiting C2 aryl substituents and the number of aromatic rings. In general, BCRP substrates share a same set of molecular properties as that of substrates to P-gp and other efflux pumps (Begley 2004; Kunta and Sinko 2004; Takano et al. 2006).

Peptide transporter 1 (Pept1)

PepT1 (*SLC15A1*), an electrogenic, H^+-dependent transporter, was first cloned from the rabbit intestine and subsequently from both rat and human (Fei et al. 1994). The cloned human PepT1 cDNA sequence encodes a 708 amino acid protein (MW 79 kDa) with an isoelectric point of 8.6 and several putative glycosylation and phosphorylation sites. There are 12 putative α-helical TM domains and a large extracellular loop between the IX and X TM domains, which possess intacellularly localized N- and C- termini (Liang et al. 1995; Rubio-Aliaga and Daniel 2008). Herrera-Ruiz *et al.* reported that PepT1 appears to be

localized predominantly in the duodenum, with decreasing expression in the jejunum and ileum (Herrera-Ruiz et al. 2001).

PepT1 has been shown to be independent of Na^+ and uses H^+-gradient and inside-negative membrane potential to provide the necessary driving force for substrate translocation. At the brush border membrane of enterocytes, an in-ward proton gradient is generated through the activity of an electroneutral proton/cation exchanger, Na^+/H^+ antiporter. This enables the uptake of PepT1 transporter substrates to be coupled with the influx of protons back into the enterocytes (Adibi 1997). The uptake of the PepT1 substrates is strongly dependent on the extracellular pH, where a pH of 4.5-6.5 (depending on the net charge of the substrate), is needed for optimal transport activity. Irie *et al.* investigated the transport mechanism of PepT1 for neutral and charged substrates by experimental studies and computational simulation (Irie et al. 2005). These uptake studies suggested that the K_m of glycylsarcosine (Gly-Sar), a neutral substrate, decreased as the pH dropped from 7.4 to 5.5, yet increased at a pH of 5.0. The K_m value of an anionic substrate, ceftibuten, declined steadily with a decreasing pH. Furthermore, the maximum transporter rate (V_{max}) values gradually increased with a fall in pH from 7.4 to 5.0, for both substrates. Consequently, the group hypothesized that unlike neutral and cationic substrates, negatively charged molecules not only require H^+ binding to H^+-binding site, but also to the substrate-binding site.

The 3D structure of the substrate binding site of PepT1 is not yet known, but its template has been proposed by the large variety of substrates (Foley et al. ; Meredith and Price 2006). It is interesting to note that the peptide bond is not required for substrate binding specificity of PepT1 transporter (Brandsch et al. 2004). Only the two oppositely charged free head groups (carboxylic carbon and amino nitrogen) separated by a 4 spacer carbon unit were identified as a minimal structural feature requirement (Doring et al. 1998). In the presence of a peptide bond, it is only the backbone carbonyl that is functional. This minimal configuration also explains the efficient transport of δ-aminolevulinic acid, which serves as a precursor for the endogenous porphyrin accumulation on which photodynamic therapy of tumors is based. In addition, the side chains provided in both di and tripeptides and in xenobiotics with charge polarity and conformation are pivotal in determining the binding affinities. It should also be emphasized that for the di and tripeptides, only the trans-configuration of the peptide bond is transported. Besides a preferred free N-terminal amino group, a high electron density around the terminal carboxylic group in dipeptide, or alternatively around the carbonyl group of the second amino acid in a tripeptide structure, is needed to ensure optimum binding affinity. Furthermore, high electron densities at the first and third side chains, as well as the presence of hydrophobic side chains, significantly contribute to overall binding affinity (Brandsch et al. 1999).

PepT1 is a low-affinity (K_m of 200 μM to 15 mM), high-capacity transporter and is known to play a pivotal role in the absorption and distribution of peptidomimetics that include β-lactam antibiotics such as cephalosporins and penicillins, angiotensin converting enzyme inhibitor such as zofenopril, fosinopril, benazepril, quinapril, trandolapril, spirapril, cilazapril, ramipril, moexipril, quinaprilat, and perindopril., selected rennin inhibitors, antitumor agents such as bestatin, and dopamine receptor antagonists such as sulpiride (Terada et al. 1997; Bretschneider et al. 1999; Watanabe et al. 2002; Watanabe et al. 2002; Watanabe et al. 2004; Knutter et al. 2008). Using PepT1 as an intestinal transporter to increase oral exposure of compounds with low oral bioavailability was shown to be an

effective strategy (Kikuchi et al. 2009). For example, acyclovir is usually associated with suboptimal oral plasma exposure (oral bioavailability 15%) that can lead to resistant viral strains. To overcome this limitation, valacyclovir, a L-valine ester prodrug of acyclovir was effectively designed to increase the oral absorption and plasma exposure of acyclovir (Ganapathy et al. 1998).

