Oral drug delivery utilizing intestinal OATP transporters

Oral drug delivery utilizing intestinal OATP transporters

Ikumi Tamai*,

Department of Membrane Transport and Biopharmaceutics, Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi Kanazawa 920-1192, Japan

*Corresponding author: Tel: +81-76-234-4479; fax: +81-76-264-6284;

E-mail address: tamai@p.kanazawa-u.ac.jp

Abstract.

Transporters play important roles in tissue distribution and urinary- and biliary-excretion of drugs and transporter molecules involved in those processes have been elucidated well. Furthermore, an involvement of efflux transporters such as P-glycoproteins, multidrug resistance associated protein 2, and breast cancer resistance protein as the intestinal absorption barrier and/or intestinal luminal secretion mechanisms has been demonstrated. However, although there are many suggestions for the contribution of uptake/influx transporters in intestinal absorption of drugs, information on the transporter molecules responsible for the intestinal absorptive process is limited. Among them, most studied absorptive drug transporter is peptide transporter PEPT1. However, utilization of PEPT1 for oral delivery of drugs may not be high due to the chemical structural requirement of PEPT1 limited to peptide-mimetics. Recently, organic anion transporting polypeptide (OATP) family such as OATP1A2 and OATP2B1 has been suggested to mediate intestinal absorption of several drugs. Since OATPs exhibit species difference in expressed tissues and functional properties between human and animals, human studies are essential to clarify the intestinal absorption mechanisms of drugs via OATPs. Recent pharmacogenomics studies demonstrated that OATP2B1 is involved in the drug absorption in human. In addition, information of drug-juice interaction in the intestine also uncovered the contribution of OATP1A2 and OATP2B1 in drug absorption. Since OATP1A2 and OATP2B1 exhibit broader substrate selectivity compared with PEPT1, their potential to be applied for oral delivery should be high. In this review, current understanding of characteristics and contribution as the absorptive transporters of OATPs in small intestine in human is described. Now, it is getting clearer that OATPs have significant roles in intestinal absorption of drugs, therefore, there are higher possibility to utilize OATPs as the tools for oral delivery.

Keywords

Absorption; OATP; Transporter; Drug-fruit juice interaction; Genetic polymorphism

Contents

1. Introduction

An involvement of transporters as the mechanism for intestinal absorption of drugs has been studied during about past five decades. Before molecular cloning of transporter genes, various *in vivo* and *in vitro* studies suggested a contribution of the carrier-mediated absorption of drugs via the nutrient transport systems for amino acids, oligopeptides, monocarboxylic acids, water-soluble vitamins, sugars, bile acids, amines, and nucleosides [1, 2]. After molecular cloning of membrane transporter genes in the middle of 1990's, more confirmative evidences which demonstrate the contribution of transporters in drug absorption have been obtained. Peptide transporter PEPT1 (*SLC15A1*) has been extensively studied as the drug transporter by demonstrating that several clinically used drugs such as beta-lactam antibiotics and valacyclovir are absorbed by PEPT1 due to their structural similarities with native peptides. Since the characteristics of PEPT1 have been clarified well, PEPT1 studies are currently in the stage for utilization of the transporter as the tool for oral delivery by mainly pro-drug approaches [3-8]. In addition to peptide transporter, we found that certain anion transporters which exhibit pH-dependent transport activity contribute to the absorption/uptake of monocarboxylic acid-type drugs such as benzoic acid [9]

and pravastatin [10]. In the beginning, monocarboxylate transporters MCTs (*SLC16A* family) were hypothesized to contribute as the responsible transporter molecules, since they accept monocarboxylates such lactic acid and nicotinic acid as substrates. When MCT1 was expressed in *Xenopus* oocytes or cultured cells, it transported benzoic acid as expected, while pravastatin was not [12]. Therefore, we had to consider that certain other transporters contribute to the absorption of such acidic drugs. Organic anion transport systems in liver and kidney had been studied well and organic anion transporting polypeptide (OATP) was molecularly identified as the anion transporter in liver [13]. Accordingly, we expected that OATP type transporters are expressed and functional as drug absorptive transporter in the small intestine. Since OATPs exhibit significantly broader substrate selectivity than PEPT1, they are more attractive to use as the transporters for oral delivery. In this review, a contribution of OATPs in the intestinal absorption of drugs mainly done by authors is described.

2. Characteristics of OATP Transporter Family

Organic anion transporting polypeptides (OATPs, *SLCO* family) are involved in the cellular uptake of numerous endogenous and xenobiotic organic anions in various tissues and affect absorption and disposition of their substrate drugs. In liver, OATP1B1 (former name is OATP-C), OATP1B3 (OATP8) and OATP2B1 (OATP-B) are expressed at the basolateral membrane of hepatocytes and contribute to hepatic uptake of various compounds [14, 15], while OATP1A2 (OATP-A), which was molecularly identified for the first time in human liver, is expressed more abundantly in brain [16]. These hepatic OATPs, OATP1B1 and OATP1B3, accept various clinically used drugs as substrates and their pharmacologically relevant observations in hepatic disposition in human are now accumulating based on the pharmacogenomic and drug-drug interaction studies. OATP1B1 has genetic variants and one of genotype called *SLCO1B1*15* shows lower transport activity by *in vitro* studies [17, 18]. The first pharmacogenomic study on OATP1B1 observed an increase of plasma concentration of pravastatin, which is extensively taken up by liver, in *SLCO1B1*15* genotype in a gene-dose dependent manner [19].

This observation is explained by a decreased hepatic uptake by impaired activity of the genotype, which results in the increased plasma concentration. Since then, it has been shown that *SLCO1B1*15* variant affects the hepatic uptake of several drugs, resulting in altered pharmacokinetics [20]. Drug-drug and drug-juice interactions also provide *in vivo* evidences for the significance of OATP transporters in drug disposition in human. For example, clinically observed effect of cyclosporine A on the cerivastatin pharmacokinetics was clearly explained by the interaction of cyclosporine A with the hepatic uptake of cerivastatin by OATP1B1 [21]. Although pharmacokinetic relevance of these hepatic OATPs in human is now accumulating, one of difficulties in OATP studies is a species difference. Figure 1 shows the similarities between typical human OATPs and rat Oatps. Multiple OATP molecules are expressed in liver of both animals and such a redundancy makes an identification of the functionally corresponding OATP/Oatp molecules between human and rat difficult only from the similarity of amino acid sequences and requires further functional studies. For example, rat Oatp1b2 is expressed in liver and its similarity in amino acid sequences with human OATP1B1 and OATP1B3 are equivalent. Accordingly, it is not easy to identify human OATPs that correspond to rat Oatp1b2, resulting in the difficult extrapolation of pharmacokinetic properties of drugs obtained in rat studies to human. We tried to identify human hepatic OATPs that correspond functionally to rat hepatic Oatps [22]. Many beta-lactam antibiotics are taken up by hepatocytes via organic anion transporters, including OATPs. Although multiple hepatic OATPs/Oatps can transport these antibiotics, a contribution of OATP1B3 and Oatp1a4 were largest in human and rat, respectively, demonstrating that amino acid sequence similarity may not be adequate to correlate human and rat OATPs/Oatps functionally. Accordingly, *in vivo* human studies such as the effects of genetic polymorphisms of transporter genes and the altered pharmacokinetics by drug-drug and drug-juice interactions should be useful for OATP studies.

As described above, since hepatic OATPs have been studied well and accept various drugs such as statins, angiotensin II receptor blockers, beta-lactam antibiotics, peptides, fluoroquinolones, angiotensin converting enzyme inhibitors, H1-antagonists, and other drugs and metabolites such as SN-38,

troglitazone sulfate, digoxin, bosentan, rifampicin, methotrexate, and glibenclamide as substrates, their pharmacological relevance is very high. Accordingly, if similar OATPs are expressed in small intestinal epithelial cells, it is expected that those OATPs may determine the bioavailability. Then, we can utilize such OATPs for oral delivery by improving intestinal membrane permeability by consideration of structural requirement of intestinal OATPs. Here, the current status of intestinal OATP studies and their possibility for oral delivery is described.

