Involvement of Specific Transport System on Uptake of Lactone Form of SN-38 in Human Intestinal Epithelial Cell Line Caco-2

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The aim of this study was to elucidate the characteristics of the transport of lactone and carboxylate forms of SN-38 (SN-38L and SN-38C, respectively), a metabolite of irinotecan hydrochloride (CPT-11), with the human intestinal epithelial cell line, Caco-2. We examined SN-38L and SN-38C uptake from the apical side into Caco-2, and the effects of various compounds on the uptake of SN-38L. SN-38L and SN-38C in the cells were determined by HPLC with a fluorescence detector. When either SN-38L (0.5 μM) or SN-38C (0.5 μM) was added extracellularly at 37°C, the accumulation of SN-38L into the cells was about 10-fold higher than that of SN-38C, suggesting a dominant role of the lactone form in the uptake of SN-38 into Caco-2. The accumulation of SN-38L in Caco-2 increased time-dependently up to 10 min at 37°C, whereas the accumulation markedly decreased at 4°C. The initial uptake rate of SN-38L approached saturation at high concentrations with Michaelis–Menten constant and ‘Hill coefficient,’ 2.84±1.00 μM and 2.13±1.14, respectively (mean±S.E.). The accumulation of SN-38L was markedly inhibited by baicalin, an active ingredient of a Chinese herbal medicine, Hange-Shashin-To, as well as CPT-11. The type of inhibition by baicalin was competitive. In contrast, concomitant sulfobromophthalein, taurocholate and estrone 3-sulfate significantly increased SN-38L uptake. These results suggest that apical uptake of SN-38 by Caco-2 is dominantly performed as a lactone form through a specific transporter, which is competitively inhibited by baicalin.

Key words SN-38; lactone form; intestinal transport; Caco-2; baicalin; irinotecan

Irinotecan hydrochloride (CPT-11) is a semisynthetic water-soluble analogue of camptothecin (CPT) that has a potent antitumor activity by inhibiting topoisomerase I.¹⁾ CPT-11 exerts its antitumor activity after transformation by carboxylesterase to its more active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which has a 100- to 1000-fold more potent antitumor activity than CPT-11.^{2,3)} SN-38 is then further metabolized by uridine diphosphate glucuronosyl transferase (UGT1A1) in the liver to form an inactive metabolite, SN-38 glucuronide (SN-38G).⁴⁾ CPT-11, SN-38 and SN-38G are chemically unstable owing to their α-hydroxy-δ-lactone ring, which undergoes reversible and pH-dependent hydrolysis in aqueous solution. Furthermore, SN-38 exists preferably as the lactone form (SN-38L) under acidic conditions, whereas carboxylate form (SN-38C) under alkaline conditions.⁵⁾ Only the SN-38L has been known to be effective as a topoisomerase I inhibitor.⁶⁾

One of the major dose-limiting toxicities of CPT-11 is severe diarrhea, which is often unresponsive to common antidiarrheal agents.⁷⁾ The cytotoxicity of SN-38L to intestinal epithelial cells may be involved in the development of delayed-type diarrhea.^{8,9)} SN-38G deglucuronidation in the intestinal lumen is responsible for the production of SN-38, which causes strong cytotoxicity.¹⁰⁾ The uptake of SN-38 in intestinal epithelial cells from the apical side is considered to be strongly affected the severe diarrhea after CPT-11 administration, however, little information is available about the mechanism of intestinal transports of SN-38L and SN-38C.

The human colon adenocarcinoma cell line Caco-2 has been used as a model to study intestinal absorption or secretion of various drugs.¹¹⁾ This cell line spontaneously differentiates in culture into polarized cell monolayers with many enterocyte-like properties of transporting epithelia and also retains ATP-binding cassette (ABC) and solute carrier (SLC) transporters

expressed in the human small intestine.^{12,13)} It remained unclear regarding the influx transport of SN-38 from the apical side of the intestinal lumen. The present investigation was undertaken to clarify the characteristics of SN-38L and SN-38C accumulation in Caco-2, and the potential contribution of intestinal SN-38L transporters expressed in the apical side to their accumulation.

