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

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Received September 26, 2008; accepted January 8, 2009

ABSTRACT:
Organic anion-transporting polypeptides (OATPs) are important uptake transporters that can have a profound impact on the systemic pharmacokinetics, tissue distribution, and elimination of several drugs. Previous in vivo studies of the pharmacokinetics of the lipophilic camptothecin (CPT) analog gimatecan suggested that the ATP-binding cassette (ABC) B1 (P-glycoprotein) and/or ABCG2 (breast cancer resistance protein) inhibitors elacridar, valsposdar, pantoprazole, and, to a lesser extent, zosuquidar and verapamil. Therefore, the effect of these ABCB1 and ABCG2 modulators on the plasma pharmacokinetics of gimatecan and BNP1350 (and possibly also other OATP1B1 substrates) may be partly because of inhibition of OATP1B1 besides inhibition of ABCB1 and/or ABCG2. The findings of this study suggest that OATP1B1 polymorphisms or coadministration with one of the ABCB1/ABCG2 inhibitors could affect drug uptake, tissue distribution, and elimination of some CPT anticancer drugs, thereby modifying their efficacy and/or safety profile.

Uptake transporters belonging to the superfamily of organic anion-transporting polypeptides (rodents: Oatps, human: OATPs) are nowadays recognized as important transporters that can have a profound impact on the systemic pharmacokinetics, tissue distribution, and elimination of a wide range of drugs. Among these OATPs, human OATP1B1 (previously called OATP-C, LST-1, or OATP2; gene symbol: SLCO1B1) is found to be specifically and highly expressed at the basolateral (sinusoidal) plasma membrane of hepatocytes and is considered to be one of the most important hepatic uptake transporters (Abe et al., 1999; Hsiang et al., 1999; König et al., 2000). The clinical importance of OATP1B1 in the plasma pharmacokinetics and elimination of substrate drugs has been confirmed by several studies focusing on commonly occurring single nucleotide polymorphisms in OATP1B1. In particular, the SLCO1B1*15 allele (c.388A>G and c.521T>C) has been associated with strongly reduced transport functionality and markedly increased plasma levels of pravastatin, pitavastatin, and simvastatin acid, sometimes resulting in fatal toxicity (Maeda and Sugiyama, 2008).

With the exception of methotrexate and SN-38 (Hagenbuch and Meier, 2004; König et al., 2006), information about anticancer drugs being transported by OATP1B1 is limited. Therefore, we investigated a number of (novel) camptothecin (CPT) analogs as possible substrates for human OATP1B1. CPT was originally isolated from the Chinese tree Camptotheca acuminata by Wall et al. (1966). The molecular mechanism of CPT has been established to be inhibition of the nuclear enzyme DNA topoisomerase I, in which the intactness of the lactone ring of CPT plays a dominant role (Adams et al., 2000). However, this lactone moiety is chemically unstable and undergoes pH-dependent reversible hydrolysis to a hydroxyl carboxylate form. Under acidic conditions, pH < 4, the lactone structure predominates, whereas at pH values > 10 the carboxylate form is exclusively present.
+ 10 μM rifampicin
(Underberg et al., 1990; Fassberg and Stella, 1992). Because CPT itself is poorly water-soluble, the initial approach in CPT analog development focused on increasing water solubility. This resulted in the development of irinotecan (CPT-11), a water-soluble precursor of the more lipophilic and potent SN-38 and topotecan (TPT), which are nowadays important and widely used anticancer drugs (Takimoto et al., 1998; Douillard et al., 2000). However, a significant limitation of CPT-11, SN-38, and TPT is their affinity for the ATP-binding cassette (ABC) drug efflux transporters ABCB1 [P-glycoprotein (P-gp)] and ABCG2 [breast cancer resistance protein (BCRP)], often resulting in tumor resistance (Hendrickx et al., 1992; Mattern et al., 1993; Laloo et al., 2004). Therefore, there is an increasing interest in the more recently developed highly lipophilic CPT derivatives, such as lurto-tecan (GH47211, NX211), gimatecan (ST1481, LBQ707), and BNP1350 (karenitecin). These drugs have been suggested to be less affected by multidrug resistance. For example, lurtotecan has less affinity for ABCG2 than TPT and SN-38, whereas BNP1350 could be classified as a poor ABCG2 substrate (Maliepaard et al., 2001). Furthermore, we recently showed that gimatecan was not a substrate for ABCB1 or ABCG2 (multidrug resistance protein 2), but our data did show that gimatecan is transported by ABCG2 (Marchetti et al., 2007). In the in vivo role of ABCG2 in gimatecan pharmacokinetics was further assessed by our group using Abcb1/Abcg2 knock out mice (S. Marchetti, R. L. Oostendorp, D. Plum, O. van Tellingen, R. Versace, J. H. Beijnen, R. Mazzanti, and J. H. M. Schellens, submitted for publication). It is interesting to note that these data revealed that coadmi-
mnistration of elacridar (GF120918, an effective ABCB1 and ABCG2 inhibitor) (Hyafil et al., 1993; Jonker et al., 2000) and pantoprazole (specific ABCG2 inhibitor) (Breedveld et al., 2004) also increased the area under the plasma concentration-time curve of gimatecan in Abcb1/Abcg2 knock out mice. This suggests that the interaction between gimatecan and elacridar or pantoprazole is partly mediated by drug transporters other than ABCB1 and ABCG2. In the present study we tested the possible role of human OATP1B1 in this drug-drug interaction.