3. Expression of OATP in Enterocytes

Table 1 shows tissue expression profiles of OATPs in human. It is well known that OATP1B1 and OATP1B3 are exclusively expressed in human liver and OATP4C1 is in human kidney [15, 23-25]. OATP1C1 is in brain and testis and others are expressed multiple tissues, including intestine [15, 26]. Accordingly, other OATPs than OATP1B1, OATP1B3, OATP1C1 and OATP4C1 are possible intestinal OATPs. Since until now drug transports have not been observed well in OATP3A1 and OATP4A1, we focused more on OATP2B1 and studied its intestinal cellular localization, which exhibits similar but relatively narrower substrate selectivity than liver-specific OATP1B1 and OATP1B3 [27]. By immunohistochemical studies in human small intestinal tissues, OATP2B1 protein was localized at the apical membrane of intestinal epithelial cells as shown in Figure 2 [28]. Therefore, it is possible that OATP2B1 contributes to the absorption of its substrates from intestinal lumen. In addition, OTP1A2 was also shown to localize at the apical membrane of intestinal epithelial cells by exhibiting the same localization with P-glycoprotein [29]. In our separate study in Caco-2 cells, we searched for the transporter molecules responsible for the uptake of fluoroquinolones, especially levofloxacin, by microarray analysis of the differentially expressed transporter genes derived from the Caco-2 sub-clones which exhibited high and low activity to transport of levofloxacin and we succeeded to identify OATP1A2 as the most responsible molecule for apical uptake of levofloxacin in Caco-2 cells [30]. At the same time, it was reported that Caco-2 cells expressed most abundantly OATP2B1 compared to

OATP3A1 or OATP4A1 [31]. Accordingly, both of OATP1A2 and OAT2B1 are considered as the intestinal OATPs. However, although expression of OATP1A2 genes and proteins are reported in human small intestine cells [29], there are several controversial observations on the expression level of OATP1A2 in the human small intestinal tissues. One observation is shown in Table 2, which examined the intestinal regional difference in the expression of transporter genes, including OATP2B1 and OATP1A2. OATP1A2 expression in intestinal tissues is very low or negligible and OATP2B1 is more abundantly expressed [32] (Table 2). We also observed an absence of mRNA expression of OATP1A2 in human small intestine, while OATP2B1 was detected significantly [15]. Our observation of OATP1A2 as the responsible transporter molecule for levofloxacin found in sub-cloned Caco-2 [30] may be explained by the alteration of expression level by long term cultivation and that Caco-2 is derived from the intestinal cancer tissues. In addition, we reported that OATP2B1 gene expression is differently regulated from that of liver specific OATP1B1 and OATP1B3 being regulated by general transcription factor Sp1 in both liver and small intestine, while HNF1alfa which regulates liver-specific OATPs is not involved [33]. Taken together, intestinal expression of OATP2B1 should be more confirmative and currently it may be possible to say that OATP2B1 is generally responsible as the functional OATP in small intestine and OATP1A2 may show a variation in expression level by an induction and/or inter-individual variability [15, 32, 34]. Further studies on expression levels of OATPs molecules in human small intestine should be needed.

In rats, Oatp2b1, a counterpart of human OATP2B1, and Oatp1a5 are expressed in small intestine [35]. However, although expression of Oatp1a5 protein at the apical membrane of rat enterocytes was demonstrated [35], intestinal cellular localization of rat Oatp2b1 has not been clarified yet. We have reported that several drugs are transported by rat Oatp1a5 (former name is Oatp3), including fexofenadine [36], pravastatin [37], pitavastatin [38], tebipenem [39], talinolol [40, 41] and endothelin receptor antagonist [42] and their transport characteristics are comparable with those observed in rat intestinal tissues. So, it is thought that Oatp1a5 is functional for drug absorption in rat. Furthermore, Oatp1a5

shows similar functionality with human OATP2B1. If rat Oatp2b1 is expressed at the apical membrane of rat enterocytes, relative contribution of Oatp1a5 and Oatp2b1 is not clear at present and further studies should be required.

4: *In Vitro* Functional Characteristics of OATP2B1

OATP2B1 is characterized by its pH-dependent transport activity. When examined the uptake of estrone-3-sulfate by *in vitro* OATP2B1-gene transfected cells, it exhibited pH dependent activity [28, 43]. Figure 3 shows the uptake of estrone-3-sulfate by HEK293 cells transfected with OATP2B1 gene at pH 5.0 and 7.4. OATP2B1 exhibited higher activity at acidic pH and the increase was due to the 7-fold increase of Vmax with only 1.5-fold increase of Km. Although the mechanism of the increase of transport activity at acidic pH is not clear, FCCP, a protonophore, decreased significantly the uptake at acidic pH to 42% of control, while the decrease at neutral pH was not significant (81% of control). Replacement of sodium or chloride ions with *N*-methylglucamine or gluconate ions, respectively, did not affect the apparent activity of OATP2B1. Furthermore, glutathione did not show any effect on OATP2B1-mediated transport of estrone-3-sulfate. Accordingly, it was suggested that proton-coupled transport (alternatively exchange transport with hydroxyl ion) was suggested as the mechanism of pH-dependent transport of OATP2B1 [43]. Since the physiological microclimate pH in the intestinal lumen is weakly acidic, pH dependence of OATP2B1 activity is not surprising as the intestinal apical membrane transporter. Similar pH dependence is well known for peptide transporter PEPT1 [8] and monocarboxylate transporter MCT1 [9, 11] that are expressed at the enterocytes. Therefore, an increase of transport activity of these transporters at acidic pH might have a physiological relevance as the intestinal transporters. In addition to estrone-3-sulafate, dehydroepiandrosterone sulfate (DHEAS), fexofenadine, and pravastatin showed higher uptakes at acidic pH (pH 5.0) than those at a neutral pH (pH 7.4) via OATP2B1, showing about 3-, 3-, and 50-folds increases at acidic pH, respectively [43]. Similar pH dependence was reported in OATP2B1-transfected cells and Caco-2 cells [44]. In addition, as

described above, we observed proton-coupled transport of pravastatin using brush-border membrane vesicles prepared from rabbit intestinal epithelial cells. Accordingly, OATP2B1 might contribute to intestinal uptake of pravastatin [10]. In addition, it is essential to functionally characterize OATP2B1-mediated transport of drugs at acidic pH to understand the physiological and pharmacological relevance, since characteristics of OATP2B1 at usually used neutral pH might be different from those at acidic pH which is more physiologically relevant.

5: Effect of Genetic Polymorphisms of *SLCO2B1*

In vivo pharmacological relevance of OATP2B1 is characterized by the effect of genetic polymorphisms of OATP2B1 gene (*SLCO2B1*). Figure 4 shows non-synonymous mutations found in genetic variants of *SLCO2B1*. Among these genetic variants, *SLCO2B1*3*, which has mutation of c.1457C>T that causes amino acid change of Ser486Phe, exhibited a decreased transport activity by *in vitro* studies [17]. When *SLCO2B1*1* (wild type) and *SLCO2B1*3* genes were expressed *in vitro* cultured cells, uptake activity of estrone-3-sulfate was decreased to 42% of wild genotype after correction of the amount of expressed OATP2B1 proteins. This change was explained by the decrease of Vmax with negligible change in affinity (Km) 2.97 uM (*1) and 2.31 uM (*3). In addition, allele frequency of *SLCO2B1*3* gene is 31% in Japanese, while it is less in Finnish (2.8%) [45]. Accordingly, allele frequency of *SLCO2B1*3* gene is relatively high in Japanese population and shows ethnic difference. When pharmacokinetics of fexofenadine after oral administration was studied in Japanese, *SLCO2B1*3*-gene dose dependent decrease of plasma concentration of fexofenadine was observed [46]. The plasma concentration-time curves are shown in Figure 5. Areas under plasma concentration time curve (AUCs) of genotypes of CC, CT, and TT are 1762, 1088, and 1136 ng·hr/mL, respectively, at a dose of 60 mg. Maximum plasma concentrations (Cmax) were 343, 224 and 179 ng/mL for those three genotypes, respectively, whereas the time to reach Cmax (1.5, 1.5 and 1.8 hr, respectively) and clearance (0.6, 1.0, and 0.8 L/hr/kg BW, respectively) were comparable among three genotypes of *SLCO2B1*

c.1457C>T. The observation demonstrated that intestinal absorption of fexofenadine is affected by genotypes of *SLCO2B1*. Similar effect of genetic polymorphism of *SLCO2B1* on the absorption of beta-blocker celiprolol was also reported [47]. The other variant of *SLCO2B1* gene, c935G>A, which causes amino acid change of Arg312Gln also showed the decreased absorption of montelukast [48, 49]. Allelic frequencies of c.935G>A genotype of *SLCO2B1* are 13.6%, 8% and 13% in Finnish, Caucasian, and African American, respectively, showing relatively high frequencies [45]. These observed correlations between intestinal absorption and variants of *SLCO2B1* genes strongly demonstrate that OATP2B1 is responsible and a determinant of the intestinal absorption of its substrate drugs. More specifically, two variants c.1457C>T and c.935G>A of *SLCO2B1* gene, decrease the plasma concentration of drugs such as fexofenadine and motelukast, which may cause a decreased efficacy of drugs. Therefore, we need to be careful for dosage regimens in patients with those genotypes. However, there is a report on the higher plasma concentration of *R*- and *S*-fexofenadine in c.1457C>T genotype than the wild type subject [50], which means that the variants exhibited higher activity than wild type. Further studies should warrant the clinical effect of *SLCO2B1* gene.

