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Featured Article

Why is it Challenging to Predict Intestinal Drug Absorption and Oral Bioavailability in Human Using Rat Model

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Purpose. To study the correlation of intestinal absorption for drugs with various absorption routes between human and rat, and to explore the underlying molecular mechanisms for the similarity in drug intestinal absorption and the differences in oral bioavailability between human and rat.
Materials and Methods. The intestinal permeabilities of 14 drugs and three drug-like compounds with different absorption mechanisms in rat and human jejunum were determined by *in situ* intestinal perfusion. A total of 48 drugs were selected for oral bioavailability comparison. Expression profiles of transporters and metabolizing enzymes in both rat and human intestines (duodenum and colon) were measured using GeneChip analysis.
Results. No correlation ($r^2 = 0.29$) was found in oral drug bioavailability between rat and human, while a correlation ($r^2 = 0.8$) was observed for drug intestinal permeability with both carrier-mediated absorption and passive diffusion mechanisms between human and rat small intestine. Moderate correlation (with $r^2 > 0.56$) was also found for the expression levels of transporters in the duodenum of human and rat, which provides the molecular mechanisms for the similarity and correlation of drug absorption between two species. In contrast, no correlation was found for the expressions of metabolizing enzymes between rat and human intestine, which indicates the difference in drug metabolism and oral bioavailability in two species. Detailed analysis indicates that many transporters (such as PepT1, SGLT-1, GLUT5, MRP2, NT2, and high affinity glutamate transporter) share similar expression levels in both human and rat with regional dependent expression patterns, which have high expression in the small intestine and low expression in the colon. However, discrepancy was also observed for several other transporters (such as MDR1, MRP3, GLUT1, and GLUT3) in both the duodenum and colon of human and rat. In addition, the expressions of metabolizing enzymes (CYP3A4/CYP3A9 and UDPG) showed 12 to 193-fold difference between human and rat intestine with distinct regional dependent expression patterns.
Conclusions. The data indicate that rat and human show similar drug intestinal 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. Therefore, a rat model can be used to predict oral drug absorption in the small intestine of human, but not to predict drug metabolism or oral bioavailability in human.

KEY WORDS: drug transporter; gene expression; inter-species correlation; intestinal permeability; metabolizing enzyme; oral bioavailability.

INTRODUCTION

The oral drug bioavailability (F) is defined as the rate and extent at which an active drug moiety becomes available at its desired sites of action. It is directly related to the drug absorption and metabolism in the gut wall and can also be elucidated by the equation: F = Fa*Fg*Fh, where Fa is the fraction of active drug that is absorbed, Fg is the fraction that escapes metabolism in the gastrointestinal tract, and Fh is the fraction that escapes first pass hepatic metabolism (1). Increasing oral drug absorption and metabolism stability will result in an increase in the bioavailability and therapeutic effect of the drug. However, it is still challenging to predict human oral drug bioavailability (F) from animal data due to the intertwined expression of transporters and drug metabolic enzymes in the intestine wall. In some cases, drug transporter (e.g., P-gp) and metabolic enzyme (e.g., P450) may act in concert in gut to further reduce F (2). Although the animal data, especially the rat permeability data, have been widely used to predict Fa in human, the correlation of drug transport and

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metabolism between the animal and human has not been extensively studied.

Oral drug absorption (Fa) is determined by two fundamental parameters: the drug's solubility and its gastrointestinal permeability (3). Several mechanisms of oral drug absorption have been shown in small intestinal regions, including passive transcellular diffusion, carrier-mediated transport processes, paracellular transport, and endocytosis. 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. Under physiological conditions, several routes may contribute to drug absorption at the same time and the fastest route dominates the absorption of a particular compound.

Many methods have been explored to predict drug absorption in human. It has previously been demonstrated that a good correlation exists between permeability and the fraction of drug absorbed in the same species (4,5). To date, one of the most commonly used assays to estimate a compound's absorption in human is to examine Caco-2 permeability. Although a general correlation does exist between Caco-2 permeability and drug absorption in humans, this model only works within certain limits. When drugs have low Caco-2 permeability or drugs are absorbed through carrier-mediated routes, many discrepancies can arise (6,7). On the other hand, the Caco-2 model is also limited because it does not account for the dose or the solubility of the drug in the intestinal lumen (8) and cannot tell the differences between cellular transport and intestinal metabolism.

When evaluating the oral drug bioavailability (F), the contribution of the metabolic enzymes expressed in enterocytes to the first-pass metabolism (Fg) could not be underestimated. The intertwined expression of metabolic enzymes and transporters in the gut wall and the interspecies physiological and metabolic differences between human and experimental animal complicate the prediction of *F* in human from preclinical animal data. However, based on the membrane structure similarity between human and rat, we hypothesize that drug intestinal absorption in rat can predict drug intestinal absorption (Fa) in human, regardless of the difference in the drug's bioavailability and metabolism in two different species. Indeed, this hypothesis has been confirmed by many reports from literatures for hundreds of compounds (5,9,10). For drugs with low solubility, it has been shown that using an Ussing chamber with rat intestinal epithelium is more effective in predicting human fraction absorbed than using Caco-2 cells (11).

However, up to date, the underlying mechanism of drug absorption similarity between the species has not been fully characterized. Therefore, the transporter expression profiles, especially for those transporters involved in carrier-mediated drug absorption process, will uncover the molecular mechanisms of drug absorption correlation between human and rat.

In this paper, we examined the oral bioavailability and intestinal permeability correlation for a variety of compounds with different absorption mechanisms between rat and human intestine. Since permeability is one of the primary factors governing absorption, a permeability correlation between the species may indicate the effectiveness of *in vivo* absorption prediction in humans. Meanwhile, we determined a variety of transporters and metabolizing enzymes for their expression profiles, which are directly related to the drug absorption and metabolism in human and rat intestine. This will reveal the underlying mechanisms for oral drug absorption correlation between the two species.

