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/C0/C0 (Oatp1a/1b knockout), Slco1a/1b/C0/C0;1B1tg, Slco1a/1b/C0/C0;1B3tg, and newly generated Slco1a/1b/C0/C0;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/C0/C0 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.
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, -1B3, and -1A2 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 OATP1A1/B transporters occur in certain tumors, they might also directly affect tumor susceptibility to methotrexate or paclitaxel treatment. We speculate that variation in OATP1A/B 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.

By using Oatp1a/1b knockout mice, we recently established an important role of mouse Oatp1a/1b transporters in the in vivo pharmacokinetics and tissue distribution of methotrexate and paclitaxel. Briefly, in the absence of Oatp1a/1b transporters, plasma levels of methotrexate and paclitaxel were markedly increased, whereas hepatic uptake and biliary excretion were significantly decreased (15, 16). Methotrexate and paclitaxel are structurally diverse anticancer drugs, with methotrexate being a charged organic anion and paclitaxel a highly hydrophobic drug. These results illustrate the broad substrate specificity of OATP1A/B transporters. However, due to the lack of direct orthology between mouse and human OATPs, together with the current lack of knowledge about species differences concerning substrate specificity, extrapolation of results obtained from Oatp1a/1b knockout mice to the human situation may at times be difficult. Moreover, in vitro to in vivo extrapolation of human transporter protein data can be challenging for various reasons (plasma protein binding of substrates, redundancy with or counteraction by other transporters, tissue- or cell-specific modification of proteins, and/or composition of the plasma membranes). To overcome these limitations, we have now generated humanized OATP1B1, OATP1B3, and OATP1A2 transgenic mice, whereas in humans, hepatic OATP1A2 is only found in cholangiocytes (9). Sko1a/1b/1A2 mice, therefore, do not mimic the normal function of OATP1A2 in the liver. However, they can be readily used to study the in vivo transport capacity of human OATP1A2 for various compounds, which is of importance given the possible role of OATP1A2 in drug uptake among others in the intestine, blood–brain barrier, and tumor cells.

In the present study, we conducted a basic characterization of the humanized strains. We then used these strains to analyze the in vivo influence of the 3 individual human OATP1A/B transporters on the disposition of the anticancer drugs methotrexate and paclitaxel, to better understand their possible relevance in human cancer chemotherapy.

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, Sko1a/1b/1A2 (Oatp1a/1b knockout; ref. 15), Sko1a/1b/1B3/1A2, Sko1a/1b/1B3, and Sko1a/1b/1A2 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. Sko1a/1b/1B3/1A2, Sko1a/1b/1B3, and Sko1a/1b/1A2 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-III; Hope Farms) and acidified water ad libitum.

Chemicals and reagents

Paclitaxel was from Sequoia Research Products, methotrexate (100 mg/mL Emthexate PF) from Pharmachemie, isoflurane (Forane) from Abbott Laboratories, and heparin (5,000 IE/mL) from Leo Pharma BV.
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 ClaI 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 XhoI. PCR products from both human crude liver membrane; Wt, wild-type; 1B1-tg, Slco1a/1b-/-;1B3tg, Slco1a/1b-/-;1B3tg, and Slco1a/1b-/-;1A2tg mice was detected by Western blotting. HCLM, human crude liver membrane; Wt, wild-type; 1B1-tg, Slco1a/1b-/-;1B1tg; 1B3-tg, Slco1a/1b-/-;1B3tg; 1A2-tg, Slco1a/1b-/-;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).

In pBluescript SK+ vector was isolated, and 3' and 5' ends of the vector were ressealed with Klenow enzyme to create blunt ends. After blunt end ligation, the vector was digested with XhoI. PCR products were used to generate the 457 bp DraI/ScaI pLIV-LE6-construct and the 7.6 kb DraI pLIV-LE6-fragment. Plasmid DNA was linearized, and irrelevant plasmid DNA was removed by restriction digestion with Sall and Drdl, followed by pronuclear injection into fertilized oocytes of FVB mice. Two-cell stage embryos were implanted into oviducts of pseudopregnant F1 fosters and carried to term. Positive founders were bred to homozygosity of the transgene locus, also in Slco1a/1b-/- background.