6: Drug-Fruit Juice Interaction on OATP2B1

Alteration of *in vivo* drug absorption by concomitantly administered drugs or food/juice provides further evidence for the contribution of transporters in intestinal absorption of drugs. In 2002, significant effect of fruit juices such as grapefruit-, orange- and apple-juices on the plasma concentration of fexofenadine after oral administration was reported [51]. The earlier reports on the increases of bioavailability of drugs by concomitant fruit juice were mainly focused on the interactions of juice with intestinal drug metabolizing enzymes such as cytochrome P450 [52]. Later, since non-metabolizable drugs showed the similar increase in plasma concentration by ingestion with fruit juice, intestinal drug efflux transporters were proposed as the second mechanism of drug-juice interaction [53]. Now, there are many reports on these drug-juice interactions on drug metabolizing enzyme (CYP3A4) and efflux

transporter (P-glycoprotein) that are expressed in enterocytes. When these enzymes and transporters in small intestine are inhibited by fruit juices, an increase of plasma concentration should be observed, which may cause an adverse effect of dugs due to the increased systemic exposure [54]. However, fruit juices caused a decrease in plasma concentrations of fexofenadine, which cannot be explained by previously known drug-juice interaction on drug metabolizing enzymes and/or drug efflux transporters in small intestine. Based on these backgrounds, new type of drug interaction on the intestinal uptake/influx transporters was proposed [51]. It was hypothesized that OATPs are contributing to the absorption of fexofenadine and *in vitro* studies showed that OATP1A2 could be responsible transporter molecule [51]. On the other hand, it was reported that talinolol, a beta-blocker, exhibited a species difference in the effect of fruit juice on the plasma concentrations after oral administration between rat and human by showing an increase and a decrease of plasma concentrations of talinolol in rat and human, respectively, by ingestion with grapefruit juice [55, 56]. It has been known that talinolol is not metabolizable in intestine and is a good substrate of efflux transporter P-glycoprotein and those characteristics cannot explain a decrease of talinolol concentration in plasma by juice. So, it was hypothesized that talinolol is a substrate of uptake/influx transporter in the intestine and the effect of grapefruit juice on intestinal uptake and efflux transporters are different between human and rat [40, 41]. To examine this hypothesis, we used naringin as the responsible ingredient in grapefruit juice, since naringin is present in grapefruit juice at high concentration (~ 1.5 mM) and has been known to affect both of drug metabolizing enzymes and P-glycoprotein [57]. Both of human and rat OATP/Oatp accepted talinolol as substrate by *in vitro* studies and OATP/Oatp-mediated transport of talinolol was inhibited by naringin at the concentration present in grapefruit juice. However, rat mdr1a but not human MDR1 was inhibited by naringin at such concentration, when examined in rat mdr1a- or human MDR1-gene-transfected cells. These studies strongly demonstrated that OATPs are responsible for the intestinal absorption of talinolol in both rat and human and that apparently differential effect of grapefruit juice on talinolol absorption between rat and human could be explained by the difference in the affinity of naringin to rat and human P-glycoprotein as

schematically shown in Figure 6 [40, 41]. There are several similar studies on the effect of grapefruit juice on drug absorption by interacting with intestinal uptake/influx transporters. Fexofenadine absorption was reduced by ingestion with grapefruit juice by inhibition of OATP1A2 [58] or OATP2B1 [46]. Intestinal absorptions of statins [59], montelukast [49] and aliskiren [60] were reduced by inhibition of OATP2B1 by grapefruit juice. All these results support that OATPs are responsible for the drug-fruit juice interaction in the process of intestinal absorption.

In addition to grapefruit juice, other fruit juices and beverages affect drug absorption by interacting with OATPs. Fexofenadine absorption is reduced by orange juice and apple juice [46, 51]. Green tea catechins such as epicatechin gallate and epigallocatechin gallate inhibit intestinal OATP1A2 and OATP2B1 as well as hepatic OATP1B1 [61]. There are several well summarized review papers on the influence of fruit juices on drug absorption due to inhibition of influx transporters OATPs as well as efflux transporters and drug metabolizing enzymes in intestine for more information [54, 62].

7: Perspective of OATP-Mediated Oral Delivery

Tebipenem pivoxyl is a prodrug of carbapenem antibiotic tebipenem and exhibits very high bioavailability by 80% of dose. Since usually observed bioavailability of ester prodrugs is about 30 to 50%, we hypothesized that tebipenem pivoxyl might include carrier-mediated transport in addition to simple diffusion in intestinal membrane permeation [39]. When examined by *in vitro* studies, tebipenem pivoxyl was shown to be a substrate of OATP1A2 and OATP2B1 but not PEPT1. Although it is not clear whether this prodrug is absorbed by intestinal OATPs *in vivo*, there is a possibility that intestinal OATPs or other influx transporters contribute to the high bioavailability of the tebipenem. We have recently reported that antiviral oseltamivir, which is an ester prodrug of active Ro64-0802, is a substrate of peptide transporter PEPT1 and it may contribute to the intestinal absorption in human [63, 64]. These observations of the increased absorption of ester prodrugs suggest that the intestinal uptake/influx

transporters are involved in the intestinal membrane permeation and determine the success of prodrug approach. We have experimentally showed the application of PEPT1 for oral deliver by three strategies depending on the chemical structures of the pharmacologically active drugs [4-6]. Compared with PEPT1, structural requirements as substrates of OATPs are not easy to clarify due to the broad selectivity, but they have more potential to be applied for variable compounds. Accordingly, at least an evaluation of transport of drug candidate compounds by intestinal OATPs, PEPT1, and other uptake/influx transporters should be valuable and the utilization of these transporters may be the next strategy for oral delivery.

References

[1] I. Tamai, A. Tsuji, Carrier-mediated approaches for oral drug delivery, Adv. Drug Delivery Rev. 20 (1996) 5-32.

[2] A. Tsuji, I. Tamai, Carrier-mediated intestinal transport of drugs, Pharm. Res. 13 (1996) 963-977.

[3] P.V. Balimane, I. Tamai, A. Guo, T. Nakanishi, H. Kitada, F.H. Leibach, A. Tsuji, P.J. Sinko, Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir, Biochem. Biophys. Res. Commun. 250 (1998) 246-251.

[4] I. Tamai, T. Nakanishi, H. Nakahara, Y. Sai, V. Ganapathy, F.H. Leibach, A. Tsuji, Improvement of L-dopa absorption by dipeptidyl derivation, utilizing peptide transporter PepT1, J. Pharm. Sci. 87 (1998) 1542-1546.

[5] T. Nozawa, H. Toyobuku, D. Kobayashi, K. Kuruma, A. Tsuji, I. Tamai, Enhanced intestinal absorption of drugs by activation of peptide transporter PEPT1 using proton-releasing polymer, J. Pharm. Sci. 92 (2003) 2208-2216.

[6] A. Kikuchi, M. Tanaka, T. Tomoyasu, M. Maeda, I. Tamai, Improvement of intestinal absorption by peptide-derivation of nonabsorbable drug via peptide transporter PEPT1, J. Pharm. Sci. 98 (2008) 1775-1787.

[7] V.J. Stella, Prodrug approaches to enhancing the oral delivery of poorly permeable drugs, In Prodrugs: Challenges and rewards, Part 1, eds V.J. Stella, R.T. Borchardt, M.J. Hageman, R. Oliyai, H. Maag, J.W. Tulley, Springer AAPS Press, New York, pp37-125, 2007.

[8] M.D. Taylor, G.L. Amidon, Peptide-based drug design: Controlling transport and metabolism, ACS, Washington, DC, 1995.