MATERIALS AND METHODS

Chemicals SN-38 and CPT-11 were kindly supplied by Daiichi-Sankyo Company, Limited (Tokyo, Japan). CPT, baicalin and sulfobromophthalein (BSP) were purchased from Wako Pure Chemicals (Osaka, Japan). Taurocholate was purchased from Nacalai Tesque (Kyoto, Japan). Estrone 3-sulfate was obtained from Sigma (St. Louis, MO, U.S.A.). SN-38 was dissolved in dimethyl sulfoxide (DMSO) (0.2% w/v final concentration for uptake study, 0.5% w/v final concentration for cytotoxicity and concentration-dependence study) owing to its hydrophobic property and poor solubility in water. SN-38 in DMSO was diluted into 50 mM phosphate buffer (pH 6.4) to prepare SN-38L solution. When preparing SN-38C solution, SN-38 in DMSO was diluted into 50 mM phosphate buffer (pH 9.0) and left at least 8 h at a concentration of 25 μM at 4°C. The solution was further diluted into 50 mM phosphate buffer (pH 6.4) to prepare a final solution. The final dilution procedures were performed just before starting experiments in order to minimize mutual conversion between SN-38L and SN-38C forms. All other chemicals used were of the highest grade available.

Cell Culture Caco-2 (ATCC HTB-37) were maintained by serial passage in plastic culture dishes in complete medium consisting of Dulbecco’s modified Eagle’s medium (Sigma)

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with 10% fetal bovine serum (Hyclone, Logan, UT, U.S.A.) and 1% non-essential amino acids (Invitrogen Life Technologies, Carlsbad, CA, U.S.A.) without antibiotics, in an atmosphere of 5% CO₂–95% air at 37°C. The cell monolayers were fed with fresh growth medium 1 week after seeding and following every 3rd to 4th day. Cells were used for experiments on the 10th to 15th days after seeding. Caco-2 cells used in this study were of a passage number between 33 and 53. For uptake experiments, 6-well plastic plates (Becton Dickinson) were inoculated with 18×10^4 cells in 2 mL of complete culture medium.

Uptake Studies Before performing uptake study of SN-38, we examined the stability of the lactone and carboxylate forms of 0.5 μ M SN-38 in the incubation medium at 37°C. The ratio of SN-38L to total SN-38 (SN-38L and SN-38C) in SN-38L solution at pH 6.4 was 98.9% after incubation for 15 min. The ratio of SN-38C to total SN-38 in SN-38C solution at pH 6.4 was 87.2% after incubation for 15 min.

The Caco-2 monolayers in 6-well plates were preincubated in 1 mL of incubation medium, pH 6.4 for 10 min. After removal of the medium, 1 mL of incubation medium containing 0.5 μ M SN-38L or SN-38C was added to the plates, and the medium was incubated for the indicated time periods at 37°C or 4°C. To characterize the concentration dependence of the SN-38L uptake by Caco-2, monolayers were incubated with 1 mL of incubation medium containing SN-38L (0.5–15 μ M) for 2 min at 37°C. To study the effects of various compounds on the uptake of SN-38L, the monolayers were incubated with 1 mL of incubation medium containing SN-38L (0.5 μ M) for 10 min at 37°C in the presence or absence of various compounds. At the end of the uptake period, the plates were washed 2 times in 2 mL of ice-cold incubation medium and adherent cells were scraped with a rubber policeman into 1 mL of extraction solution (50 mM KH₂PO₄ (pH 6.4):methanol=50:50). The extraction solution and cells were transferred to a fresh tube and mixed for 30 s. Then, they were centrifuged at 10600 $\times g$ for 15 min. To measure the concentration of total SN-38, 200 μ L of the supernatants was transferred to a fresh tube, and 200 μ L of 50 mM monobasic potassium phosphate (pH 2.5):methanol=1:1 and 50 μ L of 1 ng/mL CPT (internal standard) were added. After mixing for 30 s, the solution was filtered through a Millipore filter SGJVL (0.45 μ m), and then subjected to the determination of SN-38 by HPLC. To measure the concentration of SN-38L, 200 μ L of the supernatants was transferred to a fresh tube, and 500 μ L of diethylether and 50 μ L of 1 ng/mL CPT were added. After mixing for 30 s and flashing, the upper diethylether layer (400 μ L) was transferred to a fresh tube and evaporated to dryness. Four hundred microliters of 50 mM monobasic potassium phosphate (pH 2.5):methanol=1:1 was added to the tube and mixed for 30 s. The solution was filtered through a Millipore filter SGJVL (0.45 μ m). The filtrates were analyzed by HPLC. Protein was measured by the method of Bradford,¹³⁾ using Coomassie brilliant blue G-250 solution (Nacalai Tesque) with bovine γ -globulin as the standard.