Organic Anion-Transporting Polypeptides (OATPS)

OATPs (*SLC*O) are transmembrane solute carriers that mediate the proton-dependent transport of a wide range of amphipathic endogenous and exogenous organic compounds across the plasma membrane. Currently, 39 members of the OATP/SLCO superfamily have been identified in mammalian species (Hagenbuch and Meier 2004). The OATPs represent integral membrane proteins that contain 12 TM domains where amino and carboxy termini are oriented to the cytoplasmic spaces. There is limited information regarding the tertiary structures of OATPs, although more recent studies are beginning to address this aspect. In this book chapter , we focus on OATP2B1 (*SLCO2B1*) and OATP1A2 (*SLCO1A2*). OATP2B1 plays a key role in the uptake of various xenobiotics and was originally isolated from the human brain and named OATP-B (Tamai et al. 2000; Kullak-Ublick et al. 2001) or *SLC21A9* (Hagenbuch and Meier 2003). OATP2B1 mRNA is expressed in the human small intestine (Tamai et al. 2000; Kullak-Ublick et al. 2001; Sai et al. 2006) and its protein is immunolocalised at the apical surface of human small intestine (Kobayashi et al. 2003) and Caco-2 cell monolayers (Sai et al. 2006).

Similar to other OATPs, transport via OATP2B1 is generally considered to occur in a bidirectional fashion driven by the solute concentration gradient across the membrane. Heterologous expression of OATP2B1 produces a Na^+-independent, pH-gradient dependent transporter with a relatively narrow substrate specificity compared to other OATPs (Nozawa et al. 2004). Extracellular acidification promoted solute uptake, a property of OATP2B1 that bears relevance to the small intestinal environment in which the transporter is expressed on the apical membrane of the enterocytes. Kobayashi *et al.* studied the impact of pH on the uptake of both estrone-3-sulfate and pravastatin in OATP-2B1 transfected HEK 293 cells. The group reported that the uptake of both compounds were pH dependent, where higher uptake at pH 5.5 relative to that at 7.4 pH was reported. It is interesting to note that an increase was only observed in V_{max} with a decrease of pH from 7.4 to 5.0 and a negligible change was observed in K_m at studied pH (Kobayashi et al. 2003). In a recent study, our group examined the role of OATP2B1 in the intestinal absorption and tissue uptake of 3-hydroxy-3-methylglutaryl-CoenzymeA (HMG-CoA) reductase inhibitors (statins) (Varma et al. Accepted). We first investigated impact of extracellular pH on the functional affinity of statins to the transporter using OATP2B1-transfeced HEK293 cells. The results indicated that OATP2B1-mediated transport is significant for rosuvastatin, fluvastatin and atorvastatin, at neutral pH. However, OATP2B1 showed broader substrate specificity as well as enhanced transporter activity at acidic pH consistent with other research groups' findings (Kobayashi et al. 2003). Furthermore, uptake at acidic pH was diminished in the presence of proton ionophore, suggesting proton-gradient as the driving force for OATP2B1 activity. Notably, passive transport rates are predominant or comparable to active transport rates for statins, except for rosuvastatin and fluvastatin. Second, we studied the effect of OATP modulators on statins uptake. At pH 6.0, OATP2B1-mediated transport of atorvastatin and cerivastatin was not inhibitable, while rosuvastatin transport

was inhibited by E-3-S, rifamycin SV and cyclosporine with IC_{50} values of 19.7±3.3μM, 0.53±0.2μM and 2.2±0.4μM, respectively. Rifamycin SV inhibited OATP2B1-mediated transport of E-3-S and rosuvastatin with similar IC_{50} values at pH 6.0 and 7.4, suggesting that the inhibitor affinity is not pH-dependent. Finally, we noted that OATP2B1-mediated transport of E-3-S, but not rosuvastatin, is pH-sensitive in intestinal epithelial (Caco-2) cells. However, uptake of E-3-S and rosuvastatin by Caco-2 cells was diminished in the presence of proton ionophore (FCCP). The present results indicate that OATP2B1 may be involved in the tissue uptake of rosuvastatin and fluvastatin, while OATP2B1 may play a significant role in the intestinal absorption of several statins due to their transporter affinity at acidic pH.

The physiological and pharmacological role played by OATP2B1 in intestinal absorption may also vary between individuals. For example, a single nucleotide polymorphism (SNP) (found in 31% of the Japanese population investigated within the referenced study) leads to an amino acid change in the OATP2B1 protein (S486F), which is associated with a greater than 50% reduction in transport capacity (Nozawa et al. 2002).