[9] A. Tsuji, H. Takanaga, I. Tamai, T. Terasaki, Transcellular transport of benzoic acid across Caco-2 cells by a pH-dependent and carrier-mediated transport mechanism, Pharm. Res. 11 (1994) 30-37.

[10] I. Tamai, H. Takanaga, H. Maeda, T. Ogihara, M. Yoneda, A. Tsuji, Proton-cotransport of pravastatin across intestinal brush-border membrane, Pharm. Res. 12 (1995) 1727-1732.

[11] H. Takanaga, I. Tamai, S. Ianaba, Y. Sai, H. Higashida, H. Yamamoto, A. Tsuji, cDNA cloning and functional characterization of rat intestinal monocarboxylate transporter, Biochem. Biophys. Res. Commun. 217 (1995) 370-377.

[12] I. Tamai, Y. Sai, A. Ono, Y. Kido, H. Yabuuchi, H. Takanaga, E. Satoh, T. Ogihara, O. Amano, S. Izeki, A. Tsuji, Immunohistochemical and functional characterization of pH-dependent intestinal

absorption of weak organic acids by the monocarboxylic acid transporter MCT1, J. Pharm. Pharmacol. 51 (1999) 1113-1121.

[13] E. Jacquemin, B. Hagenbuch, B. Stieger, A.W. Wolkoff, P.J. Meier, Expression cloning of a rat liver Na(+)-independent organic anion transporter, Proc. Natl. Acad. Sci. U S A 91 (1994) 133-137.

[14] T. Mikkaichi, T. Suzuki, M. Tanemoto, S. Ito, T. Abe, The organic anion transporter (OATP) family, Drug Metab. Pharmacokinet. 19 (2004) 171-179.

[15] I. Tamai, J. Nezu, H. Uchino, Y. Sai, A. Oku, M. Shimane, A. Tsuji, Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family, Biochem. Biophys. Res. Commun. 273 (2000) 251-260.

[16] B. Gao, B. Hagenbuch, G.A. Kullak-Ublick, D. Benke, A. Aguzzi, P.J. Meier, Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier, J. Pharmacol. Exp. Ther. 294 (2000) 73-79.

[17] T. Nozawa, M. Nakajima, I. Tamai, K. Noda, J. Nezu, Y. Sai, A. Tsuji, T. Yokoi, Genetic polymorphism of human organic anion transporter OATP-C (SLC21A6) and OATP-B (SLC21A9): Allele frequencies in the Japanese population and functional analysis, J. Pharmacol. Exp. Ther. 302 (2002) 804-813.

[18] T. Nozawa, H. Minami, A. Tsuji, I. Tamai, Role of organic anion transporter OATP-C in hepatic uptake of irinotecan and its active metabolite SN-38: In vitro evidence and effect of single nucleotide polymorphisms. Drug Metab. Dispos. 33 (2005) 434-439.

[19] Y. Nishizato, I. Ieiri, H. Suzuki, M. Kimura, K. Kawabata, T. Hirota, H. Takane, S. Irie, H. Kusuhara, Y. Urasaki, A. Urae, S. Higuchi, K. Otsubo, Y. Sugiyama, Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: Consequences for pravastatin pharmacokinetics, Clin. Pharmacol. Ther. 73 (2003) 554-565.

[20] A. Kalliokoski, M. Niemi, Impact of OATP transporters on pharmacokinetics, Br. J. Pharmacol. 158 (2009) 693-705.

[21] Y. Shitara, T. Itoh, H. Sato, A.P. Li, Y. Sugiyama, inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporine A, J. Pharmacol. Exp. Ther. 304 (2003) 610-616.

[22] M. Nakakariya, T. Shimada, M. Irokawa, T. Maeda, I. Tamai, Identification and species similarity of OATP transporters responsible for hepatic uptake of beta-lactam antibiotics, Drug Metab. Pharmacokinet. 23 (2008) 347-355.

[23] T. Abe, M. Kakyo, T. Tokui, R. Nakagomi, T. Nishio, D. Nakai, H. Nomura, M. Suzuki, T. Naitoh, S. Matsuno, H. Yawo, Identification of novel gene family encoding human liver-specific organic anion

transporter LST-1, J. Biol. Chem. 274 (1999) 17519-17163.

[24] J. Konig, Y. Cui, A.T. Nies, D. Keppler, Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide, J. Biol. Chem. 275 (2000) 23161-23168.

[25] T. Mikkaichi, T. Suzuki, T. Onogawa, M. Tanemoto, H. Mizutamari, M. Okada, T. Chaki, S. Masuda, T. Tokui, N. Eto, M. Abe, F. Satoh, M. Unno, T. Hishinuma, K. Inui, S. Ito, J. Goto, T. Abe, Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney, Proc. Natl. Acad. Sci. USA 101 (2004) 3569-3574.

[26] F. Oizzagalli, B. Hagenbuch, B. Stieger, U. Klenk, G. Folkers, P.J. Meier, Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter, Mol. Endocrinol. 16 (2002) 2283-2296.

[27] I. Tamai, T. Nozawa, M. Koshida, J. Nezu, Y. Sai, A. Tsuji, Functional characterization of human organic anion transporting polypeptide OATP-B in comparison with liver-specific OATP-C, Pharm. Res. 18 (2001) 1262-1269.

[28] D. Kobayashi, T. Nozawa, K. Imai, J. Nezu, A. Tsuji, I. Tamai, Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane, J. Pharmacol. Exp. Ther. 306 (2003) 703-708.

[29] H. Glaeser, D.G. Bailey, G.K. Dresser, J.C. Gregor, U.I. Schwarz, J.S. McGrath, E. Jolicoeur, W. Lee, B.F. Leake, R.G. Tirona, R.B. Kim, Intestinal drug transporter expression and the impact of grapefruit juice in humans, Clin. Pharmacol. Ther. 81 (2007) 362-370.

[30] T. Maeda, K. Takahashi, N. Ohtsu, T. Oguma, T. Ohnishi, R. Atsumi, I. Tamai, Identification of influx transporter for the quinolone antibacterial agent levofloxacin, Mol. Pharm. 4 (2007) 85-94.

[31] Y. Sai, Y. Kaneko, S. Ito, K. Mitusoka, Y. Kato, I. Tamai, P. Artursson, A. Tsuji, Predominant contribution of organic anion transporting polypeptide OATP-B (OATP2B1) to apical uptake of estrone-3-sulfate by human intestinal Caco-2 cells, Drug Metab. Dispos. 34 (2006) 1423-1431.

[32] Y. Meier, J.J. Eloranta, J. Darimont, M.G. Ismair, C. Hiller, M. Fried, G.A. Kullak-Ublick, S.R. Vavricka, Regional distribution of solute carrier mRNA expression along the human intestinal tract, Drug Metab. Dispos. 35 (2007) 590-594.

[33] T. Maeda, M. Hirayama, R. Higashi, M. Sato, I. Tamai, Characterization of human OATP2B1 (SLCO2B1) gene promoter regulation, Pharm. Res. 23 (2006) 513-520.

[34] M. Nishimura, S. Naito, Tissue-specific mRNA expression profiles of human ATP binding cassette and solute carrier transporter superfamilies, Drug Metab. Pharmacokinet. 20 (2005) 452–477.

[35] H.C. Walters, A.L. Craddock, H. Fusegawa, M.C. Willingham, P.A. Dawson, Expression, transport properties, and chromosomal location of organic anion transporter subtype 3, Am. J. Physiol. Gastrointest. Liver Physiol. 279 (2000) G1188-1200.

[36] A. Kikuchi, T. Nozawa, T. Wakasawa, T. Maeda, I. Tamai, Transporter-mediated intestinal absorption of fexofenadine in rats, Drug Metab. Pharmacokinet. 21 (2006) 308-314.

[37] Y. Shirasaka, K. Suzuki, T. Nakanishi, I. Tamai, Intestinal absorption of HMG-CoA reductase inhibitor pravastatin mediated by organic anion transporting polypeptide, Pharm. Res. 27 (2010) 2141-2149.

[38] Y. Shirasaka, K. Suzuki, M. Shichiri, T. Nakanishi, I. Tamai, Intestinal absorption of HMG-CoA reductase inhibitor pitavastatin mediated by organic anion transporting polypeptide and P-glycoprotein/multidrug resistance 1, Drug Metab. Pharmacokinet. 26 (2011) 171-179.

