

Comparison of Human Duodenum and Caco-2 Gene Expression Profiles for 12,000 Gene Sequences Tags and Correlation with Permeability of 26 Drugs

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Purpose. To compare gene expression profiles and drug permeability differences in Caco-2 cell culture and human duodenum.

Methods. Gene expression profiles in Caco-2 cells and human duodenum were determined by GeneChip[®] analysis. *In vivo* drug permeability measurements were obtained through single-pass intestinal perfusion in human subjects, and correlated with *in vitro* Caco-2 transport permeability.

Results. GeneChip[®] analysis determined that 37, 47, and 44 percent of the 12,559 gene sequences were expressed in 4-day and 16-day Caco-2 cells and human duodenum, respectively. Comparing human duodenum with Caco-2 cells, more than 1000 sequences were determined to have at least a 5-fold difference in expression. There were 26, 38, and 44 percent of the 443 transporters, channels, and metabolizing enzymes detected in 4-day, 16-day Caco-2 cells, and human duodenum, respectively. More than 70 transporters and metabolizing enzymes exhibited at least a 3-fold difference. The overall coefficient of variability of the 10 human duodenal samples for all expressed sequences was 31% (range 3% to 294%) while that of the expressed transporters and metabolizing enzymes was 33% (range 3% to 87%). The *in vivo* / *in vitro* drug permeability measurements correlated well for passively absorbed drugs ($R^2 = 85\%$). The permeability correlation for carrier-mediated drugs showed 3- 35-fold higher in human above the correlation of passively absorbed drugs. The 2- 595-fold differences in gene expression levels between the Caco-2 cells and human duodenum correlated with the observed 3- 35-fold difference in permeability correlation between carrier-mediated drugs and passively absorbed drugs.

Conclusions. Significant differences in gene expression levels in Caco-2 cells and human duodenum were observed. The observed differences of gene expression levels were consistent with observed differences in carrier mediated drug permeabilities. Gene expression profiling is a valuable new tool for investigating *in vitro* and *in vivo* permeability correlation.

KEY WORDS: gene expression; transporter; GeneChip[®]; permeability; *in vivo/in vitro* correlation.

INTRODUCTION

Caco-2 cells, which are polarized epithelial cells, can form a differentiated monolayer that resembles the human intestinal epithelium. Numerous efforts have been made to use *in vitro* Caco-2 cells' permeability data to predict the fraction of drug absorbed in humans (1). However, the gene expression profiles of the Caco-2 cells and human intestine might be different. One report appeared in which correlation of the mRNA levels of 10 drug efflux transporters was performed between Caco-2 cells and human jejunum by RT-PCR (2). However, a comprehensive comparison of gene expression and an extensive *in vivo/in vitro* permeability correlation between Caco-2 cells and human intestine has not been reported.

While attempts to predict fraction drug absorbed *in vivo* using *in vitro* Caco-2 permeability data have had some success for passively absorbed drugs, the results for carrier-mediated drugs have been less successful (3-5). Lipophilic drugs, which are mainly absorbed through passive membrane diffusion, have shown good correlation between *in vitro* Caco-2 cells and *in vivo* intestinal permeability. However, hydrophilic drugs, which are absorbed by paracellular or carrier-mediated transport processes show a significantly different *in vivo/in vitro* permeability correlation. It has been suggested that many clinically used drugs including cephalexin, amoxicillin, lisinopril, enalapril, cimetidine, L-dopa, and glucose are absorbed, at least in part, through transporter-mediated absorption process (6-10).

In this study, the expression profiles of 12,559 gene sequences including 443 transporters, channels, and metabolizing enzymes were measured using GeneChip[®] analysis to determine the differences mRNA expression between Caco-2 cells and human duodenum. In addition, the *in vivo* intestinal permeabilities of 26 drugs determined in healthy human subjects are correlated with the *in vitro* Caco-2 cell permeability determined in this laboratory and from the literature (1,3,4,11-25). This report summarizes the differences that were observed in the mRNA expression profiles and in the observed permeabilities.

The expression results, far too extensive to present in this report, are also available online for the all transporters and metabolizing enzymes on the chip (http://www.sitemaker.umich.edu/amidonlab/genechip_data).

MATERIALS AND METHODS

Materials

TRIzol reagent and SuperScript Choice System for cDNA synthesis kit were purchased from Gibco BRL (Grand Island, NY, USA). BioArray high yield RNA transcript labeling kit was purchased from Enzo Biochem (New York, NY, USA). Genechips were purchased from Affymetrix (Santa Clara, CA, USA). The genechip hybridization and scanning was performed at the Genomic Information Support Facility at the Michigan State University (E. Lansing, MI, USA). Healthy male or female volunteers between 21 and 45 years of age and within 20% of their ideal body weight were

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enrolled in the study. 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). Tissue culture plates were purchased from Becton Dickinson Labware (Bedford, MA, USA). All cell culture medium and reagents were from Gibco BRL (Grand Island, NY, USA).

GeneChip[®] Analysis

Human duodenum tissues or Caco-2 cells (passage 32–40) were homogenized in TRIzol reagent (Gibco) and the RNA phase was separated by chloroform, total RNA was precipitated with isopropyl alcohol, and washed with 80% ethanol. Total RNA was further cleaned with RNeasy mini kit (Qiagen). First-strand cDNA was transcribed from total RNA using T7-(dT)₂₄ oligomer primer and SSII reverse transcriptase at 42°C. The second strand cDNA 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), then cleaned with Phase-Locking gel. Biotin-labeled cRNA synthesized from the double strand cDNA using T7 RNA polymerase-catalyzed *in vitro* transcrip-

tion in the presence of biotin-labeled NTP (BioArray high yield RNA transcription labeling kit, Enzo Biochem) then fragmented at 95°C. Biotin-labeled cRNA was heated at 99°C for 5 min in 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 non-stringent 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 and Data Mining Tool software.

Western Blot Analysis and RT-PCR

For Western blot analysis, Caco-2 cells were washed twice with phosphate-buffered saline (PBS) and collected in 0.5 ml PBS with 1 mM PMSF (phenylmethanesulfonyl fluoride) and 60 µg/ml of soybean trypsin inhibitor, and sonicated three times for 20 s. Human duodenum tissue was homogenized in PBS with 1 mM PMSF and 60 µg/ml of soybean trypsin inhibitor. The cell suspension or tissue homogenate were incubated with 2× SDS loading buffer for 30 m at room tempera-

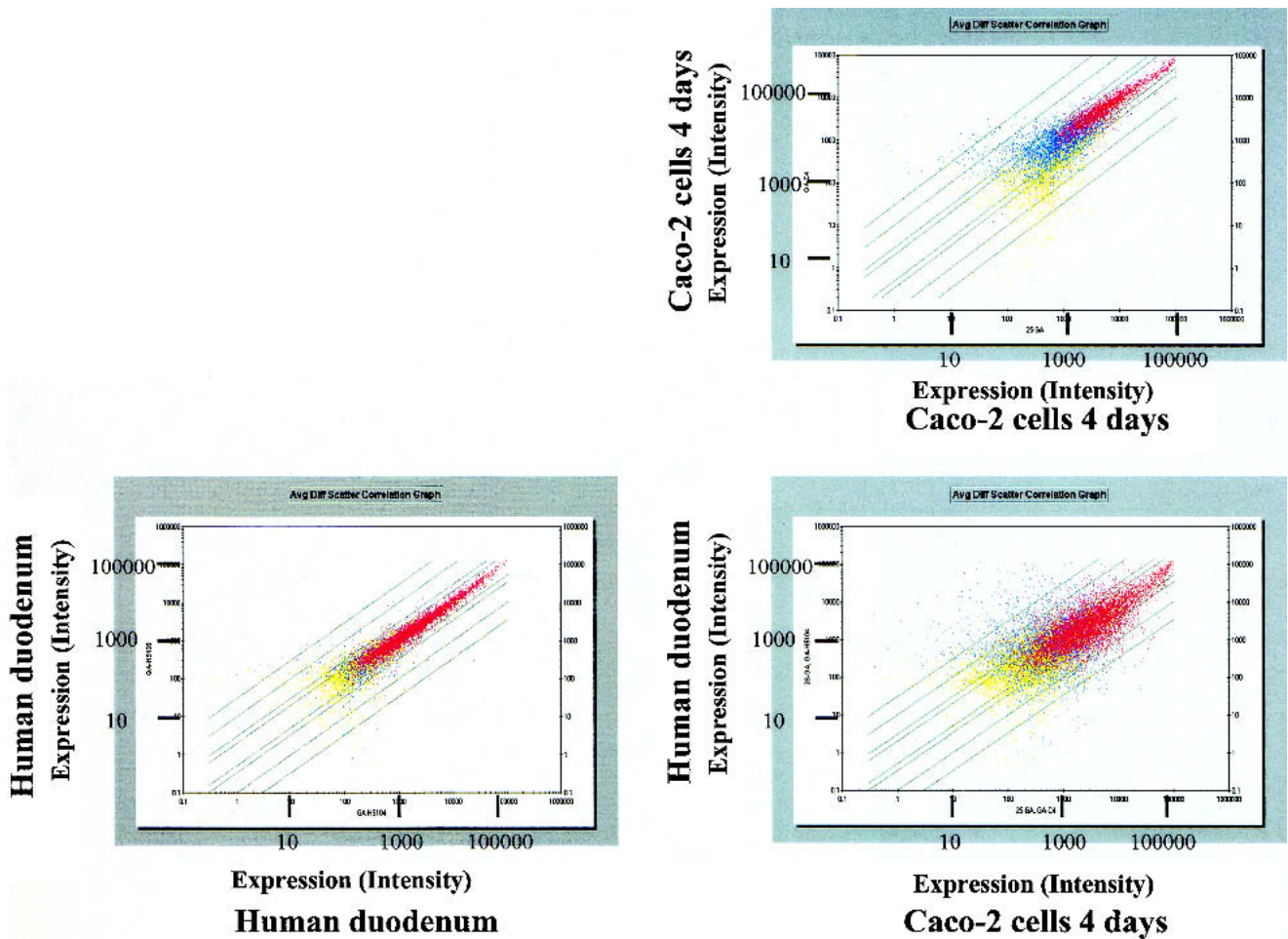


Fig. 1. Scatter analysis of the expression of 12,000 genes in human duodenum and Caco-2 cells. The X and Y axis represent the expression levels of genes by its hybridization signal density in human duodenum or Caco-2 cells. Red color indicates the genes are present in both samples. Blue color indicates the genes are present in one sample, but absent in the other. Yellow color indicates the genes are absent in both samples. Green lines on both sides of the dots indicate the genes with expression levels 2-fold, 3-fold, 5-fold, or 10-fold more in one sample than in the other, respectively.