The aim of this study was to establish whether human OATP1B1 could transport the CPT analogs CPT-11, SN-38, TPT, lurtotecan, gimatecan, and BNP1350. We also tested the pH dependence of the OATP1B1-mediated transport of these compounds. Furthermore, several ABCB1 and/or ABCG2 inhibitors, including elacridar, zosuqui-
dar, verapamil, valsaparol, and pantoprazole, were screened as possible OATP1B1 modulators.

Materials and Methods

Chemicals and Reagents. [3H]ulinin (0.78 Ci/mmol) and [14C]ulinin car-bosylic acid (54 mCi/mmol) were purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). [3H]estradiol 17β-D-glucuronide (E2G; 44 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). TPT (Hyacanthin) and [3H]TPT (48 mCi/mmol) were obtained by GlaxoSmithKline (Welwyn Garden City, Hertfordshire, UK). [3H]Gimate-
can (40 μCi/mg), valsaparol, and cyclosporin A were provided by Novartis (Basel, Switzerland). Lurtotecan (NX211) was provided by Gilead Sciences (Foster City, CA), BNP1350 by BioNumerator Pharmaceuticals, Inc. (San Antonio, TX), zosuquidar trihydrochloride by Dr. P. Multani of Kanisa Pharmaceuticals (San Diego, CA), and elacridar by GlaxoSmithKline. SN-38 was purchased from Sequoia Research Products (Pangbourne, UK). Pantoprazole (Pantozol 40 mg; Altana Pharma, Zwanenburg, The Netherlands) and CPT-11 (Campto; Pfizer BV, Capelle aan den IJsdel, The Netherlands) were obtained from the pharmacy of The Netherlands Cancer Institute/Slotervaart Hospital. Other chemicals and drugs were of analytical grade or better and were purchased from Sigma-Aldrich (St. Louis, MO) or Invitrogen (Breda, The Netherlands).

Cell Lines and Culture Conditions. Polarized Madin-Darby canine kidney II (MDCKII) epithelial cells, parental and stably expressing functional human OATP1B1, were provided by Dr. Y. Sugiyama and were cultured as described previously (Sasaki et al., 2002).

Transport Assays. Transcellular transport studies were performed as described previously (Schinkel et al., 1995) with minor modifications. In brief, MDCKII-parental and -OATP1B1 cells were grown on microporous polycarbonate membrane filters (3.0-μm pore size, 24-mm diameter, Transwell 3414; Corning Life Sciences, Acton, MA) at a density of 1.5 × 105 cells/well for 3 days. The expression level of OATP1B1 was induced with 5 mM sodium butyrate for 24 h before the start of the transport study. As control, in all the transport experiments [3H]E2G (1 μM) was used as OATP1B1 substrate, and the results were in line with data reported previously by Sasaki et al. (2002). To this use, transport data (percentage of totally added drug) were converted to picomole per milligram of protein, which is described in the legend of Fig. 1. Rifampicin (10 μM) was used as an OATP1B1 inhibitor (Vavricker et al., 2002). When inhibitors were used, cells were incubated with one of the respective inhibitors for 2 h before the start and throughout the transport experiments.