Since the unavailability of crystal structures of OATPs and relative difficulties in validating their homology models, pharmacophore models have helped elucidate the key molecular features involved in the substrate/inhibitor and protein interactions. These models have demonstrated good structure and activity correlation within the studied chemical space. The proposed OATP2B1 pharmacophores may share the similar molecular features for the consideration of the substrate binding at the positively-charged region. Its substrates may have features such as a hydrophobic core to form the π-stacking interaction with the imidazole ring of H579, or a HBD to directly interact with the nitrogen atom of the imidazole ring, both of which should be oriented at the energetically favored position inside the pore. To model these interactions structurally using molecular docking and dynamics, the minimal requirement will be a validated homology model of OATP2B1. To date, the strategy to elucidate the SAR of OATP2B1 is the combination of QSAR, pharmacophore, and structure-based modeling with the support of *in vitro* and cell-based experimental data.

Another OATP transporter that plays a role in the intestinal absorption of xenobiotics is OATP1A2 (also known as human OATP-A or *SLCO1A2*). This transporter consists of 670 amino acids and is expressed in the brain, kidney and apical membrane of the enterocytes (Kullak-Ublick et al. 1995). Unlike other OATP transporters, OATP1A2 possesses perhaps the broadest spectrum of solutes in that compounds of acidic, basic, and neutral character are substrates. It has been reported to transport bile salts and bromosulfophtalein (BSP), steroid sulfates, thyroid hormones [triiodothyronine (T3), thyroxine (T4), and reverse T3], prostaglandin E2, fexofenadine, opioid peptides [e.g., deltorphin II and (*d*-penicillamine)enkephalin], rocuronium, *N*-methylquinine and *N*-methylquinidine, ouabain, the endothelin receptor antagonist BQ-123, talinolol, and the thrombin inhibitor CRC-220 (Ianculescu et al. ; Loubiere et al. ; Kullak-Ublick et al. 1995; Hsiang et al. 1999; Gao et al. 2000; Geyer et al. 2004; Schwarz et al. 2005; Shimizu et al. 2005; Kalliokoski and Niemi 2009). Similar to OATP2B1, genetic variations has been reported in *SLCO1A2*. For example, Lee *et al.* identified six non-synonymous SNPs in the coding region of SLCO1A2. The c.516A>C (p.Glu172Asp) variant had markedly reduced uptake capacity for the OATP1A2 substrates estrone 3-sulfate and the d-opioid receptor agonists, deltorphin II and [D-penicillamine2,5]-enkephalin *in vitro*. The group concluded that considering its substrate specificity and expression in organs such as the brain, kidney and intestine, genetic variations in *SLCO1A2*

may be an important contributor to inter-individual variability in drug disposition and central nervous system entry of OATP1A2 substrate drugs (Lee et al. 2005).

In the clinic, the effect of grapefruit juice on the oral exposure of fexofendadine was evaluated. The oral plasma exposure of fexofenadine was decreased 63%. This seems likely to be mediated by inhibition of intestinal absorption via OATP1A2. (Dresser et al. 2005; Bailey et al. 2007). Similar findings were reported in a study that evaluated the effect of single and repeated grapefruit juice ingenstion on the oral plasma exposure of talinolol in humans. The decrease in the oral plasma exposure of talinolol (44%) was attributed to the inhibition of OATP1A2 (Shirasaka et al.; Schwarz et al. 2005). Overall, these findings identify OATP1A2 as a potential site for diet-drug interactions and clearly demonstrate the potential role of OATP1A2 in the absorption of xenobiotics.

Monocarboxylate transporter 1 (MCT1)

The bi-directional movement of monocarboxylic acids across the plasma membrane is catalyzed by a family of proton-linked monocarboxylate transporters (MCTs). MCTs are encoded by the *SLC16A* gene family, of which there are 14 known members that were identified through screening genomic and expressed sequence tag databases (Halestrap and Meredith 2004). Only MCTs 1-4 have been shown to catalyze the proton-coupled transport of metabolically important monocarboxylates such as lactate and pyruvate (Halestrap and Meredith 2004). This book chapter will focus on the first member of the MCT family, MCT1 (*SLC*16A1), which is well characterized and known to play a role in the intestinal drug absorption.

MCT1 consists of 12 TM α-helical domains with a large intracellular loop between TM segments VI and VII and intracellular C- and N- termini (Poole et al. 1996; Halestrap and Price 1999). MCT1 is expressed in most tissues and is especially prominent in the heart, red skeletal muscle, erythrocytes, and all cells under hypoxic conditions, where it can either be involved in the uptake or efflux of glycolytically produced lactic acid. MCT1 is also highly expressed in the small and large intestine (Gill et al. 2005), where it is responsible for the absorption of short chain fatty acids such as acetate, propionate and butyrate, produced from microbial fermentation of dietary fiber (Cummings and Macfarlane 1991).