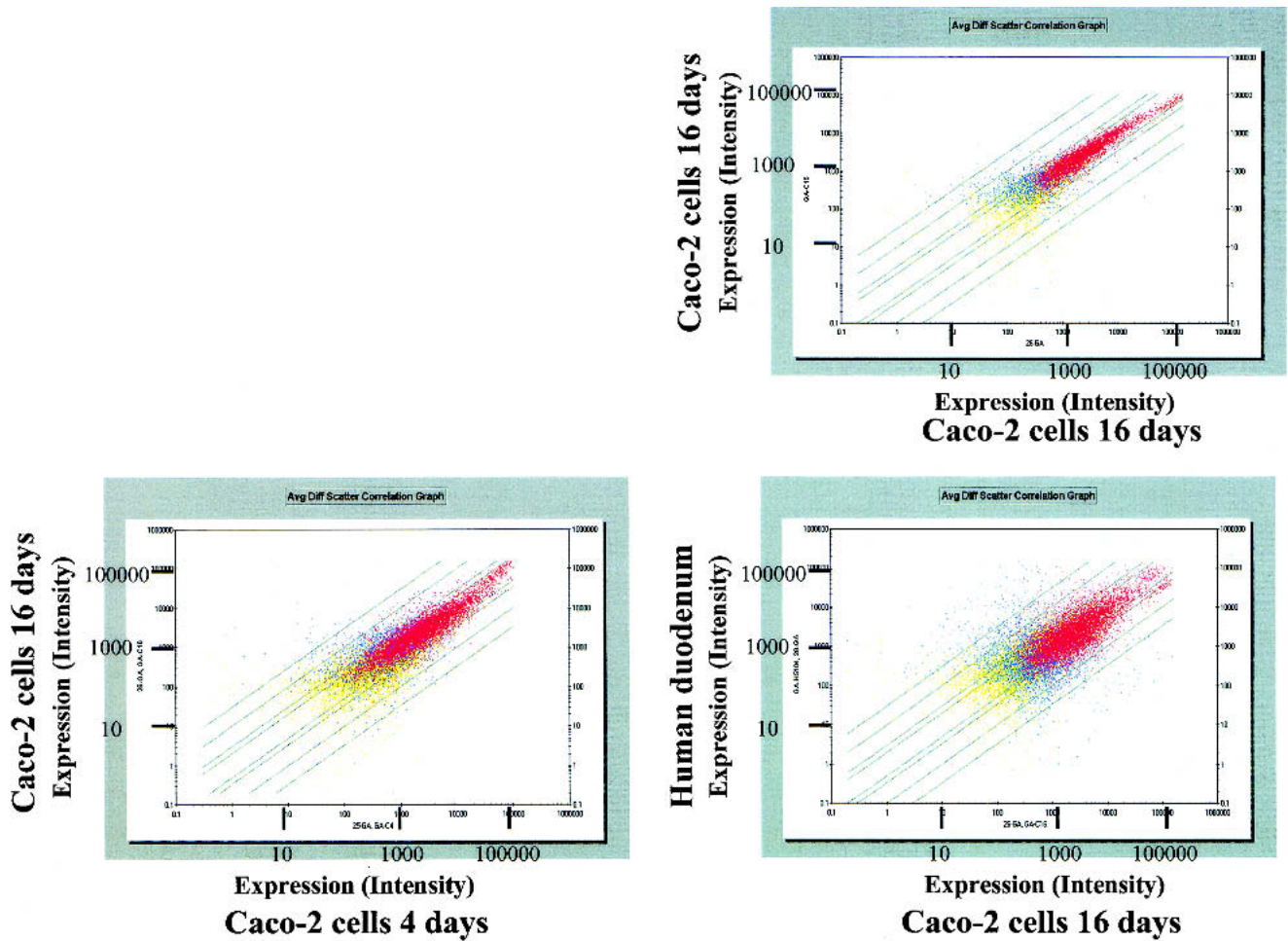


Fig. 2. Scatter analysis of the expression of 12,000 genes in human duodenum and Caco-2 cells. The X- and Y-axis represent the expression levels of genes by its hybridization signal density in human duodenum or Caco-2 cells. Red color indicates the genes are present in both samples. Blue color indicates the genes are present in one sample, but absent in the other. Yellow color indicates the genes are absent in both samples. Green lines on both sides of the dots indicate the genes with expression levels 2-fold, 3-fold, 5-fold, or 10-fold more in one sample than in the other, respectively.

ture and then 15 μg of protein was subjected to electrophoresis in 4–20% SDS-polyacrylamide gels (Bio-Rad). The protein was transferred to Hybond-P PVDF transfer membrane for 1.5 h under 250 mA. The membrane was incubated with an anti-hPepT1 polyclonal antibody (1:1000 in 2.5% milk Tris-buffered saline with 0.1% tween-20 (TBS-T), provided by Dr. Wolfgang Sadee at UCSF) at room temperature for 1 h, washed 4 times with TBS-T for 15 min then incubated with horseradish peroxidase conjugated secondary antibody (Jackson ImmunoResearch Labs, West Grove, PA, USA) for 1 h at room temperature. An enhanced chemiluminescence system ECL+Plus (Amersham) was used to detect hPepT1 expression level.

For RT-PCR, total RNA from the tissue and Caco-2 cell samples was purified using Trizol reagent (Gibco). One microgram of total RNA from each sample was subjected to RT-PCR (PCR Access system, Promega) using hPepT1 specific primers. The primers corresponded to nucleotide positions 342–360 (forward primer) and 1575–1592 (reverse primer) of the cDNA. The first strand cDNA was synthesized using AMV reverse transcriptase (AMV RT) at 48°C for 45

min. This was followed by a 2 min cycle at 94°C to inactivate AMV RT and to denature the primers and cDNA. The PCR was performed for 25 cycles of 94°C for 30 s, primer annealing for 1 min at 54°C, extension at 68°C for 2 min, and a final extension at 68°C for 7 min. The conditions were established to obtain linear amplification of PCR product. The expected hPepT1 PCR fragment was 1.2 kb. The reaction mixture was separated on a 4%–20% TBE -polyacrylamide gel (Novex) and visualized with SYBR Green nucleic acid gel stain (Molecular Probes).

Table I. The Number of Genes Expressed in Caco-2 Cells and Human Duodenum

	Number of gene sequences detected	Total number of gene sequences
Caco-2 4 days	4635 \pm 2926	12,559
Caco-2 16 days	5908 \pm 871	12,559
Human duodenum	5882 \pm 1035	12,559

Table II. The Number of Genes Expressed with More than 5-Fold Difference in Comparison of Human Duodenum and Caco-2 Cells

	Number of genes with more than 5-fold expression difference	Number of genes with more than 5-fold expression increase	Number of genes with more than 5-fold expression decrease
Caco-2 16 days vs. Caco-2 4 days	239 ± 106	155 ± 0.7	83 ± 103
Human duodenum vs. Caco-2 4 days	1112 ± 449	614 ± 176	460 ± 330
Human duodenum vs. Caco-2 16 days	925 ± 147	516 ± 119	408 ± 39

Clinical Human Intestinal *in Situ* Perfusion Study

Human subjects were fasted for approximately 7 h, and then underwent esophagogastroduodenoscopy (EGD). Ten biopsy samples were taken from the duodenum with endoscopy guidance. Subsequently, a 6-lumen perfusion tube (Loc-I-Gut®), which contained 2 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 non-absorbed compound, was used to correct for water secretion or absorption in the perfused segment. Total of 26 drugs were tested, which included cephalexin, hydrochlorothiazide, furosemide, atenolol, cimetidine, amoxicillin, lisinopril, losartan, metoprolol, propranolol, desipramine, naproxen, ketoprofen, *D*-glucose, proxicam, ranitidine, verapamil, cyclosporin, enalapril, phenylalanine, antipyrine, terbutaline, *L*-dopa, *L*-leucine, creatine, and manitol (26–35). The permeability was calculated using the following equation: $P_{eff} = Q (1 - C_{out}/C_{in}) / 2\pi RL$. Where P_{eff} is effective permeability, Q is perfusion rate (ml/min), C_{out} and C_{in} 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).

Table III. The Number of the Transporters, Channels and Metabolizing Enzymes Expressed in Caco-2 Cells and Human Duodenum

	Caco-2 cells, 4 days	Caco-2 cells, 16 days	Human duodenum	Total genes
peptide transporters	2	2	3	4
Na ⁺ /H ⁺ exchangers	2	3	3	7
Na ⁺ /K ⁺ ATPase	2	4	3	7
Amino acid transporters	7	8	8	25
Nucleoside transporters	2	3	3	5
Organic cation transporters	1	2	2	7
Organic anion transporters	4	5	11	17
Glucose transporters	5	7	6	11
Ion exchangers	1	2	1	6
Phosphate transporters	2	3	2	6
P-gp	2	2	2	5
MRP	4	3	4	7
Other ABC transporters	0	0	0	3
Fatty acid transporters	1	1	2	3
Bile acid and bile salt transporters	1	0	0	3
Amine transporters	1	1	1	7
Citrate transporters	2	2	2	2
Mitochondria solute carrier	2	4	3	6
Ubiquitin carriers	3	3	3	3
Other transporters	7	12	11	19
Proton pumps and H ⁺ ATPase	7	8	9	10
Other ion ATPase	6	7	7	15
Ion transporters	1	1	1	5
Channels	8	17	20	111
Junction proteins	3	3	4	7
Villin	1	3	3	4
Mucin	2	3	6	19
Alkaline phosphatase	0	0	2	3
Esterase and other related	7	9	8	15
Cytochrome P-450	6	11	25	44
Phase II metabolism enzymes	25	37	39	57
Total	117	166	194	443

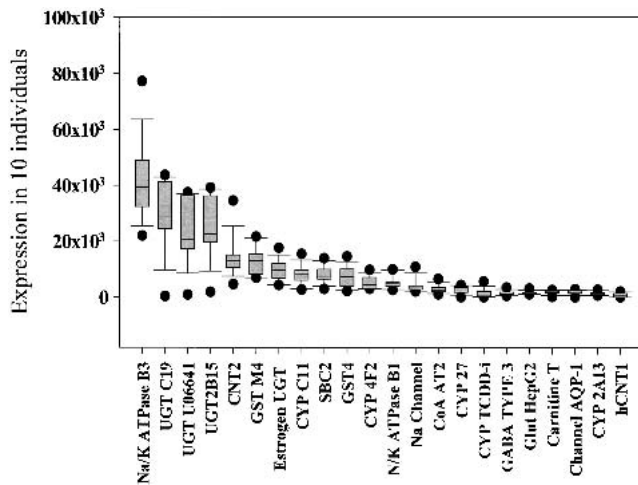


Fig. 3. Box Whisker plot of the expression levels of transporters and metabolizing enzymes with CV more than 50% in 10 human individuals. Shaded box indicated 25–75% of expression range, and error bar indicated 10–90% of expression range. UGT, UDT-glucuronosyltransferase; CNT2, Na/purine transporter (CNT2); GST, Glutathione S-transferase; CYP, Cytochrome P450; SBC, Sodium/bicarbonate transporter. CoA-AT2, CoA acetyltransferase 2; GLUT, Glucose transporter; GABA type 3, GABA transporter type 3; Carnitine T, Carnitine transporter; hCNT1, human nucleoside transporter 1.

Uptake Permeability of Cephalexin in Caco-2 Cells

Caco-2 cells were grown at 37°C, 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% nonessential amino acids, 1 mM sodium pyruvate, 1% L-glutamine. Caco-2 cells were seeded into 6-well culture plates at the density of 10⁴ cells/cm². Three days after seeding, cells were washed twice with 2 ml of pH 6.5 uptake buffer containing 1 mM CaCl₂, 1 mM MgCl₂, 150 mM NaCl, 3 mM KCl, 1 mM NaH₂PO₄, 5 mM D-glucose and 5 mM MES. Then 1 ml uptake buffer with 0.1 mM cephalexin was added in each well and agitated on a plate shaker at 37°C. At different time point (10–120 min), cells were washed 3 times with ice-cold pH 6.5 uptake buffer, and 1 ml water was added into each well. Cells were collected in an eppendorf tube, sonicated for 5 min and protein was precipitated with 4% trifluoroacetic acid (TFA). After centrifugation at 3000 rpm for 5 min, the supernatant was filtered using a membrane filter (0.45 μm). The cephalexin concentration was measured by reverse-phase HPLC assay (SUPELCOSIL LC-8-DB column, 5C18, 4.6 × 250 mm, SUPELCO) at 250 nm. Protein concentrations before adding TFA were determined using Bio-Rad protein dye reagent (Bio-Rad, Hercules, CA). The cephalexin uptake permeability was calculated by the following equation: $P_{eff} = (dQ/dt) / A C_o Pt$. Where dQ/dt is the uptake rate (mmol/min), A is surface area of the well, C_o is initial drug concentration in each well, and Pt is the amount of cell protein in each well.

RESULTS

Summary of Expression for 12,559 Sequences in Caco-2 Cells and Human Duodenum

The scatter plots for the 12,559 sequences are showed in Fig. 1 and Fig. 2. Significantly greater scatter is observed when

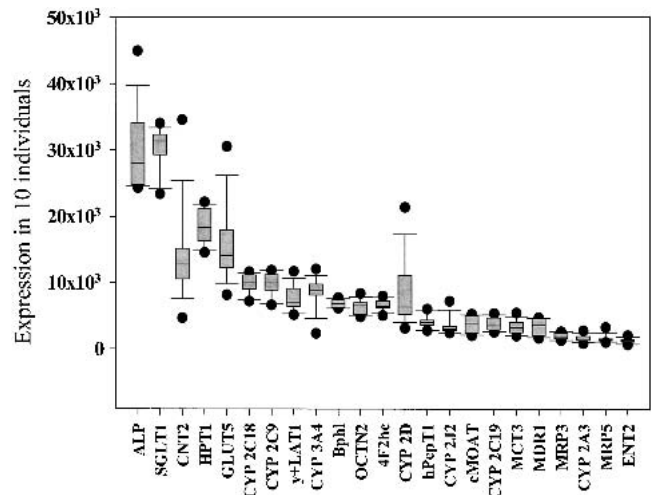


Fig. 4. Box Whisker plot of the expression levels of partial common transporters and metabolizing enzymes with CV less than 50% in 10 human individuals. Shaded box indicated 25–75% of expression range, and error bar indicated 10–90% of expression range. ALP, Alkaline phosphatase; SGLT1 Na/glucose transporter (SGLT1); CNT2, Na/purine transporter (CNT2); HPT1, human peptide transporter 1 (Cadherin family); NADC1, Na/dicarboxylate cotransporter 1; y+LAT1, Amino acid transporter (y+LAT1); BPHL, Biphenyl hydroxylase like protein; OCTN2, organic cation transporter (OCTN2); GLUT5, Sodium-independent Glucose transporter 5; 4F2hc, Amino acid transporter (4F2hc); MRP, multi-drug resistant associated protein; CYP, Cytochrome P450; hPepT1, human di-, tri-peptide transporter 1; cMOAT (ABCC2), organic anion transporter (cMOAT); MCT3, monocarboxylate transporter 3; MDR1, multidrug resistance protein; ENT2, nucleoside transporter (ENT2).

comparing the expression profiles for human duodenum vs. Caco-2 cells than when comparing human-human or Caco-2-Caco2 expression.