MATERIALS AND METHODS

Materials

SD rats were purchased from Charles River laboratories (Wilmington, MA). Valacyclovir was generously provided by Glaxosmithkline Inc. (Research Triangle Park, NC). All other drugs were purchased from Sigma (St. Louis, MO) (14). C-PEG was from Amersham. All drugs were dissolved in MES perfusion buffer (NaCl 140 mM, KCl 5 mM, MES 10 mM, NaOH 5 mM). The clinical study protocols were approved by the IRB of the University of Michigan. A Loc-I-gut® perfusion tube was purchased from Pharmacia (Peapack, NJ, USA).

Single-Pass *in Situ* Intestinal Perfusion

Rats were anesthetized with ketamine/xylazine (1 ml/kg) and pentobarbital (40 mg/kg). Surgery was performed to isolate 10 cm segment of jejunum. The perfusion tubes were connected to each 10 cm segment and MES buffer with 100 μM drug solution and 7 μci/l (14) C-PGE 4000 was perfused through the intestinal segment at a flow rate of 0.16 ml/min. All solutions and animals were maintained at 30°C during the perfusion. After absorption reached steady state in 60 min perfusion, the outlet flow was collected every 15 min for 1 h and 15 min. Small portion of perfusate (0.4 ml) was used for (14) C-PEG concentration measurement by scintillation count to correct for water absorption and secretion during perfusion. The rest of the perfusate was immediately frozen on dry ice for later HPLC analysis. After perfusion, the duodenum and colon were recovered, cut open and washed in saline five times. The mucosal layer was scrapped off using a glass slide. The mucosal sample was frozen in liquid N_2 and saved for later RNA isolation.

HPLC Analysis of Drug Concentration

The perfusate was centrifuged at 15,000 rpm for 15 min, and then the supernatant was filtered with a 0.45 μm filter, and 50 μl of sample was injected onto HPLC for concentration analysis. The column was a C18 supelco column (5 μm, 4.6×250 mm). The pump was from Waters (model 515), and auto injector was from Waters (WISP model 712). Gradient mobile phase acetonitrile(4–35%) in pH 3.5 ammonium acetate buffer was used. The PDA detector (Waters 996 photodiode array detector) was used for the detection.

Intestinal Permeability Calculation

The intestinal effective permeability is calculated under steady-state perfusion status. The perfusate was collected and analyzed after 60 min perfusion at a flow rate of 0.16 ml/min.

The inlet drug concentration was measured by sham perfusion tube at 60, 75, 90, 105, and 120 min, and the outlet drug concentrations were measured at the perfusion tube after passing through 10 cm of isolated intestinal segment at 60, 75, 90, 105, and 120 min. The outlet drug concentration was normalized by the nonabsorbable marker (14) C-PEG 4000 outlet and inlet concentration to correct for water absorption or secretion.

$$C'out(drug) = Cout(drug) \cdot Cin(PEG4000)/Cout(PEG4000).$$
$$Peff = Q(1 - C'out/Cin)/2\pi RL,$$

where *Peff* is effective permeability, Q is perfusion flow rate (0.16 ml/min), *C'out* is corrected outlet drug concentration, *Cin* is inlet drug concentration, R is the radius of rat small intestine (0.18 cm), and L is the 10 cm intestinal segment.

Clinical Human Intestinal *in Situ* Perfusion Study

Healthy male or female volunteers between 21 and 45 years of age and within 20% of their ideal body weight were enrolled in the study. Human subjects were fasted for approximately 7 h, and then underwent esophagogastroduodenoscopy (EGD). Ten biopsy samples were taken from the duodenum with endoscope guidance for RNA isolation and gene expression. Subsequently, a 6-lumen perfusion tube (Loc-I-Gut®), which contained two inflatable balloons, was introduced orally into the upper jejunum with the guidance of EGD. Twenty to thirty milliliters of air was used to inflate each balloon to create a 10 cm closed intestinal segment. The segment was then perfused with a solution containing 10 μM test drug, 1 mg/l *dl*-propranolol, 10 mg/l *l*-phenylalanine, 5 g/l PEG 4000, 5 g/l PEG 400, 5.4 mM KCl, 45 mM NaCl, 21 mM Na2HPO4, 49 mM NaH2PO4.H2O, 35.1 mM *D*-mannitol and 10 mM *D*-glucose at a flow rate of 3 ml/min for 120 min. The pH and osmolarity of the perfusate solution were adjusted to 6.5 and 300 mOsm/l, respectively. PEG 4000, a nonabsorbed compound, was used to correct for water secretion and absorption in the perfused segment. A total of 17 drugs were from literature (3,4,12–28), which included verapamil, L-leucine, Phenylalanine, L-dopa, Valacyclovir, Enalapril, Cephalexin, Methyldopa, Propranolol, Cimetidine, Atenolol, Furosemide, Ketoprofen, Naproxen, Antipyrine, Metoprolol, Tacrolimus. The permeabilities were calculated using the following equation: $Peff = Q\ (1-Cout/Cin)/2\pi RL$. Where *Peff* is effective permeability, Q is perfusion rate (ml/min), *Cout* and *Cin* are the outlet and inlet drug concentrations, R is the radius of human jejunum (1.75 cm), and L is the length of perfusion segment (10 cm).

Correlation of Oral Drug Bioavailability Between Rat and Human

Data for oral bioavailability in rat and human were obtained from Appendix II of Goodman-Gilman's *The Pharmacological Basis of Therapeutics*, 10th edition (29), and an extensive Medline database search (30–62). A total of 48 drugs were selected for the inter-species correlation.