First, the CPTs CPT-11 (5 μM), SN-38 (5 μM), [14C]TPT (5 μM), lurtotecan (5 μM), 5H]gimatecan (1 μM), and BNP1350 (0.2 μM) were tested for OATP1B1-mediated transport. After 2 and 4 h, aliquots of medium were taken, and the amount of the tested drugs appearing in the compartment (apical (A) or basal (B)) opposite to which the drug was added was measured. Radiolabeled drugs were analyzed by liquid scintillation counting (Tri-Carb 2120 CS Liquid Scintillation Analyzer; Canberra Industries, Meriden, CT) and the nonradiolabeled drugs by fluorescence measurements at specific excitation/ emission wavelengths of 370/480 (CPT-11), 380/535 (SN-38), 380/420 (lur-
totecan), or 370/420 (BNP1350) using a microplate reader (Infinite M200; Tecan Trading AG, Männedorf, Switzerland). The tested concentrations of the nonradiolabeled CPTs were chosen within the linear range of the calibration curve of the fluorescence measurements, and the concentrations of the radio-
labeled drugs were based on our experience with these compounds (Breedveld et al., 2007; Marchetti et al., 2007). Second, cellular accumulation of 1 μM [3H]E2G in MDCKII-OATP1B1 cells 4 h after B to A-directed transport in the absence and presence of different concentrations of the CPT analogs CPT-11 (10 and 100 μM), SN-38 (10 and 100 μM), TPT (10 and 100 μM), lurtotecan (1, 10, and 100 μM), gimatecan (1 and 4 μM), and BNP1350 (1 and 10 μM) was tested.

Third, the effect of extracellular pH 6.5 and 8.0 on the cellular accumulation of 1 μM [3H]E2G and several CPTs in MDCKII-OATP1B1 cells 4 h after B to A-directed transport was investigated. Herein, the (serum-free) Opti-MEM medium (Invitrogen) was adjusted to pH 6.5 or 8.0 by the addition of HCl (37% w/w) and NaOH (5 mM), respectively, immediately before addition of the (non)-radiolabeled drug.

Finally, the ABCB1 and/or ABCG2 inhibitors elacridar (5 μM), zosuquidar (10 μM), valsaparol (5 μM), and pantoprazole (500 μM) were tested in this assay for the inhibition of OATP1B1-mediated transport of E2G and CPTs. The concentrations we used for the different ABCB1/ABCG2 inhibitors are based on our comprehensive experience with these inhibitors (Bardelmeijer et al., 2004; Breedveld et al., 2005).

Statistical Analysis. Statistical evaluation was performed using the two-sided unpaired Student’s t test to assess the statistical significance of difference.
OATP1B1-dependent transport of gimatecan and BNP1350 is some-
what higher than the positive control E2G (E2G 9.2%, gimatecan 14.4%, BNP1350 14.3%; Supplemental Data 1). As both E2G and gimatecan were used at 1 μM this indicates that gimatecan transport was in the same order as for E2G. BNP1350 was used at 0.2 μM, so the absolute transport is approximately 3-fold lower than for E2G. Thus, OATP1B1-dependent transport of both gimatecan and BNP1350 was of the same order of magnitude as that of E2G. BNP1350 was used at 0.2 μM, so total transport after 4 h. The respective modulators were present 2 h before the start and throughout the experiments.

