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

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Received August 19, 2004; accepted December 16, 2004

ABSTRACT:

Irinotecan hydrochloride (CPT-11) is a potent anticancer drug that is converted to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), and other metabolites in liver. The disposition and gastrointestinal toxicity of irinotecan exhibit a wide interpatient variability. Here, we examined the contribution of an organic anion-transporting polypeptide, OATP1B1 (OATP-C), which transports a variety of drugs and their metabolites from blood to liver in humans, to the hepatic disposition of irinotecan, SN-38, and its glucuronide conjugate (SN-38G) by using HEK293 cells stably transfected with SLCO1B1*1a (OATP-C*1a) coding wild-type OATP1B1. We further examined the effect of single nucleotide polymorphisms in OATP1B1 by measuring uptake activity in Xenopus oocytes expressing OATP1B1*1a and three common variants. In all cases, transport activity for SN-38 was observed, whereas irinotecan and SN-38G were not transported. Moreover, SN-38 exhibited a significant inhibitory effect on OATP1B1-mediated uptake of [3H]estrone-3-sulfate. Among the variants examined, OATP1B1*15 (N130D and V174A; reported allele frequency 10–15%) exhibited decreased transport activities for SN-38 as well as pravastatin, estrone-3-sulfate, and estradiol-17β-glucuronide. This study is the first to yield evidence that OATP1B1 is involved in the hepatic disposition of SN-38 and that genetic polymorphisms of OATP1B1 may contribute to the known interpatient variability in disposition of irinotecan.

Irinotecan is a derivative of camptothecin, an antitumor alkaloid isolated from Camptotheca acuminata and an inhibitor of topoisomerase I (Fig. 1). The disposition and adverse effects of irinotecan exhibit a marked interpatient variability. Irinotecan is converted by carboxylesterase to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which has a 100- to 1000-fold higher antitumor activity than irinotecan itself. SN-38 is subjected to glucuronidation by hepatic UDP-glucuronosyltransferase to afford SN-38 glucuronide (SN-38G), but SN-38G excreted into bile is again deconjugated to SN-38 by β-deglucuronidase in the intestinal lumen (Mathijsen et al., 2001). Thus, we need to consider many factors as possible causes of the interindividual variations of the pharmacological and toxicological effects of irinotecan.

Many researchers have suggested that functional alterations of several factors affect the metabolism and disposition of irinotecan. For example, carboxylesterase activity in proliferating tissues and cell lines is associated with chemosensitivity to irinotecan (van Ark-Over et al., 1998). In patients with Gilbert’s syndrome and Crigler-Najjer type I syndrome, a UDP-glucuronosyltransferase (UGT1A1) gene mutation, which is associated with a decreased activity, results in impaired detoxification of SN-38 to SN-38G and an increased risk of irinotecan-induced toxicity (Monaghan et al., 1996; Iyer et al., 1998). Moreover, the reduction of β-glucuronidase activity by antibiotics leads to a lower concentration of SN-38 in the intestine, with a consequent reduction of intestinal toxicity (Takasuna et al., 1996). As regards transporters, multidrug resistance-associated protein (MRP) and P-glycoprotein (P-gp), expressed at the bile canalicular membrane, appear to be responsible for the biliary excretion of irinotecan, SN-38, and SN-38G (Chu et al., 1997a,b, 1998). The inhibition of biliary secretion via MRP and/or P-gp by cyclosporin A and probenecid increases the plasma concentration and prolongs the terminal disposition half-life of irinotecan and its metabolites (Gupta et al., 1996; Horikawa et al., 2002; Arimori et al., 2003). Moreover, a single nucleotide polymorphism (SNP) in the MDR1 gene was found to be associated with increased exposure to irinotecan and SN-38 (Mathijsen et al., 2003). Recently, breast cancer resistance protein (BCRP) was reported to play a role in the efflux of SN-38 from cancer cells, leading to the acquisition of resistance to anticancer drugs (Kawabata et al., 2001; Doyle and Ross, 2003), and Imai et al. (2002) suggested that the SNP (C421A) in BCRP was associated with a low expression level. The inhibition of BCRP and reduced resistance to SN-38. These results suggested the importance of metabolic and transport processes of irinotecan on its efficacy or adverse effects. The hepatic basolateral uptake process