RNA Isolation from Human and Rat Intestinal Mucosa Tissues

Rat intestinal mucosa tissues were immediately scraped with a glass slide from duodenum and colon after *in situ* intestinal perfusion, transferred to a new frozen vial and dipped into liquid N_2. The human colon mucosa was scraped from colon segment, which was obtained from surgery. A total of 50 mg of tissue was added to 1 ml TRIzol reagent (Gibco), homogenized with tissue razor at a maximum speed for 20 s×3 times on ice. The homogenate was transferred to a new ependorff tube and then 200 ul of chloroform was added to the TRIzol mixture, vortexed, and then centrifuged at 12,500 rpm for 15 min at 4°C. The aqueous phase was transferred to a new tube. Then 500 μl of isopropanol was added to the aqueous phase. The mixture is vortexed, then centrifuged at 12,500 rpm for 15 min at 4°C. The supernatant is discarded and the pellet is washed with 80% ethanol. The RNA pellet is resuspended in 30–50 μl of DNase/RNase free-water, and the concentration is measured at 260 nm UV. The RNA was further purified after isolation with an RNase Mini kit (Qiagen). The absorbance ratio of the RNA at 260/280 nm should be 1.7–1.8. The RNA was checked in Agarose/formaldehyde gel for quality before further cRNA labeling. Total of 5 μg of RNA was mixed with RNA loading buffer and heated at 75°C for 15 min. After cooling down on ice for 5 min, the RNA was loaded on 1% agarose/formaldehyde gel in 1×MOPS buffer. The gel was run at 80 V for 50 min and RNA quality should be checked under UV and two sharp 18S and 28S band should be visible.

cRNA Labeling and GeneChip Analysis

First-strand cDNA was transcribed from 8 μg of total RNA using T7-$(dT)_{24}$ oligomer primer, the primer was annealed at 70°C for 10 min, and then SSII reverse transcriptase was used for reverse transcription at 42°C for 1 h. The second strand cDNA was synthesized from first-strand cDNA using DNA ligase, DNA polymerase I and T4 DNA polymerase at 16°C (SuperScript Choice System for cDNA synthesis kit, Gibco) for 2 h, the reaction was stopped by adding 10 ul of 0.5 M EDTA. The dsDNA was then cleaned by phenol/chloroform extraction with Phase-Locking gel and ethanol precipitation in the presence of 1 μg of glycogen. Biotin-labeled cRNA was synthesized from the double strand cDNA using T7 RNA polymerase-catalyzed *in vitro* transcription in the presence of biotin-labeled NTP (BioArray high yield RNA transcription labeling kit, Enzo Biochem) at 37°C for 5 h. The labeled cRNA was purified using RNase mini kit (Qiagen). The concentration of labeled cRNA was measured at 260 nm. A total of 20 μg of labeled cRNA was fragmented at 95°C. Biotin-labeled cRNA was heated at 99°C for 5 min in a hybridization cocktail including hybridization control (Bio B, C, D, and Cre) and hybridized with GeneChip® (Affymetrix) at 42°C for 16 h. The GeneChip® was then washed with nonstringent wash buffer at 50°C and stained with streptavidin phycoerythrin (SAPE) solution. After washing at 25°C, the GeneChip® was scanned with a laser scanner (Affymetrix). The gene expression profiles were analyzed by Affymetrix Microarray Suite.

Data Analysis

All of the gene chip data was normalized by the gene expression levels of all the genes in the entire GeneChip. The expression levels of transporters and metabolizing enzymes examined in this paper were then normalized by a housekeeping gene expression (GAPDH) to give a ratio of interest gene expression/GAPDH expression. These normalized ratios were then used in the bar plots to compare gene expressions in human and rat. Only those transporters and enzymes which were homologous and present in both the human U95 and rat U34 GeneChip were compared in this study. Homology comparisons of each transporter and metabolizing enzyme were confirmed using UniGene from the NCBI database (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Evaluation of Oral Drug Bioavailability between Rat and Human

The oral bioavailability (F) values of the 48 drugs studied in rat and human were obtained from Goodman-Gilman's book and an extensive literature search. Results of the *F* values comparison are shown in (Fig. 1). No correlation (r^2 = 0.29) was found for oral drug bioavailability between rat and human. In contrast, Chiou and Buehler observed low correlation of 35 drugs' bioavailability between monkey and human with r^2 = 0.502 (63), 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.

Correlation of Drug Permeability in Rat and Human Intestine

In order to depict the oral drug absorption process, permeabilities of 17 drugs were evaluated in rat and human jejunum. Since permeability is one of the primary factors governing absorption (3), studying the permeability correlation should be useful when predicting human absorption from rat permeability. *In situ* intestinal perfusion was used to measure the drug permeability of 14 drugs and three drug-like compounds in rats. The drug permeabilities in human intestine were reported in our previous study (3,4,12–28). The tested drugs are absorbed by carrier-mediated processes as well as passive diffusion. For instance, valacyclovir, enalapril and cephalexin are 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 absorbed through passive diffusion. The drug permeabilities in the rat jejunum were then correlated with the drug permeabilities in the human jejunum (Fig. 2). It showed that drug permeability in the rat is generally 5 to 10-fold lower than the permeability in the human. However, both carrier-mediated and passively diffusing drugs showed 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.

Our data are in agreement with what was previously reported, that the percentage of absorption of 98 drugs was correlated between rat and human with a correlation of r^2 = 0.88 (9). *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 (11). 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 (10). However, another 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 (13). These discrepancies might be due to the different numbers of transporter substrates that are used in the correlation analysis. Our data, together with others, indicate that reasonable permeability correlation between human and rat can be used to predict drug absorption in humans.

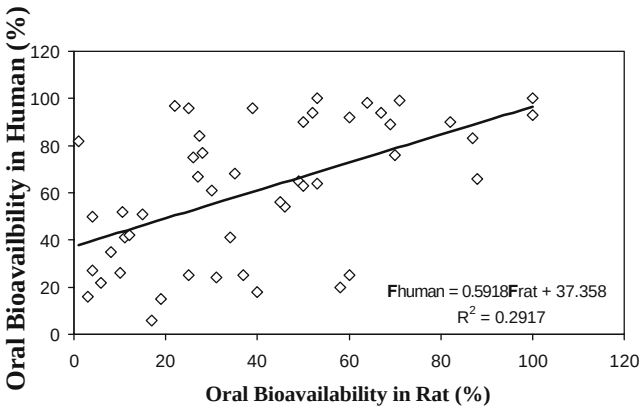

Fig. 1. Correlation of oral bioavailability between rat and human. Total of 48 drugs were plotted. The equation describes the correlations for rat oral bioavailability (Frat) and human oral bioavailability (Fhuman).