Results
Transport of Several CPTs across MDCKII-Parental and MDCKII-OATP1B1-Overexpressing Monolayers. Transport of E2G (control), TPT, CPT-11, SN-38, lurtotecan, gimatecan, and BNP1350 by OATP1B1 was studied in MDCKII-OATP1B1 and MDCKII-parental cells. We found an increased transport of 1 μM gimatecan and 0.2 μM BNP1350 from the B-to-A compartments compared with the transport in the opposite direction (A to B) in MDCKII-OATP1B1 cells (ratio B to A/A to B after 4 h is 2.7 ± 0.6 and 2.7 ± 0.1, respectively) (Fig. 1; Table 1), whereas the parental cell line did not show transport (ratio B to A/A to B after 4 h is 1.1 ± 0.02 and 1.0 ± 0.2, respectively) (Fig. 1; Table 1). The additional OATP1B1-dependent transport of gimatecan and BNP1350 is somewhat higher than the positive control E2G (E2G 9.2%, gimatecan 14.4%, BNP1350 14.3%; Supplemental Data 1). As both E2G and gimatecan were used at 1 μM this indicates that gimatecan transport was in the same order as for E2G. BNP1350 was used at 0.2 μM, so the absolute transport is approximately 3-fold lower than for E2G. Thus, OATP1B1-dependent transport of both gimatecan and BNP1350 was of the same order of magnitude as that of E2G. Moreover, like for E2G, gimatecan and BNP1350 transport was completely inhibited in MDCKII-OATP1B1 monolayers in the presence of the OATP1B1 inhibitor rifampicin (10 μM; Fig. 1).

SN-38 showed complex transport behavior, with increased B to A-directed transport by OATP1B1 compared with parental cells; however, A to B-directed transport was dominant, presumably because of the presence of an apical uptake transporter or a basolateral efflux transporter for SN-38 (Fig. 1). Addition of rifampicin resulted in inhibition of the B to A-directed (OATP1B1-mediated) transport, but it also increased the A to B-directed transport and the transport of SN-38 in the parental cells (Fig. 1). It may be that reduction of basolateral SN-38 reuptake by inhibiting OATP1B1 (and possibly a related endogenous basolateral SN-38 uptake system in parental cells) with rifampicin results in higher net A to B-directed transport by the endogenous basolaterally directed SN-38 transport system. Indeed, a qualitatively similar shift (increased basolaterally directed transport) was seen on inhibiting OATP1B1-mediated gimatecan transport with rifampicin (Fig. 1; see also Table 1).

No net transport was found for CPT-11, TPT, and lurtotecan in MDCKII-OATP1B1 and parental cell line monolayers (data not shown), and there was no difference in cellular accumulation (after 4-h transport) between MDCKII-OATP1B1 cells and parental cells (Supplemental Data 2). Therefore, we concluded that CPT-11, TPT, and lurtotecan are no OATP1B1 substrates.

Effects of Several CPTs on the OATP1B1-Mediated Transport of E2G. The inhibitory effect of two concentrations of the CPT analogs CPT-11, SN-38, TPT, lurtotecan, gimatecan, and BNP1350 on the OATP1B1-mediated transport of E2G was assessed. Our aim was not to establish IC50 values; this might be part of future studies.

MDCKII-OATP1B1 cells showed a 2.4-fold (P < 0.05) increased B to A transport of 1 μM E2G after 4 h compared with A to B-directed transport (Table 1), whereas parental cells did not show any transport of E2G. The effects of various CPTs on the uptake of E2G by MDCKII-OATP1B1 cells are presented in Fig. 2, which shows the cellular accumulation of E2G in MDCKII-OATP1B1 cells 4 h after addition of E2G to the basal compartment without (i.e., control) and with addition of several CPTs (presented as percentage of control). However, the first bar represents the cellular accumulation of E2G in parental cells. This bar shows that complete inhibition will maximally

<table>
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<tr>
<th>Inhibitors</th>
<th>E2G (1 μM) BA/AB</th>
<th>Gimatecan (1 μM) BA/AB</th>
<th>BNP1350 (0.2 μM) BA/AB</th>
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<tr>
<td></td>
<td>%</td>
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<tr>
<td>Rifampicin (10 μM)</td>
<td>15.1 ± 0.7</td>
<td>10.7 ± 0.8</td>
<td>4.1 ± 2.4</td>
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<tr>
<td>Elacridar (5 μM)</td>
<td>5.7 ± 0.6</td>
<td>16.6 ± 1.1</td>
<td>22.3 ± 3.6</td>
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<tr>
<td>Zosuquidar (5 μM)</td>
<td>18.2 ± 0.4</td>
<td>163.3 ± 0.04</td>
<td>15.0 ± 0.4</td>
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<tr>
<td>Verapamil (10 μM)</td>
<td>22.3 ± 0.6</td>
<td>142.2 ± 1.7</td>
<td>16.2 ± 2.1</td>
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<tr>
<td>Valproprazole (5 μM)</td>
<td>15.0 ± 3.6</td>
<td>11.4 ± 0.8</td>
<td>9.7 ± 2.3</td>
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<tr>
<td>Pantoprazole (500 μM)</td>
<td>11.1 ± 2.4</td>
<td>12.4 ± 1.5</td>
<td>1.2</td>
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*P < 0.05 compared with OATP1B1-mediated transport of E2G, gimatecan, and BNP1350 without inhibitor.

