Influence of Human OATP1B1, OATP1B3, and OATP1A2 on the Pharmacokinetics of Methotrexate and Paclitaxel in Humanized Transgenic Mice

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Abstract

Purpose: Organic anion-transporting polypeptide (OATP) drug uptake transporters are thought to play an important role in drug pharmacokinetics and toxicokinetics. We aimed to determine the influence of the individual human OATP1B1, OATP1B3, and OATP1A2 transporters on the in vivo disposition of the anticancer drugs methotrexate and paclitaxel by using liver-specific humanized OATP1A/1B transgenic mice.

Experimental Design: Wild-type, Slco1a/1b−/− (Oatp1a/1b knockout), Slco1a/1b−/−;1B1tg, Slco1a/1b−/−;1B3tg, and newly generated Slco1a/1b−/−;1A2tg (humanized OATP1B1, OATP1B3, and OATP1A2 transgenic) mice were characterized biochemically and physiologically, and subsequently intravenously dosed with methotrexate or paclitaxel (2 or 10 mg/kg each) for pharmacokinetic analyses.

Results: Humanized OATP1B1, OATP1B3, and OATP1A2 transgenic mice all showed partial or complete rescue of increased plasma bilirubin levels, but also of the increased plasma levels and decreased liver and small intestinal accumulation of methotrexate observed in Slco1a/1b−/− mice. Furthermore, hepatic expression of OATP1B3 and OATP1A2, but not OATP1B1, resulted in increased liver uptake of paclitaxel (2 mg/kg). At 10 mg/kg, a modest effect of only OATP1A2 on paclitaxel liver uptake was observed.

Conclusion: Human OATP1A/1B transporters play an important role in plasma and tissue distribution of the structurally diverse chemotherapeutics methotrexate (organic anion) and paclitaxel (hydrophobic, bulky). Variation in OATP1A/1B activity due to genetic variation and pharmacologic inhibition, or differences in tumor-specific expression levels might therefore affect plasma, tissue, and tumor levels of these drugs in patients, and hence their therapeutic efficacy. Humanized transgenic OATP1A/1B mice will provide excellent tools to further study these aspects in vivo for many (anticancer) drugs. Clin Cancer Res; 19(4); 821–32. ©2012 AACR.

Introduction

Organic anion-transporting polypeptides (OATP; gene name: SLCO) are transmembrane transporters that can mediate the cellular uptake of a broad range of substrates. Many are expressed in various tissues important for pharmacokinetics (1). OATPs are classified into 6 distinct families and currently 11 human and 15 mouse OATPs/Oatps have been identified (2, 3). Especially within the OATP family, based on amino acid homology, no clear and direct orthologs between individual human and mouse OATPs exist. While humans only have one OATP1A transporter (OATP1A2), mice have at least 4 (Oatp1a1, Oatp1a4, Oatp1a5, and Oatp1a6; refs. 2, 4). On the other hand, while humans have 2 OATP1B transporters (OATP1B1 and OATP1B3), mice only have one, Oatp1b2 (2).

Human OATP1B1 and OATP1B3 are highly and predominantly expressed at the basolateral membrane of the liver. They can therefore make an important contribution to the hepatic uptake and hence systemic clearance of drugs and toxins after intravenous and oral administration (5–7). OATP1A2, on the other hand, is more widely expressed in several tissues like brain (8), kidney (9), liver (in cholangiocytes, not hepatocytes; ref. 9), and in small intestine (10), where its effect on drug absorption and/or elimination needs further investigation. Besides their physiologic expression, many OATPs have been found expressed in certain malignancies like breast, gastrointestinal, and lung cancers (6, 11–14), where they may well be involved in the tumor uptake of anticancer drugs.

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localization of OATP1B1 and OATP1B3 in humans, and liver expression and basolateral (sinusoidal) hepatocyte cells (hepatocytes) was chosen in view of the exclusive expression, can be an important factor in determining treatment efficacy and/or toxicity of these and likely many other anticancer drugs.

To overcome tissue- or cell-specific modification of proteins, and/or redundancy with or counteraction by other transporters, various reasons (plasma protein binding of substrates, species differences concerning substrate specificity, OATP1B3, and OATP1A2 humanized transgenic mice, respectively) of comparable genetic background (>99% FVB) between 9 and 14 weeks of age. Generation of OATP1B1 transgenic mice has been described (18), and OATP1B3 and OATP1A2 transgenic mice were generated as described below. Scl1a1/1b−/−;B1G8, Scl1a1/1b−/−;B3G8, and Scl1a1/1b−/−;A2G8 humanized mice were generated by cross-breeding and by breeding the transgenes to homozygosity. Animals were kept in a temperature-controlled environment with a 12-hour light/dark cycle. They received a standard diet (AM-II; Hope Farms) and acidified water ad libitum.