Correlation Analysis of the Expression Levels of Transporters and Metabolizing Enzymes between Rat and Human Intestine

To understand the underlying mechanisms for the similarity in drug intestinal absorption between humans and rats, we determined the correlation in the gene expression levels of a group of transporters and metabolism enzymes between these two species in the regions of duodenum and colon. The choice of duodenum is based on previous reports that the duodenum has the highest expression levels of CYP genes, and the UGT genes are expressed throughout the whole intestine (64,65). While the distribution of efflux transporters, such as Pgp, is not homogenous throughout the intestinal tract but rather has a regional dependence with a progressive increase from proximal to distal (66). Gene expression profiles were measured using GeneChip analysis in the duodenum and colon of human and rat. The expression for

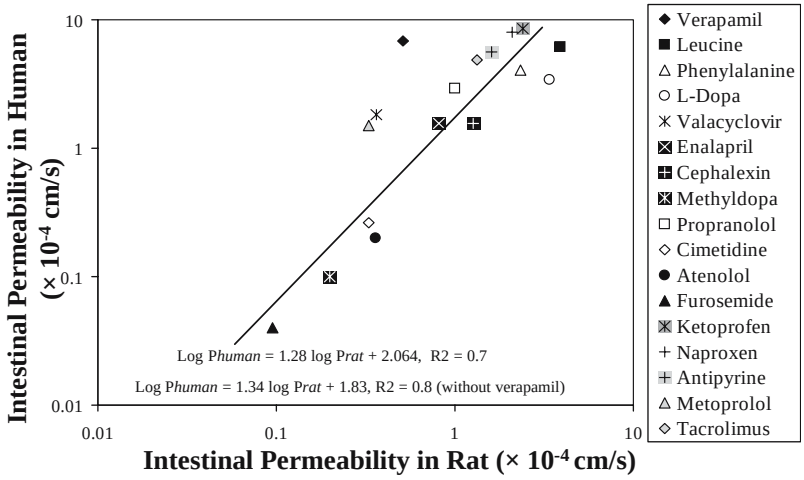

Fig. 2. Correlation of drug permeability in rat jejunum and in human jejunum. Permeability coefficients (Peff) were determined by *in situ* intestinal perfusion. The equations describe the correlations for rat permeability (Prat) and human permeability (Phuman).

each individual gene was normalized by the average levels of gene expressions of all genes in the entire GeneChip in human and rat, respectively. In order to compare the expression levels between human and rat, the expressions of transporters and metabolizing enzymes were then normalized by a housekeeping gene's expression (GAPDH) to give a ratio of relative expression levels of interest gene to GAPDH expression. GAPDH is involved in the glucose metabolism pathway and provides primary energy sources for many physiological functions. The expression of GAPDH was assumed to be the same although it might be expressed differently between human and rat or in different sections of intestine, which is one of the limitations for using GAPDH. The gene homology was searched in the UniGene database to confirm the orthologs of each transporter or metabolizing enzyme between human and rat.

Carrier-mediated transport is an important pathway of drug absorption. When examining transporter expression, we found a moderate correlation in transporter expression levels in the duodenum (Fig. 3) between human and rat with correlation coefficient $r^2 = 0.5687$. However, the correlation of transporter expressions in colon between two species is relatively poor with $r^2 = 0.4099$ (Fig. 4). The reasonable correlation in transporter expression levels between rat and human small intestine confirms the similarity of drug permeability correlation. These data indicate that animal absorption in the small intestine is sufficient for predicting human absorption in the small intestine.

The transporters are found in both human genechip (U95) and rat genechip U34 for correlation analysis as follows:

Oligopeptide transporter 1 (PepT1)
Dibasic and neutral amino acid transporter (SLC3A2)
Glial high affinity glutamate transporter (SLC1A3)
Neuronal/epithelial high affinity glutamate transporter (SLC1A1)
Nucleoside transporter 1 (NT1)
Nucleoside transporter 2 (NT2)
Sodium coupled nucleoside transporter (Na Coupled NT)
Glucose transporter 1 (GLUT1)
Glucose transporter 3 (GLUT3)
Glucose transporter 5 (GLUT5)
Sodium dependent glucose cotransporter 1 (SGLT1)
Multidrug resistance protein (MDR1)
Multidrug resistance associated protein 3 (MRP3)
Organic cation transporter (OCTN2)
Multidrug resistance associated protein 2 (cMOAT–MRP2)
Pancreas sodium bicarbonate cotransporter 2 (Pancreas SBC)
Monocarboxylate transporter 3 (MCT3)

On the other hand, fraction of drug metabolized in intestinal epithelium is another important component of oral drug bioavailability. Therefore, we also examined the expression

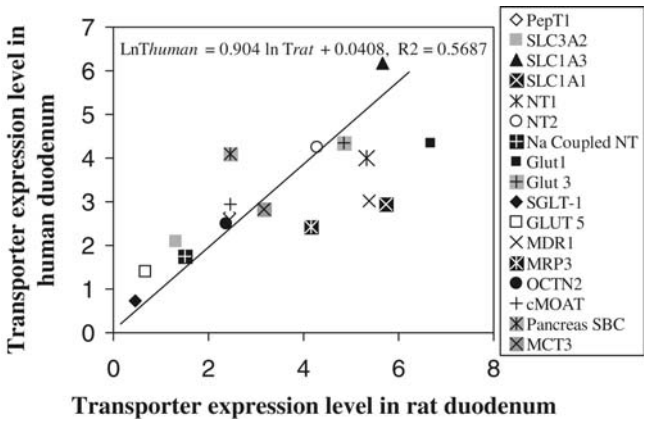

Fig. 3. Correlation plot of normalized transporter expression in duodenum between rat and human. Gene expressions of indicated transporters were measured using GeneChip analysis. The expression levels of the transporters in duodenum were then normalized by GAPDH expressions and transformed by natural logarithm and the absolute value were used in the correlation analysis. The equations describe the correlations for rat transporter expression level (Trat) and human transporter expression level (Thuman) in duodenum.