ABBREVIATIONS: SN-38, 7-ethyl-10-hydroxycamptothecin; OATP, organic anion-transporting polypeptide; MRP, multidrug resistance-associated protein; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; SNP, single nucleotide polymorphism; SN-38G, SN-38 glucuronide; CPT-11, irinotecan hydrochloride; cRNA, complementary RNA; HPLC, high-performance liquid chromatography.
should also be important in the drug disposition and metabolism. However, the transporter that mediates transfer of irinotecan and/or its metabolites from the systemic circulation into hepatocytes has not been identified.

The aim of the present study was, therefore, to identify the transporter(s) involved in the hepatic uptake of irinotecan and its metabolites. Among various transporters expressed in human hepatocytes, we focused on organic anion-transporting polypeptide OATP1B1 (formerly termed OATP-C; gene symbol SLCO1B1). Many members of the OATP family have been identified and functionally analyzed. Among them, OATP1B1 is specifically expressed at the basolateral membrane of human hepatocytes and is involved in hepatic handling of a variety of endogenous and xenobiotic compounds, including estrone-3-sulfate, estradiol-17β-glucuronide, and pravastatin, whereas OATP2B1 (formerly termed OATP-B) and OATP1B3 (formerly termed OATP8), which are also expressed at the hepatic basolateral membranes, exhibit a comparatively restricted substrate spectrum and lower expression, respectively (Hsiang et al., 1999; König et al., 2000; Abe et al., 2001; Kobayashi et al., 2003; Nozawa et al., 2004).

Moreover, many genetic variants of OATP1B1 have been found, and some of them exhibited altered uptake activity in the in vitro studies using transfected cells (Tirona et al., 2001, 2003; Nozawa et al., 2002; Nishizato et al., 2003). In the present study, to clarify the involvement of OATP1B1 in hepatic handling of irinotecan, we examined, first, the transport of irinotecan and its metabolites in HEK293 cells stably transfected with SLCO1B1*1a and, second, the uptake of these and other substrates in Xenopus oocytes expressing each of the OATP1B1 genetic variants.

Materials and Methods

Materials. [3H]Estrone-3-sulfate, ammonium salt (1702.0 GBq/mmol) and [3H]estradiol-17β-glucuronide (1665.0 GBq/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [14C]Pravastatin (529.1 MBq/mmol) was kindly supplied by Sankyo Co. (Tokyo, Japan). pcDNA3 vector was obtained from Invitrogen (Carlsbad, CA). Irinotecan, SN-38, and SN-38G were kindly provided by the Yakult Honsha Co. Ltd. (Tokyo, Japan). HEK293 cells were obtained from Health Science Research Resources Bank (Tokyo, Japan). Xenopus were purchased from Saitama Experimental Animal Supply Co. Ltd. (Saitama, Japan). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan).