Data are mean ± S.D., n = 3.
result in ~35% of control E₂G accumulation values. Indeed, the addition of rifampicin (10 μM), an established OATP1B1 inhibitor that we used as a control, resulted in ~40% of control E₂G accumulation in MDCKI-OATP1B1 cells. This illustrates that rifampicin can virtually completely inhibit E₂G uptake by OATP1B1, as it resulted in cellular accumulation levels of E₂G roughly similar to that in parental cells. Lurtotecan and BNP1350 were identified as strong inhibitors of OATP1B1-mediated transport of E₂G because at 1 μM these compounds significantly inhibited cellular accumulation of E₂G (P < 0.001). Lurtotecan was initially tested at a concentration of 10 and 100 μM, which did show inhibition but not concentration dependence. Therefore, we tested an additional concentration of 1 μM. In contrast, 1 μM gimatecan did not inhibit cellular accumulation of E₂G; however, at a concentration of 4 μM, it significantly inhibited the cellular accumulation of E₂G in MDCKI-OATP1B1 cells (P < 0.01). Gimatecan precipitated at concentrations >4 μM, which therefore was the highest testable concentration. Furthermore, 10 μM SN-38 had only a minimal (but significant) inhibitory effect (P < 0.05), whereas at a higher concentration (100 μM) inhibition was more profound (~50% of control, P < 0.001). CPT-11 and TPT could be classified as less potent OATP1B1 inhibitors, only inhibiting E₂G transport by MDCKI-OATP1B1 cells at a concentration of 100 μM (P < 0.05 and P < 0.001, respectively). No effect of the tested CPTs was seen on the transport of E₂G in parental cells (data not shown).

**pH-Dependent Uptake of E₂G, Gimatecan, and BNP1350 by Human OATP1B1.** We tested whether the OATP1B1-mediated transport of gimatecan and BNP1350 differed between the carboxylate and lactone form of these CPT analogs. Therefore, the effect of acidic (pH 6.5) and basic (pH 8.0) conditions on the OATP1B1-mediated uptake of gimatecan, BNP1350, and E₂G (used as a control) was investigated. Cellular accumulation of gimatecan and BNP1350 was not changed between these different pH values (data not shown). However, cellular uptake of E₂G, the molecular shape of which is chemically not affected by the tested pH values (Ngheim et al., 2004), was significantly higher at acidic pH (pH 6.5) compared with control pH (pH 7.2) as shown in Fig. 3 (0.58 ± 0.13 versus 0.34 ± 0.04% of totally added E₂G accumulation 4 h after B to A-directed transport in MDCKI-OATP1B1 cells at pH 6.5 and 7.2, respectively; P < 0.05). This shows that OATP1B1 transports E₂G more efficiently at acidic pH, illustrating a pH dependence of OATP1B1. No effect of the different pHs was seen on cellular accumulation of E₂G in parental cells (Fig. 3).

**Effects of Several Classic ABCB1/ABCG2 Inhibitors on the Transport of E₂G, Gimatecan, and BNP1350 by OATP1B1.** Using MDCKII-parental and -OATP1B1 monolayers we investigated whether the classic ABCB1 and/or ABCG2 inhibitors elacridar (5 μM), zosuquidar (5 μM), verapamil (10 μM), valspodar (5 μM), and pantoprazole (500 μM) were capable of inhibiting the transport of E₂G, gimatecan, and BNP1350 by OATP1B1. OATP1B1 expression resulted in a 2.4-, 2.6-, and 3.4-fold (P < 0.05) increased transport of E₂G, gimatecan, and BNP1350, respectively, from B to A compared with the transport from A to B (Table 1). Interestingly, the tested ABCB1 and/or ABCG2 inhibitors elacridar, valspodar, and pantoprazole fully inhibited the OATP1B1-mediated transport of E₂G, gimatecan, and BNP1350 (Table 1). Zosuquidar completely inhibited gimatecan, but to a minor extent E₂G and BNP1350, transport by OATP1B1. Verapamil only had a minimal (but significant) inhibitory effect on the OATP1B1-mediated transport of E₂G and gimatecan, but had no inhibitory effect on the transport of BNP1350 (Table 1). No effect of the tested ABCB1 and/or ABCG2 inhibitors was found on the disposition of E₂G by parental cells (Table 1).