[39] K. Kato, Y. Shirasaka, E. Kuraoka, A. Kikuchi, M. Iguchi, H. Suzuki, S. Shibasaki, T. Kurosawa, I. Tamai, Intestinal absorption mechanism of tebipenem pivoxil, a novel oral carbapenem: involvement of human OATP family in apical membrane transport, Mol. Pharm.7 (2011) 1747-1756.

[40] Y. Shirasaka, Y. Li, Y. Shibue, E. Kuraoka, H. Spahn-Langguth H, Y. Kato, P. Langguth, I. Tamai, Concentration-dependent effect of naringin on intestinal absorption of beta(1)-adrenoceptor antagonist talinolol mediated by p-glycoprotein and organic anion transporting polypeptide (Oatp), Pharm. Res. 26 (2009) 560-570.

[41] Y. Shirasaka, E. Kuraoka, H. Spahn-Langguth, T. Nakanishi, P. Langguth, I. Tamai, Species difference in the effect of grapefruit juice on intestinal absorption of talinolol between human and rat, J. Pharmacol. Exp. Ther. 332 (2010) 181-189.

[42] T. Tani, L.K. Gram, H. Arakawa, A. Kikuchi, M. Chiba, Y. Ishii, B. Steffansen, I. Tamai, Involvement of organic anion transporting polypeptide 1a5 (Oatp1a5) in the intestinal absorption of endothelin receptor antagonist in rats, Pharm. Res. 25 (2008) 1085-1091.

[43] T. Nozawa, K. Imai, J. Nezu, A. Tsuji, I. Tamai, Functional characterization of pH-dependent organic anion transporting polypeptide OATP-B in human, J. Pharmacol. Exp. Ther. 308 (2004) 438-445.

[44] O. Kis, J.A. Zastre, M. Ramaswamy, R. Bendayan, pH Dependence of organic anion-transporting polypeptide 2B1 in Caco-2 cells: potential role in antiretroviral drug oral bioavailability and drug-drug interaction, J. Pharmacol. Exp. Ther. 334 (2010) 1009-1022.

[45] A. Laitinen, M. Niemi, Frequencies of single-nucleotide polymorphisms of SLCO1A2, SLCO1B3 and SLCO2B1 genes in a Finnish population, Basic Clin. Pharmacol. Toxicol. 108 (2010) 9-13.

[46] J. Imanaga, T. Kotegawa, H. Imai, K. Tsutsumi, T. Yoshizato, T. Ohyama, Y. Shirasaka, I. Tamai, T. Tateishi, K. Ohashi, The effects of the SLCO2B1 c.1457C>T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans, Pharmacogenet. Genomics 21 (2011) 84-93.

[47] I. Ieiri, Y. Doi, K. Maeda, T. Sasaki, M. Kimura, T. Hirota, T. Chiyoda, M. Miyagawa, S. Irie, K. Iwasaki, Y. Sugiyama, Microdosing clinical study: Pharmacokinetic, pharmacogenomic (SLCO2B1), and interaction (Grapefruit Juice) profiles of celiprolol following the oral microdose and therapeutic dose, J. Clin. Pharmacol. in press (2011).

[48] E.B. Mougey, H. Feng, M. Castro, C.G. Irvin, J.J. Lima, Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response, Pharmacogenet. Genomics 19 (2009) 129-138.

[49] E.B. Mougey, J.E. Lang, X. Wen, J.J. Lima, Effect of citrus juice and SLCO2B1 genotype on the pharmacokinetics of montelukast, J. Clin. Phramacol. 51 (2011) 751-760.

[50] Y. Akamine, M. Miura, S. Sunagawa, H. Kagatya, N. Yasui-Furukori, T. Uno, Influence of drug-transporter polymorphisms on the pharmacokinetics of fexofenadine enantiomers, Xenobiotica 40 (2010) 782-789.

[51] G.K. Dresser, D.G. Bailey, B.F. Leake, U.I Schwarz, P.A. Dawson, D.J. Freeman, R.B. Kim, Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine, Clin. Pharmacol. Ther. 71 (2002) 11-20.

[52] D.G. Bailey, J.D. Spence, C. Munoz, J.M.O. Arnold, Interaction of citrus juices with felodipine and nifedipine, Lancet 337 (1991) 268-269.

[53] H. Takanaga, A. Ohnishi, H. Matsuo, Y. Sawada, Inhibition of vinblastine efflux mediated by P-glycoprotein by grapefruit juice components in Caco-2 cells, Biol. Pharm. Bull. 21 (1998) 1062-1066.

[54] D.G. Bailey, Fruit juice inhibition of uptake transport: a new type of food-drug interaction, Br. J. Clin. Pharmacol. 70 (2010) 645-655.

[55] U.I. Schwarz, D. Seemann, R. Oertel, S. Miehlke, E. Kuhlisch, M.F. Fromm, R.B. Kim, D.G. Bailey, W. Kirch, Grapefruit juice ingestion significantly reduces talinolol bioavailability, Clin. Pharmacol. Ther. 77 (2005) 291-301.

[56] H. Spahn-Langguth, P. Langguth, Grapefruit juice enhances intestinal absorption of the P-glycoprotein substrate talinolol, Eur. J. Pharm. Sci. 12 (2001) 361-367.

[57] W.V. de Castro, S. Mertens-Talcott, H. Derendorf, V. Butterweck, Grapefruit juice-drug interactions: Grapefruit juice and its components inhibit P-glycoprotein (ABCB1) mediated transport of talinolol in Caco-2 cells, J. Pharm. Sci. 96 (2007) 2808-2817.

[58] D.G. Bailey, G.K. Dresser, B.F. Leake, R.B. Kim, Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice, Clin. Pharmacol. Ther. 81 (2007) 495-502.

[59] Y. Shirasaka, K. Suzuki, T. Nakanishi, I. Tamai, Differential effect of grapefruit juice on intestinal absorption of statins due to inhibition of organic anion transporting polypeptide and/or P-glycoprotein, J. Pharm. Sci. 100 (2011) 3843-3853.

[60] T. Tapaninen, P.J. Neuvonen, M. Niemi, Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren, Clin. Pharmacol. Ther. 88 (2010) 339-342.

[61] M. Roth, B.N. Timmermann, B. Hagenbuch, Interaction of green tea catechins with organic anion-transporting polypeptides, Drug Metab. Dispos. 39 (2011) 920-926.

[62] D. Farkas, D.J. Greenblatt, Influence of fruit juices on drug disposition: discrepancies between in vitro and clinical studies, Expert Opi. Drug Metab. Toxicol. 4 (2008) 381-393.

[63] T. Ogihara, T. Kano, T. Wagatsuma, S. Wada, H. Yabuuchi, S. Enomoto, K. Morimoto, Y. Shirasaka, S. Kobayashi, I. Tamai, Oseltamivir (TamifluTM) is a substrate of PEPT1, Drug Metab. Dispos. 37 (2009) 1676-1681.

[64] K. Morimoto, K. Kishimura, T. Nagami, N. Kodama, Y. Ogama, M. Yokoyama, S. Toda, T. Chiyoda, R. Shimada, A. Inano, T. Kano, I. Tamai, T. Ogihara, Effect of milk on the pharmacokinetics of oseltamivir in healthy volunteers, J. Pharm. Sci. 100 (2011) 3854-3861.

Table 1

Tissue expression profiles of OATPs in human

Protein Name	Former Name	Major Expressed tissues
OATP1A2	OATP-A	Intestine, Brain and various tissues
OATP1B1	OATP-C	Liver (No expression in intestine)
OATP1B3	OATP8	Liver No expression in intestine)
OATP2B1	OATP-B	Intestine, Liver, Placenta, Muscle etc.
OATP3A1	OATP-D	Intestine and various tissues
OATP4A1	OATP-E	Intestine and various tissues
OATP1C1	OATP-F	Brain, Testis (No expression in intestine)
OATP4C1	OATP-R	Kidney (No expression in intestine)

Table 2

Regional difference of expression levels of transporter mRNA in human intestine.