MCT1 catalyses the facilitative diffusion of substrate across the plasma membrane, coupled with the translocation of a proton. The driving force for transport is provided by both the substrate and H^+-concentration gradients, with the pH gradient determining the extent of transport activity (Juel 1997; Halestrap and Price 1999). Based on the reported crystal structures of two members of the major facilitator superfamily, the Escherischia coli glycerol-3-phosphate transporter (G1pT) and lactose permease (Lac Y) (Abramson et al. 2003; Huang et al. 2003), the structure of MCT1 has been modelled (Manoharan et al. 2006). Futhermore, site-directed mutagenesis identifying key substrate-binding residues together with structural modeling has lead to the suggestion of a translocation cycle as the mechanism of transport for MCT1 (Wilson et al. 2009). This mechanism of transport is consistent with the "Rocker Switch" mechanism (Law et al. 2008). This model describes MCT1 existing in an open and closed conformation, with the N- and C-terminal halves tilting against each other along an axis that separates the two domains, allowing the substrate binding site alternating access to the either side of the membrane (Wilson et al. 2009). MCT1 also requires an ancillary protein, CD147, for correct trafficking to the plasma

membrane as well as functional activity (Wilson et al. 2005). CD147 is a member of the immunoglobulin gene superfamily, and has been shown to closely interact with both MCT1 and MCT4 (Kirk et al. 2000).

MCT1 is a low affinity, high capacity transporter that has been shown to transport unbranched aliphatic monocarboxylates such as acetate and proprionate and substituted monocarboxylates pyruvate, lactate, acetoacetate and β-hydroxybutyrate, with the K_m values for pyruvate and lactate about 0.7 and 3-5 mM, respectively (Halestrap and Meredith 2004). Other MCT1 monocarboxylate substrates include the branched chain keto-acids (formed from the transamination of leucine, isoleucine and valine) and the ketone bodies acetoacetate, β-hydroxybutyrate and acetate (Poole and Halestrap 1993), and exogenous acids p-aminohippuric acid, benzoic acid, γ-hydroxy butyrate, foscarnet, mevolonic acid, and salicylic acid (Enerson and Drewes 2003; Lam et al. 2010). MCT1 is also thought to be responsible for the intestinal absorption of the β-lactam antibiotics such as carbenicillin indanyl sodium as well as phenethicillin and propicillin (Li et al. 1999). The targeting of MCT1 by pharmacologically active drugs has been shown to result in enhanced intestinal drug uptake. For example, XP13512 is rapidly absorbed along the length of the intestine via MCT1 (as well as the SMVT). XP13512 is an anionic compound produced by the reversible modification of the amine group of gabapentin (which has limited oral absorption), with an acyloxyalkylcarbamate promoeity (Cundy et al. 2004). Overall, prototypical substrates of MCT1 generally consist of weak organic acids with the carboxyl group attached to a relatively small R group containing lipophilic or hydrophilic properties (Enerson and Drewes 2003).

5.2 Physicochemical factors that impact oral drug absorption

Our group recently investigated the interrelation of physicochemical properties and individual parameters for a database comprised of Fa, Fg, Fh, and F values for 309 drugs in humans (Varma et al. 2010). The aim is to define the physicochemical space for optimum human oral bioavailability. The current data set suggested an even distribution of the bioavailability values, with about 17% of compounds showing F less than 0.2 and 34% of compounds showing F more than 0.8. However, the vast majority of compounds showed Fa (71%), Fg (70%), and Fh (73%) more than 0.8. The current data set indicated that bioavailability is mainly limited by absorption as evident from the subset of compounds showing bioavailability less than 0.2, where mean and median values suggest the rank-order of limiting parameters as Fa > Fg > Fh.

The distribution of the data set in physicochemical space is heterogeneous and thoroughly covered the range of conventional small molecule marketed drugs. Trend analysis clearly indicate that ionization state, molecular weight (MW), lipophilicity, polar descriptors, and free rotatable bonds (RB) influence bioavailability. For example, ionization state analysis of compounds studied indicate that although bases tend to have higher Fa, they are relatively less bioavailable as compared to acids and neutrals. MW trends suggest that increasing the size of molecules above 400 g/mol will on average lead to a steady decline in bioavailability, mainly due to the effect on Fa. Lipophilicity (cLog P and cLog $D_{pH7.4}$) trends indicate that very hydrophilic compounds have drastically reduced intestinal absorption. On the other hands, RB and polar descriptors such as PSA, hydrogen bonding count (HBA + HBD) showed inverse relationship with Fa, in particular for compounds with RB > 12, PSA greater than 125 $A^{\circ 2}$, and hydrogen bond count more than 9.