For the 12,559 sequences analyzed, there were 4635 ± 2926 , 5908 ± 871 , and 5882 ± 1035 genes determined to have significant expression in Caco-2 cells (4 days), Caco-2 cells (16 days), and human duodenum respectively. While similar numbers of sequences were detected in Caco-2 cells and human duodenum, more than 1000 (17%) of the gene sequences were determined to have at least a 5-fold difference when comparing human duodenum with Caco-2 cells (Table I, II).

Expression of Transporters, Channels, and Metabolizing Enzymes

The expression of 443 transporters, channels, and metabolizing enzymes was measured (Table III), of which, 117, 166, and 194 were detected in Caco-2 (4 days), Caco-2 (16 days), and human duodenum respectively. When comparing human duodenum with Caco-2 cells, more than 70 transporters, channels, and metabolizing enzymes were determined to be at least 3-fold different in expression, while only 28 were seen with at least a 3-fold difference when comparing Caco-2 cells (16 days) with Caco-2 cells (4 days).

The Variability of Gene Expression in Human Duodenum

Although some genes exhibited high expression variability with a CV greater than 50% and difference (maximum/

minimum) greater than 5 fold (Fig. 3), the majority of the 443 transporters, channels, and metabolizing enzymes showed relatively low expression variability among the 10 duodenal samples from normal human subjects. The average of CV (%) for expression is $33 \pm 20.22\%$ (range 3.30% to 86.88%). The overall coefficient of variability for all expressed sequences was $31.02 \pm 19.92\%$ (range 3.30% to 294.27%). Several metabolizing enzymes including CYP TCDD inducible, CYP c11, CYP X02612, CYP 2A13, CYP 27, CYP 5, UGT U06641, UGT 2B, and UGT 2B15 showed a larger variability with more than 3.2–45-fold differences among 10 individuals. Several transporters including GABA transporter type 3, glucose transporter HepG2, Na/purine transporter (CNT2), carnitine transporter, and Na/bicarbonate cotransporter2 (SBC2) showed a high variability (CV more than 50%) with 3- to 14-fold difference (Fig. 3). Figure 4 shows the variability of the common transporters and metabolizing enzymes with less than 50% CV in 10 human duodenal samples. The gene expression variabilities in duodenum using GeneChip[®] analysis are in agreement with the report for the variabilities of 10 drug efflux proteins in jejunum using RT-PCR (2).

In Vivo/in Vitro Drug Permeability Correlation

The *in vivo* intestinal permeability measurements of 26 drugs were obtained at the University of Michigan (Ann Arbor, MI, USA) and the University of Uppsala (Sweden). The *in vivo* permeability measurements were correlated with *in vitro* Caco-2 cell transport permeabilities obtained from literature at both pH 6.5 and pH 7.4 (1,3,4,11–25) (Fig. 5, Fig. 6). The correlation coefficient (r^2) of permeability of all 24 drugs determined at pH 7.4 was 0.5126 ($\log P_{eff, human} = 0.4926 \times \log P_{eff, Caco-2} - 0.1454$), while the permeability correlation coefficient (r^2) of the 20 drugs assayed at pH 6.5 was 0.7276 ($\log P_{eff, human} = 0.6532 \times \log P_{eff, Caco-2} - 0.3036$).

The *in vivo/in vitro* permeability correlation of the drugs including cephalexin, enalapril, lisinopril, losartan, amoxicillin, phenylalanine, L-leucine, L-dopa, D-glucose, cyclosporin, and verapamil, which are transporter substrates deviated from the trend of linear correlation at both pHs (Fig. 5, 6). Removing the carrier-mediated absorbed drugs from the *in vivo/in vitro* permeability correlation improves the correlation at both pHs. The correlation coefficient (r^2) for the 15

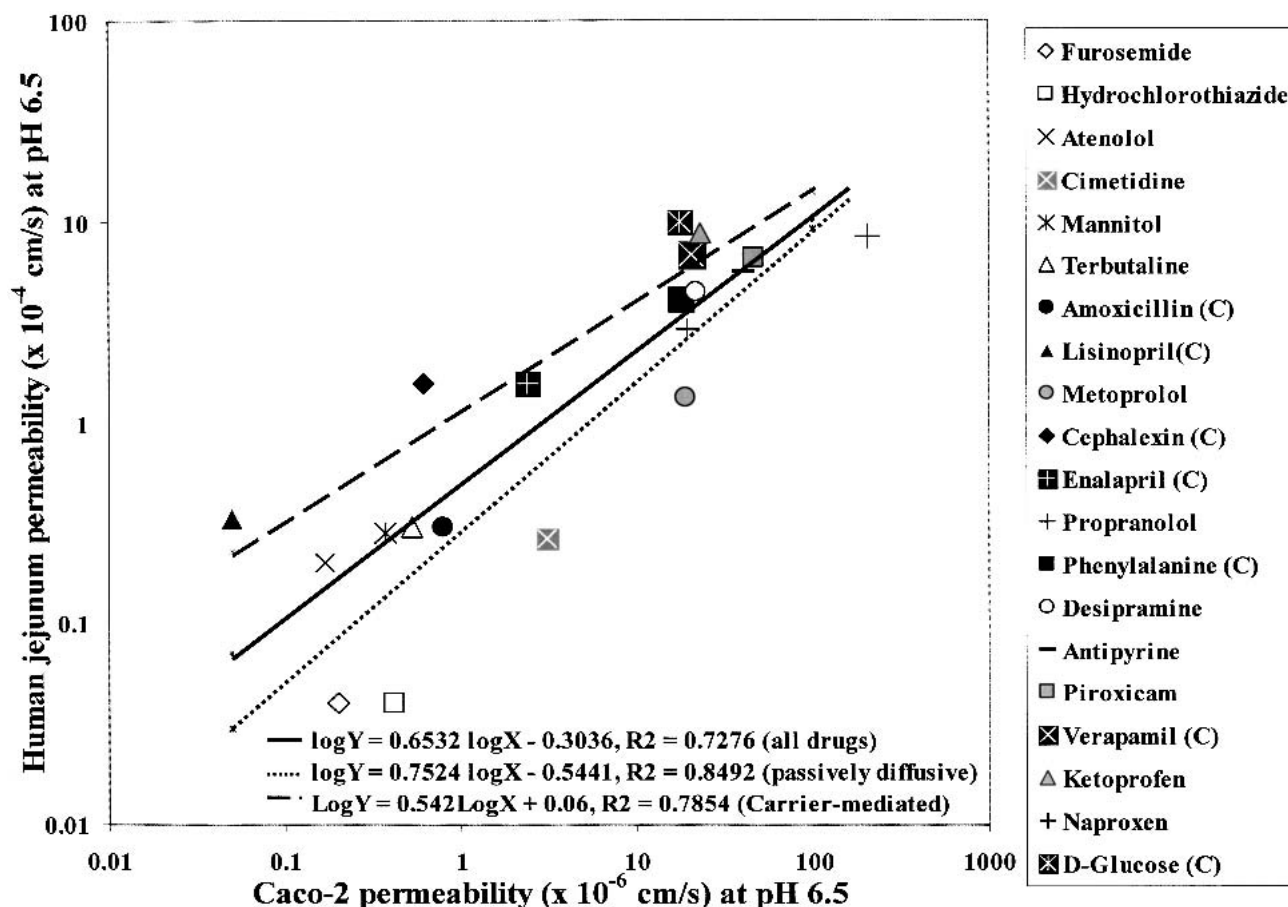


Fig. 5. *In vivo/in vitro* permeability correlation of 20 drugs at pH 6.5. Human jejunum permeability was obtained through a single-pass *in situ* intestinal perfusion study at the University of Michigan (USA) and Uppsala University (Sweden). *In vitro* Caco-2 cell permeability was obtained from the literature (1–3,10–24). Correlation coefficient ($r^2 = 0.7276$) was calculated from the permeability correlation of all 20 drugs. Correlation coefficient ($r^2 = 0.8492$) was calculated from the permeability correlation of the following drugs: furosemide, hydrochlorothiazide, atenolol, cimetidine, mannitol, terbutaline, metoprolol, propranolol, desipramine, antipyrine, piroxicam, ketoprofen, and naproxen. Correlation coefficient ($r^2 = 0.7854$) was calculated from the permeability correlation of the following drugs: cephalexin, enalapril, lisinopril, losartan, amoxicillin, phenylalanine, L-leucine, L-dopa, D-glucose, cyclosporin, and verapamil. Drugs are labeled with different symbols. Black symbols are carrier-mediated absorbed drugs, while gray and open symbols are passively absorbed drugs.