	Duodenum	Ileum	Colon Ascendens
PEPT1	3.87* (2.52)	3.79* (2.19)	0.14* (0.12)
OCTN1	0.53* (0.54)	1.27* (1.33)	0.43* (0.40)
CNT2	0.32* (0.26)	0.15* (0.11)	0.003 (0.003)
OCTN2	0.09* (0.07)	0.01* (0.05)	0.34* (0.20)
ASBT	0.09* (0.05)	0.42* (0.25)	0.007 (0.003)
CNT1	0.06* (0.06)	0.06* (0.04)	0.0002 (0.0001)
OATP2B1	0.02* (0.02)	0.06* (0.10)	0.02* (0.02)
ENT2	0.009 (0.006)	0.006 (0.005)	0.02* (0.01)
OAT2	0.006 (0.006)	0.001 (0.001)	0.0006 (0.001)
OCT1	0.0004 (0.0004)	0.0004 (0.0004)	0.0002 (0.001)
OATP1A2	0.0002 (0.0001)	0.003 (0.004)	0.0004 (0.0004)

The data is cited from report by Meier et al. (reference 32) and modified. The numbers represent the copy numbers relative to villin and the number in parenthesis is S.D. *: significant expression when the number is larger than 0.01. Transporters shown by italic are significantly expressed in small intestine.

Figure legends

Figure 1:
Similarities in amino acid sequences between typical human OATPs and rat Oatps.
The numbers represent percentage of similarities. Human OATPs are shown by capital letter and rat Oatps are shown by lowercases. Parenthesis show former names of each transporter.

Figure 2:
Immunohistochemical localization of OATP2B1 in human small intestine.
OATP2B1 (a, c) and Normal IgG (b, d) represent the result using anti-OATP2B1 serum and rabbit normal IgG, respectively. Expression of OATP2B1 is indicated by brown coloration with the immune-peroxidase method. OATP2B1 protein was localized at luminal surface. The result is cited from report by Kobayashi *et al*. (reference 28) and modified.

Figure 3
Concentration- and pH-dependences of estron-3-sulfate uptake by OATP2B1.
Uptake of estrone-3-sulfate by OATP2B1 at acidic (pH 5.0: closed circles) and neutral (pH 7.4: open circles) pH was measured at its increasing concentrations. Inset: Eadie-Hofstee plot of the result and obtained kinetic parameters (Km and Vmax) are shown. The data is cited from report by Nozawa *et al.* (reference 43) and modified.

Figure 4.
Locations of non-synonymous changes of amino acid residues in OATP2B1 variants.
Membrane spanning model of OATp2B1 and upper and lower area mean the extracellular and intracellular domains. The locations of mutated amino acid residues are shown by star and each circles represents each amino acid residues.

Figure 5.
Effect of *SLCO2B1*3* gene on plasma concentration-time curve of fexofenadine after oral administration in subjects with c.[1457C]/c.[1457C], c.[1457C]/c.[1457C>T], and c.[1457C>T]/c.[1457C>T] for circles, squares, and triangles, respectively.
The data is cited from report by Imanaga *et al.* (reference 46) and modified.

Figure 6.
Scheme of the species difference of grapefruit juice effect on change of plasma concentration of talinolol between human and rat. Left: *In vitro* inhibitory effect of increasing concentration of naringin on OATP

and MDR1 in rat (a) and human (b). Dotted area means the naringin concentration present in grapefruit juice. Right: *In vivo* talinolol blood concentration ingested with water (closed squares with dotted line) and with grapefruit juice (open squares with solid line). Both of human OATP and rat Oatp are inhibited by naringin at the concentration present in grapefruit juice, while only rat mdr1a but not human MDR1 is inhibited. Differential effect of naringin (grapefruit juice) on MDR1/mdr1a causes the differential change in absorption of talinolol between human and rat, while influx was commonly mediated by OATP/Oatp in human and rat.

Figure 1

	Human OATP						Rat Oatp						
	1A2 (A)	2B1 (B)	1B1 (C)	3A1 (D)	4A1 (E)	1B3 (8)	1a1 (1)	1a4 (2)	1a5 (3)	1b2 (4)	2b1 (9)	3a1 (11)	4a1 (12)
1A2 (A)	100	34	44	36	32	42	67	73	72	42	34	36	34
2B1 (B)		100	35	36	34	35	35	33	34	35	77	35	34
1B1 (C)			100	37	31	80	44	46	46	64	36	38	35
3A1 (D)				100	36	38	36	37	38	35	37	97	35
4A1 (E)					100	34	35	35	34	31	32	37	76
1B3 (8)						100	46	45	46	66	33	36	35
1a1 (1)							100	77	80	43	34	36	35
1a4 (2)								100	82	44	33	37	33
1a5 (3)									100	44	33	38	34
1b2 (4)										100	33	36	33
2b1 (9)											100	37	34
3a1 (11)												100	36
4a1 (12)													100