The scholarship outlined above is consistent with the finding of Lipinski et al, who introduced the rule of 5 (RO5), which is one of the most widely used concepts to qualitatively predict oral drug absorption. The group analyzed 2245 compounds from the World Drug Index (WDI) database that were either considered for, or entered into, Phase II clinical trials. Results indicate that good oral absorption is more likely with drug molecules that have less than 5 hydrogen bond donors (defined as NH or OH groups)/10 hydrogen bond acceptors (defined as oxygen or nitrogen atoms, including those that are part of hydrogen-bond donors), a molecular weight that is smaller than 500, and a calculated lipophilicity (cLog P) that is smaller than 5 (Lipinski 1995; Lipinski 2000; Lipinski et al. 2001). Poor bioavailability is more likely when the compounds violate two or more of the RO5. Using the current data set, we evaluated the relationships between number of violations and bioavailability and the individual processes. From Figure 1, it is evident that median bioavailability dropped considerably from 0.70 to 0.35 ($p < 0.005$) for the compound subsets with no violation and two violations, respectively. Compounds with three violations showed a further decline in median bioavailability (0.05). However, similar relationship was observed only with Fa but not with Fg and Fh, suggesting that relationship of rule-of-five and bioavailability is associated mainly with intestinal absorption.

5.3 Biopharmaceutical factors that impact oral drug absorption

5.3.1 Particle size

Drug dissolution rate is an important parameter that affects oral drug absorption (Chaumeil 1998; Boobis et al. 2002; Hilgers et al. 2003). A drug is defined as being poorly soluble when its dissolution rate is so slow that dissolution takes longer than the transit time past its absorptive sites, resulting in incomplete oral absorption. Based on the Noyes-Whitney equation, many factors can affect a drug's dissolution rate (Healy 1984; Frenning and Stromme 2003):

$$DR = \frac{A \cdot D}{h}(C_s - C) \tag{6}$$

Where DR is the dissolution rate, A is the surface area available for dissolution, D is the diffusion coefficient of the drug, h is the thickness of the boundary diffusion layer adjacent to the dissolving drug surface, C_s, is the saturation solubility of the drug in the diffusion layer, C is the concentration of the drug in the bulk solution at time t. As shown in the equation above, the drug dissolution rate is directly proportional to the surface area of the drug particle, which in turn is increased with decreasing particle size. This can be accomplished by micronization or by the use of nanosuspension to reduce the particle size of the drug and therefore increases drug dissolution rate, which usually is associated with an increase in the extent as well as rate of oral absorption (Chaumeil 1998; Li et al. 2005; Borm et al. 2006). Examples on a drug for which reducing its particle size had significant impact on its dissolution rate is griseofulvin. This molecule has a particularly low solubility and was thus studied as a micronized powder with a median particle size of 3 μM (Nystrom et al. 1985; Nystrom and Bisrat 1986). Measurement of the amount dissolved in water *versus* time using a micronized powder showed that the rate of dissolution depended on the area of contact, which is related to the particle size. Increasing this area was an effective way of increasing the rate of dissolution of this drug (Sjökvist et al. 1989).

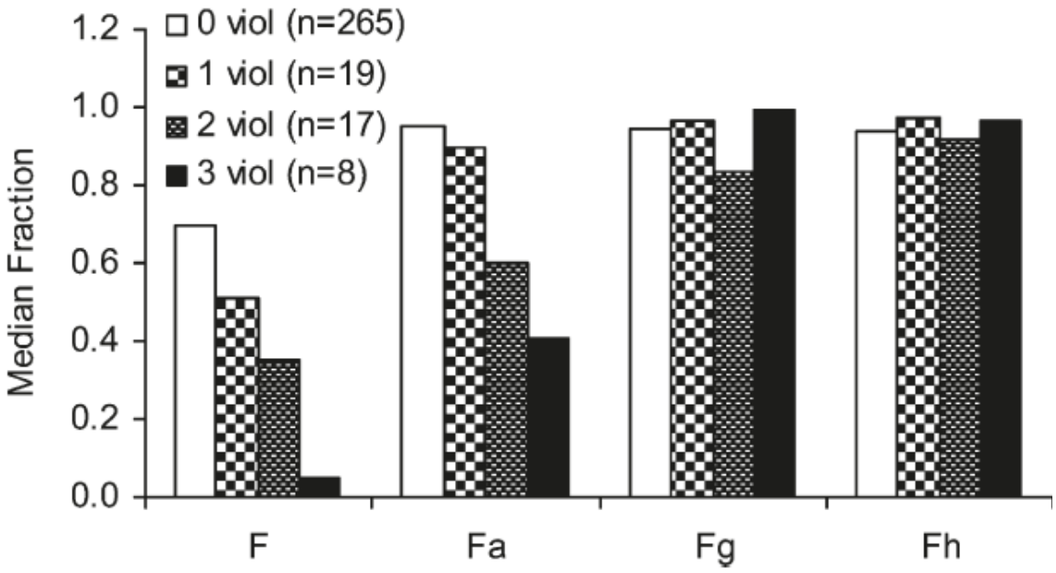

Fig. 1. Relationship between number of violations of rule-offive and bioavailability and individual processes. "n" is the number of compounds in each bin (Varma et al. 2010).