Pharmacokinetic Analysis The kinetic parameters for the concentration dependence of SN-38 uptake by Caco-2 monolayers were calculated using the following equation: $V = V_{\max} S^n / (K_m^n + S^n)$, where V is the transport rate (pmol/mg protein per 2 min), S is the substrate concentration in the medium (μ M), K_m is the Michaelis–Menten constant (μ M), $V_{\max}$

is the maximum velocity by the saturable process (pmol/mg protein per 2 min), and n is the ‘Hill coefficient’ for fitting to the concentration uptake of the SN-38L curve.

Analytical Procedures An HPLC system (LC-10AD, Shimadzu, Kyoto, Japan) equipped with a fluorescence (RF-10AXL, Shimadzu, Kyoto, Japan) detector was used to measure SN-38L and SN-38C in the cells according to a study reported previously^{14,15)} with a minor modification. The column was a C8 column (250 $\times$ 4.6 mm, 5 μ m; GL Sciences, Tokyo, Japan). A mobile phase consisting of [50 mM monobasic potassium phosphate (pH 2.5), 7.5 mM tetrabutylammonium bromide]:acetonitrile=72:28 was used. The column temperature and flow rate were 40°C and 1.2 mL/min, respectively. The fluorescence detector was operated at excitation and emission wavelengths of 375 nm and 560 nm, respectively. The lower limit of quantitation for SN-38 was 0.5 nM.

RESULTS

Uptake of Lactone and Carboxylate Forms of SN-38 by Caco-2 Monolayers

The accumulation of SN-38L and SN-38C in Caco-2 monolayers was measured after administration of 0.5 μ M SN-38L and SN-38C, respectively. Figure 1 shows the time courses of the uptake of both forms of SN-38 added to the apical medium. When exposed to SN-38L solution, the uptake of SN-38L increased in a time-dependent manner and almost reached saturation at 10 min after the start of the incubation. The uptake of SN-38L was markedly decreased at 4°C compared with that at 37°C. Meanwhile, the uptake of SN-38C was about 10-fold lower than that of SN-38L.

To clarify the characteristics of SN-38L transport, the concentration dependence of SN-38L uptake by Caco-2 monolayers was measured. Figure 2 shows the concentration-dependence of uptake by Caco-2 monolayers at 37°C and 4°C for 2 min. The inset shows the result of analysis using the Michaelis–Menten equation with the ‘Hill coefficient’ for SN-38L uptake obtained by subtraction of SN-38L uptake at 4°C from that at 37°C. The data was fitted to the above equation by a nonlinear least squares regression method. The initial uptake rate of SN-38L approached saturation at high concentrations. The apparent K_m and $V_{\max}$ values for the saturable uptake of SN-38L were $2.84 \pm 1.00 \mu$ M and 35.65 ± 6.03 pmol/mg protein, respectively. In addition, the apparent ‘Hill coefficient’ ob-

Fig. 1. Time Course of Uptake of SN-38L (A) and SN-38C (B) in Caco-2 Monolayers

Caco-2 monolayers were incubated with either 0.5 μ M SN-38L (A) or 0.5 μ M SN-38C (B) for the periods specified at 37°C (○) and 4°C (●). Each value represents the mean $\pm$ S.E. of 6 monolayers from two separate experiments. *Significant difference ($p < 0.05$) between 37°C and 4°C by Student's t -test.

Fig. 2. Concentration-Dependence of SN-38L Uptake by Caco-2 Monolayers

Caco-2 monolayers were incubated at 37°C (○) or 4°C (●) for 2 min with various concentrations of SN-38L. Inset shows the result of analysis by Michaelis-Menten equation for temperature-sensitive components of SN-38L uptake obtained by subtraction of SN-38L uptake at 4°C from that at 37°C. Each symbol represents the mean ± S.E. of 6 monolayers from two separate experiments.