Transport Experiments in HEK293 Cells. For the transport experiments, HEK293 cells were transfected with SLCO1B1*1a using plasmid vector pcDNA3 (pcDNA3/SLCO1B1*1a) to establish stably OATP1B1-expressing cells. After 3 weeks of G418 selection (600 μg/ml), single colonies were screened for SLCO1B1 expression by a polymerase chain reaction method and also for [3H]estrone-3-sulfate transport activity. HEK293 cells transfected with pcDNA3 vector alone were used to obtain the background activity (termed mock). Transfected HEK293 cells were routinely grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, penicillin, streptomycin, and G418 in a humidified incubator at 37°C under 5% CO2. Sodium butyrate (5 mM) was added to the culture medium 24 h before the uptake study (Cui et al., 1999). For the uptake measurement, HEK293 cells were harvested and suspended in uptake medium containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM Na-gluconate, 1.2 mM CaCl2, 1.2 mM KH2PO4, 12 mM MgSO4, and 25 mM HEPES, adjusted to pH 7.4. Irinotecan, SN-38, and SN-38G were dissolved in 50 mM phosphate buffer (pH 9.0) and left overnight at 1 mM as stock solution. HEK293 cells were routinely grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, penicillin, streptomycin, and G418 in a humidified incubator at 37°C under 5% CO2. Sodium butyrate (5 mM) was added to the culture medium 24 h before the uptake study (Cui et al., 1999). For the uptake measurement, HEK293 cells were harvested and suspended in uptake medium containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM Na-gluconate, 1.2 mM CaCl2, 1.2 mM KH2PO4, 12 mM MgSO4, and 25 mM HEPES, adjusted to pH 7.4. Irinotecan, SN-38, and SN-38G were dissolved in 50 mM phosphate buffer (pH 9.0) and left overnight at 1 mM as stock solution. For uptake study, the stock solutions were diluted by uptake medium (pH 7.4) to the appropriate concentrations. The cell suspension was precultured at 37°C for 20 min in the uptake medium, then centrifuged, and the resultant cell pellets were mixed with the uptake medium containing a test compound to initiate uptake. At appropriate times, aliquots of the mixture were withdrawn and the cells were separated from the uptake medium by centrifugation through a layer of a mixture of silicone oil (SH550; Toray Dow Corning Co., Tokyo, Japan) and liquid paraffin (Wako Pure Chemical Industries), with a density of 1.03 on 3 M KCl solution. One hundred fifty microliters of 0.1 M HCl was added to each cell pellet in 3 M KCl, and the cells were solubilized by sonication and deproteinized by adding an equal volume of methanol, followed by centrifugation for 5 min at 15,000 rpm. The resultant supernatant was
evaporated to dryness and dissolved in phosphate buffer (pH 3.0) for quantitation by HPLC. In the present study, we obtained HEK293 cells that stably expressed OATP1B1 and exhibited approximately 2-fold higher uptake of \[^{[3]H}\]estrone-3-sulfate at 3 min compared with the mock cells.

**Expression of OATPs in Xenopus Oocytes.** cDNA of SLCO1B1*1a, SLCO1B1*1b, SLCO1B1*5, SLCO1B1*15, SLCO1B3, or SLCO2B1 was sub-cloned into pcDNA3 vector for the synthesis of complementary RNA (cRNA). For transport experiments, Xenopus oocytes were injected with in vitro synthesized cRNA of each SLCO1B1 variant, SLCO1B3, or SLCO2B1 using T7 RNA polymerase as previously described (Tamai et al., 2001). Briefly, for standard experiments, defolliculated oocytes were injected with 50 nl of water for RNA polymerase as previously described (Tamai et al., 2001). Briefly, for standard experiments, defolliculated oocytes were injected with 50 nl of water containing 25 ng of cRNA, cultured for 3 days in modified Barth’s solution [96 mM NaCl, 1 mM KCl, 2.4 mM NaHCO\(_3\), 0.82 mM MgSO\(_4\), 0.33 mM Ca(NO\(_3\))\(_2\), 0.41 mM CuCl\(_2\), and 10 mM HEPES, adjusted to pH 7.4] and used for the uptake experiments. Uptake was initiated by incubating the oocytes at 25°C in modified Barth’s solution containing a test compound. At appropriate times, the oocytes were washed with ice-cold modified Barth’s solution. For quantitation of test compounds, the oocytes were solubilized in 5% sodium dodecyl sulfate solution for radioactive compounds. For nonradioactive compounds, the cells were sonicated in 0.1 M HCl, mixed with methanol to make 50% final concentration, and centrifuged, and the resultant supernatant was analyzed by HPLC. For the control, the same volume of water was injected into oocytes, incubated in modified Barth’s solution in the same manner as OATP-expressing cells, and used for the evaluation of background uptake activity.