5.3.2 Salt form

As noted above, many drug molecules can be classified as either weak acids or bases that tend to form strong ionic interaction with an oppositely charged counter-ion and maintain that interaction through crystallization. The resulting solid comprises charged drug molecules and their associated oppositely charged counter-ions and is usually referred to as salt. The use of salt forms as active pharmaceutical ingredients is well established in the literature (Berge et al. 1977; Chowhan 1978). A salt form of a drug molecule changes the coulombic attraction between the drug molecule and its counterion and alters the potential energy of the solid state. This is usually associated with alteration of the pH of the diffusion layer at the surface of the dissolving solid, and therefore significantly increases the solubility of the parent drug molecule (C_s), in that layer over its inherent solubility at the pH of the dissolution medium (C). In general, these changes can result in a significant increase in the dissolution rates and higher apparent solubility of the drug molecules in physiologically relevant timescales. Overall, if other relevant factors such as chemical stability, permeability, intestinal and liver metabolism remain constant, the dissolution rate of a compound should determine the rate of build-up of blood levels with time and the maximal levels achieved (Nelson 1957; Chowhan 1978; Hendriksen et al. 2003; Huang and Tong 2004; Li et al. 2005).

In summary, the drug salt form usually alters the drug dissolution rate by modifying the diffusion layer pH at the surface of the dissolving solid (Nelson 1957). Nelson was the first to report this phenomenon in which the salts of acidic theophylline with high diffusion layer pH's had greater *in vitro* dissolution rates than those exhibiting a lower diffusion layer pH. In fact, the rank order of dissolution rates of theophylline was closely correlated with the clinical blood exposure. This report led many additional studies that demonstrated the influence of the salt form on drug dissolution and the benefit of changing nonionized drug to salts (Nelson 1957; Nelson 1958; Berge et al. 1977; Nang et al. 1977; Chowhan 1978; Chen et al. 2002; Hendriksen et al. 2003; Huang and Tong 2004; Strickley 2004; Li et al. 2005)

5.3.3 Polymorphism and drug amorphous form

Polymorphs of a drug substance are chemically identical. However, due to the differences in their molecular packing, they have different physical properties such as crystal shape, molecular density, melting temperature, hygroscopicity, and enthalpy of fusion (Huang and Tong 2004; Li et al. 2005). Albeit these differences, the various polymorphs tend to have comparable solubility profile. Pudipeddi and Serajuddin evaluated the effect of various polymorphs of drug molecules reported in the literature on their solubility profiles. The group reported that the solubility values of various polymorphs for these drug molecules did not differ more than two-folds. This difference in the solubility value is not expected to have profound impact on the compound biopharmaceutical profile depending on the doses used, particle sizes, and solubility values (Pudipeddi and Serajuddin 2005). However, polymorphism may influence the physical and chemical stability of various drug molecules by influencing the rate and mechanism of decay (Cohen and Green 1973; Matsuda et al. 1993; Singhal and Curatolo 2004). Examples are carbamezepine (Matsuda et al. 1993), indomethacin (Chen et al. 2002), furosemide (De Villiers et al. 1992), and enalapril maleate (Cohen and Green 1973; Eyjolfsson 2002).

There are significant differences between crystalline polymorphs and the amorphous form of a drug. In general, the amorphous form tends to have significantly higher dissolution rate and solubility compared to their crystalline forms, which may significantly increase their rate and extent of oral absorption. However, the amorphous form is generally less chemically stable due to the lack of a three dimensional crystalline lattice, higher free volume, and greater molecular mobility. The chemical stability of amorphous systems has been discussed in detail elsewhere (Craig et al. 1999; Doelker 2002; Kaushal et al. 2004; Singhal and Curatolo 2004).

5.3.4 Drug complexation

The drug complexes of interest are generally divided into two major categories based on the energy of attraction between the components of the complexes. They are (1) covalently linked complexes, (2) ionic/inclusion complexes. It is interesting to note that the energy of attraction of covalently linked complexes is about 100 kcal/mol. Whereas; the latter type of complexes is less than 10 kcal/mol. Examples on covalently linked complexes are prodrugs that are prepared by chemical modification of the drug through the addition of a labile moiety, such as ester group (Van Gelder et al. 2000). This approach is widely used to increase drug solubility/permeability and thus improving drug bioavailability. The labile groups are usually broken by enzymatic action, and the parent drug is freed to produce its pharmacological action. The prodrug approach has been widely used in the development of bacampicillin, chloramphenicol, pivampicillin, and enalapril (Van Gelder et al. 2000; van De Waterbeemd et al. 2001; Beaumont et al. 2003).