Figure 2

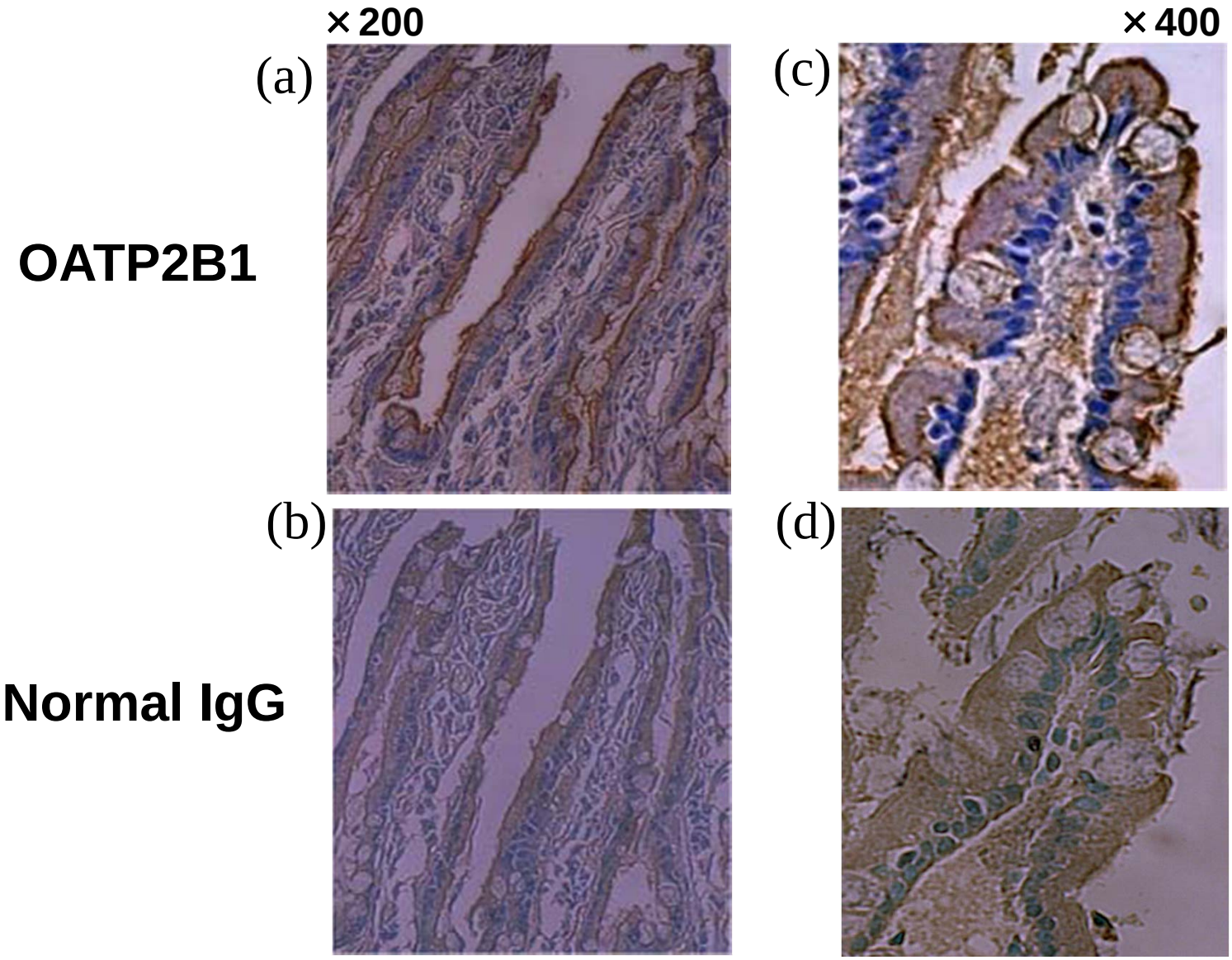


Figure 3

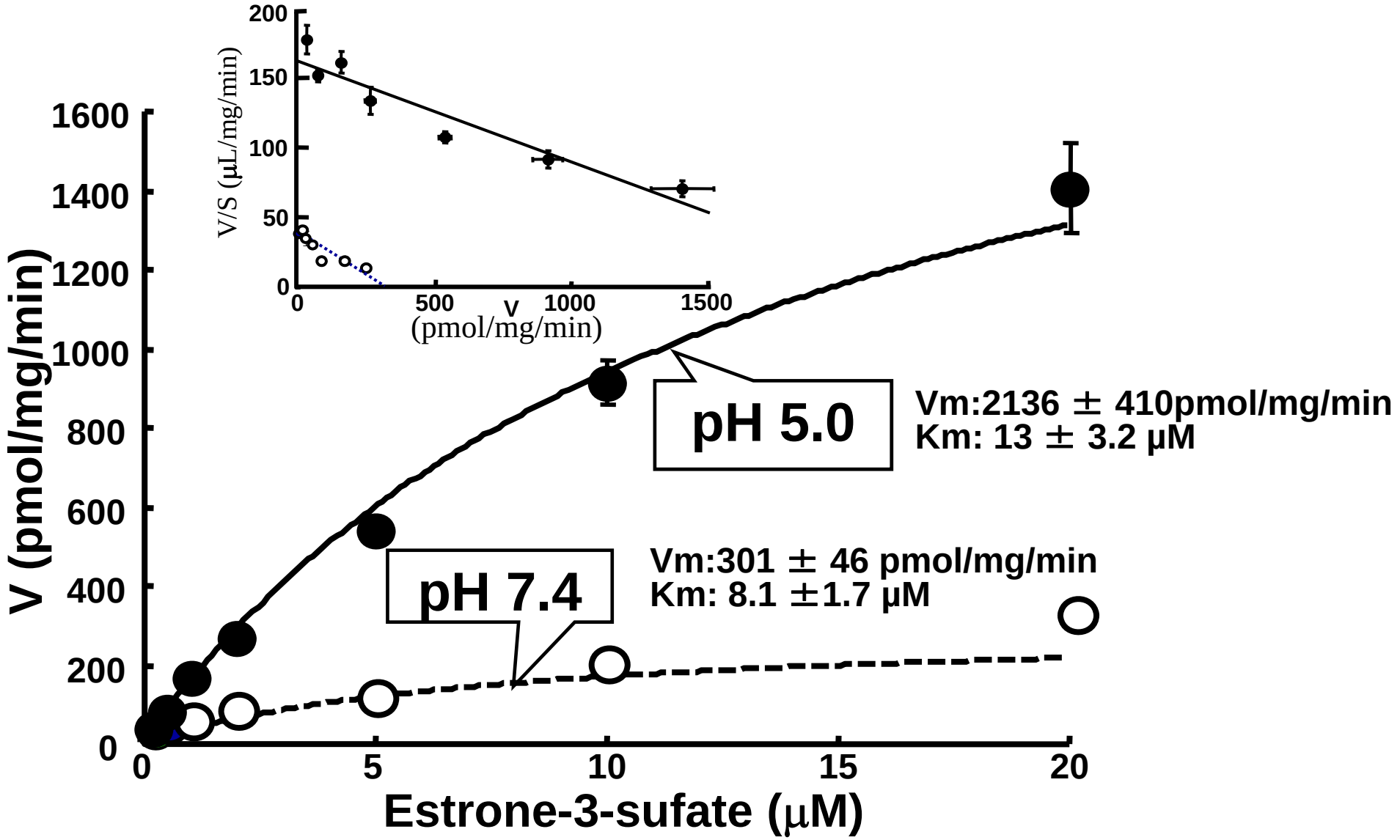

V (pmol/mg/min)
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
Estrone-3-sufate (μM)
pH 5.0
Vm:2136 ± 410pmol/mg/min
Km: 13 ± 3.2 μM
pH 7.4
Vm:301 ± 46 pmol/mg/min
Km: 8.1 ±1.7 μM
V/S (μL/mg/min)
200
150
100
50
0
0
500
1000
1500
V
(pmol/mg/min)

Figure 4

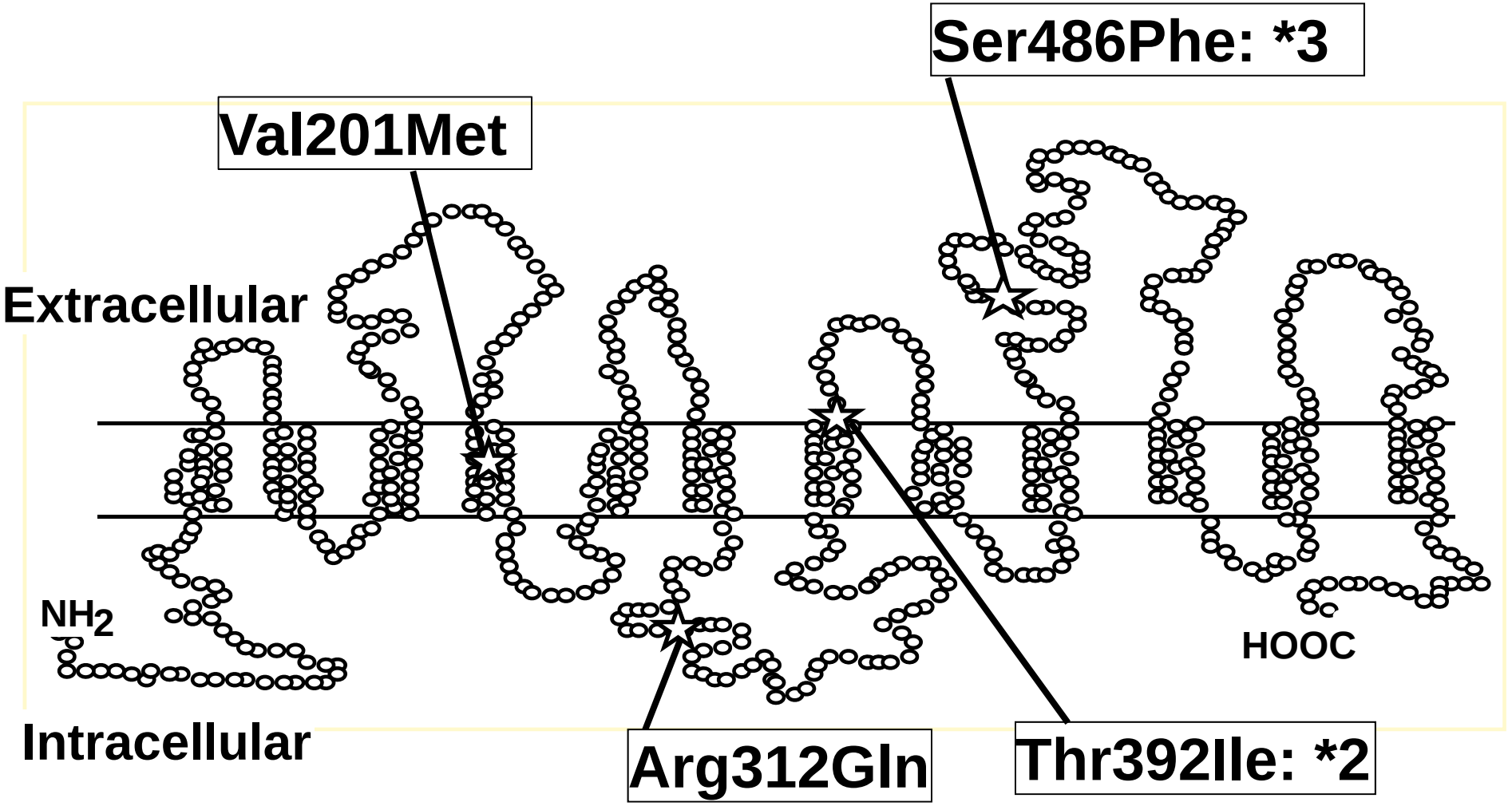

Ser486Phe: *3
Val201Met
Extracellular
NH2
HOOC
Intracellular
Arg312Gln
Thr392Ile: *2