**Analytical Methods.** Cellular protein content was determined according to the method of Bradford (1976) by using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard. Irinotecan, SN-38, and SN-38G were quantified by HPLC using an ODS 80Ts reversed-phase analytical column (4.6 mm × 15 cm; Tosoh, Tokyo, Japan) as previously described (Sparreboom et al., 1998). Briefly, the mobile phase was methanol/0.1 M ammonium acetate containing 10 mM tetrabutylammonium (40:60) and was used at a flow rate of 1.0 ml/min. The analytical column was kept at 40°C, and the eluent was monitored with a fluorescence detector (Tosoh, model FS-8010) at excitation and emission wavelengths of 380 and 556 nm for irinotecan and SN-38, and 370 and 430 nm for SN-38G, respectively. The uptake of radioactive compounds was quantified with a liquid scintillation counter (LSC-5100; Aloka, Tokyo, Japan). All data are expressed as means ± S.E.M. of at least three experiments, and statistical analysis was performed by the use of Student’s t test with p < 0.05 as the criterion of significance. Cell-to-medium ratio was obtained by dividing the cellular uptake amount by the concentration of test compound in the uptake medium.

**Results**

**Uptake of Irinotecan, SN-38, and SN-38G by OATP1B1 Expressed in HEK293 Cells.** To clarify whether OATP1B1 transports irinotecan, SN-38, and SN-38G, we performed an uptake study using HEK293 cells stably transfected with SLCO1B1*1a. The time courses of the uptake of irinotecan, SN-38, and SN-38G at 10 μM are shown in Fig. 2. The uptake of SN-38 by HEK293 cells expressing OATP1B1 was increased significantly compared with that by mock cells after 5 min. In contrast, the uptake of irinotecan and SN-38G by HEK293 cells expressing OATP1B1 was comparable with those by mock cells.

**Inhibitory Effects of Irinotecan, SN-38, and SN-38G on OATP1B1.** The affinity of OATP1B1 for irinotecan, SN-38, and SN-38G was evaluated in terms of the inhibitory effect on estrone-3-sulfate uptake by Xenopus oocytes injected with cRNA of SLCO1B1*1a. Oocytes injected with 25 ng of cRNA of SLCO1B1*1a showed significantly increased uptake of \[^{[3]H}\]estrone-3-sulfate compared with that by water-injected oocytes (OATP1B1, 5.75 ± 0.37 μl/oocyte; water-injected, 0.05 ± 0.007 μl/oocyte). As shown in Fig. 3, SN-38 at 1 and 10 μM, and irinotecan at 10 μM significantly inhibited OATP1B1-mediated uptake of \[^{[3]H}\]estrone-3-sulfate, whereas SN-38G was not inhibitory.

**Uptake of SN-38 by OATP1B1, OATP2B1, or OATP1B3 in Xenopus Oocytes.** Since OATP2B1 and OATP1B3, as well as OATP2B1 were expressed at the basolateral membrane of human hepatocytes, we evaluated their transport activity for SN-38. The uptake of SN-38 by Xenopus oocytes expressing OATP1B1, OATP2B1, or OATP1B3 was measured for 60 min (Fig. 4). The uptake of SN-38 by oocytes expressing OATP1B1 was significantly greater than that by water-injected oocytes, whereas the uptake by oocytes expressing OATP2B1 or OATP1B3 is comparable with that by water-injected oocytes. These results demonstrated that SN-38 is transported only by OATP1B1 among OATP transporters expressed at basolateral membrane of human hepatocytes, and OATP1B1 may transport SN-38 from blood to liver and/or liver to blood.

**Transport Activity of OATP1B1 Variants and Gene-Dose Effect.** For the evaluation of SNP variants, the expression level of each

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**Fig. 2.** Time courses of uptake of irinotecan, SN-38, and SN-38G by HEK293 cells expressing OATP1B1. Uptake of irinotecan and its metabolites (10 μM) by HEK293 cells stably transfected with SLCO1B1*1a (closed symbols) or mock (open symbols) over 40 min was measured at 37°C and pH 7.4. Triangles, circles, and squares represent the uptake of irinotecan, SN-38, and SN-38G, respectively. Each result represents the mean ± S.E.M. (n = 3 or 4) and * indicates a significant difference from the uptake by mock (p < 0.05).