Fig. 3. Effects of Various Compounds on SN-38L Uptake in Caco-2 Monolayers

Caco-2 monolayers were incubated with 0.5 μM SN-38L for 5 min at 37°C in the presence or absence (control) of various compounds. Each column represents the mean ± S.E. of 6 monolayers (two separate experiments) and 3 monolayers (single experiment) for 37°C and 4°C, respectively. *** Significant difference ($p < 0.05$, $p < 0.01$, respectively) from control by Dunnett's multiple comparison test.

tained was 2.13 ± 1.14 .

Effects of Various Compounds on the Uptake of SN-38L by Caco-2 Monolayers The effects of various compounds on the uptake of 0.5 μM SN-38L in Caco-2 were examined. Compounds were added simultaneously with SN-38L into the medium at 37°C. As shown in Fig. 3, the uptake of SN-38L was significantly decreased by 1 mM CPT-11, and 0.5 mM and 1 mM baicalin. In contrast, 0.5 mM and 1 mM BSP, 1 mM taurocholate and 1 mM estrone 3-sulfate significantly increased the uptake of SN-38L.

To elucidate the inhibition mechanisms of baicalin on SN-38L uptake, we performed Dixon plot analysis for the inhibitory effects of baicalin. As shown in Fig. 4, the apparent

Fig. 4. Dixon Plots for the Inhibitory Effect of Baicalin on SN-38L Uptake

Caco-2 were incubated in 0.5 μM (○), 1.0 μM (△) or 1.5 μM (□) SN-38L in the absence or presence of the baicalin at the indicated concentrations. The level of SN-38 uptake was determined. Data are expressed as $1/\text{uptake}$ (pmol/mg protein/min). Each point represents the mean ± S.E. of 4 monolayers from two separate experiments.

inhibition constant (K_i) value was approximately 0.2 mM for baicalin, indicating that baicalin inhibited SN-38L transport in a competitive manner.

DISCUSSION

One of the major dose-limiting toxicities of CPT-11 is a severe diarrhea, which is considered to be mainly caused by SN-38. The uptake of SN-38 in the intestinal epithelial cells should be important to induce cytotoxicity, however, little is known about the transport mechanisms that contribute to the intestinal absorption of SN-38. In the present study, the characteristics of SN-38L and SN-38C transport in the Caco-2, a model of the intestinal epithelial cells, were investigated.

We mainly used 0.5 μM of SN-38L to examine characteristics of its transport into Caco-2. This concentration was employed because the reported clinical plasma concentrations of SN-38 are mostly between 0.02 μM and 0.22 μM.¹⁶⁾ In the preliminary experiments, we evaluated the cytotoxicity of SN-38 in the Caco-2 monolayers. We observed no cytotoxicity of the cells by the exposure to 5 μM SN-38L for 2 min, although the number of floating cells and the amount of lactate dehydrogenase released into culture medium were significantly elevated by the exposure of the cells for 24 h at the concentrations higher than 10 μM and 5 μM, respectively (data not shown). Therefore, 0.5 μM SN-38 did not affect the viability of the cells.

Compared with the uptake into Caco-2 of 0.5 μM SN-38L and 0.5 μM SN-38C at 37°C. The uptake of SN-38L into Caco-2 was about 10-fold higher than that of SN-38C at 37°C. Because the main form responsible to SN-38 uptake into Caco-2 is SN-38L, and SN-38L is more cytotoxic than SN-38C, we focused on SN-38L. The accumulation of SN-38L in the cells increased time-dependently and was markedly low at 4°C compared with that at 37°C (Fig. 1). The accumulation of SN-38L at 2 min approached saturation at a concentration higher than 5 μM (Fig. 2). These findings clearly suggest that a specific transporter(s) is (are) involved in the SN-38L influx into Caco-2 cells. When kinetics were analyzed using the Michaelis-Menten equation containing the 'Hill coefficient,'

the coefficient value obtained was about 2, suggesting the possibility of cooperative transport of SN-38L into the Caco-2, such as the coupling of two molecules of SN-38L when they are transported across plasma membranes of Caco-2 from the apical side. Although the influx transport of SN-38L was not concentrative at 37°C, the characteristics of SN-38L transport such as temperature dependence, concentration dependence, kinetics and inhibitory characteristics shown in the present study suggested the involvement of influx transporters. However, it is also likely that the apparent sigmoidal shape of the concentration-dependence curve is attributable to the presence of an efflux transporter with a higher affinity than that of the specific influx transporter(s) for SN-38L expressed on the apical membranes of Caco-2.