Materials and Methods

Animals

Mice were housed and handled according to the Institutional guidelines complying with the Dutch legislation. Animals used in this study were wild-type, Scl1a1/1b−/− (Oatp1a1/1b knockout; ref. 15), Scl1a1/1b−/−;B1G8, Scl1a1/1b−/−;B3G8, and Scl1a1/1b−/−;A2G8 mice (OATP1B1, OATP1B3, and OATP1A2 humanized transgenic mice, respectively) of comparable genetic background (>99% FVB) between 9 and 14 weeks of age. Generation of OATP1B1 transgenic mice has been described (18), and OATP1B3 and OATP1A2 transgenic mice were generated as described below. Scl1a1/1b−/−;B1G8, Scl1a1/1b−/−;B3G8, and Scl1a1/1b−/−;A2G8 humanized mice were generated by cross-breeding and by breeding the transgenes to homozygosity. Animals were kept in a temperature-controlled environment with a 12-hour light/dark cycle. They received a standard diet (AM-II; Hope Farms) and acidified water ad libitum.

Chemicals and reagents

Paclitaxel was from Sequoia Research Products, methotrexate (100 mg/ml), Emethexate Ph from Pharmachemie, isoflurane (Forane) from Abbott Laboratories, and heparin (5,000 IE/mL) from Leo Pharma BV.

Translational Relevance

Interindividual variation in drug response and drug toxicity and drug–drug interactions complicate anticancer drug treatment. Given their tissue distribution and broad substrate specificity, organic anion transporting polypeptide (OATP) drug uptake transporters may play a marked role in these processes. Using humanized OATP1B1, -B3, and -A2 transgenic mice, we found that all 3 human proteins transport the anticancer drug methotrexate in vivo, whereas only OATP1B3 and OATP1A2 transport paclitaxel. Methotrexate and paclitaxel are commonly used in cancer treatment, but they are also associated with severe and sometimes lethal toxicity in patients. Moreover, as many of the OATP1A/1B transporters occur in certain tumors, they might also directly affect tumor susceptibility to methotrexate or paclitaxel treatment. We speculate that variation in OATP1A/1B activity, because of genetic variation or drug–drug interactions, and tumor-specific expression, can be an important factor in determining treatment efficacy and/or toxicity of these and likely many other anticancer drugs.
Transgene construction and generation of transgenic mice

To achieve liver-specific expression of human OATP1B3 and OATP1A2, we generated transgene constructs as follows (Fig. 1A). pLIV-LE6 (kindly provided by Dr. J. Taylor, Gladstone Institute, University of California, San Francisco, CA; ref. 19) was completely digested with Clal followed by a partial digestion with Asp718. The 7.6 kb fragment of the vector was isolated, and 5′- and 3′-ends of the vector were filled up with Klenow enzyme to create blunt ends. After blunt end ligation, the vector was digested with Xhol. PCR was used to generate the SLCO1B3 and SLCO1A2 constructs with Xhol recognition sites at the 5′ and 3′ ends, respectively. The templates, human SLCO1B3 or SLCO1A2 cDNA in pBluescript SK−, were a kind gift by Dr. T. Abe (University Graduate School of Medical Science, Sendai, Japan). Inserted cDNA ranged from 5′-CACCTGATCTCCTGA-3′ (position −23–9) to 5′-ATGCTGTCGCAAC-3′ (position 2092–2106) for SLCO1B3 and 5′-CACCCTGAAGAG-3′ (position −16–2) to 5′-TTGAAAACTAAATTG-3′ (position 1996–2010) for SLCO1A2 (positions correspond to scale. Translational start (ATG) and stop (TAA) codons, and reading frame direction (bold arrow) for the transgenes are indicated. Small arrows indicate the initial detected by PCR screen using the following primers: forward 5′-GAAGGCTAACCTGGGGTGAG-3′ and reverse 5′-CAGTTCCCATAAGGAGACAACC-3′ for OATP1B3 transgenics (yielding a 457 bp fragment), and forward 5′-GAAGGCTAACCITGGGTAG-3′ and reverse 5′-GCACATGTTAAGCAGTAACAG-3′ for OATP1A2 transgenics (yielding a 217 bp fragment; Fig. 1A). Southern blot analysis was used for the definitive identification of transgenic founders, as well as for the differentiation between heterozygous and homozygous individuals. DNA was extracted from the tail tips of mice, digested with Ncol, and probed with an approximately 1.5-kb DraI pLIV-LE6-SLCO1B3 fragment or approximately 1.9 kb DraI/Scal pLIV-LE6-SLCO1A2