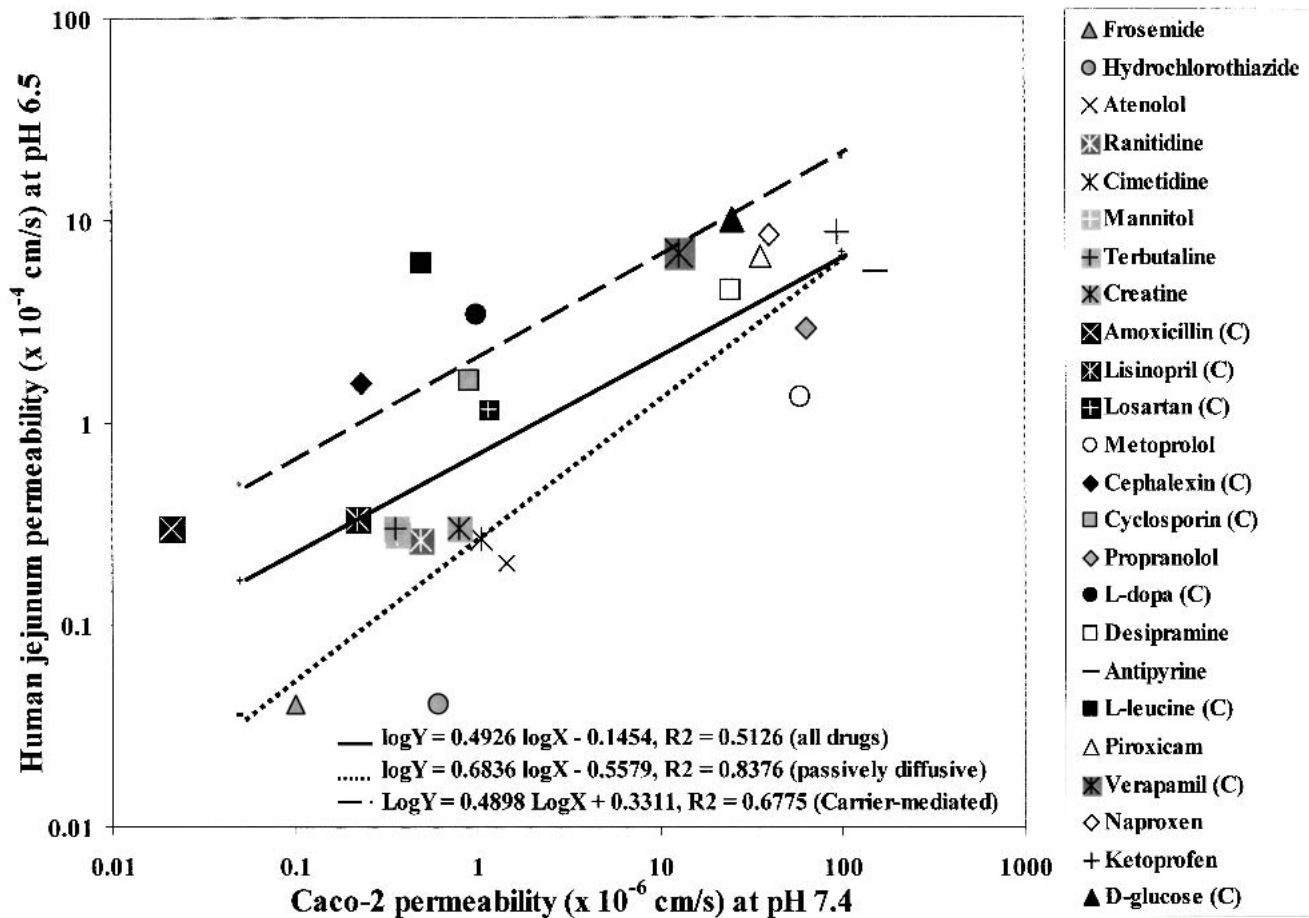


Fig. 6. *In vivo/in vitro* permeability correlation of 24 drugs at pH 7.4. Human jejunum permeability was obtained through a single-pass *in situ* intestinal perfusion study at the University of Michigan (USA) and Uppsala University (Sweden). *In vitro* Caco-2 cell permeability was obtained from the literature (1–3,10–24). Correlation coefficient ($r^2 = 0.5126$) was calculated from the permeability correlation of all 24 drugs. Correlation coefficient ($r^2 = 0.8376$) was calculated from the permeability correlation of the following drugs: furosemide, hydrochlorothiazide, atenolol, ranitidine, cimetidine, mannitol, terbutaline, creatine, metoprolol, propranolol, desipramine, antipyrine, piroxicam, ketoprofen, and naproxen. Correlation coefficient ($r^2 = 0.6775$) was calculated from the permeability correlation of the following drugs: cephalexin, enalapril, lisinopril, losartan, amoxicillin, phenylalanine, L-leucine, L-dopa, D-glucose, cyclosporin, and verapamil. Drugs are labeled with different symbols. Black symbols are carrier-mediated absorbed drugs, while gray and open symbols are passively absorbed drugs.

passively absorbed drugs at pH 7.4 and 13 passively absorbed drugs at pH 6.5 were 0.8376 ($\log P_{eff, human} = 0.6836 \times \log P_{eff, Caco-2} - 0.5579$) and 0.8492 ($\log P_{eff, human} = 0.7524 \times \log P_{eff, Caco-2} - 0.5441$) respectively. The *in vivo/in vitro* permeability for only those carrier-mediated absorbed drugs also showed reasonable correlation with a correlation coefficient (r^2) of 0.6775 ($\log P_{eff, human} = 0.4898 \times \log P_{eff, Caco-2} + 0.3311$) at pH 7.4 and 0.7854 ($\log P_{eff, human} = 0.542 \times \log P_{eff, Caco-2} + 0.06$) at pH 6.5, respectively. However, compared to the correlation of passively absorbed drugs, the permeability of the carrier-mediated absorbed drugs are with an average of 11-fold (3- to 35-fold) higher in human than Caco-2 cells at pH 7.4, and with an average of 4-fold higher in human than Caco-2 cells at pH 6.5.

Prediction of Fraction of Drug Absorbed with Human Permeability and Caco-2 Permeability

Prediction of human drug fraction is shown in Fig. 7, 8, 9, using human jejunal permeability (pH 6.5) and Caco-2 permeability at pH 6.5 and pH 7.4. A reasonable prediction can be made from human jejunum permeability. However, the

prediction of fraction absorbed for the carrier-mediated drugs with low Caco-2 permeability and high fraction absorbed is poor.

DISCUSSION

The Expression of Di- and Tri-Peptide Transporters and Comparison with Substrate Permeability

Four di- and tri-peptide transporters were determined (Fig 10). The peptide transporter (hPepT1) expression was very low in Caco-2 cells, but its expression in human duodenum was 15-fold higher. Peptide transporter (HPT1), which is related to the cadherin superfamily (36), was detected in high levels in both Caco-2 cells and human duodenum. The hPepT2 expression was undetectable in both Caco-2 cells and human duodenum. The pH sensitive regulatory factor of peptide transporter was also very low in both Caco-2 cells and human duodenum tissue.

Even though drug permeability showed a better *in vivo/in vitro* correlation at pH 6.5 than that at pH 7.4, the cephalexin, amoxicillin, enalapril, and lisinopril permeability in hu-

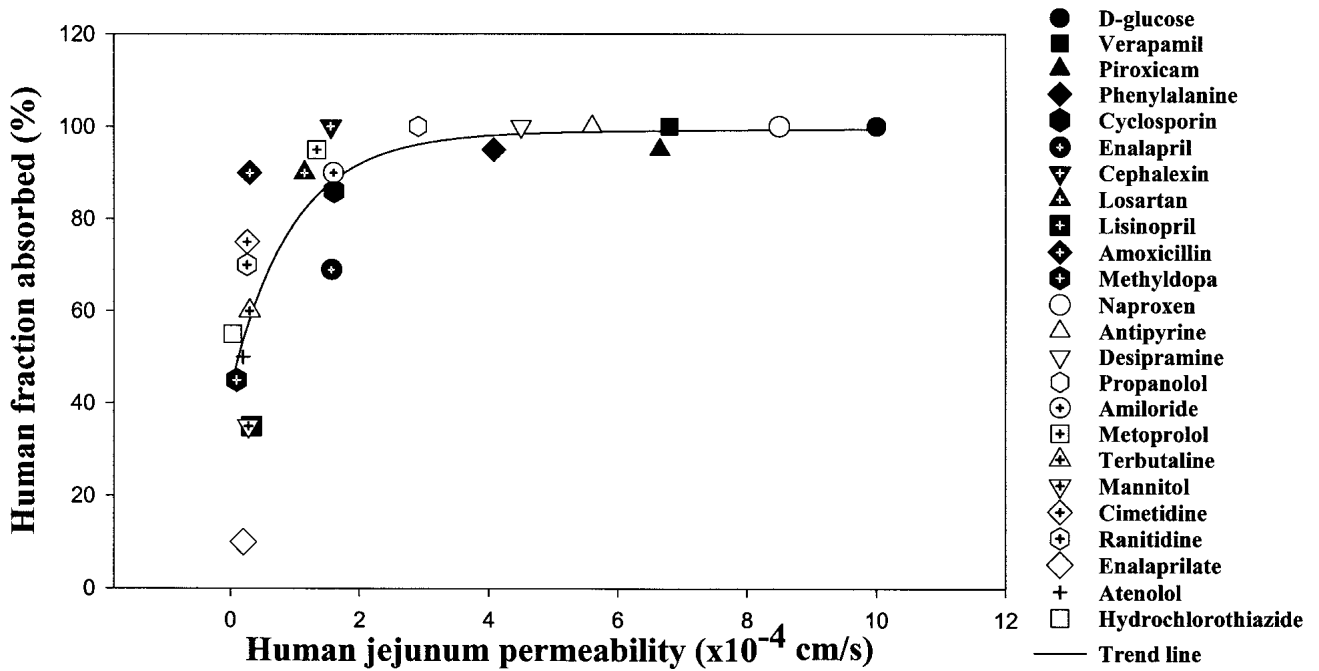


Fig. 7. Prediction of drug fraction absorbed using human jejunum permeability. Drugs are labeled with different symbols. Closed symbols are carrier-mediated absorbed drugs, while open symbols are passively absorbed drugs.

man deviated above the trend of linear correlation (Figs. 5 and 6). If their permeabilities were normalized by propranolol permeability in both human jejunum and Caco-2 cells, respectively, the human permeability ratios (drug permeability/propranolol permeability) of cephalixin, amoxicillin, enalapril, and lisinopril are always significantly higher (2.5- to 148-fold higher) than Caco-2 permeability ratios (Fig. 10). It has been suggested that oral absorption of those drugs is mediated by hPepT1 transporter (6–8). The expression results suggest that low hPepT1 expression is one of the main causes for the low permeability in Caco-2 cells.

The expression levels of hPepT1 in Caco-2 cells and human duodenum were also determined by Western blot using anti-hPepT1 antibody and RT-PCR analysis. There were undetectable levels of hPepT1 protein in Caco-2 cells (4 days and 16 days), while human duodenum showed much higher (8-fold) hPepT1 protein level than Caco-2 cells measured by western blot analysis (Fig. 11A). However, the mRNA levels of hPepT1 in Caco-2 cells were detected in Caco-2 cells (4 days and 16 days) through RT-PCR analysis, even though its expression in Caco-2 cells was much lower (2.5-fold) than in human duodenum (Fig. 11B). These hPepT1 expression pro-

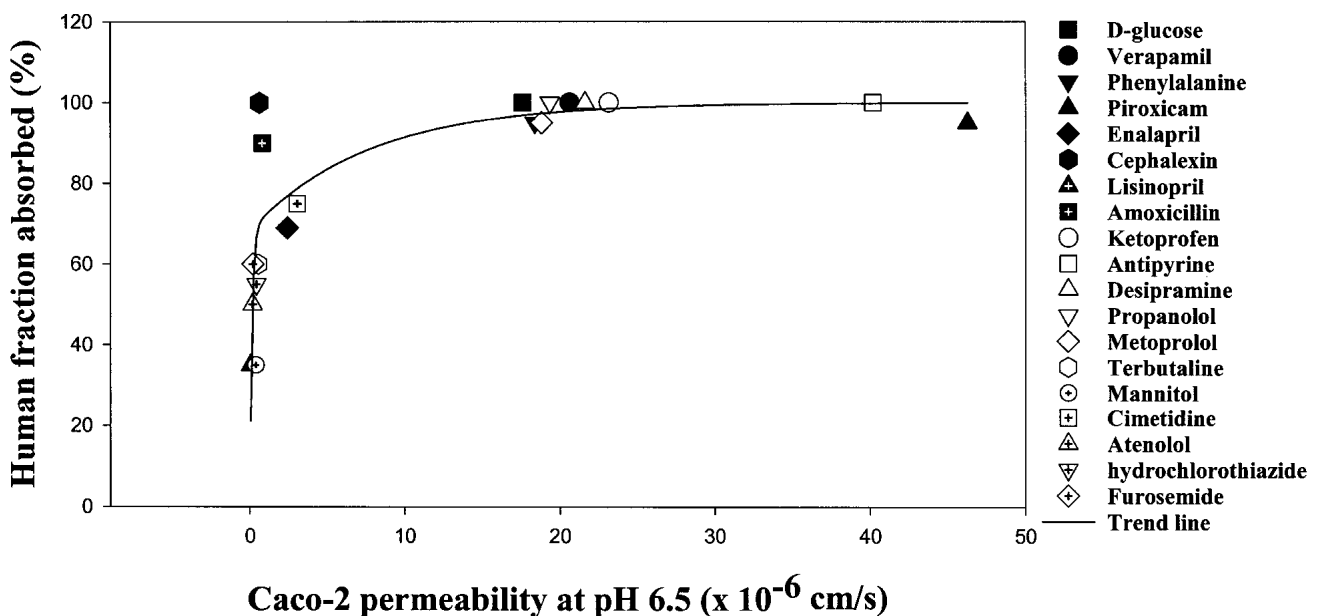


Fig. 8. Prediction of drug fraction absorbed using Caco-2 permeability at pH 6.5. Drugs are labeled with different symbols. Closed symbols are carrier-mediated absorbed drugs, while open symbols are passively absorbed drugs.