Inclusion compounds, which form the second category of complexes, result more from the architecture of molecules than from their chemical interaction. One of the constituents of the complex is trapped in the cage-like molecular structure of the other to yield a stable arrangement. Cyclodextrins have been most widely used for this purpose, since they can trap lipophilic drugs in their molecular envelope and form a complex having a comparatively more hydrophilic character (Shimpi et al. 2005). It is well established in the

literature that a complex formation of a drug with cyclodextrin is known to improve drug solubility or dissolution rate, and thereby its oral bioavailability (Irie and Uekama 1997; Loftsson et al. 2002; Strickley 2004; Shimpi et al. 2005).

It should be stressed that the drug molecules can also form complexes that may adversely affect their oral bioavailability. One widely reported example is the complexation of tetracycline with aluminum, calcium, or magnesium ions to form an insoluble complex that cannot be absorbed (Kakemi et al. 1968; Kakemi et al. 1968). Before the complexation phenomenon was known, the administration of antacids with tetracycline was suggested to minimize the gastrointestinal disturbance (nausea and vomiting) caused by the antibiotic (Gugler and Allgayer 1990). As most antacids contain aluminum or magnesium hydroxide and/or calcium carbonate ions, such coadministration have reduced greatly the bioavailability of the antibiotic. However, complexation can also arise due to the calcium ions present in milk and other dairy products (Jung et al. 1997). For example, for democycline, only 13% was absorbed when administered with milk. Doxycycline has been reported to be less prone to complexation with dairy products, yet only 10% was absorbed when coadministered with aluminum hydroxide gel (Gugler and Allgayer 1990).

6. BCS and BDDCS

Solubility and permeability are the fundamental properties determining the bioavailability of an orally active drug. Based on these properties Amidon *et al*. proposed biopharmaceutic classification system (BCS), which in present times is serving as a guide for regulatory and industrial purposes (Amidon et al. 1995). This concept exploring dose number, dissolution number, and absorption number of an orally administered drug clearly dictate its systemic availability. These three numbers are associated with a number of multifaceted hurdles, which include (i) physicochemical properties of the molecule (solubility/dissolution) (ii) stability of drug in GI environment (acid degradation) (iii) enzymatic stability in GI lumen, epithelium and liver (iv) permeability (molecular weight, log P, H-bonding efficiency) and (v) substrates specificity to various uptake and efflux transporters. The US FDA, other regulatory agencies, and healthcare organizations have implemented the BCS to enable the use of in vitro solubility and permeability data to waive conducting expensive bioequivalence clinical studies (BE) of high solubility-high permeability (Class I) drugs. While the pharmaceutical industry has taken advantage of BCS-based biowaivers, its principles are used throughout the drug discovery and development to drive oral active programs. On the basis of the apparent correlation between intestinal permeability rate and extent of drug metabolism, Benet and coworkers proposed biopharmaceutics drug disposition classification system (BDDCS), and suggested that the extent of drug metabolism may be used for characterizing high intestinal permeability drugs (Wu and Benet 2005; Benet 2009).

7. Intestinal metabolism

Small intestine has an ability to metabolize drugs by several pathways involving both phase I and phase II reactions and may lead to limited oral bioavailability. CYP3A4, the most abundant cytochrome P450 present in human hepatocytes and intestinal enterocytes is implicated in the metabolic elimination of many drugs (Paine et al. 2006; Thummel 2007). It has also been proposed that drug interactions involving CYP3A inhibition and induction may be largely occurring at the level of the intestine (Hebert et al. 1992; van Waterschoot et

al. 2009). In a recent analysis of 309 drugs with intravenous and oral clinical pharmacokinetic data, we noted that roughly 30% of the drugs in the data set show more than 20% intestinal extraction, underscoring the importance of considering intestinal metabolism in predicting bioavailability and dose projections in drug discovery and development settings (Varma et al. 2010). Although, the average human intestinal content of CYP3A has been estimated to be only about 1% of the average hepatic content (Paine et al. 2006), the data set indicated that intestinal metabolism may contribute to first-pass extraction more than the hepatic metabolism for certain drugs. This could be a result of better access to the enzymes in the enterocytes; a function of transcellular flux and the large absorptive area, and/or due to reduced access to hepatic enzymes because of potential plasma protein binding (Thummel 2007).