Figure 1. Generation and characterization of Slco1a1b−/−;1B1tg, Slco1a1b−/−;1B3tg, and Slco1a1b−/−;1A2tg mice. A, structure of the ApoE promoter-HCR1-driven expression cassette, containing human SLCO1B1, SLCO1B3, or SLCO1A2 cDNA. Functional elements are presented approximately to scale. Translational start (ATG) and stop (TAA) codons, and reading frame direction (bold arrow) for the transgenes are indicated. Small arrows indicate the primers used for PCR detection (F, forward; R, reverse). Probes used for Southern blot analysis (1.9 kb for SLCO1B3 detection and 1.5 kb for SLCO1A2 detection) are indicated below the cDNA. Expression of human OATP1B1 (A), 1B3 (C), and -1A2 (D) in liver, kidney, and small intestine of male (m) or female (f) wild-type, Slco1a1b−/−;1B1tg, Slco1a1b−/−;1B3tg, and Slco1a1b−/−;1A2tg mice was detected by Western blotting. HCLM, human crude liver membrane; WT, wild-type; 1B1-tg, Slco1a1b−/−;1B1tg; 1B3-tg, Slco1a1b−/−;1B3tg; 1A2-tg, Slco1a1b−/−;1A2tg. Crude membrane protein (20 μg) was analyzed for all fractions. A molecular mass marker of 85 kDa is indicated. Total protein staining (Ponceau S) confirmed equal loading across the lanes (Supplementary Fig. S1).
fragment for detection of the transgene in OATP1B3 and OATP1A2 transgenics, respectively (Fig. 1A).

RNA isolation, cDNA synthesis, and real-time PCR
RNA isolation from mouse liver, kidney, and small intestine and subsequent cDNA synthesis and real-time PCR (RT-PCR) were conducted as described (21). Specific primers (QIAGEN) were used to detect expression levels of the following mouse genes: Slco1a1, Slco1a2, Slco1b1, Slco1b3, and Slco2b1. Expression levels of the transgenes were confirmed by Ponceau S staining of the membranes after transfer. Human OATP1B3 was detected with the polyclonal ESL and SKT antibodies, respectively, kindly provided by Prof. Dr. D. Keppler (Deutsches Krebsforschungszentrum, Heidelberg, Germany; refs. 7, 22). Human OATP1B1 and OATP1B3 were detected with antibody OATP1 (sc-18428; dilution 1:1,000) from Santa Cruz.

Western blot analysis
Isolation of crude membrane fractions from liver, kidney, and small intestine, and Western blot analysis were conducted as described previously (18). Equal protein loading was confirmed by Ponceau S staining of the membranes after transfer. Human OATP1B1 and OATP1B3 were detected with the polyclonal ESL and SKT antibodies, respectively, kindly provided by Prof. Dr. D. Keppler (Deutsches Krebsforschungszentrum, Heidelberg, Germany; refs. 7, 22). Human OATP1A2 was detected with antibody OATP1 (sc-18428; dilution 1:1,000) from Santa Cruz.

Immunohistochemical staining of OATP1B3
Livers from wild-type and Sko1a1/b<sup>−/−</sup>;1B3<sup>tg</sup> mice were fixed in 4% phosphate-buffered formalin, embedded in paraffin, sectioned at 4 μm, and incubated with the rabbit polyclonal SKT antibody (1:250 in PBS) followed by horse-radish peroxidase-labeled secondary antibody (Dako Denmark A/S). Nuclei were stained with hematoxylin and eosin according to the standard procedures.

Analysis of bilirubin in mouse plasma and urine
For the detection of bilirubin levels in wild-type, Sko1a1/b<sup>−/−</sup>, Sko1a1/b<sup>−/−</sup>;1B1<sup>tg</sup>, Sko1a1/b<sup>−/−</sup>;1B3<sup>tg</sup>, and Sko1a1/b<sup>−/−</sup>;1A2<sup>tg</sup> mice, we isolated hepatic plasma by cardiac puncture and urine by spot-collection. Ten microliters of ascorbate (100 mg/mL) was added to all samples to prevent the oxidation of bilirubin. All samples were immediately protected from the light, snap-frozen, and stored at −80°C until further analysis. Concentrations of bilirubin mono-glucuronides (BMG), bilirubin diglucuronide (BDG), and unconjugated bilirubin (UCB) in plasma, bile, and urine were determined as described (23).