Figure 5

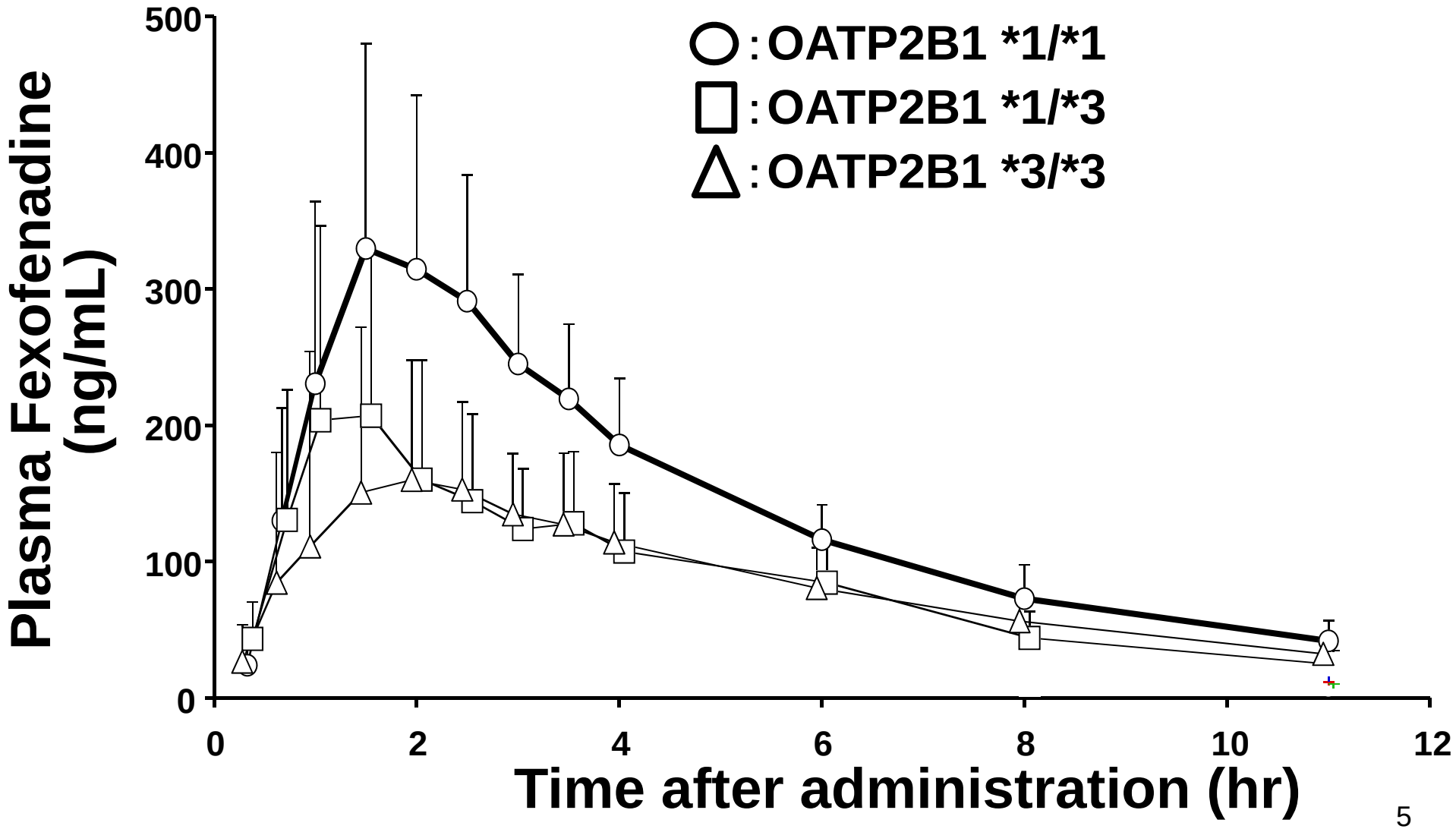
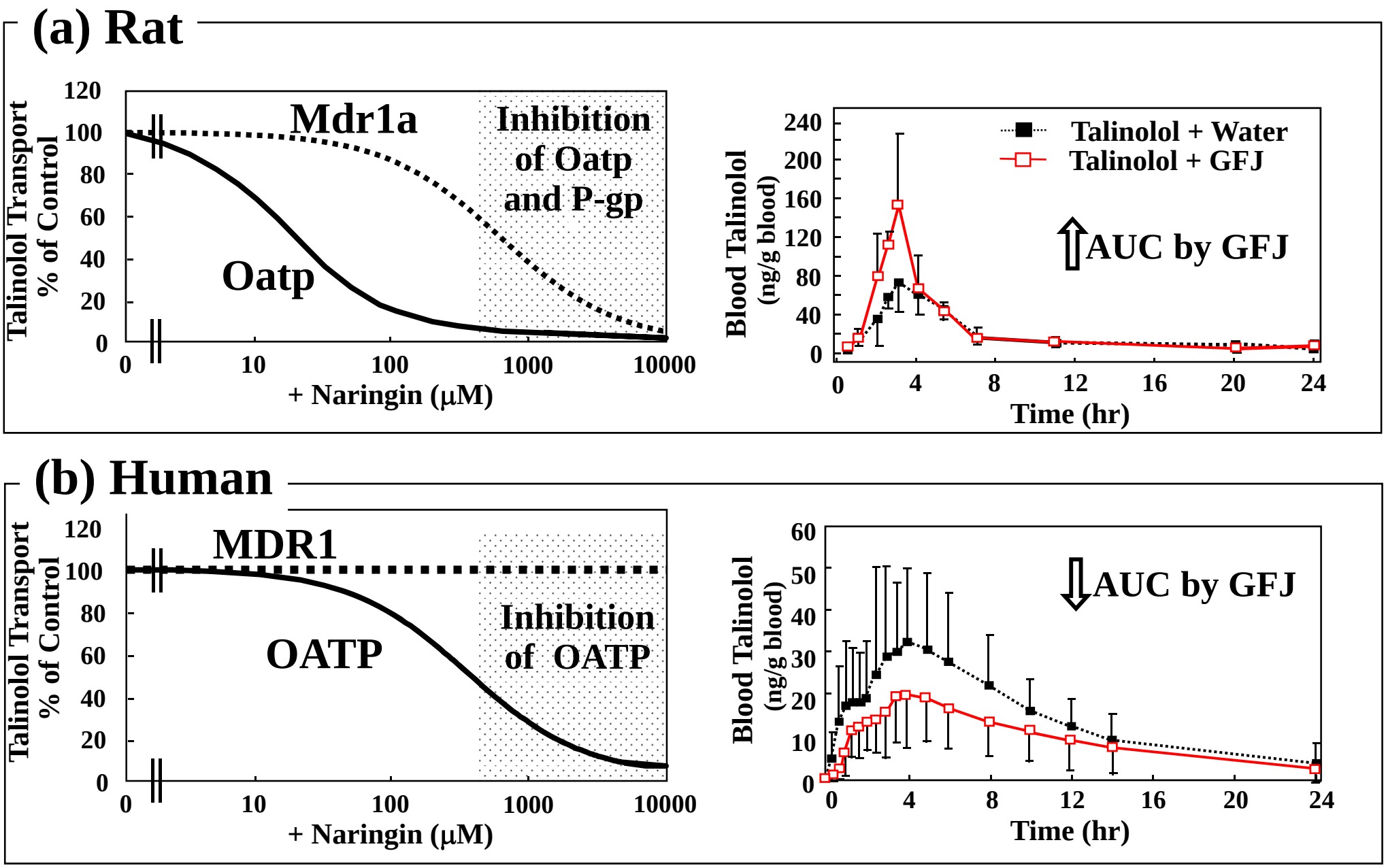

Plasma Fexofenadine (ng/mL)
500
400
300
200
100
0
0
2
4
6
8
10
12
Time after administration (hr)
:OATP2B1 *1/*1
:OATP2B1 *1/*3
:OATP2B1 *3/*3

Figure 6


(a) Rat
Talinolol Transport
% of Control
120
100
80
60
40
20
0
Mdr1a
Inhibition
of Oatp
and P-gp
Oatp
0
10
100
1000
10000
+ Naringin (μM)
Blood Talinolol
(ng/g blood)
240
200
160
120
80
40
0
Talinolol + Water
Talinolol + GFJ
AUC by GFJ
0
4
8
12
16
20
24
Time (hr)
(b) Human
Talinolol Transport
% of Control
120
100
80
60
40
20
0
MDR1
Inhibition
of OATP
OATP
0
10
100
1000
10000
+ Naringin (μM)
Blood Talinolol
(ng/g blood)
60
50
40
30
20
10
0
AUC by GFJ
0
4
8
12
16
20
24
Time (hr)