PCR and Southern blot analysis

OATP1B3 and OATP1A2 transgenic founder lines were initially detected by PCR screen using the following primers: forward 5'-GAAGGCTAACCTGGGGTGAG-3' and reverse 5'-CATGTTCCCATAGGAGACAACC-3' for OATP1B3 transgenics (yielding a 457 bp fragment), and forward 5'-GAAGGCTAACCTGGGGTGAG-3' and reverse 5'-GCCACTGTTATGCGCAAAG-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/ScaI pLIV-LE6-SLCO1A2.
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: Sloc2b1, Sloc10a1, Abcc2-4, Abcb1a, Abcb1b, Abcb11, Abcg2, Aox1, Aox2, and Ugt1a1.

**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. Keppeler (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 Sloc1a1b−/−;B3G 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, Sloc1a1b−/−;B3G mice, we isolated hepatic plasma by cardiac puncture under isoflurane anesthesia and urine by spot-collection. Ten microliters of blood was immediately protected from the light, snap-frozen, and stored at −80°C until further analysis. Concentrations of bilirubin monoglucononides (BMG), bilirubin diglucononide (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 Sloc1a1b−/−;Sloc1a1b−/−;B1G, Sloc1a1b−/−;B3G, and Sloc1a1b−/−;A2G mice, we isolated hepatic plasma by cardiac puncture and urine by spot-collection. Ten microliters of blood was immediately protected from the light, snap-frozen, and stored at −80°C until further analysis. Concentrations of bilirubin monoglucononides (BMG), bilirubin diglucononide (BDG), and unconjugated bilirubin (UCB) in plasma, bile, and urine were determined as described (23).

**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 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 Sloc1a1b−/− 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). Life span, body weight, and liver weight of Sloc1a1b−/−;B1G, Sloc1a1b−/−;B3G, or Sloc1a1b−/−;A2G 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
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S1). Only Abcc3/Slco3a1 mRNA was substantially (2- to 4-fold) downregulated in all the humanized strains relative to the wild-type and Slco1a1/C0/C0 mice, 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 Slco1a1/C0/C0;1B1tg and Slco1a1/C0/C0;1A2tg mice, respectively. Only after very long exposure, minimal amounts of OATP1B1 were detected in kidneys of Slco1a1/C0/C0;1B1tg and Slco1a1/C0/C0;1B3tg mice (not shown). Slco1a1/C0/C0;1B3tg mice 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 Slco1a1/C0/C0;1B3tg mice in the abundant hepatocytes.

Immunohistochemical staining confirmed basolateral (sinusoidal) localization and disperse distribution of human OATP1B3 throughout the liver lobules of Slco1a1/C0/C0;1B3tg mice (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 Slco1a1/C0/C0;1B3tg mice, 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 Slco1b1/C0/C0 transgenic mice was basolateral and throughout the liver lobule, albeit with stronger staining of OATP1B1 around the portal vein (18). Immunohistochemical staining confirmed a similar basolateral localization and lobular distribution of OATP1B1 in the liver of Slco1a1/C0/C0;1B1tg mice (not shown). The basolateral localization of these OATPs throughout the liver lobule supports the physiologic relevance of these humanized models. OATP1A2 antibodies available to us did not work well in immunohistochemistry, but subsequent physiologic and pharmacologic analyses (see below) were in line with the basolateral expression and transport activity of transgenic OATP1A2 in hepatocytes, analogous to OATP1B1 and OATP1B3.

Analysis of plasma and urine levels of unconjugated and conjugated bilirubin

Preliminary partial physiologic characterization of Slco1a1/C0/C0;1B1tg and Slco1a1/C0/C0;1B3tg mice has shown almost complete reversal of the increased plasma and urine levels of bilirubin glucuronide seen in Slco1a1/C0/C0;1B1tg mice (15, 17). Measurement of these parameters in Slco1a1/C0/C0;1A2tg mice and comparison with the known results for Slco1a1/C0/C0;1B1tg and Slco1a1/C0/C0;1B3tg mice 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 Slco1a1/C0/C0;1B3tg mice 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, Slco1a1/C0/C0, Slco1a1/C0/C0;1B1tg, Slco1a1/C0/C0;1B3tg, and Slco1a1/C0/C0;1A2tg mice, and analyzed plasma, liver, and
intestinal (tissue plus contents) levels. The effect of removal of mouse Oatp1a/1b transporters on methotrexate disposition 15 minutes after dosing to \textit{Slco1a/1b}/\textit{C0}\textit{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 \textit{Slco1a/1b}/\textit{C0}\textit{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 \textit{Slco1a/1b}/\textit{C0}\textit{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 or OATP1B3 or OATP1A2 humanized transgenic mice 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−/− 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−/− mice was restored to wild-type levels in Slco1a/1b−/−;1B3tg and Slco1a/1b−/−;1A2tg mice, but not in Slco1a/1b−/−;1B1tg 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