In the clinical studies, it was reported that the coadministration of Hange-Shashin-To, a Chinese herbal medicine that contains baicalin, a β -glucuronidase inhibitor, prevented the onset of CPT-11-induced diarrhea.⁹ Thus, the medicine is used in the clinical settings in Japan in order to ameliorate or prevent CPT-11-induced diarrhea through the prevention of deglucuronidation of SN-38G.⁹ In the present study, SN-38L uptake into Caco-2 was inhibited by CPT-11 as well as baicalin. The SN-38 was reported to be the specific substrate of organic anion transporter polypeptide 1B1 (OATP1B1).¹⁷ Furthermore, it has been reported that baicalin¹⁸ and CPT-11¹⁹ have a potential for an inhibitor of OATPs. In Caco-2 cells, mRNAs of the uptake transporters such as OATP2A1 and OATP2B1 were found to be expressed, however, that of OATP1B1 were not found.^{12,20} These facts led to the impression that SN-38L was transported by OATP2A1 or OATP2B1, which is inhibited by baicalin and CPT-11. Because baicalin and SN-38 do not share chemical structures with each other, it is likely that the transporter(s) mediating apical influx of SN-38L may have multispecific characteristics.

Our study indicated that BSP, the substrate of organic anion transporter, added to the same side significantly increased SN-38L accumulation in the cells, that was contrary to the study by Itoh *et al.*⁸) and Itagaki.²¹) Similarly as BSP, taurocholate and estrone 3-sulfate, the substrates of organic anion transporters, increased the accumulation of SN-38L in Caco-2. The concentrations of SN-38 used by Itoh *et al.* was 25 μ M SN-38, whereas we used 0.5 μ M SN-38L for uptake experiments; it is near the clinical concentration reported.¹⁶) Therefore, apparent discrepancy between the results by Itoh *et al.* and ours seems to be due to the difference of SN-38 concentration. SN-38 appears to be transported by a low affinity, and presumably high capacity, influx transporter as well as a high affinity, and presumably low capacity, efflux transporter. In other words, BSP seems to dominantly inhibit the efflux transporter rather than influx transporter, when low concentrations of SN-38L were exposed, and thereby increased apparent accumulation of SN-38. Taurocholate and estrone 3-sulfate were reported to be specific substrates of OATPs.²²) It was reported that estrone 3-sulfate was transported by OATP1B1 and was inhibited by SN-38.²³) In the present study, estrone 3-sulfate did not decrease apparently the accumulation of SN-38L into Caco-2 cells. Therefore, the transporter(s) mediating SN-38 influx into the cells might have higher affinity for SN-38 than estrone 3-sulfate. In addition, taurocholate and estrone 3-sulfate were reported to inhibit Breastcancer resistance protein (BCRP).²⁴) Because SN-38L is transported by BCRP, it is reasonable to

deduce that SN-38L accumulation into the cells increased due to inhibition of the efflux transport of SN-38L by taurocholate and estrone 3-sulfate.

In conclusion, the present study demonstrates that SN-38 uptake from apical side of Caco-2 cells dominantly undergoes as a lactone form through a specific transporter(s), which is sensitive to temperature and inhibited by baicalin as well as CPT-11 in a competitive manner.