Pharmacokinetic experiments
For intravenous administration of methotrexate, the stock solution (100 mg/mL) was diluted with saline for administration of dose levels of 2 and 10 mg/kg, yielding final drug concentrations of 0.4 (2 mg/kg) and 2 mg/mL (10 mg/kg). A total of 5 μL/g body weight was injected as single bolus into the tail vein of mice (n = 3–6 for each group). Animals were sacrificed at indicated time points (15 or 30 minutes after methotrexate and paclitaxel dosage, respectively) by terminal bleeding through cardiac puncture under isoflurane anesthesia and tissues were isolated. Blood samples were centrifuged at 5,000 rpm for 5 minutes at 4°C, and plasma was collected and stored at −20°C until analysis.

Drug analysis
Amounts of methotrexate and its hydroxylated metabolite 7-hydroxymethotrexate (7OH-MTX) in plasma and organs [homogenized in ice-cold 4% (w/v) BSA] were determined by high-performance liquid chromatography (HPLC) analysis as described (24). Amounts of paclitaxel in plasma and organs [homogenized in ice-cold 4% (w/v) BSA] were determined using liquid–liquid and solid-phase extraction followed by reversed-phase HPLC with UV detection, as described before (25).

Statistical analysis
Statistical significance of differences between wild-type and Sko1a1/b<sup>−/−</sup>, Sko1a1/b<sup>−/−</sup>;1B1<sup>tg</sup>, Sko1a1/b<sup>−/−</sup>;1B3<sup>tg</sup>, or Sko1a1/b<sup>−/−</sup>;1A2<sup>tg</sup> mice, or between Sko1a1/b<sup>−/−</sup> mice and Sko1a1/b<sup>−/−</sup>;1B1<sup>tg</sup>, Sko1a1/b<sup>−/−</sup>;1B3<sup>tg</sup>, or Sko1a1/b<sup>−/−</sup>;1A2<sup>tg</sup> mice was assessed by one-way ANOVA followed by Dunnett multiple comparison test (or as indicated otherwise). Results are presented as the mean ± SD. Differences were considered to be statistically significant when P < 0.05.

Study approval
All mouse studies were ethically reviewed and carried out in accordance with Dutch legislation, European Directive 86/609/EEC, and the GlaxoSmithKline policy on the Care, Welfare, and Treatment of Laboratory Animals. Mouse experiments were approved by the Animal Experimentation Committee (DEC) of the Netherlands Cancer Institute (Amsterdam, the Netherlands).

Results
Generation and characterization of humanized Oatp1a1/b<sup>−/−</sup> knockout mice with liver-specific expression of human OATP1B1, OATP1B3, or OATP1A2
OATP1B3 and OATP1A2 transgenic mice were generated using an ApoE promoter-driven expression cassette, as described before for OATP1B1 liver-specific transgenic mice (18), and crossed back with Sko1a1/b<sup>−/−</sup> mice to obtain homozygous humanized strains with predominant liver-specific expression of the transgenes (Fig. 1A). A partial physiologic characterization of the OATP1B1- and OATP1B3-(but not OATP1A2-) humanized mice was published recently (17). Lifespan, body weight, and liver weight of Sko1a1/b<sup>−/−</sup>;1B1<sup>tg</sup>, Sko1a1/b<sup>−/−</sup>;1B3<sup>tg</sup>, or Sko1a1/b<sup>−/−</sup>;1A2<sup>tg</sup> mice were not different from those of wild-type mice. The expression levels of various endogenous transporters and metabolic enzymes (see Materials and Methods) in livers of the different mouse strains were analyzed by RT-PCR (Supplementary Methods and Supplementary Table...
Only Abcc3/Mrp3 mRNA was substantially (2- to 4-fold) downregulated in all the humanized strains relative to the wild-type and S1co1a/1b/C0 mices, but subsequent protein immunoblot analyses failed to reveal differences at the protein level (not shown).