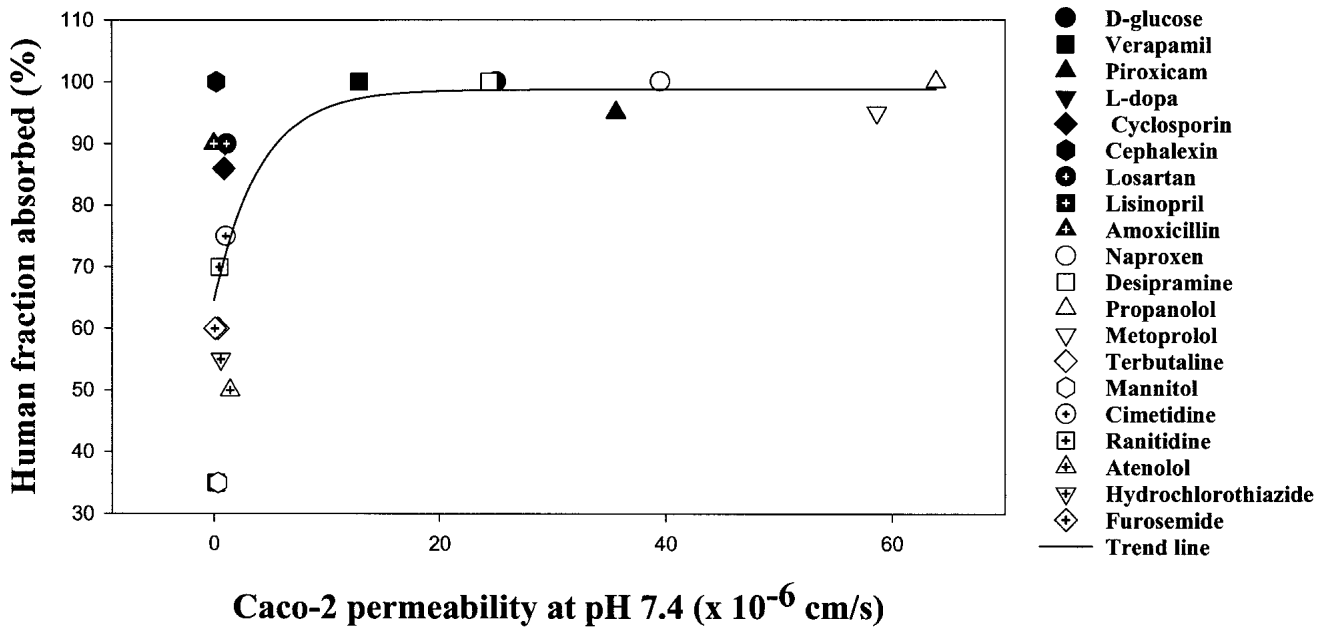


Fig. 9. Prediction of drug fraction absorbed using Caco-2 permeability at pH 7.4. Drugs are labeled with different symbols. Closed symbols are carrier-mediated absorbed drugs, while open symbols are passively absorbed drugs.

files obtained by Western blot and RT-PCR analysis were similar to the results obtained by the GeneChip[®] assay. Figure 11C showed the comparison of the three different methods for the detection of hPepT1 expression. The GeneChip[®] showed a 15-fold difference in hPepT1 expression in human duodenum compared to Caco-2 cells (4 days), while RT-PCR and Western blot analysis showed 2.5-fold and 8-fold differences respectively.

The Expression of Amino Acid Transporters and Comparison with Substrate Permeability

GeneChip[®] analysis showed that seven out of twenty-five amino acid transporters were detected in both Caco-2 cells and human duodenum (Fig. 12). Both glycoprotein associated amino acid transporter (y+LAT1) and membrane glycoprotein 4F2 antigen heavy chain (4F2hc) were expressed higher (2.5–4-fold) in Caco-2 cells than in human duodenum. These two transporters were reported to form a heterodimer for a functional complex to transport cationic and neutral amino acids (10). Caco-2 cells expressed neutral amino acid transporter B (ATB⁰) at more than 12-fold higher levels than that in human duodenum. Proline transporter (hPROT) expression was relatively abundant in both Caco-2 cells and human duodenum. However, GABA/noradrenaline transporter was detected at a more than 20-fold higher level in human duodenum than Caco-2 cells.

L-dopa is thought to be absorbed by amino acid transporters (10). Phenylalanine and leucine are also the substrates for amino acid transporters. However, their permeability deviated above the linear trend in human intestine (Fig. 6). Their human normalized permeability ratio (drug permeability/propranolol permeability) is 1.5- to 273-fold higher than normalized Caco-2 permeability ratio (Fig. 12), even though the expression of all the amino acid transporters (on the GeneChip[®]) were higher in Caco-2 cells compared to human duodenum, with the exception of GABA transporter. It is

unlikely that L-dopa is absorbed by the GABA transporter and other factors, such as the involvement of transporters not on the chip or as yet unidentified may play a role in L-dopa transport. Further, amino acid transporters usually have broad substrate specificity, in which one amino acid might be transported by several different amino acid transporters complicating any correlation analysis.

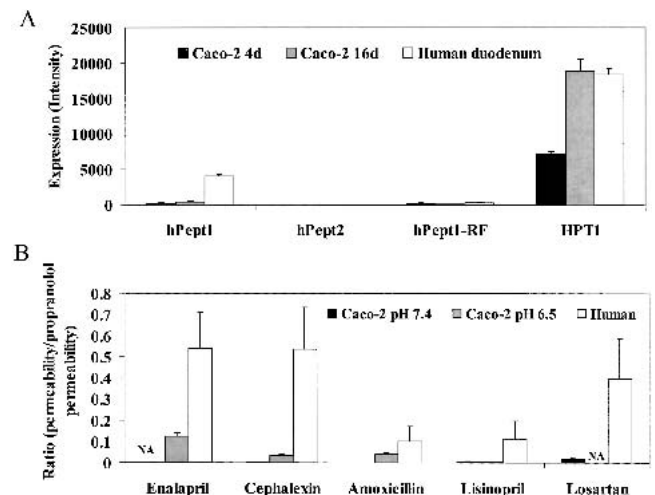


Fig. 10. (A). Expression of di- and tri-peptide transporters in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal. hPepT1, human proton/peptide transporter 1; hPepT2, human proton/peptide transporter 2; hPepT1-RF, pH-sensitive regulatory factor of peptide transporter; HPT1, peptide transporter (LI-cadherin). (B). Normalized drug permeability ratio in Caco-2 cells (pH 7.4, black bar), Caco-2 cells (pH 6.5, gray bar), and human jejunum (blank bar). Normalized permeability ratio was calculated by drug permeability/propranolol permeability in human and Caco-2 cells, respectively. NA, data not available.

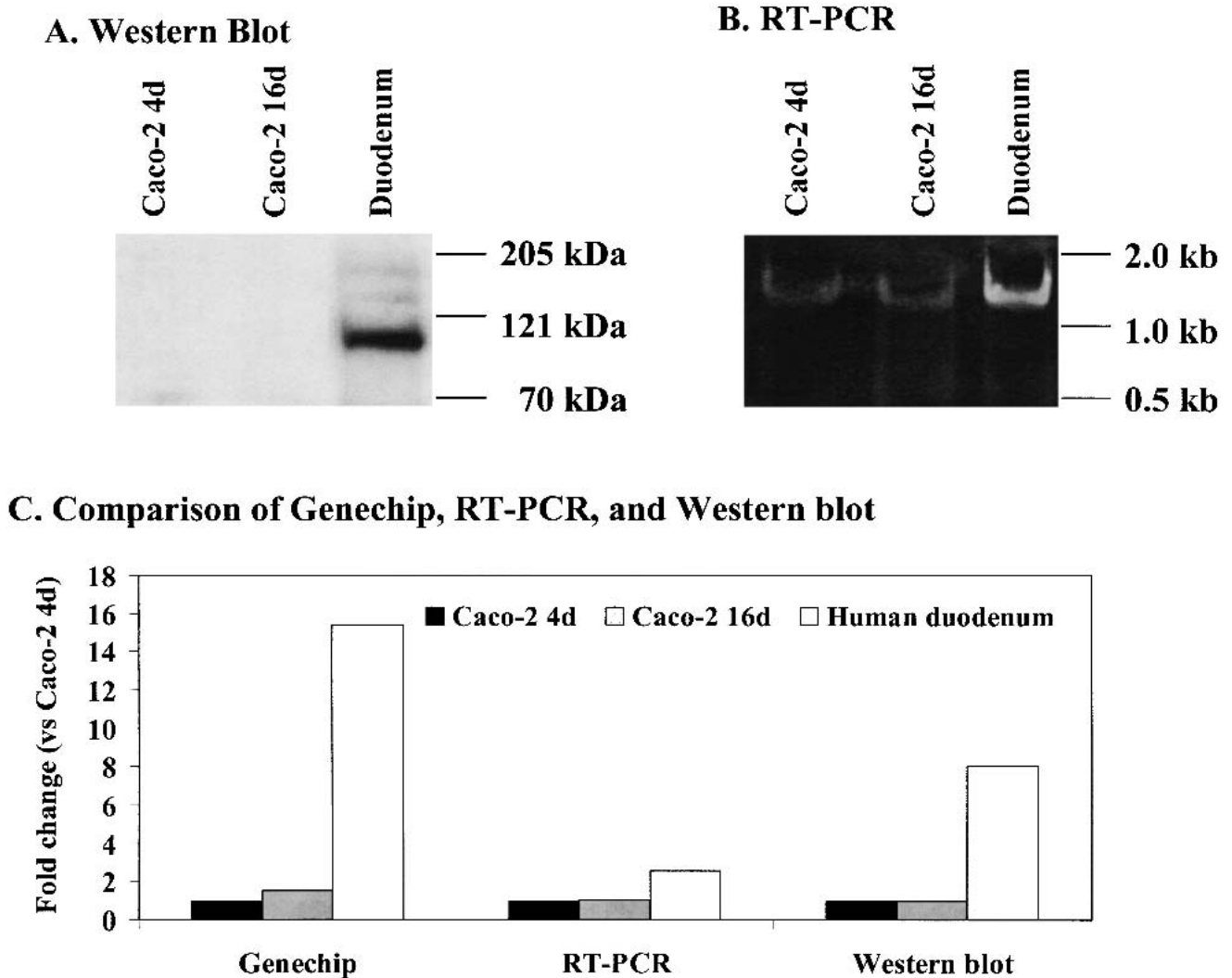


Fig. 11. Expression of hPepT1 in Caco-2 cells and human duodenum by Western blot analysis and RT-PCR. (A). Western blot analysis of hPepT1. hPepT1 (~100 kDa) was detected only in human duodenum by rabbit anti-human hPepT1 antibody. (B). RT-PCR amplify 1.2 kb hPepT1 fragment from Caco-2 cells and human duodenum. (C). Comparison of Genechip, Western blot and RT-PCR analysis for hPepT1 expression. The fold change was calculated by the intensity of the band/intensity of the band in Caco-2 cells (4 days). The intensity of genechip analysis was obtained by the fluorescence of hybridization signal, and intensity of the band in Western blot and RT-PCR was obtained using Metaphor software.

The Expression of Organic Cation and Organic Anion Transporters and Cimetidine Permeability

Only two organic cation transporters (OCTN1 and OCTN2) out of seven organic cation transporters were detected in human duodenum and Caco-2 cells (Fig. 13). Both were expressed at higher levels in the human duodenum than in Caco-2 cells. Human duodenum tissue had 2.8-fold higher OCTN1 expression and 3-fold higher OCTN2 expression than in Caco-2 cells (Fig. 13).

Nine out of seventeen organic anion transporters were detected in human duodenum and Caco-2 cells. Three monocarboxylate transporters (MCT U59185, MCT U59299, and MCT3) and two multispecific organic anion transporters (ABC transporter: MOAT-C (ABCC5) and cMOAT (ABCC2)) were expressed at similar levels in both human duodenum and Caco-2 cells. However, human duodenum ex-

pressed very high levels of sodium/dicarboxylate transporter (NADCL, 7-fold higher), sodium/bicarbonate cotransporters (SBC) (78-fold higher SBC2, 7-fold higher pancreas SBC, 19-fold higher HNBC1) compared to Caco-2 cells (Fig. 13).

It has been reported that cimetidine might be a substrate of the organic cation transporter (9). Yet, its permeability showed reasonable *in vivo/in vitro* correlation even though the human intestine expressed higher levels of organic cation transporters (Fig. 5, 6). If cimetidine was only absorbed by organic cation transporters, then we would expect that cimetidine would have higher permeability in the correlation curve in human intestine than Caco-2 cells due to the differences in expression levels of the organic transporters. These data suggest that the absorption of cimetidine *in vivo* is not exclusively mediated by these two organic cation transporters (OCNT1 and OCNT2).