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REFERENCES

- 1) Kuhn JG. Pharmacology of irinotecan. *Oncology* (Huntington), **12** (Suppl. 6), 39—42 (1998).
- 2) Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res.*, **51**, 4187—4191 (1991).
- 3) Kojima A, Shinkai T, Saijo N. Cytogenetic effects of CPT-11 and its active metabolite, SN-38 on human lymphocytes. *Jpn. J. Clin. Oncol.*, **23**, 116—122 (1993).
- 4) Atsumi R, Suzuki W, Hakusui H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. *Xenobiotica*, **21**, 1159—1169 (1991).
- 5) Fassberg J, Stella VJ. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. *J. Pharm. Sci.*, **81**, 676—684 (1992).
- 6) Wani MC, Ronman PE, Lindley JT, Wall ME. Plant antitumor agents. 18. Synthesis and biological activity of camptothecin analogues. *J. Med. Chem.*, **23**, 554—560 (1980).
- 7) Abigeres D, Armand JP, Chabot GG, Da Costa L, Fadel E, Cote C, Hérait P, Gandia D. Irinotecan (CPT-11) high-dose escalation using intensive high-dose loperamide to control diarrhea. *J. Natl. Cancer Inst.*, **86**, 446—449 (1994).
- 8) Itoh T, Itagaki S, Sumi Y, Hirano T, Takemoto I, Iseki K. Uptake of irinotecan metabolite SN-38 by the human intestinal cell line Caco-2. *Cancer Chemother. Pharmacol.*, **55**, 420—424 (2005).
- 9) Mori K, Kondo T, Kamiyama Y, Kano Y, Tominaga K. Preventive effect of Kampo medicine (Hangeshashin-to) against irinotecan-induced diarrhea in advanced non-small-cell lung cancer. *Cancer Chemother. Pharmacol.*, **51**, 403—406 (2003).
- 10) Tukey RH, Strassburg CP, Mackenzie PI. Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. *Mol. Pharmacol.*, **62**, 446—450 (2002).
- 11) Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology*, **96**, 736—749 (1989).
- 12) Maubon N, Le Vee M, Fossati L, Audry M, Le Ferrec E, Bolze S, Fardel O. Analysis of drug transporter expression in human intestinal Caco-2 cells by real-time PCR. *Fundam. Clin. Pharmacol.*, **21**, 659—663 (2007).
- 13) Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248—254 (1976).
- 14) Irie M, Terada T, Okuda M, Inui K. Efflux properties of basolateral peptide transporter in human intestinal cell line Caco-2. *Pflugers Arch.*, **449**, 186—194 (2004).
- 15) Itoh T, Takemoto I, Hata Y, Takaoka K, Sugawara M, Iseki K, Miyazaki K. Determination of the lactone forms and carboxylate forms of irinotecan and its active metabolite, glucuronide by high-performance liquid chromatography. *Jpn. J. Ther. Drug Monit.*, **17**,

- 383—389 (2000).
- 16) Takasuna K, Hagiwara T, Hirohashi M, Kato M, Nomura M, Nagai E, Yokoi T, Kamataki T. Inhibition of intestinal microflora beta-glucuronidase modifies the distribution of the active metabolite of the antitumor agent, irinotecan hydrochloride (CPT-11) in rats. *Cancer Chemother. Pharmacol.*, **42**, 280—286 (1998).
- 17) Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I. Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: *in vitro* evidence and effect of single nucleotide polymorphisms. *Drug Metab. Dispos.*, **33**, 434—439 (2005).
- 18) Fan L, Zhang W, Guo D, Tan ZR, Xu P, Li Q, Liu YZ, Zhang L, He TY, Hu DL, Wang D, Zhou HH. The effect of herbal medicine baicalin on pharmacokinetics of rosuvastatin, substrate of organic anion-transporting polypeptide 1B1. *Clin. Pharmacol. Ther.*, **83**, 471—476 (2008).
- 19) Oostendorp RL, van de Steeg E, van der Kruijsen CM, Beijnen JH, Kenworthy KE, Schinkel AH, Schellens JH. Organic anion-transporting polypeptide 1B1 mediates transport of Gimatecan and BNP1350 and can be inhibited by several classic ATP-binding cassette (ABC) B1 and/or ABCG2 inhibitors. *Drug Metab. Dispos.*, **37**, 917—923 (2009).
- 20) Rodrigues AC, Curi R, Genvigir FD, Hirata MH, Hirata RD. The expression of efflux and uptake transporters are regulated by statins in Caco-2 and HepG2 cells. *Acta Pharmacol. Sin.*, **30**, 956—964 (2009).
- 21) Itagaki S. Intestinal absorption and secretion mechanism of carboxylate drugs. *Yakugaku Zasshi*, **129**, 1341—1349 (2009).
- 22) Abe T, Kakyō M, Tokui T, Nakagomi R, Nishio T, Nakai D, Nomura H, Unno M, Suzuki M, Naitoh T, Matsuno S, Yawo H. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J. Biol. Chem.*, **274**, 17159—17163 (1999).
- 23) Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I. Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: *in vitro* evidence and effect of single nucleotide polymorphisms. *Drug Metab. Dispos.*, **33**, 434—439 (2005).
- 24) Imai Y, Asada S, Tsukahara S, Ishikawa E, Tsuruo T, Sugimoto Y. Breast cancer resistance protein exports sulfated estrogens but not free estrogens. *Mol. Pharmacol.*, **64**, 610—618 (2003).