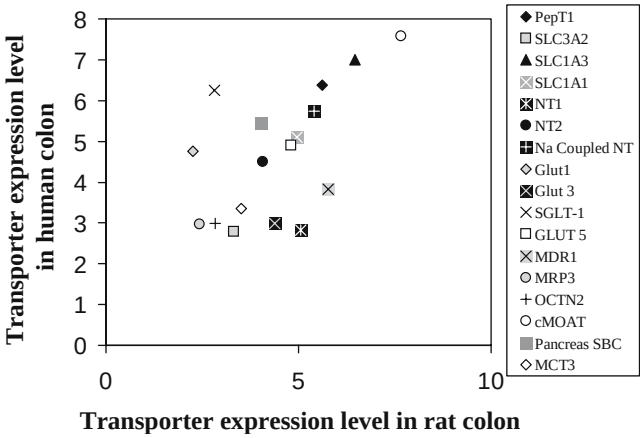

Fig. 4. Correlation plot of normalized transporter expression in colon between rat and human. Gene expressions of indicated transporters were measured using GeneChip analysis. The expression levels of the transporters in colon were then normalized by GAPDH expressions and transformed by natural logarithm and the absolute value were used in the correlation analysis.

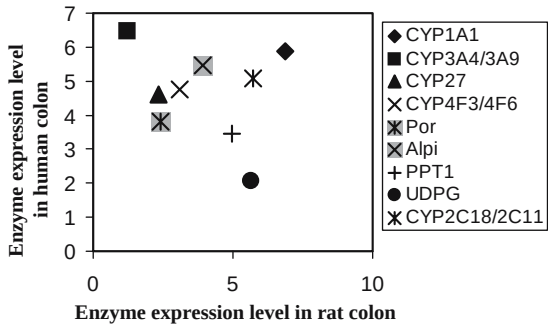

Fig. 6. Correlation plot of normalized metabolism enzymes expression in colon between rat and human. Gene expressions of indicated metabolism enzymes were measured using GeneChip analysis. The expression levels of the transporters were then normalized by GAPDH expressions in colon and transformed by natural logarithm and the absolute value were used in the correlation analysis.

levels of metabolizing enzymes in the intestine for the correlation analysis between the two species. Interestingly, we found that no correlation exists in enzyme expressions in both the duodenum (Fig. 5) and colon (Fig. 6) between human and rat. These data indicate that drug metabolism in rat would be very different from that in human. The drug metabolism and bioavailability in rat cannot be used to predict drug metabolism and bioavailability in human.

The enzymes were found in both human GeneChip (U95) and rat GeneChip (U34) for correlation analysis as follows:

Cytochrome P450 1A1 (CYP1A1)
Cytochrome P450 3A4/3A9 (CYP3A4/3A9)
Cytochrome P450 27 (CYP27)
Cytochrome P450 4F3/4F6 (CYP4F3/4F6)
Cytochrome P450 2C18 (CYP2C18/2C11)
Cytochrome P450 oxidoreductase (Por)
Alkaline phosphatase 1, intestinal (Alpi)
Palmitoyl protein thioesterase (PPT1)
UDP-glucuronosyltransferase (UDPG).

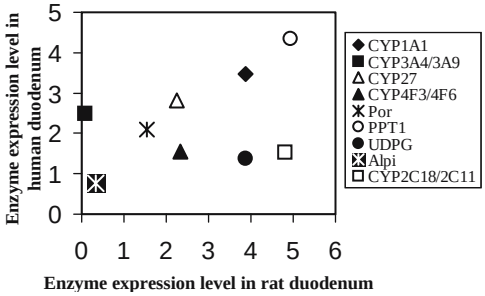

Fig. 5. Correlation plot of normalized metabolism enzymes expression in duodenum between rat and human. Gene expressions of indicated metabolism enzymes were measured using GeneChip analysis. The expression levels of the transporters were then normalized by GAPDH expressions in duodenum and transformed by natural logarithm and the absolute value were used in the correlation analysis.

Comparison of Gene Expression of Transporters and Metabolizing Enzymes in the Duodenum and Colon between Human and Rat Intestine

To study the regional and species dependence of transporter expressions in duodenum and colon between human and rat, we examined the gene expressions of several selected transporters and metabolizing enzymes related to drug absorption and metabolism.

Peptide Transporter (PepT1)

PepT1 is the main transporter involved in oligopeptide absorption from the intestine (67). It is expressed and localized at the brush border membrane in the duodenum, jejunum, and ileum while not detected in the esophagus, stomach, or colon in rat (68). The expression of PepT1 is higher in the small intestine than that in the large intestine because the small intestinal region accounts for more nutrient absorption. Fig. 7A shows the PepT1 expression level in duodenum and colon in both human and rat. The expression of PepT1 was 38-fold higher in the human duodenum, 24-fold higher in the rat duodenum when compared to the colon of the same species. Similar levels of expression were observed in both species in the two regions studied. The results suggest that PepT1 expression is similar in both species, with rat and human displaying the same levels of expression and regional dependence. As for substrates absorbed through PepT1, such as valacyclovir, enalapril and cephalexin, rat absorption could be correlatively used to predict drug absorption in humans.