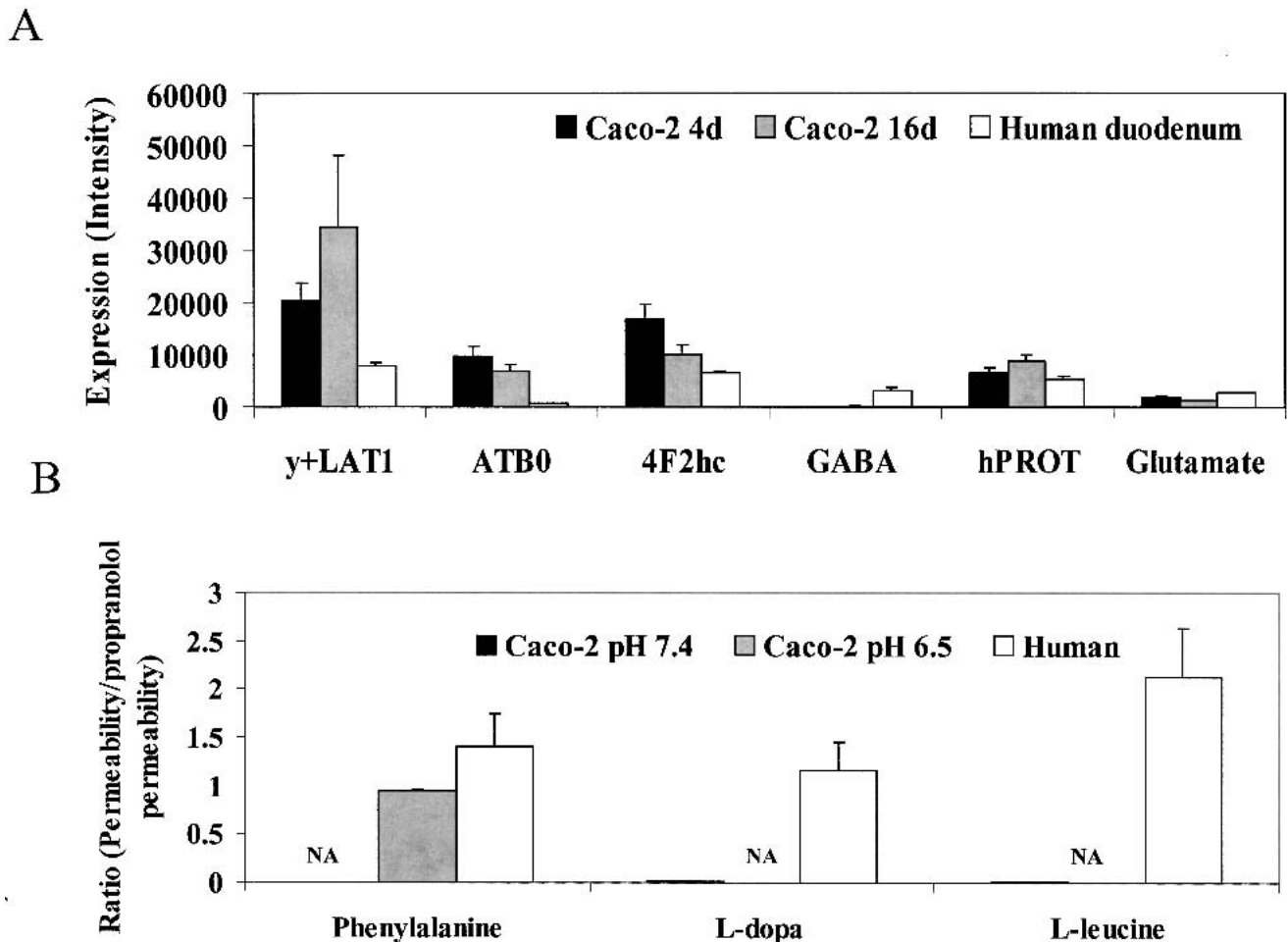


Fig. 12. (A). Expression of amino acid transporters in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal. y+LAT1, glycoprotein associated amino acid transporter; ATB⁰, neutral amino acid transporter B; 4F2hc, membrane glycoprotein 4F2 antigen heavy chain; GABA, GABA/noradrenaline transporter; hPROT, proline transporter; Glutamate, glutamate transporter (accession number: U08989). (B). Normalized drug permeability ratio in Caco-2 cells (pH 7.4, black bar), Caco-2 cells (pH 6.5, gray bar), and human jejunum (blank bar). Normalized permeability ratio was calculated by drug permeability/propranolol permeability in human and Caco-2 cells, respectively. NA, data not available.

The Expression of Glucose Transporters and Glucose Permeability

Five out of eleven glucose transporters were detected in both Caco-2 cells and human duodenum (Fig. 14). There were undetectable levels of the apical membrane localized sodium-dependent glucose transporter (SGLT1) and apical membrane localized sodium-independent glucose transporter (GLUT5) in Caco-2 cells, while human duodenum expressed more than 600-fold higher SGLT1 levels and 20-fold higher GLUT5 than in Caco-2 cells. However, sodium-independent glucose transporter (GLUT3), which is usually expressed in neuron cells in human (37), was expressed more than 150-fold higher in Caco-2 cells (4 days) than in human duodenum. Similar levels of glucose transporter (HepG2) and UDP-galactose transporter were detected in both human duodenum and Caco-2 cells (Fig. 14). These results suggested that Caco-2 cells are using a different mechanism for glucose uptake than human duodenum.

Intestinal glucose absorption is mediated by glucose transporters (SGLT1, GLUT5, and GLUT2). Its *in vivo* intestinal permeability is the highest among the 26 drugs we

tested, but its permeability in human was deviated above the trend of linear correlation (Figs. 5 and 6). The human normalized glucose permeability ratio (glucose permeability/propranolol permeability) is 3- to 8-fold higher than Caco-2 permeability ratio (Fig. 14). This might be due to the fact that human intestine expresses different types of glucose transporters thereby using a different or multiple glucose uptake pathways.

MDR, MRP Expression, and Cyclosporin Verapamil Permeability

Five multidrug resistance genes (*mdr*) and seven multidrug resistance associated proteins (MRP) were measured by GeneChip[®] analysis, six were detected in Caco-2 cells and human duodenum (Fig. 15). Human duodenum and Caco-2 cells (4 days) expressed similar levels of multiple drug resistance protein (p-glycoprotein, p-gp; multidrug resistance gene, *mdr*), however, human duodenum has 5-fold more p-gp expression than Caco-2 cells (16 days) (Fig. 15). The human normalized permeabilities ratio (drug permeability/propranolol permeability) of verapamil and cyclosporin,

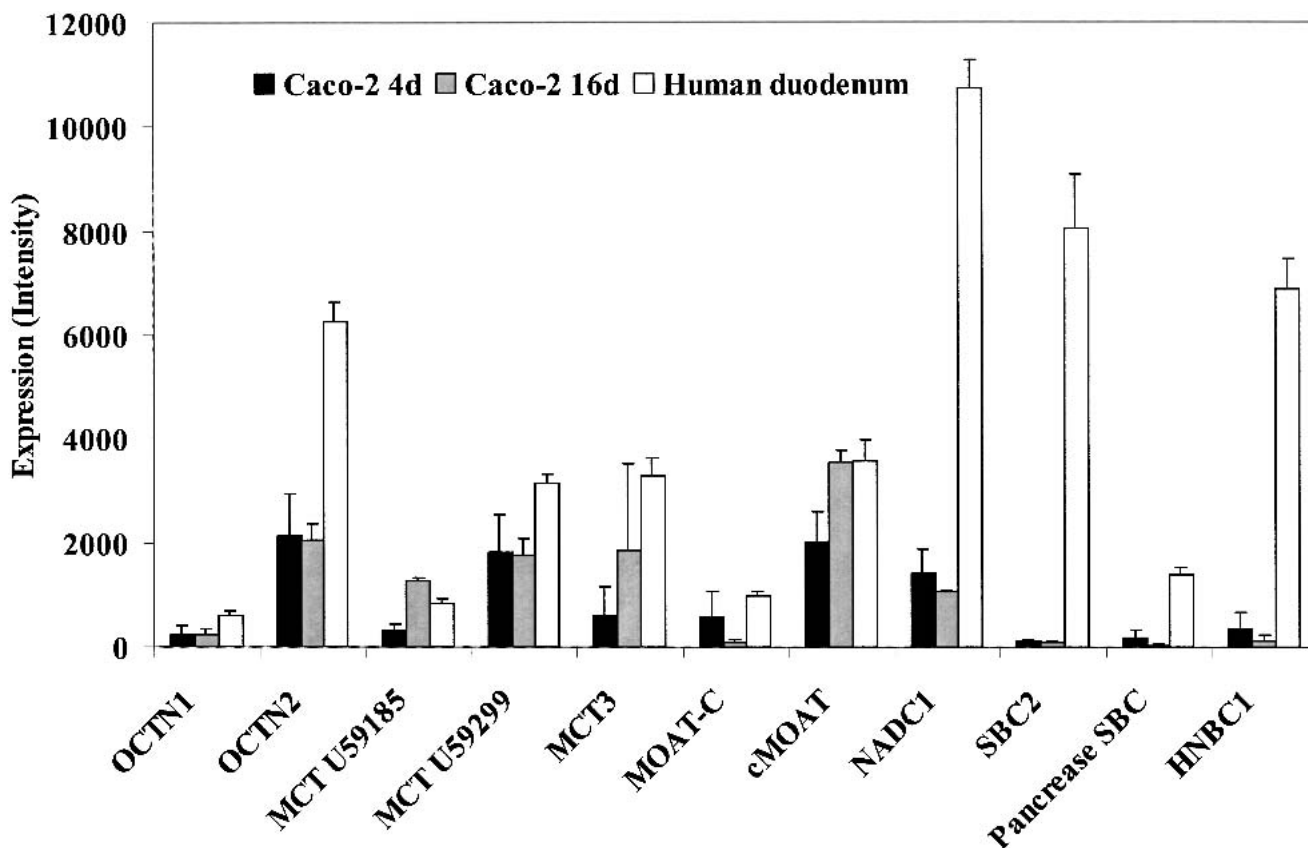


Fig. 13. Expression of organic cation and organic anion transporters in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal. OCTN1 and OCTN2, organic cation transporter 1 and 2; MCT U59185, monocarboxylate transporter (accession number: U59185); MCT U59299, monocarboxylate transporter (accession number: U59299); MCT3, monocarboxylate transporter 3; MOAT-C, ABC transporter MOAT-C; cMOAT, multispecific organic anion transporter; NADC1, sodium/dicarboxylate cotransporter NADC1; SBC2, sodium/bicarbonate cotransporter 2; pancreas SBC, pancreas sodium/bicarbonate cotransporter; HNBC1, sodium/bicarbonate cotransporter HNBC1.

which are substrates of p-gp, are 2- to 10-fold higher than Caco-2 normalized permeability ratios (Fig. 15). The difference of p-gp expression between human intestine and Caco-2 cells (16 days) could not explain their *in vivo/in vitro* permeability differences, which might suggest that there are other unknown factors involved in their intestinal absorption. The difference of *mdr1* expression in 16 days Caco-2 cells and human duodenum using GeneChip[®] analysis was not in agreement with the report of *mdr1* expression difference in 21 days Caco-2 cells and human jejunum using RT-PCR (2). This might be due to the different culture conditions and the passage number of Caco-2 cells, the regional difference of duodenum and jejunum, as well as the different detection sensitivity and amplification of mRNA by these two different methods.

RHOA proton-oncogene multidrug resistance protein (RHOA) was expressed at similar level in both human duodenum and Caco-2 cells. All other multidrug resistance associated proteins (MRP3B, MRP5, and MRP17) were expressed at similar levels in human duodenum and Caco-2 cells, with exception of MRP3, which was expressed 6-fold more in human duodenum than in Caco-2 cells (4 days) (Fig. 15). The expression differences of MRP3 and MRP5 in Caco-2 cells (16 days) and human duodenum detected by GeneChip[®] analysis were in agreement with the report of their gene expression differences in Caco-2 cells (21 days) and human jejunum detected by RT-PCR (2).

The Expression of Other Transporters and Ion Channels

All five nucleoside transporters, six phosphate transporters, three fatty acid transporters, three bile acids transporters, two citrate transporters, a creatinine transporter, a sulfate transporter, a co-enzyme transporter, a ADP/ATP transporter, a carnitine transporter, a glutarate transporter, a sterol transporter, a serotonin transporter, and 111 ion channels were also analyzed. The expression profiles of some of these transporters were at dramatically different levels in human duodenum compared to Caco-2 cells. Human duodenum had 25-fold less nucleoside transporter (hENT1), 110-fold higher sodium/purine transporter (CNT2), 360-fold more intestine fatty acid binding protein (FABP), 3-fold more liver fatty acid binding protein, 6-fold more creatine transporter, 2–3-fold more sterol transporter, 4-fold more K⁺ channel KLQT1, 26-fold more K⁺ channel TWK-1, and 350-fold more calcium-dependent chloride channel-1 expression than in Caco-2 cells.