**Fig. 3.** Inhibitory effects of irinotecan and its metabolites on uptake of \[^{[3]H}\]estrone-3-sulfate by Xenopus oocytes expressing OATP1B1. Uptake of \[^{[3]H}\]estrone-3-sulfate (9.2 nM) by Xenopus oocytes injected with cRNA of SLCO1B1*1a was measured at 25°C for 30 min. The results are shown as a percentage of control uptake measured in the absence of inhibitor after subtracting the uptake of water-injected oocytes. The inhibitor concentrations are indicated by the numbers in the figure. Each column represents the mean ± S.E.M. (n = 6–10) and * indicates a significant difference from the control (p < 0.05).
variant protein in the in vitro cultured cells is important. In the present study, the uptake activity of OATP1B1 by Xenopus oocytes was evaluated by introducing 25 ng of cRNA from each SLCO1B1 variant in 50 nl of water into each oocyte. However, apparent transport activity is affected by the amount of protein expressed as a result of introduction of each SLCO1B1 variant, and the activity should be evaluated under conditions where stable expression levels can be maintained for all the SNP variants. Therefore, we examined the effect of doses of cRNA on the apparent uptake activity by changing the cRNA concentration in the range from 0.2 to 1.0 μg/μl (10–50 ng/oocyte). As shown in Fig. 5, the uptake activity of \(^{[3]H}\)estrone-3-sulfate by OATP1B1*1a initially increased with the dose of injected cRNA and attained the apparent maximum at a dose of 25 ng of cRNA/oocyte. Moreover, the uptake activity of OATP1B1*15 was dose dependently increased in the same manner as that of OATP1B1*1a, although the uptake activity was about half that of OATP1B1*1a. Since the expressed amount of transporter protein is apparently saturated and thus comparable at higher doses of cRNA, the difference in uptake among OATP1B1 variants is concluded to be due to a difference in the intrinsic transport activity rather than a difference in the expressed level of transporter. Therefore, in the following studies, the intrinsic activities of OATP1B1 variants were compared in Xenopus oocytes injected with 25 ng of cRNA.

To evaluate the effect of SNPs on the uptake activities of OATP1B1, the uptake of SN-38 (10 μM) \(^{[14]C}\)pravastatin (60 μM), \(^{[3]H}\)estrone-3-sulfate (12 nM), and \(^{[3]H}\)estradiol-17β-glucuronide (20 nM) by OATP1B1 variants was measured for 60, 120, 30, and 120 min, respectively (Fig. 6). Each cRNA was injected at 25 ng/oocyte. OATP1B1*1a provided significantly increased uptake of all the test compounds compared with water-injected oocytes. The uptake by OATP1B1*1a and OATP1B1*1b was comparable for all compounds tested. Although the difference in uptake between OATP1B1*1a and OATP1B1*5 was not statistically significant for any compound examined, OATP1B1*5 tended to show lower uptake activities than OATP1B1*1a. On the other hand, the uptake activities of OATP1B1*15 were significantly decreased to approximately 50% of those of OATP1B1*1a. Therefore, patients with SLCO1B1*15 may exhibit lower hepatic extractions of various clinically used drugs, such as SN-38 and pravastatin, and this may be one factor contributing to interpatient variability of the drug effects.