**Discussion.** We have identified two novel CPT analog anticancer drugs, gimatecan and BNP1350, as substrate drugs for human OATP1B1. It is interesting to note that OATP1B1-mediated transport of these compounds could be efficiently inhibited by elacridar, valspodar, pantoprazole, and to a lesser extent also by zosuquidar and verapamil, which were originally developed and classified as ABCB1 and/or ABCG2 inhibitors.

To study the role of OATP1B1 in the disposition of several CPT analogs, we made use of MDCKII cells stably expressing OATP1B1. In these cells, which were previously established and validated by Sasaki et al. (2002), we showed that gimatecan, BNP1350, and SN-38 are substrate drugs for OATP1B1. CPT-11, TPT, and lurtotecan could not be identified as OATP1B1 substrates. Concerning CPT-11 and SN-38, our results were in line with previously described data (Nozawa et al., 2005). In this study by Nozawa et al. (2005), SN-38 (but not CPT-11) was identified as a substrate for human OATP1B1, using human embryonic kidney 293 cells and Xenopus oocytes expressing OATP1B1. Our results revealed increased B to A-directed transport of SN-38 in MDCKII-OATP1B1 cells compared with parental MDCKII cells, but A to B-directed transport was dominant in both cell lines. From this we could conclude that SN-38 is transported by OATP1B1, but it also suggested that MDCKII cells express one or more unidentified endogenous apical uptake transporters (or basolateral active efflux transporters) with affinity for SN-38. The existence of an apical uptake transporter for SN-38 has been observed before, although these studies only focused on a human intestinal cell line (Caco-2) and hamster or human intestinal epithelial cells, respectively (Kobayashi et al., 1999; Itoh et al., 2005). Furthermore, inhibition experiments indicated that the reduced transport of SN-38 in MDCKII-OATP1B1 cells by rifampicin is related to more complex mechanisms because it also affected A to B-directed transport of SN-38 and the transport of SN-38 by parental cells.

Several independent studies confirmed the in vivo importance of OATP1B1 in systemic pharmacokinetics and elimination of several drugs (i.e., several statins, fexofenadine, atrasentan, torsemide, and irinotecan) by investigating single-nucleotide polymorphisms. Especially the commonly occurring SLCO1B1*15 allele (c.388A>G and c.521T>C) is associated with reduced transport capacity and increased plasma area under the plasma concentration-time curve.
(Maeda and Sugiyama, 2008). Nozawa et al. (2005) showed that OATP1B1*15 displayed 50% decreased transport activity for SN-38 in vitro. It is noteworthy that OATP1B1*15 has also been associated with higher systemic exposure to SN-38, lower clearance of this compound, and even severe toxicity in patients treated with irinotecan (Kweekel et al., 2008). Together, this shows that genetic polymorphisms of OATP1B1 may contribute to the interpatient variability in the efficacy and toxicity of several drugs, including CPTs. Therefore, this might also have clinical implications for the (highly toxic) anticancer drugs gimatecan and BNP1350. It would be interesting and of high importance to further investigate this in vivo.

In a recent study, additional novel CPTs have been developed to improve oral uptake, intracellular accumulation, lactone stability, drug-target interaction, and lipophilicity (Teicher, 2008). This has led to the development of seven-position modified lipophilic CPT derivatives (e.g., gimatecan and BNP1350) (Huang et al., 2007) and stable five-membered E-ring ketone CPTs (Lansaix et al., 2007). In the present study, we show that gimatecan and BNP1350 are both transported by human OATP1B1. Interestingly, De Cesare et al. (2001) reported that gimatecan was more potent than TPT against liver metastasis in mice, suggesting the importance of Oatp-mediated hepatic uptake. It will be interesting to investigate whether other newly developed CPTs, besides ABCB1 and ABCG2, are also OATP1B1 substrate drugs.