Alkaline Phosphatase and Esterase Expression

All 13 carboxylesterases and 3 alkaline phosphatase were measured for their expression, six of which were detected in Caco-2 cells and human duodenum (Fig. 16). Human duodenum expressed 100-fold greater alkaline phosphatase, 140-

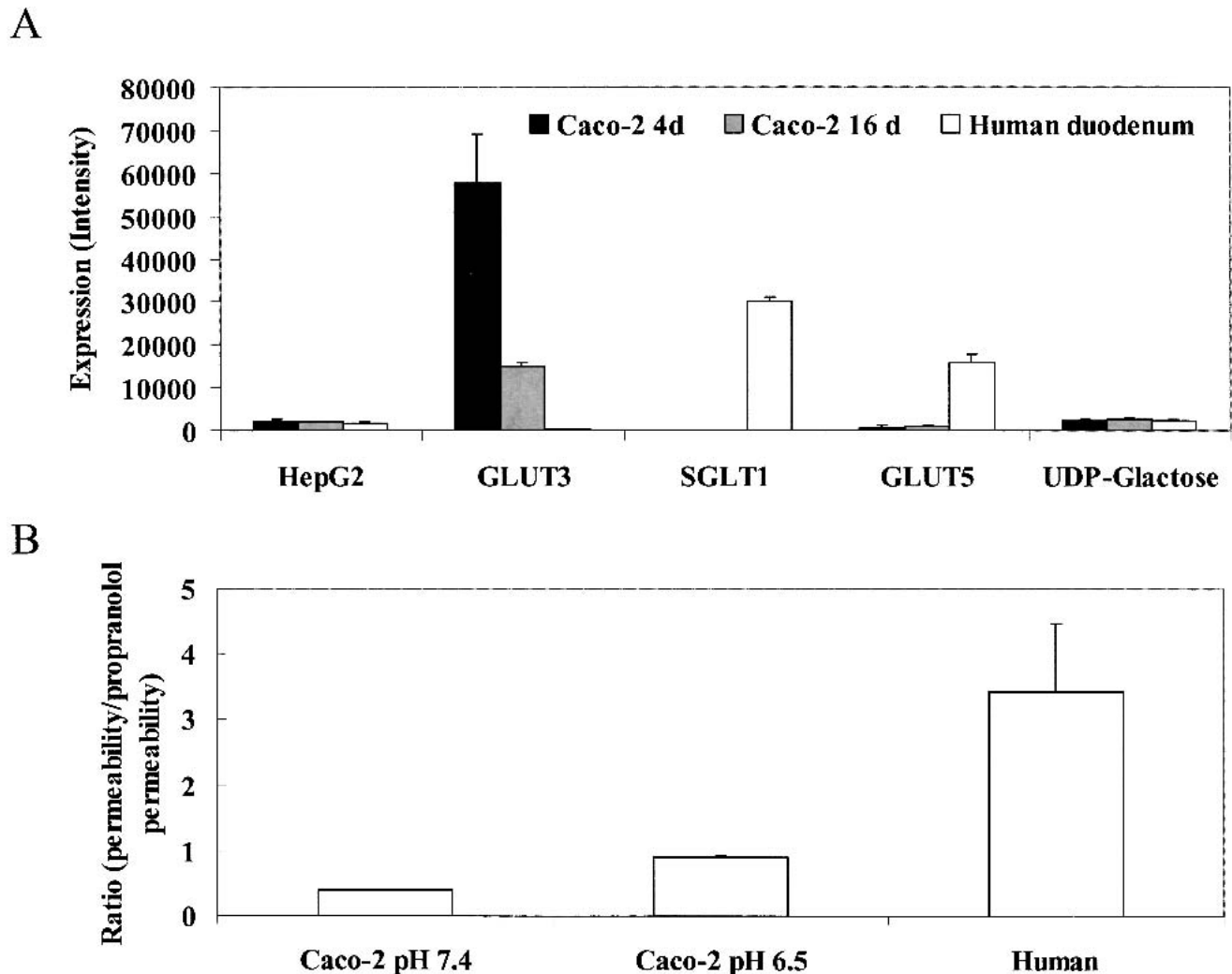


Fig. 14. (A). Expression of glucose transporters in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal. HepG2, human (HepG2) glucose transporter; GLUT3, sodium-independent glucose transporter-like protein III; GLUT5, sodium-independent glucose transporter-like protein 5; SGLT1, sodium-dependent glucose cotransporter; UDP-galactose, UDP-galactose transporter related isoenzyme 1. (B). Normalized drug permeability ratio in Caco-2 cells (pH 7.4, black bar), Caco-2 cells (pH 6.5, gray bar), and human jejunum (blank bar). Normalized permeability ratio was calculated by drug permeability/propranolol permeability in human and Caco-2 cells, respectively. NA, data not available.

fold more putative carboxylesterase (Y09616), and 6-fold less carboxylesterase (L07765) than Caco-2 cells. These results suggest that phosphate prodrug and ester prodrug might have different absorption and metabolism profiles in human intestine and Caco-2 cells.

The Expression of Phase I and Phase II Metabolizing Enzymes

The expression profiles of 44 cytochrome P-450 (CYP) enzymes and 57 phase II metabolizing enzymes were analyzed. Only 20 CYPs and 32 phase II metabolizing enzymes were detected in Caco-2 cells or human duodenum. Human duodenum showed 49-fold more CYP h2B1, 17-fold more CYP 2C9, 6-fold more CYP 2C18, 10-fold more CYP 2C19, 10-fold more CYP 2D, 20-fold more CYP 2J2, 160-fold more CYP 3A4, 18-fold more CYP 4F2, 30-fold more CYP c11,

28-fold more CYP LTBV, 322-fold more CYP PCN3, and 37-fold more CYP (X65962) than Caco-2 cells (Fig. 17). The difference in CYP 3A4 expression in Caco-2 cells and human duodenum determined by GeneChip® analysis is in agreement with the report of the difference of its expression in Caco-2 cells and human jejunum by RT-PCR (2).

Phase II metabolism enzymes including UDP-glucuronosyltransferase (UGT), glutathione S-transferase (GST), sulfotransferase (ST), *N*-acetyltransferase (AT), and acyltransferase (AcyT) in human duodenum and Caco-2 cells were also dramatically different. Human duodenum had 17-fold higher UGT2, 5-fold more UGT 2B15, 7-fold more UGT (U06641), 8-fold more UGT C19, 7-fold more UGT (J05428), 117-fold more GST4, 10-fold more GST M4, 3-fold less GST P1c, 4-fold more sulfotransferase 1A3, 4-fold more sulfotransferase P2, and 5-fold more *N*1-acetyltransferase (N1-AT) than that of Caco-2 cells.

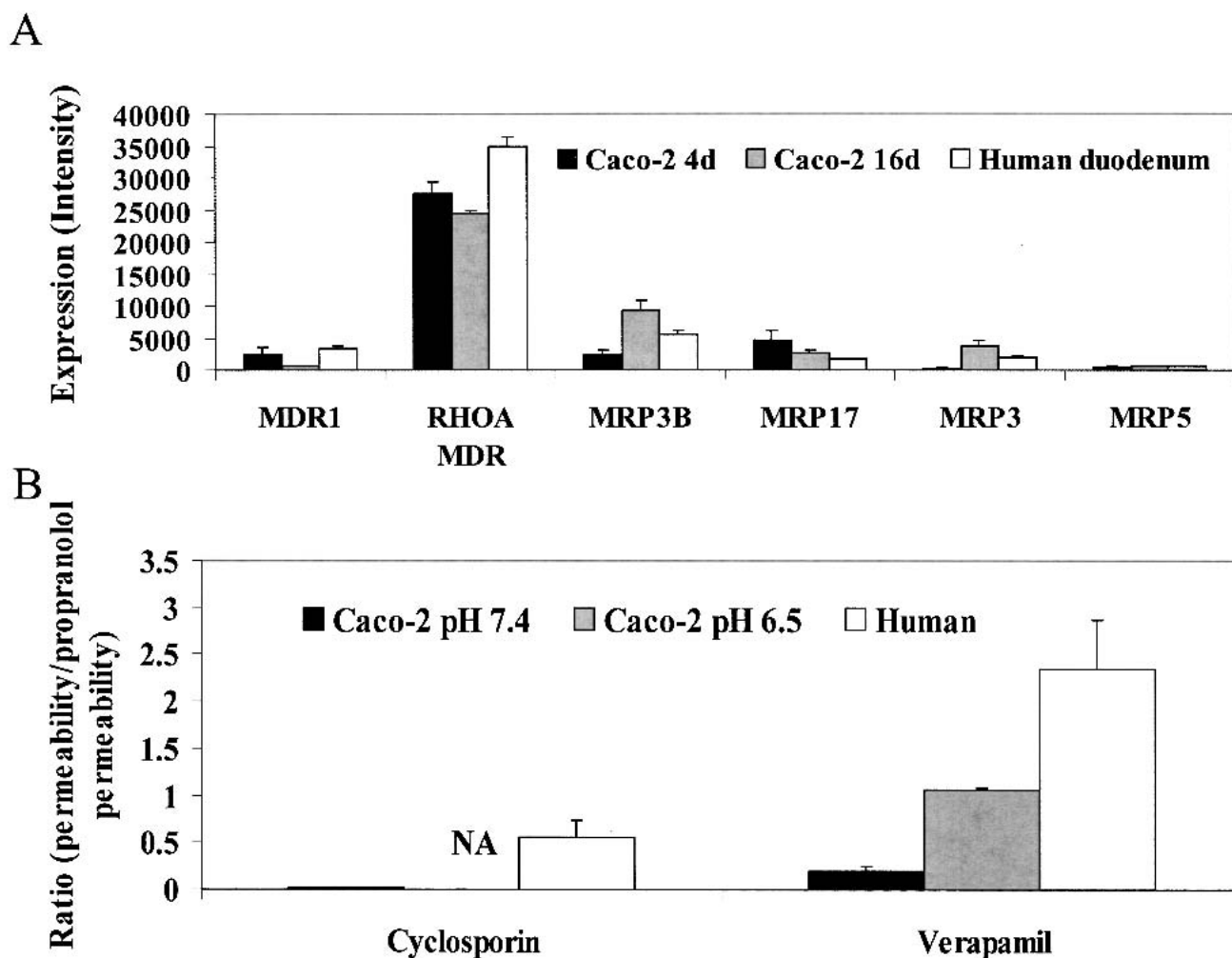


Fig. 15. (A). Expression of MDRs and MRPs in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal. MDR1, multidrug resistance gene 1 (p-glycoprotein, p-gp); RHOA MDR, RHOA proton-oncogene multidrug resistance protein; MRP, multidrug resistance associated protein. (B). Normalized drug permeability ratio in Caco-2 cells (pH 7.4, black bar), Caco-2 cells (pH 6.5, gray bar), and human jejunum (blank bar). Normalized permeability ratio was calculated by drug permeability/propranolol permeability in human and Caco-2 cells, respectively. NA, data not available.

SUMMARY

The enormous amount of data obtained with the GeneChip® precludes a comprehensive analysis in this report. Additional results are presented at the following link (http://www.sitemaker.umich.edu/amidonlab/genechip_data). Overall, there were 37–44% of 12,559 gene sequences expressed in Caco-2 cells and human duodenum with the chip comprising about one third of the genes in the estimated human genome. Comparing human duodenum with Caco-2 cells, more than 1000 genes were determined to have at least a 5-fold difference in expression. A 2- to 595-fold difference was observed in expression of transporters, channels, and metabolizing enzymes between human duodenum and Caco-2 cells. The expression differences generally correlated with the 3- to 35-fold higher permeability correlation in human for carrier-mediated absorbed drugs than passively absorbed drugs and with the limited expression data in the literature. The GeneChip® and the related technologies provide an invaluable new tool for investigating drug transport at a molecular level and comparisons of transport in tissue culture and human.