Figure 4. Influence of human OATP1B1, OATP1B3, and OATP1A2 transporters on methotrexate (MTX) disposition in vivo. Female wild-type, Slco1a/1b−/−, Slco1a/1b−/−;1B1tg, Slco1a/1b−/−;1B3tg, and Slco1a/1b−/−;1A2tg mice were i.v. dosed with methotrexate [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) 15 minutes after administration were determined. All data are presented as mean ± SD (n = 3–5; *, P < 0.05; **, P < 0.01; †††, P < 0.001 when compared with wild-type mice; †, P < 0.05; ††, P < 0.01; †††, P < 0.001 when compared with Slco1a/1b−/− mice).
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Figure 5. Influence of human OATP1B1, OATP1B3, and OATP1A2 transporters on tissue distribution of 7OH-MTX in vivo. Female wild-type, Slco1a/1b−/−;Slco1a/1b−/−;1B19, Slco1a/1b−/−;1B30, and Slco1a/1b−/−;A29 mice were dosed with methotrexate (10 mg/kg i.v.) and 7OH-MTX concentrations in liver (A) and small intestine (including contents; B) 15 minutes after administration were determined. All data are presented as mean ± SD (n = 3–5; †, P < 0.05; ††, P < 0.01; †††, P < 0.001 when compared with wild-type mice; 1, P < 0.05; 11, P < 0.01; 111, P < 0.001 when compared with Slco1a/1b−/− mice). ND, not detectable; detection limit was 0.02 μg/mL, that is, approximately 0.03% of dose in liver and small intestine. Statistical significance of differences of data compared with Slco1a/1b−/− mice was calculated by assuming Slco1a/1b−/− values to be 0.03 ± 0.03% of dose. Plasma levels of 7OH-methotrexate were below the limit of detection in all strains.

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, thus affecting the plasma and tissue levels of these compounds. The same applies to hepatic uptake and plasma levels of methotrexate, whereas OATP1B3 and OATP1A2, but not OATP1B1, 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 OATP1B-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 into the liver, and that both transporters can at least partly compensate for the loss of the murine Oatp1a/1b transporters.

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 OATP1B3 and OATP1A2, but not OATP1B1, 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.
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 μmol/L 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 OATP1B1 and OATP1B3 are together involved in the hepatocellular uptake of methotrexate, whereas transporter-mediated liver uptake of paclitaxel may be primarily mediated by OATP1B3. Letschert and colleagues (2006) found that paclitaxel is not a substrate for human OATP2B1 in vitro.
is expressed in hepatocytes in Because of the transgenic promoter used (ApoE), OATP1A2 the cholangiocytes, the epithelial cells of the bile duct (9). uptake.

murine Oatp2b1 is unlikely to contribute much to this (2009) have shown

barrier of gliomas (44). Recently, Takano and colleagues

OATP2B1) have also been localized in the blood–tumor

nant breast cell lines (14, 43), and OATP1A2 (and

tumor cells as well. For example, OATP1B3 was found

sorption of urinary and intestinal compounds and/or drugs

role of this transporter in brain penetration and the reab-

abundant expression of OATP1A2 is found at the blood–

this model can therefore only be used to qualitatively study

results suggest a potential link between expression of OATPs

improved effectiveness of tamoxifen therapy (46). These

activity of OATP1B3 in breast carcinomas was inversely cor-

OATP1B1 or OATP1B3 alone. Such full deficiencies may

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 phar-

macokinetics and toxicokinetics of administered OATP

substrate drugs. Note also that such individuals, unlike

Roto 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 pharmacoki-

netic behavior of 2 anticancer drugs that are near the

extremes of the OATP1A/1B substrate spectrum: methotrex-

e 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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Development of methodology: E. Wagenaar

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. van de Steeg, E. Wagenaar

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. van de Steeg, A. van Esch, E. Wagenaar, A.H. Schinkel

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. van de Steeg, A. van Esch, E. Wagenaar

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