**Discussion**

We examined the transport of irinotecan and its metabolites by the hepatic organic anion transporter, OATP1B1, and the effect of SNPs in OATP1B1 on the transport activity, as a part of our studies to elucidate the causes of the interpatient variability in the pharmacological and toxicological effects of irinotecan. Several transporters are involved in the biliary excretion of irinotecan and its metabolites across the bile canalicular membrane of hepatocytes and the efflux of SN-38 from cancer cells, including P-gp, MRP1, MRP2, and BCRP (Chu et al., 1997a,b, 1998; Doyle and Ross, 2003). MRP2 and P-gp expressed at the bile canalicular membrane are responsible for the biliary excretion of irinotecan, SN-38, and SN-38G. Moreover, BCRP plays a role in the efflux of SN-38 from cancer cells, leading to the acquisition of drug resistance (Kawabata et al., 2001). Thus, several
transporters play important roles in the disposition of irinotecan, although the transporter responsible for hepatic uptake of irinotecan from blood has not been identified. In the present study, we, first of all, demonstrated that OATP1B1 transports SN-38, the active metabolite of irinotecan, but not irinotecan itself or other metabolites, since OATP1B1 is abundantly expressed in liver and exhibits broad substrate selectivity (Hsiang et al., 1999; König et al., 2000). Moreover, it was suggested that OATP1B3 and OATP2B1, which are also expressed at the basolateral membrane of human hepatocytes and transport various organic anions, are unlikely to be involved in the hepatic disposition of SN-38. After i.v. administration, irinotecan was excreted into both urine and feces, and SN-38G mainly into urine. In contrast, the cumulative fecal excretion of SN-38 was 8.24% of the dose, which is much higher than the renal excretion (0.18–0.43%) (Mathijssen et al., 2001). Since OATP1B1 cannot transport irinotecan or SN-38G, SN-38 may be preferentially taken up by liver via OATP1B1 expressed at the basolateral membrane of human hepatocytes.

The plasma concentration of irinotecan was much higher than that of SN-38, and irinotecan is metabolized mainly in liver to SN-38 (Mathijssen et al., 2001). Therefore, to understand the overall pharmacokinetics of irinotecan, the hepatic uptake mechanism of irinotecan itself is important, although, since OATP1B1 did not transport irinotecan itself, other transporter(s) may be involved in its hepatic disposition. In contrast, the disappearance of SN-38 from the systemic circulation should be mediated by OATP1B1. Since SN-38 is produced in the liver, it is also possible that SN-38 is effluxed out of liver cells to the blood via OATP1B1. We have preliminary evidence for the bidirectional transport of OATP1B1. The efflux of estrone-3-sulfate from the Xenopus oocytes tended to be increased by the expression of OATP1B1, although further studies should be required to be confirmative. Moreover, it was reported that coadministration of cyclosporin A, which is known as an inhibitor of MRP2 and P-gp, elevated the plasma concentration and the hepatic accumulation of SN-38 after i.v. administration of irinotecan to rats, suggesting the inhibition of the efflux by MRP2 and P-gp (Arimori et al., 2003). However, it may also be possible that the increase of plasma concentration and hepatic accumulation of SN-38 is caused by inhibition of OATP1B1-mediated hepatic uptake and/or efflux of SN-38 by cyclosporin A, since OATP1B1 is strongly inhibited by cyclosporin A (10 μM) (Nozawa et al., 2003). Thus, since OATP1B1 could be involved in the hepatic disposition of SN-38, the effect of genetic polymorphisms of OATP1B1 on SN-38 transport should be important for the clarification of the cause of interpatient variability in efficacy and adverse effects of irinotecan. Investigation of genetic polymorphisms of OATP1B1 has uncovered many variants so far. Among them, we focused on SLC01B1*1a, SLC01B1*1b, SLC01B1*5, and SLC01B1*15, since their allele frequencies are comparatively high at 32.5 to 35.2%, 45.8 to 53.7%, 0 to 14.0%, and 10.3 to 15.0%, respectively, and some of them may affect the pharmacokinetics of pravastatin (Tirona et al., 2001; Nozawa et al., 2002; Nishizato et al., 2003; Mwinyi et al., 2004). Therefore, attention should be given to the effects of these variants should be clinically relevant.