Our group previously showed that ABCG2 transports substrate drugs more efficiently at low pH (Breedveld et al., 2007). Here, we also show a pH-sensitive activity of human OATP1B1, which was shown by increased transport of E2G at acidic pH, pH 6.5, compared with control pH, pH 7.2. In contrast to gimatecan and BNP1350, whose structures can change from the lactone to the carbonylate form on pH changes, the chemical structure of E2G is not chemically affected by this pH difference because the pK<sub>a</sub> of E2G is 10.4 (Ngkiem et al., 2004). No effect of different pH values was found on the transport of gimatecan and BNP1350 by OATP1B1. This is possibly masked by a difference in affinity between the lactone and the carbonylate form of gimatecan and BNP1350 because the molecular conversion of CPTs is pH-dependent.

The pH sensitivity of OATP1B1 has not been reported before; however, transport of estrone-3-sulfate by human OATP2B1 and OATP1A2 has been reported to be increased by low pH as well (Kobayashi et al., 2003; Badagnani et al., 2006). Unlike OATP2B1 and OATP1A2, the expression of human OATP1B1 is liver-specific (König et al., 2000; Tamai et al., 2001) and therefore is not found at the intestinal epithelial cells, where the luminal pH ranges from 4 to 7 (Badagnani et al., 2006). Consequently, the physiological impact of pH sensitivity of OATP1B1 might be limited, as the extracellular pH around hepatocytes is neutral.

Based on our previous in vivo results (Oostendorp et al., 2009), we found that elacridar and pantoprazole, two well known ABCB1 and ABCG2 modulators, most likely inhibit one or more transporters distinct from ABCB1 and ABCG2. In the present study, we hypothesized that the inhibitory effect of elacridar and pantoprazole could be partly because of modulation of the uptake transporter OATP1B1. The effect of several classic ABCB1 and/or ABCG2 modulators, elacridar, zosuquidar, verapamil, valsodar, and pantoprazole, was tested. Our data show, to our knowledge, for the first time that elacridar, valsodar, pantoprazole, and to a lesser extent zosuquidar and verapamil are besides ABCB1 and ABCG2 inhibitors also OATP1B1 inhibitors (at levels of exposure that are commonly applied to inhibit ABCB1 and ABCG2). This most likely explains our previous in vivo results regarding the effects of elacridar and pantoprazole in Abcb1/Abcg2 knockout mice (Oostendorp et al., 2009). Coadministration of CPTs, or other drugs that are substrates for OATP1B1, and one of these well known ABCB1/ABCG2 inhibitors could affect drug uptake, tissue distribution, and/or elimination, thereby modifying the efficacy and/or safety profile of these drugs. Pantoprazole, for example, belongs to the class of proton pump inhibitors that have emerged as the most important class of drugs for the management of a variety of acid-related disorders of the upper gastrointestinal tract, including gastric and duodenal ulcers and gastroesophageal reflux disease accompanied by esophagitis (Jungnickel, 2000). Pantoprazole is clinically a widely used drug, and intake of this drug is rarely discontinued before treatment with other (anticancer) drugs. Although in this study we used high concentrations of pantoprazole, this might indicate that this combined treatment could lead to drug-drug interactions at the level of OATP1B1, possibly resulting in increased plasma levels of the drug, which could lead to undesirable side effects.

In conclusion, in the present study we show that gimatecan and BNP1350 are novel OATP1B1 substrate drugs. Because the clinical importance of OATP1B1 polymorphisms has been widely established nowadays, these might therefore have marked implications for these (highly toxic) anticancer drugs in patients. In addition, the OATP1B1-mediated transport of gimatecan and BNP130 could be inhibited by several classic ABCB1 and/or ABCG2 modulators. Therefore, the effect of these ABCB1 and/or ABCG2 modulators on the plasma pharmacokinetics of these CPT analogs (and possibly also other OATP1B1 substrate drugs) may not be solely ascribed to inhibition of ABCB1 and/or ABCG2. The results of this study add to a better insight into the pharmacokinetic behavior of CPT analogs, including drug-drug interactions that can affect the efficacy and/or safety profile of these drugs.

References