APPENDIX

The list of Transporters and Metabolizing Enzymes Exhibiting at Least 3-Fold Difference

Comparing human duodenum with Caco-2 cells (4 days), the following are the transporters, channels, and metabolizing enzymes with at least a 3-fold expression difference:

I. Gene expression with more than a 3-fold increase (3- to 595-fold): (a) Peptide transporters, amino acid transporter, and related proteins: hPepT1, Na/K ATPase α , GABA transporter. (b) Organic cation and organic anion transporters: MCT (U59185), MCT3, NADC1, SBC2, pancreas SBC, and HNBC1. (c) Glucose transporters and nucleoside transporters: SGLT1, GLUT5, and Na/purine transporter. (d) Other transporters: Na/PO₄ cotransporter, MRP3, intestinal fatty acid binding protein (FABP), liver FABP, UDP-acetylglucosamine transporter, creatinine transporter, glutarate transporter, and H⁺ pump 116 kDa. (e) Channels: K⁺ channel KLQT1, K⁺ channel TWK-1, Cl channel-1, Na chan-

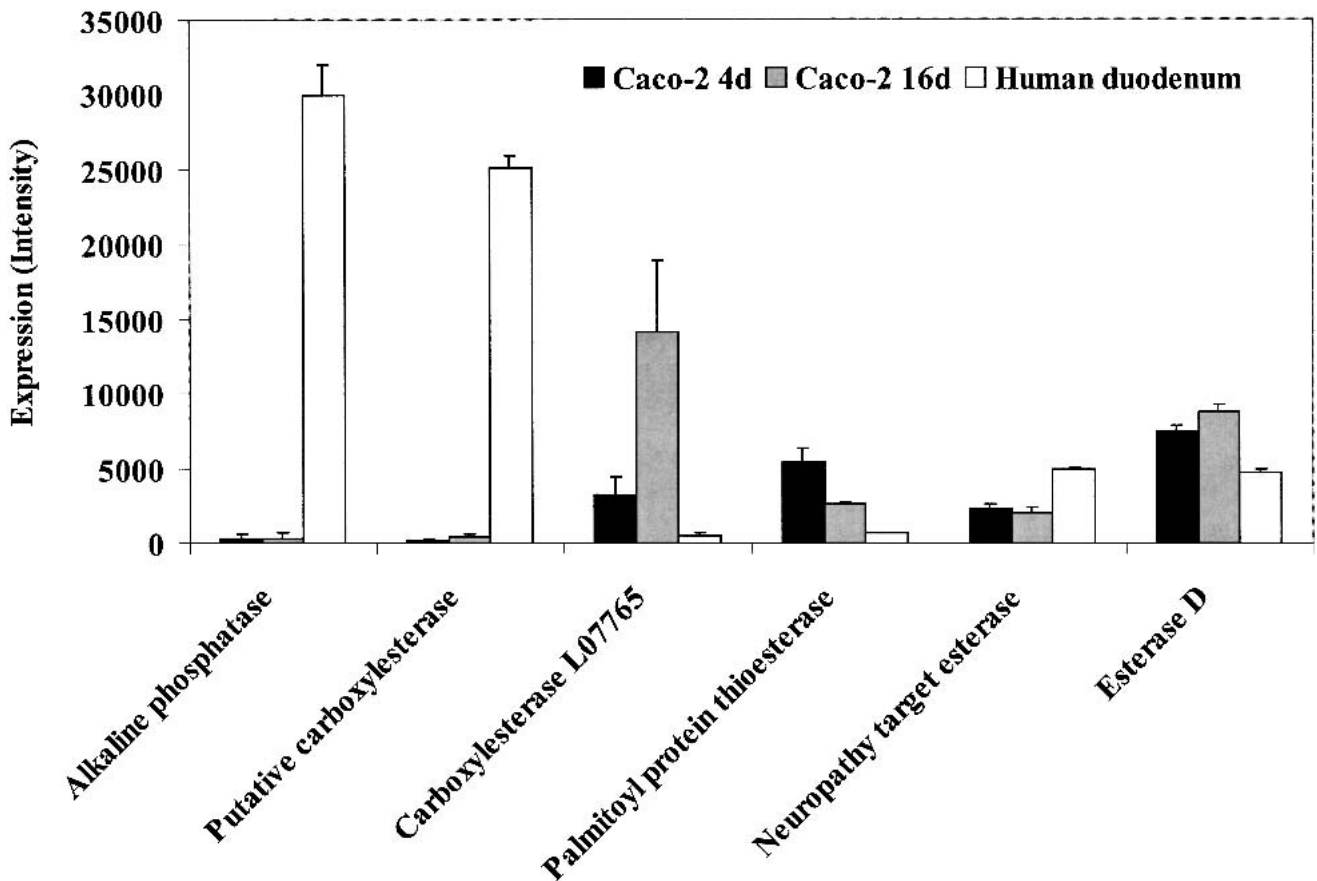


Fig. 16. Expression of alkaline phosphatase and esterases in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal. Putative carboxylesterase (accession number: Y09616).

nel, AQP-1, and water channel. (f) Alkaline phosphatase and esterase: Alkaline phosphatase and putative carboxylesterase. (g) Cytochrome P-450: 2A3, h2B1, 2C9, 2C18, 2C19, C11, 2D, 2J2, 3A4, 4F2, LTBV, PCN3, CYP reductase, CYP 27, TCDD inducible CYP, and CYP (X65962). (h) Phase II metabolizing enzymes: UDP-glucuronosyltransferase (UGT) including UGT2, UGT 2B15, UGT (U06641), UGT C19, UGT (J05428); acyltransferase (AcyT) including AcyT beta; glutathione S-transferase (GST) including GST Ha2, GST4, GST M4; sulfotransferase (ST) including ST 1A3, ST-P2, cerebroside ST, SULT 2B1b; and N-acetyltransferase (AT) including N1-AT, AT PDC-E2. (i) Junction protein and microvilli related protein: Claudin-7, villin like protein, mucin 2, mucin 3, mucin 6, pancrease mucin, and brush border myosin.

II. Gene expression with more than a 3-fold decrease (3- to 172-fold): Amino acid transporter ATB⁰, glucose transporter (GLUT3), cyclin-selected ubiquitin carrier, ubiquitin carrier E2-EPF, Fe transporter, hydrogen carrier, carboxylesterase (L07765), palmitoyl thioesterase, CYP51, and 6-ST.

Comparing human duodenum with Caco-2 cells (16 days), the following are the transporters, channels, and metabolizing enzymes with at least a 3-fold difference:

I. Gene expression with more than a 3-fold increase (3- to 595-fold): (a) Peptide transporters, amino acid transporters and related proteins: hPepT1, NHE1, Na/K ATPase α , and GABA transporter. (b) Organic cation and organic anion transporters: OCTN2, MCT3, MOAT-C, NADC1, SBC2,

pancrease SBC, and HNBC1. (c) Glucose transporters and nucleoside transporters: SGLT1, GLUT5, and Na/purine transporter. (d) Other transporters: Na/PO₄ cotransporter, mdr1, MRP5, intestinal FABP, liver FABP, creatinine transporter, and H⁺ pump 116 kDa. (e) Channels: K channel TWK-1, K channel KLOT1, K channel MaxiK, Cl channel-1, Na channel, and AQP-1. (f) Alkaline phosphatase and esterase: Alkaline phosphatase, neuropathy protein esterase, and putative carboxylesterase. (g) Cytochrome P-450: h2B1, 2C9, 2C18, 2C19, C11, 2D, 2J2, 3A4, 4F2, LTBV, AKA, PCN3, P-(1)-450 (X02612), CYP TCDD inducible, and CYP (X65962). (h) Phase II metabolizing enzymes: UGT2, UGT 2B15, UGT (U06641), UGT C19, UGT (J05428), GST4, GST M4, ST 1A3, ST-P2, and N1-AT. (i) Junction protein and microvilli related protein: Cardiac gap junction protein, claudin-7, mucin 2, mucin 3, mucin 6, and brush border myosin.

II. Gene expression with more than a 3-fold decrease (3- to 79-fold): Amino acid transporter (y+LAT1), amino acid transporter ATB⁰, glutamate transporter (D26443), glucose transporter (GLUT3), mitochondrial solute carrier, cyclin-selected ubiquitin carrier, ubiquitin carrier E2-EPF, Fe transporter, sulfate transporter, hydrogen carrier, carboxylesterase (L07765), palmitoyl protein thioesterase, Ca⁺⁺ channel α 1, Cl channel CLC-7, CYP51, and GST P1c.

Comparing Caco-2 cells (16 days) with Caco-2 cells (4 days), the following are the transporters, channels, and me-

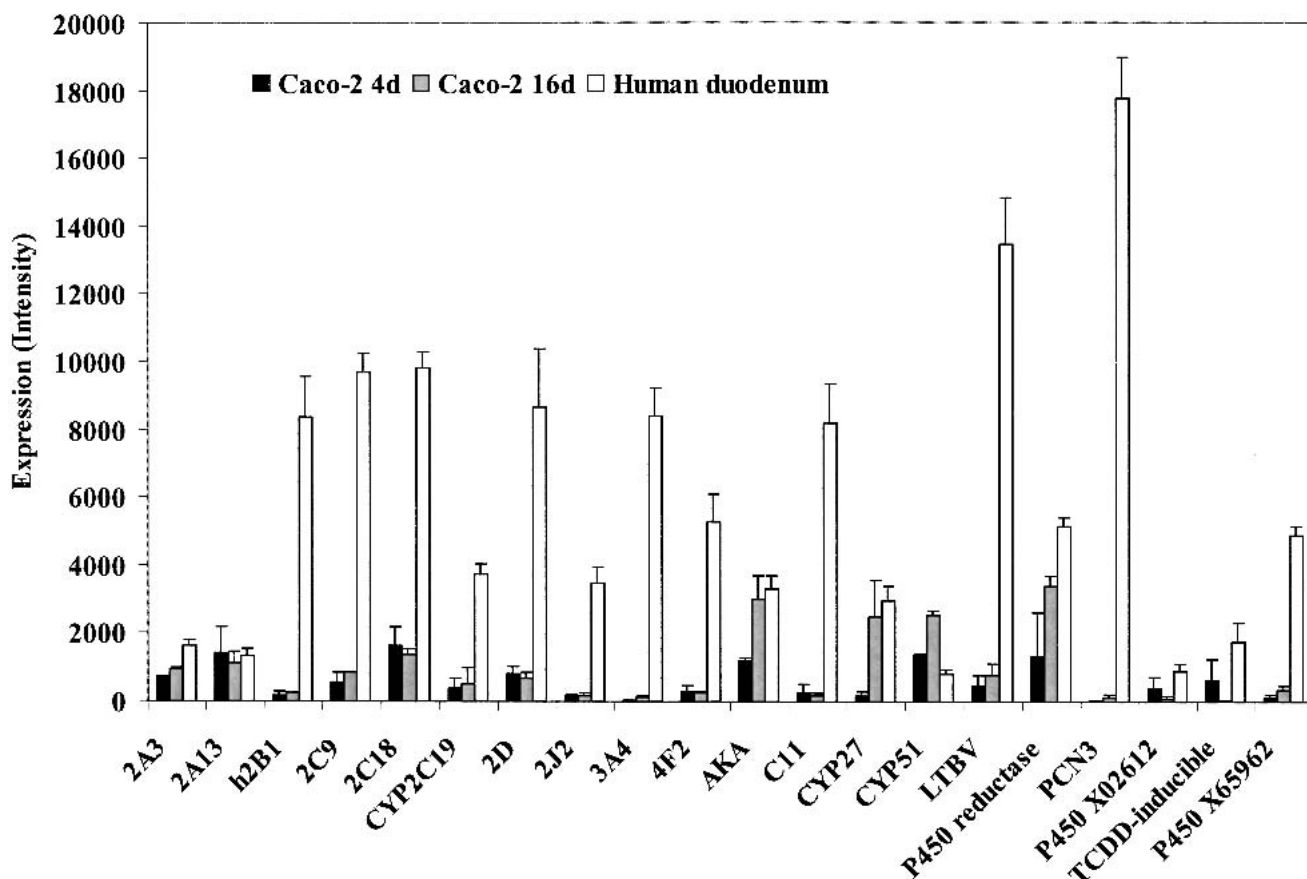


Fig. 17. Expression of cytochrome P-450 in Caco-2 cells (4 days, black bar), Caco-2 cells (16 days, gray bar), and human duodenum (blank bar). Expression levels were quantified by the intensity of hybridization signal.

tabolizing enzymes with at least a 3-fold expression difference:

I. Gene expression with more than a 3-fold increase (3- to 22-fold): (a) peptide transporters and related proteins: Na/H exchanger KIAA. (b) Organic anion transporters and MRPs: MCT U59185, MCT3, MRP3B, and MRP3. (c) Other transporters: mitochondrial Fe transporter, Ca^{++} transporting ATPase, liver fatty acid binding protein (FABP), ubiquitin carrier E2-PEF, creatinine transporter, and sulfate transporter. (d) Channels: water channel. (e) Metabolizing enzymes: carboxylesterase L07765, CYP reductase, CYP 27, UGT C19, AcyT2, GST Ha2, ST 1A3, ST-P2, and cerebroside ST. (f) Others: villin like protein, brush border myosin, and mucin 2.

II. Gene expression with more than a 3-fold decrease (3- to 12-fold): Glucose transporter (GLUT3), mdr1, MRP5, Glutamate transporter D26443, GST-M4.

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