Crude membrane fractions of liver, kidney, and small intestine of the humanized strains were analyzed for protein expression of the transgenes on the Western blots (Fig. 1B). Results have shown specific expression of OATP1B1 and OATP1A2 in livers of S1co1a/1b/C0;1B1/C0 and S1co1a/1b/C0;1A2/C0 mices, respectively. Only after very long exposure, minimal amounts of OATP1B1 were detected in kidneys of S1co1a/1b/C0;1B1/C0 mices (not shown). S1co1a/1b/C0;1B3/C0 mices showed abundant expression of OATP1B3 in the liver, whereas some expression of the transgene was detected in the kidney as well (Fig. 1C). Expression of the transgenic proteins was similar in male and female mice. Expression of OATP1B1 and OATP1B3 in livers of humanized mice was roughly comparable with that in a pooled human crude liver membrane fraction, but OATP1A2 expression in humanized mice was much higher than that in human liver (Fig. 1). Low levels of OATP1A2 in a total human liver preparation reflect the fact that this protein is restricted to cholangiocytes, which only form a small fraction of the total liver cell pool (9). In contrast, the transgenic promoter used by us (ApoE) drives expression of OATP1A2 in S1co1a/1b/C0;1B3/C0 mices in the abundant hepatocytes.

Immunohistochemical staining confirmed basolateral (sinusoidal) localization and disperse distribution of human OATP1B3 throughout the liver lobules of S1co1a/1b/C0;1B3/C0 mices (Fig. 2). This is similar to human liver, although, often a more zonal lobular distribution of OATP1B3 with higher expression around the central vein has been observed (22, 26, 27). In the kidney, we mainly observed cytoplasmic staining of OATP1B3 toward the basolateral side of the proximal tubular epithelium of S1co1a/1b/C0;1B3/C0 mices, with possibly some protein expression in the basolateral membrane, but not in the apical (luminal) membrane (Supplementary Fig. S2). We previously showed that the expression of human OATP1B1 in the livers of SLC10A1 transgenic mice was basolateral and throughout the liver lobule, while OATP1B1 showed stronger staining (Fig. 3).

Analysis of plasma and urine levels of unconjugated and conjugated bilirubin

Preliminary partial physiologic characterization of S1co1a/1b/C0;1B3/C0 and S1co1a/1b/C0;1B3/C0 mices has shown almost complete reversal of the increased plasma and urine levels of bilirubin glucuronide seen in S1co1a/1b/C0 mices (15, 17). Measurement of these parameters in S1co1a/1b/C0;1A2/C0 mices and compared with the known results for S1co1a/1b/C0;1B1/C0 and S1co1a/1b/C0;1B3/C0 mices showed that the transgenic OATP1A2 also reversed the increased plasma and urine levels of bilirubin mono- and diglucuronide, but not as strongly as OATP1B1 and OATP1B3 (Fig. 3). Interestingly, however, the modestly but reproducibly increased plasma levels of UCB in S1co1a/1b/C0;1B3/C0 mices were reversed at least as efficiently by OATP1A2 as by OATP1B1 or OATP1B3, and highly significantly (Fig. 3D). These results suggest that transgenic OATP1A2 transports bilirubin glucuronides into hepatocytes in vivo, but less efficiently than OATP1B1 and OATP1B3. In contrast, the clear decrease in plasma UCB suggests that OATP1A2 is a more efficient uptake transporter of UCB than OATP1B1 or OATP1B3 in the livers of the transgenic mice.