Amino Acid Transporter-Neuronal/Epithelial High Affinity Glutamate Transporter

Due to the increased nutrient absorption that occurs in the small intestine, we expected to find higher expression of amino acid transporters in the duodenum. When examining regional expression differences, we found that rat and human displayed the same regional dependence. Expression was found to be more than 2.3-fold higher in duodenum when compared to the colon (Fig. 7B). It is surprising that the expression of amino acids carrier is not larger between duo-

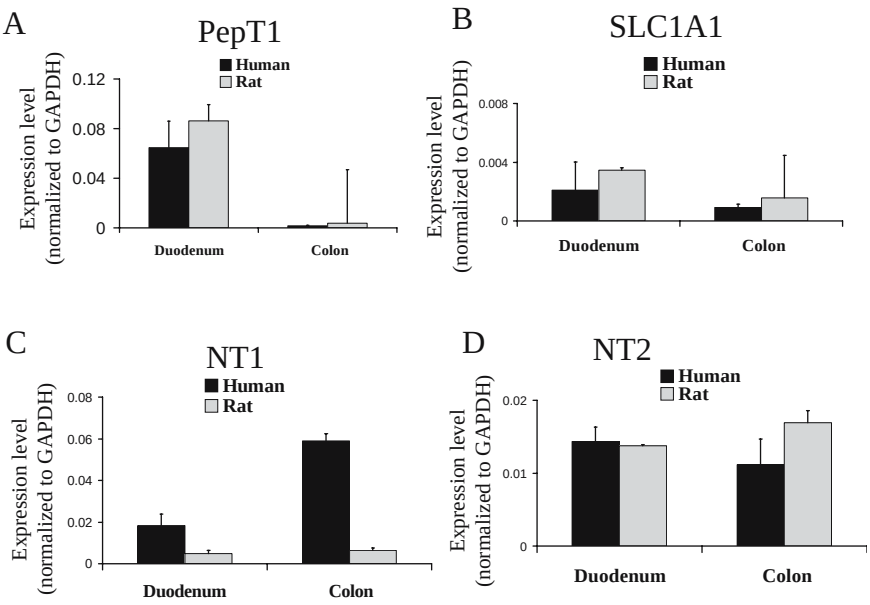

Fig. 7. Comparison of oligopeptide, glutamate, and neucleoside transporter gene expression in duodenum and colon of human and rat intestine. (A) Oligopeptide transporter1 (PepT1); (B) Neuronal/epithelial high affinity glutamate transporter; (C) Nucleoside transporter 1(NT1); (D) Nucleoside transporter 2 (NT2). Gene expressions of indicated transporters were measured using GeneChip analysis. The expression levels of the transporters were then normalized by GAPDH expressions in duodenum and colon, respectively. *Columns*, means from at least three replicates; *bars*, SD.

denum and colon. This could be explained by that some nutrient transporters are even higher expressed in jejunum due to the fact the digestive enzymes are mainly secreted in the end of duodenum and the major uptake actually occurs in jejunum and ileum. However, the absolute expression level of amino acid carrier in small intestine is much higher than that in colon based on the much longer length of small intestine. An interspecies comparison showed that expression levels were similar in both regions of the intestine in both species. Based on the data obtained, we would conclude that uptake of amino acid like compounds by this transporter, such as L-Dopa and methyldopa, would be similar in both species.

Nucleoside Transporters

Nucleoside transporters are essential for cell survival. Characterization of the nucleoside transporter expression can provide critical data for nucleoside drug absorption. Nucleoside transporters, NT1 and NT2, selectively transport purine and pyrimidine nucleosides, respectively.

When examining regional NT1 expression, our data showed that NT1 was expressed at a similar level in the duodenum and colon of rat intestine. However, NT1 was expressed 3.2-fold higher in the colon than in the duodenum in human (Fig. 7C). Interspecies expression differences were also observed for NT1 expression. NT1 expression was 3.7-fold and 9.2-fold higher in human duodenum and colon than that in rat intestine, respectively.

In contrast, NT2 expression was at similar levels in both duodenum and colon of human and rat (Fig. 7D). It is worth noting that there is higher inter-individual variability in NT2 expression levels. Thus, the absorption of a compound mediated by NT2 would have more accurate correlation between rat and human than that mediated by NT1 based on the different expression profiles of NT1 and NT2.

Glucose Transporters

Glucose transport is a critical process of the intestinal glucose absorption as glucose is one of the primary energy sources in many physiological functions. Similar to a previous report that GLUT1 is not expressed in the normal rat small intestinal epithelium (69), our data showed very low levels of GLUT1 in rat duodenum while 81-fold higher level in the rat colon (Fig. 8A). However for human, it is GLUT3 that showed four-fold higher expression in colon than in duodenum (Fig. 8B). These data suggest that GLUT1 may be the dominant glucose transporter in the rat large intestine while GLUT3 is the dominant glucose transporter in human large intestine to mediate complete glucose absorption in the entire intestine. SGLT-1 and GLUT5 are also involved in the uptake of sugars in the intestines. Our data showed that SGLT-1 and GLUT5 have the same expression pattern in rat and human. Both transporters were more highly expressed in the duodenum than in the colon in both species (Fig. 8C and D). These data suggest that GLUT5 and SGLT-1 are dominantly expressed in the small intestine. These findings provide molecular mechanisms for accurate correlation of glucose absorption between human and rat.

Efflux Transporters

Efflux transporters, which are the ATP-binding cassette (ABC) superfamily, are known to affect pharmacokinetics

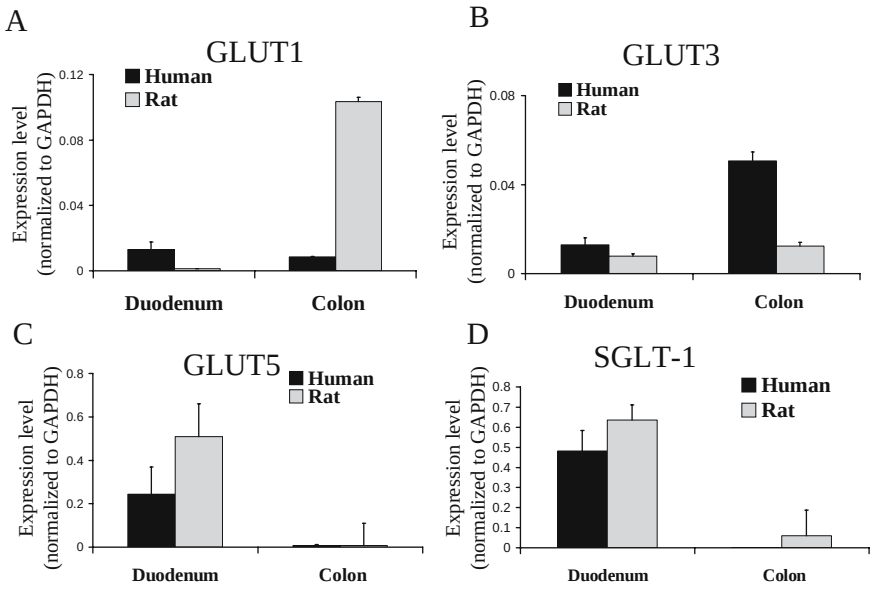

Fig. 8. Comparison of gene expression for various glucose transporters in duodenum and colon of the human and rat intestine. (A) Sodium independent glucose transporter 1 (GLUT1); (B) Sodium independent glucose transporter 3 (GLUT3); (C) Sodium independent glucose transporter 5 (GLUT5); (D) Sodium dependent glucose transporter 1 (SGLT-1). Gene expressions of indicated transporters were measured using GeneChip analysis. The expression levels of the transporters were then normalized by GAPDH expressions in duodenum and colon respectively. *Columns*, means from at least three replicates; *bars*, SD.