The intestinal first-pass metabolism in humans is indirectly estimated under certain assumption, by comparing the plasma AUCs following intravenous and oral dosing. Early studies in liver transplant patients during the anhepatic phase indicated the relative importance of the gut extraction to the first-pass metabolism for drugs such as midazolam and cyclosporine (Paine et al. 1996). Further clinical evidences were obtained in the grape-fruit juice interaction studies, where coadministration of grape-fruit juice result in the inhibition of gut CYP3A4 without significantly affecting the hepatic metabolism of drugs like felodipine (Gertz et al. 2008). However, assessment of the quantitative contribution of intestinal and hepatic extraction in first-pass metabolism is limited by ethical and technical challenges. There exist gaps in predicting the gut extraction before the clinical development stage due to shortcomings in the *in vitro-in vivo* extrapolation (Eg. utilizing human intestinal microsomal stability). Also species differences exist where rat and monkey typically under-predicts the fraction escaping gut extraction (Fg) in human (Cao et al. 2006; Nishimuta et al. 2010). Recently, transgenic mice model with constitutive expression of human CYP3A4 in liver or intestine that provides quantitative estimation of the contribution of hepatic and gut extraction to the first-pass metabolism has been generated (van Waterschoot et al. 2009). Overall, due to limited access to the sophisticated models and complexities with *in vitro in vivo* extrapolation and species differences, intestinal metabolic disposition is far from consistently predictable.

Recent studies demonstrated that efflux transporters present on the apical membrane of enterocytes, in particular Pgp, can affect the intestinal metabolism by prolonging the enterocytic transit time and consequent exposure to CYP3A enzymes (Wacher et al. 2001). A significant overlap has also been identified between substrates and inhibitors of CYP3A4 and Pgp, suggesting that these two proteins may act complementarily in further limiting Fg of CYP3A substrates. Due to the complexity in these biochemical processes and the lack of availability of extensive experimental models, application of physiologically-based pharmacokinetic (PBPK) models and systems biology seem to provide quantitative prediction of first-pass metabolism. These emerging tools aim towards appropriate reconstruction of the physicochemical, anatomical and biochemical complexities in mathematical terms.

8. Conclusions

Reliable delivery of drugs via oral administration is most sort after in drug industry. Consequently, the design and development of orally active drugs has to take into account a

plethora of factors which may include the physicochemical, biopharmaceutical and physiological determinants. While, solubility and permeability, are fundamental biopharmaceutical parameters that determine the oral absorption, physicochemical and drug substance properties are directly or indirectly associated with these parameters. Lipophilicity, hydrogen bonding ability and number of rotatable bonds are generally identified as critical molecular properties of drugs influencing the rate of membrane transport and thus the intestinal absorption (Fa). However, for drugs with low membrane permeability, role of uptake and efflux transporters may become significant and thus need appropriate characterization. It is believed that targeting intestinal uptake transporter and circumventing efflux transporters may be an useful strategy to design drugs with oral activity. Understanding the contribution of intestinal metabolism to the oral bioavailability is also key in projecting clinical pharmacokinetics and doses. Modeling intestinal absorption and metabolism is complicated due to variability in the physiology and gradient enzyme and transporter localization. Nevertheless, better characterization of factors influencing intestinal absorption and metabolism might result in improved pharmacokinetic optimization in discovery and development settings.

9. References

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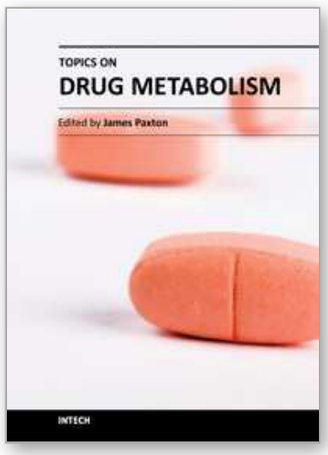

Topics on Drug Metabolism
Edited by Dr. James Paxton

ISBN 978-953-51-0099-7
Hard cover, 294 pages
Publisher InTech
Published online 22, February, 2012
Published in print edition February, 2012

In order to avoid late-stage drug failure due to factors such as undesirable metabolic instability, toxic metabolites, drug-drug interactions, and polymorphic metabolism, an enormous amount of effort has been expended by both the pharmaceutical industry and academia towards developing more powerful techniques and screening assays to identify the metabolic profiles and enzymes involved in drug metabolism. This book presents some in-depth reviews of selected topics in drug metabolism. Among the key topics covered are: the interplay between drug transport and metabolism in oral bioavailability; the influence of genetic and epigenetic factors on drug metabolism; impact of disease on transport and metabolism; and the use of novel microdosing techniques and novel LC/MS and genomic technologies to predict the metabolic parameters and profiles of potential new drug candidates.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ayman El-Kattan and Manthena Varma (2012). Oral Absorption, Intestinal Metabolism and Human Oral Bioavailability, Topics on Drug Metabolism, Dr. James Paxton (Ed.), ISBN: 978-953-51-0099-7, InTech, Available from: http://www.intechopen.com/books/topics-on-drug-metabolism/oral-absorption-intestinal-metabolism-and-human-oral-bioavailability-

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