Role of OATP1A and 1B transporters in MTX disposition in vivo

To study the effect of human OATP1B1, OATP1B3, and OATP1A2 on methotrexate disposition in vivo, we intravenously administered 2 or 10 mg/kg of this drug to wild-type, S1co1a/1b/C0, S1co1a/1b/C0;1B1/C0, S1co1a/1b/C0;1B3/C0, and S1co1a/1b/C0;1A2/C0 mices, and analyzed plasma, liver, and
The effect of removal of mouse Oatp1a/1b transporters on methotrexate disposition 15 minutes after dosing to Slco1a/1b/C0/C0 mice was very clear at both 2 and 10 mg/kg: plasma levels were 3.9- and 5.0-fold increased, liver accumulation was 17.6- and 23.8-fold decreased, and small intestinal accumulation was 7.5- and 20.3-fold decreased, respectively, compared with wild-type mice (Fig. 4A–F). In general, hepatic expression of OATP1B1, OATP1B3, or OATP1A2 in the transgenic strains resulted in substantial and highly significant, albeit not complete, rescue of the impaired liver uptake of methotrexate in Slco1a/1b/C0/C0 mice after intravenous administration of 2 and 10 mg/kg methotrexate, with liver levels 2.7- to 9.2-fold increased. Plasma levels of methotrexate were concomitantly 1.6- to 2.2-fold decreased in the transgenic strains, whereas small intestinal amounts of methotrexate were 1.6- to 5.6-fold increased (Fig. 4A–F). Reversal of liver and small intestinal methotrexate levels was more pronounced in the OATP1B3 and OATP1A2 transgenic strains than in the OATP1B1-humanized mice. The increased small intestinal methotrexate levels likely reflect higher hepatobiliary excretion due to the higher liver methotrexate levels, as intestinal excretion of methotrexate directly from blood is negligible in mice (28, 29). Very similar effects of transgenic OATP1B1, OATP1B3, and OATP1A2 were observed for the reversal of the strongly reduced liver and small intestinal levels of the main hepatic (and toxic) metabolite of methotrexate, 7-hydroxymethotrexate (7OH-MTX) in Slco1a/1b/C0/C0 mice after dosing of methotrexate at 10 mg/kg (Fig. 5). Tissue and plasma levels of 7OH-MTX at 2 mg/kg methotrexate and plasma levels at 10 mg/kg methotrexate were too low for quantification in all strains.

Collectively, the bilirubin and methotrexate data described above support functional activity of each of the 3 human OATPs in the basolateral membrane of the transgenic hepatocytes.
Role of OATP1A and 1B transporters in PTX disposition in vivo

To study the possible independent effects of OATP1B1, OATP1B3, and OATP1A2 on paclitaxel disposition in vivo, OATP1B1, OATP1B3, or OATP1A2 humanized transgenic mouse strains were intravenously treated with 2 or 10 mg/kg paclitaxel. As we found before (16), liver levels of paclitaxel 30 minutes after administration were about 2-fold reduced in Slco1a/1b−/−/C0 mice compared with wild-type mice (Fig. 6B and E). Concurrently, plasma levels at this time point were modestly, but significantly, increased in the absence of Oatp1a/1b transporters (Fig. 6A and D). However, at 2 mg/kg paclitaxel, the clearly reduced hepatic uptake of paclitaxel in Slco1a/1b−/−/C0 mice was restored to wild-type levels in Slco1a/1b−/−/1B38 and Slco1a/1b−/−/1A28 mice, but not in Slco1a/1b−/−/1B18 mice (Fig. 6B). At 10 mg/kg paclitaxel, only the OATP1A2 transgenic mice showed partial reversal of the reduced hepatic paclitaxel uptake, but not the OATP1B1- or OATP1B3-humanized mice, suggesting saturation of transgenic OATP1B3, but not OATP1A2 at this dosage. Modest alterations in the levels of paclitaxel in the small intestine of the various strains were, like the plasma levels, quite erratic and variable (Fig. 6C and F). This may reflect a combination of biliary excretion and the substantial direct intestinal...
excretion of paclitaxel (from blood) by P-glycoprotein (16, 30). Nonetheless, especially the liver accumulation data at 2 mg/kg paclitaxel clearly indicate that transgenic OATP1B1 and OATP1A2, but not OATP1B3, can substantially transport paclitaxel in vivo into the liver, and that both transporters can at least partly compensate for the loss of the murine Oatp1a/1b transporters.

Discussion

We show here that human OATP1B1, OATP1B3, and OATP1A2 are functionally expressed in the basolateral membrane of hepatocytes of the 3 liver-specific humanized mouse strains we generated from Slco1a/1b mice, each of which could, to varying extents, rescue the markedly reduced hepatic uptake of bilirubin glucuronides and in part of UCBLB in Slco1a/1b mice, thus affecting the plasma and tissue levels of these compounds. The same applies to hepatic uptake and plasma levels of methotrexate, whereas OATP1B1 and OATP1A2, but not OATP1B3, could rescue the reduced liver uptake of paclitaxel. These data show the substantial in vivo activity of the human OATP1A/1B transporters in hepatocellular uptake of 2 structurally diverse anticancer drugs, directly affecting plasma and tissue pharmacokinetics. These observations also imply that the expression of one or more of the human OATP1A/1B transporters in various tumors in vivo may directly affect the drug uptake, and hence susceptibility of the tumor to the wide range of anticancer drugs that are substrates of these transporters (31). Moreover, genetic polymorphisms that affect the activity of these transporters may alter both tumor susceptibility and the plasma disposition, tissue distribution, and elimination of substrate anticancer drugs, and hence their overall therapeutic efficacy and risk of toxicity (32). The same applies to coadministered drugs that substantially inhibit activity of OATP1A/1B transporters through drug–drug interactions, or induce or repress the expression of these transporters.