and drug–drug interactions. We examined the expressions of MDR1, MRP2 and MRP3 in different regions of human and rat intestine.

Our data show that the level of MDR1 is 2.7-fold higher in human duodenum than in the colon. These data are in agreement with literature reports. For instance, with GAPDH as an endogenous RNA control, Nakamura *et al.* investigated the expression of three ABC transporters in human duodenal and colorectal tissues. They showed a decrease MDR1 and much lower MRP2 expression level in colon when compared

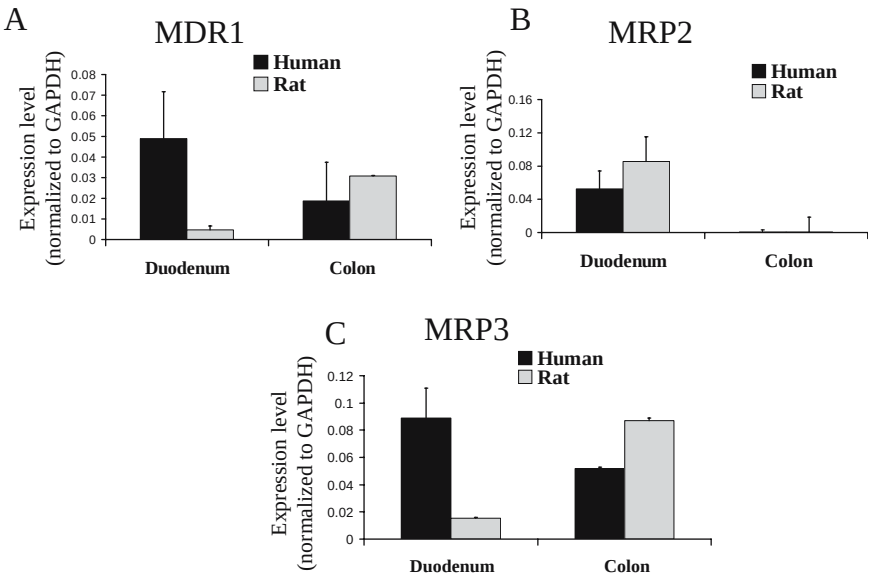

Fig. 9. Comparison of gene expression levels for various efflux proteins in duodenum and colon of the human and rat intestine. (A) Multidrug resistance 1 (MDR1 or P-gp); (B) Multidrug resistance-associate protein 2 (MRP2); (C) Multidrug resistance-associate protein 3 (MRP3). Gene expressions of indicated transporters were measured using GeneChip analysis. The expression levels of the transporters were then normalized by GAPDH expressions in duodenum and colon, respectively. *Columns*, means from at least three replicates; *bars*, SD.

to duodenum (70). However, Zimmermann *et al.* reported that a slightly higher MDR1 expression level in human colon over duodenum was observed when the intra-individual villin expression level was used as a control (71). These differences might be due to the different house keeping genes expression level of GAPDH and villin as well as the inter-individual variabilities. In contrast, MDR1 expression in rat was seven-fold higher in colon when compared to the duodenum (Fig. 9A).

MRP2 expression is 181-fold higher in rat duodenum and 103-fold higher in human duodenum when compared to the colon, respectively (Fig. 9B). These data are in agreement with the findings for the regional dependence in humans for MRP2 (70). In addition, human and rat share similar expression levels of MPR2.

However, MRP3 expression does not follow the same pattern of regional dependence between the two different species. Our data showed that MRP3 was 5.6-fold higher in rat colon when compared to the duodenum (Fig. 9C). These data have been confirmed by other reports that MRP3 expression has low expression levels in the duodenum and jejunum but is dramatically increased in the rat large intestine (72). Our results together with others, may suggest that MRP3 is an important component of drug efflux in rat large intestine. In contrast, human intestine has similar high expressions of MRP3 in both the duodenum and colon, which is also consistent with the reports from other laboratories that MRP3 is highly expressed in the human small intestine and colon (71,73).

The distribution of efflux proteins may be related to their substrate specificity and the distribution drug metabolizing enzymes. For example, MDR1 preferentially extrudes large hydrophobic, positively charged molecules and CYP3A4 is reported to have an overlapping substrate specificity (74). The distribution pattern of MDR1 is opposite to that of CYP3A4, especially in rat (Fig. 9A and Fig. 10B). This may suggest that higher levels of MDR1 expression in colon are important in expelling xenobiotics not yet metabolized by CYP3A4. In contrast, the expression pattern of MRP2 is consistent with the expression of phase II metabolizing enzymes (Fig. 9B and Fig. 10C). Since the substrate specificity of MRP2 includes glutathione conjugates, bilirubin gluronides, and a number of drugs and their conjugated drug metabolites (75), this confirms that the phase II metabolizing enzymes provide the conjugated compounds for subsequent export by MRP2. Diet and physiological differences may decide the interspecies differences of the efflux proteins and metabolizing enzymes between rat and human.

Drug Metabolizing Enzymes

Cytochrome P450s are a class of drug metabolizing enzymes, which are expressed in intestine and liver to regulate drug pharmacokinetics and oral bioavailability. Therefore, examining the differences in expression levels of these enzymes may provide insight into the differences in PK and drug bioavailability between rat and human.