To assess the effect of SNPs on transport activity, we first evaluated the intrinsic activity of the SNP variants using Xenopus oocytes by changing the injected amount of each cRNA. Since the transport activities of OATP1B1*1a and OATP1B1*15 were saturated over 25 ng of cRNA injected (Fig. 5), the expressed amounts of protein were considered to be saturated. Therefore, the uptake activity of each variant in Xenopus oocytes injected with a 25-ng dose of cRNA should represent the intrinsic activity, because the expression level is expected to be stable and maximal. Since the uptake of 3H]estrone-3-sulfate, which is a typical substrate of OATP1B1, by OATP1B1*1a was approximately 2-fold greater than that by OATP1B1*15 (statistically significant difference), the intrinsic activity of OATP1B1*15 appears to be lower than that of OATP1B1*1a.

We next evaluated the uptake activity of OATP1B1 variants for SN-38, pravastatin, estrone-3-sulfate, and estradiol-17β-glucuronide. The intrinsic activity expressed at the dose of 25 ng of cRNA by SLC01B1*15 was lower than that by SLC01B1*1a for all of the tested compounds. This is the first direct demonstration that the overall activity of OATP1B1*15 is lower than that of OATP1B1*1a in vitro cultured cells using Xenopus oocytes expressing OATP1B1. These results support the previous proposal that SLC01B1*15 is likely to be associated with altered pharmacokinetics of pravastatin (Nishizato et al., 2003) and further suggest that it may influence the pharmacokinetic properties of SN-38, as well. In the case of OATP1B1*5, the present study suggested a decrease in the uptake activity for all tested compounds, although the difference between OATP1B1*1a and OATP1B1*5 was not statistically significant. These results are consistent with the previous findings that OATP1B1*5 showed reduced fractional plasma membrane expression, diminished transport activity of estrone-3-sulfate, estradiol-17β-glucuronide, and rifampin in HeLa cells, and decreased uptake activity of estrone-3-sulfate after correction for the expressed amount of the transporter protein in HEK293 cells (Nozawa et al., 2002; Tirona et al., 2001, 2003). Recently, it was reported that SLC01B1*5 carriers had a higher oral bioavailability of pravastatin in comparison with SLC01B1*1a subjects (Mwinyi et al., 2004). This result is also consistent with the previous and present observations (Nozawa et al., 2002; Tirona et al., 2003). Moreover, the transport activity of OATP1B1 variants may be substrate-dependent, since the transport activities of OATP1B1*1b for rifampin and taurocholate were decreased, and the activity for sulfobromophthalein was increased, whereas those for estrone-3-sulfate and estradiol-17β-glucuronide were not changed (Michalski et al., 2002; Nozawa et al., 2002; Tirona et al., 2003). The present study, however, did not show substrate-dependent alteration, and consistent results were obtained for the uptake of SN-38, pravastatin, estrone-3-sulfate, and estradiol-17β-glucuronide for all of the tested OATP1B1 variants. Although the mechanism of intersubstrate differences of the effect of SNPs observed in the previous studies is not clear, amino acid substitutions in extracellular loop 2 of OATP1B1, including the polymorphism of A388G (N130D), are likely to be important for substrate recognition (Michalski et al., 2002). Moreover, OATP1B1*15 is a haplotype of N130D (A388G) and V174A (T521C), and exhibited functional alteration, whereas each individual mutation, N130D or V174A, produced no significant alteration. Most recently, Niemi et al. (2004) suggested that a novel SNP in the promoter region of SLC01B1 (G-11187A) is also associated with the alteration of pharmacokinetics of pravastatin. Thus, we need a comprehensive and careful evaluation of the functional alterations of OATP variants for a range of substrates.

In conclusion, this study is the first to provide evidence that OATP1B1 is involved in the hepatic disposition of SN-38 and that genetic polymorphisms of OATP1B1 may contribute to the interpatient variability in the efficacy and toxicity of clinically used drugs such as irinotecan and pravastatin. Although, to clarify the clinical importance of OATP1B1 and its SNPs in the hepatic disposition of SN-38, further studies will be required for the determination of the relative contribution of OATP1B1-mediated transport compared with the other transport mechanisms, such as passive diffusion, the present observation would be an important step toward understanding the reasons for interpatient variability in drug effects.

Acknowledgments. We thank M. Tada for technical assistance.
References


col Exp Ther 302:804–813.


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