We show that all 3 human OATP1A/1B transporters can substantially transport methotrexate in vivo, in line with, and extending previous in vitro results (6, 33, 34). Plasma levels were similarly decreased by the 3 transgenic transporters, but at both dose levels the effect of OATP1B1 on methotrexate liver and intestinal disposition was about 2- to 3-fold lower than that of OATP1B3 and OATP1A2 (Fig. 4). Although absolute quantification of the expression of the transgenes at the plasma membrane has not been carried out, our data suggest that in human liver both OATP1B1 and OATP1B3 can contribute to methotrexate pharmacokinetics. Note that the observed partial rescue in liver uptake of methotrexate by singly expressed OATP1B1 or OATP1B3 in the humanized transgenic mice probably underestimates the actual OATP1B1-mediated liver uptake in humans, as in human liver both proteins function at the same time, likely resulting in additive effects. Presumably, this would also apply to the effects of these transporters on plasma and small intestinal (content) levels of methotrexate in humans.

Paclitaxel is used in the treatment of various types of malignancies (e.g., breast, ovarian, and lung cancer; refs. 35, 36). Our results with the humanized OATP1A/1B transgenic mice indicate that OATP1B3 and OATP1A2, but not OATP1B1, can substantially transport paclitaxel in vivo, and that both transporters can at least partly compensate for the loss of the murine Oatp1a/1b transporters. This is partly in line with in vitro studies that have identified paclitaxel as a substrate of OATP1B1 (Km ~7 μmol/L), but not of OATP1B1 (37–39). In addition, although a previous in vitro study failed to reveal paclitaxel transport by OATP1A2 (38), our data indicate relatively high in vivo transport compared with OATP1B3. In fact, while there was no significant effect of transgenic OATP1B3 (or OATP1B1) on liver uptake of paclitaxel after 10 mg/kg administration, Slco1a/1b mice still showed a significant 4-fold increase in hepatic uptake of paclitaxel compared with Slco1a/1b;1A2mice (liver-to-plasma ratios were also significantly increased; P < 0.01). Possibly, the transgenic OATP1B3 was saturated at this higher dosage, whereas OATP1A2 was not, implying that OATP1A2 has a higher Km for paclitaxel transport. Given a reported Km of...
approximately 7 μmol/L (~6 μg/mL) for paclitaxel transport by OATP1B3 (37), it is not surprising that OATP1B3 activity may have been saturated over the time period before 15 minutes after intravenous administration, when plasma levels must have been (far) higher than the approximately 7 μg/mL observed at 15 minutes (Fig. 6D). In the clinic, patients are generally treated with 5 to 6 doses of 135 or 175 mg/m² paclitaxel as a 3- or 24-hour infusion, every 3 weeks (40). Peak plasma concentrations after high-dose treatment (175 mg/m²) are about 5 or 0.4 μg/mL in patients receiving 3- or 24-hour infusions, respectively (41). In addition, despite extensive binding to plasma proteins (95%–98%), paclitaxel is readily cleared from the plasma. Plasma levels of paclitaxel in patients are thus generally well below the K_m value of paclitaxel transport by OATP1B3 (and presumably also by OATP1A2), implying that these transport proteins could be significant determinants of paclitaxel pharmacokinetics and toxicokinetics (note that neutropenia in patients is related to paclitaxel plasma levels of more than 0.08 μg/mL; refs. 41, 42).

OATP1B1 and OATP1B3 are, in addition to OATP2B1, highly expressed at the basolateral membrane of hepatocytes and are, therefore, thought to be some of the most important hepatic (drug) uptake transporters (3, 32). Results from the present study indicate that human OATP1A/1Bs in Paclitaxel and Methotrexate Disposition

Figure 6. Influence of human OATP1B1, OATP1B3, and OATP1A2 transporters on paclitaxel (PTX) disposition in vivo. Female wild-type, Slco1a/1b⁻⁻;Slco1a/1b⁻⁻;1B1⁻⁻;1B3⁻⁻, and Slco1a/1b⁻⁻;1A2⁻⁻ mice were intravenously dosed with paclitaxel [2 mg/kg (A–C) or 10 mg/kg (D–F)] and drug concentrations in plasma (A, D), liver (B, E), and small intestine (including contents; C, F) 30 minutes after administration were determined. All data are presented as mean ± SD (n = 3–6; **, P < 0.05; †††, P < 0.001) when compared with wild-type mice; †, P < 0.05; ††, P < 0.01; †††, P < 0.001 when compared with Slco1a/1b⁻⁻ mice).
Expression of OATP1A2 in the human liver is restricted to the cholangiocytes, the epithelial cells of the bile duct (9). Because of the transgenic promoter used (ApoE), OATP1A2 is expressed in hepatocytes in Slo1a1b−/− mice (Fig. 4), however, at least murine Oatp2b1 is unlikely to contribute much to this uptake.