When examining CYP1A1, a 15-fold and a 20-fold higher expression of CYP1A1 in the duodenum were found compared to the colon of human and rat, respectively. In addition, both rat and human showed similar low expression levels and similar regional dependent patterns. Our data are also confirmed with RT-PCR in other reports that only weak CYP1A1 signals are detected in human intestine (76).

CYP3A is the primary CYP subfamily in humans, responsible for metabolism of more than 50% of administered drugs. It has been previously reported that CYP3A4 is the major

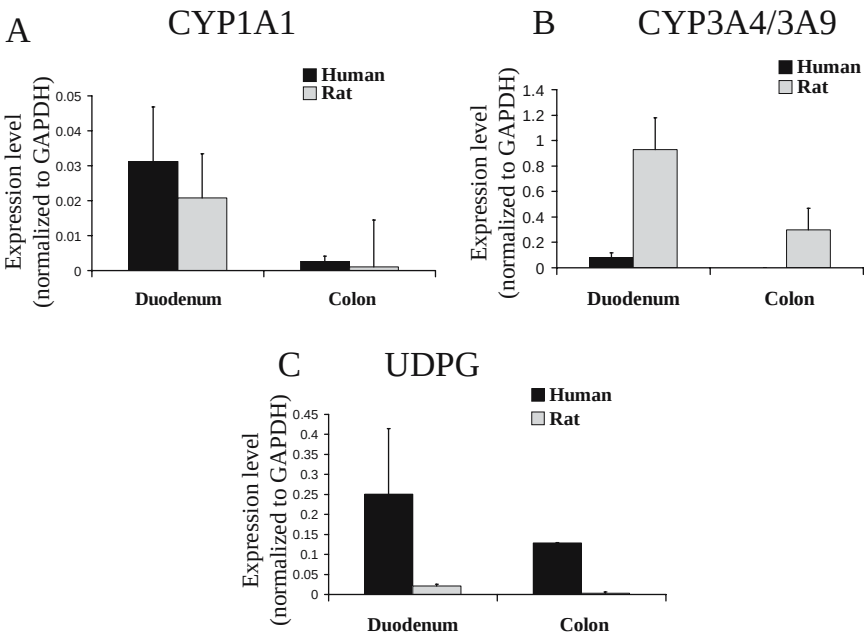

Fig. 10. Comparison of gene expression levels of various enzymes in the duodenum and colon of the human and rat intestine. (A) Cytochrome P450 1A1 (CYP1A1); (B) Human Cytochrome P450 3A4 (CYP3A4) and the homology rat CYP3A9; (C) UDP-glucuronsyltransferase. Gene expressions of indicated enzymes were measured using GeneChip analysis. The expression levels of the enzymes were then normalized by GAPDH expressions in duodenum and colon, respectively. *Columns*, means from at least three replicates; *bars*, SD.

CYP form and highly expressed in the human small intestine (76–79). CYP3A4 in human is corresponding to the ortholog CYP3A9 in rat based on the Unigene database. Our data showed that human CYP3A4 and rat CYP3A9 have much higher expression levels in the intestine when compared to CYP1A1. In the human duodenum, CYP3A4 expression was found to be 8% of GAPDH expression, which was 41-fold higher than that in human colon. Rat CYP 3A9 was expressed three-fold higher in rat duodenum than in the colon.

Very interestingly, CYP3A9 was expressed at extremely high levels in rat intestine in comparison to its ortholog CYP3A4 in human. For instance, CYP3A9 expression level in rat duodenum was similar to GAPDH expression, and was 11-fold higher than that in the human duodenum (Fig. 10B). In the colon, rat CYP3A9 expression was 193-fold higher than that in the human colon. Our data have been confirmed by the report from Takara *et al.* (80). It was reported that CYP3A9 expression levels were at or even higher than expression of GAPDH in the rat small intestine. It was also reported that the CYP2 family, such as CYP2B1, CYP2C6, CYP2C11 and CYP2D1, are more highly expressed in rat intestine compared to the rat liver (81). However, when compared to CYP3A9, CYP2 family only takes 0.3% expression level of CYP3A9 in rat duodenum (data not shown). Our data together with others suggest that CYP3A4 and CYP3A9 show a regional and species dependent expression. CYP3A4 and 3A9 are the dominant CYPs in both human and rat intestine. Due to the high expression levels and dramatic difference in CYP3A4/CYP3A9 expression between rat and human, the drug metabolism in the intestine in these two species will surely be different. This explains why the oral bioavailability of many drugs differs in these two species.

One of the phase II drug metabolizing enzymes is UDP-glucuronsyltransferase (UDPG). Our results showed a six-fold and two-fold higher UDPG expression levels in the duodenum than that in the colon in rat and human, respectively. However, when comparing rat and human for UDPG expression, human duodenum was 12-fold higher human colon was 36-fold higher than that in rat duodenum and colon, respectively (Fig. 10C). These data are also in consistence with other reports. It has been shown that the activity of UDPG decreases along the GI tract from the duodenum to the colon of the rat intestine (82,83). These data suggest that the drug metabolism mediated by UDPG would therefore be significantly different between human and rat.

CONCLUSIONS

In summary, various drugs with different absorption mechanisms were studied for their oral bioavailability and intestinal permeability in both human and rat. No correlation was found in the bioavailability between rat and human, while a correlation was observed with $r^2 = 0.8$ between human and rat intestinal permeability of drugs with both carrier-mediated absorption and passive diffusion mechanisms. The expression of all known transporters and metabolizing enzymes were determined in both human and rat duodenum and colon. 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 expressions 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 (Fa) 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 challenge in predicting Fg and F from rat to human.

Taken together, our data indicate that rat and human show similar drug absorption profiles and similar transporter expression patterns in the small intestine, while two species exhibit distinct expression levels and patterns for metabolizing enzymes in the intestine. Therefore, rat model can be used to predict oral drug absorption (Fa) in the small intestine of human, but not to predict drug metabolism (Fg and Fh) and oral bioavailability (F) in human.

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