Importantly, expression of OATPs has been detected in tumor cells as well. For example, OATP1B3 was found expressed in gastrointestinal cancers (gastric, pancreatic, and colon cancer; ref. 6), hepatocellular carcinomas (11), breast carcinomas (13), and lung cancer (12). In addition, expression of OATP1B1 and OATP1A2 was found in malignant breast cell lines (14, 43), and OATP1A2 (and OATP2B1) have also been localized in the blood–brain barrier and at the apical membranes of the kidney tubules and small intestine (8–10), suggesting a possible role of this transporter in brain penetration and the reabsorption of urinary and intestinal compounds and/or drugs (including, e.g., methotrexate and paclitaxel).

Moreover, the immunoreactivity of OATP1B3 in breast carcinomas was inversely correlated with the tumor size and directly associated with improved effectiveness of tamoxifen therapy (46). These results suggest a potential link between expression of OATPs and tumor development and/or susceptibility to chemotherapy. The presence or absence of OATPs in tumor cells, therefore, might codetermine the sensitivity of these cells to methotrexate, paclitaxel, and many other anticancer drugs that are OATP substrates, as postulated earlier for methotrexate sensitivity of gastrointestinal cancers (6). Further studies with our humanized OATP1A/1B mice may help to assess the in vivo importance of human OATP1A/1B transporters in pharmacokinetics of many other anticancer drugs and their implications for therapeutic efficacy.

Many genetic polymorphisms affecting the functionality of OATP transporters have been identified (32). A recent genome-wide study showed that several SNPs in SLCO1B1 associated with reduced transport activity were linked with decreased plasma clearance and decreased gastrointestinal toxicity in children treated with methotrexate (47). Moreover, we recently discovered that individuals who suffer from the Rotor syndrome are fully deficient of both OATP1B1 and OATP1B3 activity (17). While the incidence of Rotor syndrome is very low (roughly estimated around 1 in 106 individuals, depending on the population analyzed), we also found indications for a much higher incidence of single full OATP1B1 or OATP1B3 deficiencies in various populations (17). Again, roughly estimated, it would not surprise us if at least 1 in 10,000 individuals, if not more, in many human populations would be fully deficient for either OATP1B1 or OATP1B3 alone. Such full deficiencies may substantially affect the plasma, tissue, and tumor cell pharmacokinetics and toxicokinetics of administered OATP substrate drugs. Note also that such individuals, unlike Rotor subjects, will not be immediately recognizable from obvious jaundice (17).

The present study illustrates that human OATP1B1, OATP1B3, and/or OATP1A2 affect the in vivo pharmacokinetic behavior of 2 anticancer drugs that are near the extremes of the OATP1A/1B substrate spectrum: methotrexate as a polar, constitutively charged anion, and paclitaxel as a highly lipophilic, uncharged compound. Very likely, many drugs in the vast array of OATP1A/1B substrates with intermediate physicochemical properties will be similarly affected. The OATP1A/1B-humanized mice will therefore present important models to study such in vivo functions of OATP1A/1B proteins.

Disclosure of Potential Conflicts of Interest
A.H. Schinkel has a commercial research grant from GlaxoSmithKline (S2918) and his research group receives revenue from the commercial distribution of some of the mouse strains used in this study. No potential conflicts of interest were disclosed by the other authors.

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Grant Support
E. van de Steeg, A. van Esch, E. Wagenaar, and A.H. Schinkel were supported in part by the Dutch Cancer Society (NKB 2000–2143 and 2007–3764), GlaxoSmithKline (S2918), and the Technical Sciences Foundation of the Netherlands Organization for Scientific Research (NWO/STW; BFA.6165).

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Received June 25, 2012; revised November 13, 2012; accepted December 4, 2012; published OnlineFirst December 14, 2012.

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superfamily, new nomenclature and molecular/functional properties.
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