Short Communication

Methotrexate Pharmacokinetics in Transgenic Mice with Liver-Specific Expression of Human Organic Anion-Transporting Polypeptide 1B1 (SLCO1B1)^S

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ABSTRACT:

Human organic anion-transporting polypeptide 1B1 (OATP1B1) is an important hepatic uptake transporter that can transport a wide variety of drugs. In the present study, we have generated and characterized a transgenic mouse model with specific and functional expression of human OATP1B1 (SLCO1B1) in the liver. Immunohistochemical staining revealed basolateral localization of transgenic OATP1B1 in the liver, whereas no expression of OATP1B1 was found in the kidney and small intestine. Using this transgenic model, the in vivo role of human OATP1B1 in the disposition of the anticancer drug methotrexate (MTX) was studied. In mice on a semisynthetic diet, the area under the plasma concentration-time curve for intravenous methotrexate in SLCO1B1 transgenic mice was 1.5-fold decreased compared with wild-type mice. Furthermore, the amount of MTX in the liver was markedly higher (2-fold) in the SLCO1B1 transgenic mice compared with wild-type mice, resulting in 2- to 4-fold higher liver-plasma ratios of MTX. Some murine liver Slc genes were markedly down-regulated on the semisynthetic diet compared with a standard diet, which probably reduced murine Oatp-mediated MTX uptake in the liver and therefore facilitated detection of the function of the transgenic OATP1B1. Taken together, these data demonstrate a marked and possibly rate-limiting role for human OATP1B1 in MTX elimination in vivo. Variation in OATP1B1 activity due to genetic polymorphisms, drug-drug interactions, and possibly dietary conditions may therefore play a role in the severity of MTX-related toxicity. SLCO1B1 transgenic mice could be a useful tool in studying the in vivo role of human OATP1B1 in drug pharmacokinetics.

Human OATP1B1 (previously called OATP-C, LST-1, or OATP2; gene name, SLCO1B1) is highly expressed at the basolateral (sinusoidal) plasma membrane of hepatocytes and could play a key role in the uptake of compounds into the human liver (Abe et al., 1999; König et al., 2000; Tamai et al., 2000). In a recent study, however, SLCO1B1 mRNA was also detected in human enterocytes (Glæser et al., 2007). OATP1B1 has a broad substrate specificity and seems to be involved in the transport of bile salts, bromosulphalein, steroid conjugates, the thyroid hormones T3 and T4, and drugs like benzylpenicillin, rifampicin, pravastatin, pitavastatin, rosuvastatin, fexofenadine, and methotrexate (König et al., 2006; Matsushima et al., 2008). The importance of OATP1B1 in the therapeutic efficacy and toxic side effects of substrate drugs has been confirmed by several studies focusing on genetic polymorphisms in SLCO1B1. For example, a commonly occurring haplotype, SLCO1B1*15, containing single nucleotide polymorphisms A388G and T521C, has been associated with a strongly reduced transport functionality, markedly increased plasma levels (2fold), and drastically reduced nonrenal clearance of the drugs pravastatin and pitavastatin in Japanese, Korean, and white populations (Nogawa et al., 2002; Nishizato et al., 2003; Niemi et al., 2004; Chung et al., 2005; Ho et al., 2007).

Methotrexate (MTX), a folate antimetabolite and a bicarboxylic organic anion, is widely used for the treatment of various types of cancer (i.e., breast cancer, head and neck cancer, lung cancer, and non-Hodgkin’s lymphoma). It is also used to treat nonmalignant diseases, including psoriasis and rheumatoid arthritis (van Outryve et al., 2002; Wessels et al., 2008). Two independent studies show that OATP1B1 is able to transport MTX in vitro, suggesting the impor-
In this study, we have generated and characterized a transgenic mouse model that shows substantial and functional expression of human OATP1B1 specifically in the liver. Using this transgenic model, the role of human OATP1B1 in MTX disposition in vivo was studied. Our results indicate that, in vivo, OATP1B1 can be a rate-limiting factor in the clearance of MTX, illustrating the potential use of this model in assessing drug pharmacokinetics.

Materials and Methods

Animals. Mice were housed and handled according to institutional guidelines complying with Dutch legislation. The animals used in this study were male SLOCO1B1 transgenic and wild-type mice of identical genetic background (FVB) between 9 and 14 weeks of age. Animals were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle. Mice received a standard diet (AM-II; Hope Farms, Woerden, The Netherlands) and acidified water ad libitum. Three weeks before specified experiments, mice were fed a semisynthetic diet (Reference diet 20% casein, 4068.02; Hope Farms).

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Chemicals and Reagents. Methotrexate (100 mg/mL, Emethexate PF) was obtained from Pharmachemie (Haarlem, The Netherlands). Methotrexate (Mefotec) was from Medical Developments Australia (Springvale, Victoria, Australia) and heparin (5000 UI/mL) was from Leo Pharma BV (Breda, The Netherlands). Bovine serum albumin, Fraction V, was obtained from Roche Diagnostics (Mannheim, Germany). Drug-free human plasma was obtained from healthy volunteers. The polyclonal antibody against human OATP1B1 was purchased from AbD Serotec (Mannheim, Germany) (Ko¨nig et al., 2000).

Western Blot Analysis. Crude membrane fractions from liver, kidney, and small intestine were prepared as described previously (Ogihara et al., 1996). The microsomal protein was quantified by the Bio-Rad protein assay based on the Bradford (1976) method (Bio-Rad, Hilden, Germany) for mouse Crude liver membrane; Wt, wild-type; Tg, SLOCO1B1 transgenic. Crude membrane protein (20 μg) was analyzed for all fractions. A molecular mass marker of 85 kD is indicated. Total protein staining (Ponceau S) confirmed equal loading across the lanes (bottom panel).

Immunohistochemical Analysis. The two-sided unpaired t test was used to assess the statistical significance of differences between two sets of data. Results are presented as the means ± S.D. Differences were considered to be statistically significant when P < 0.05. Averaged

MTX Pharmacokinetics. MTX (100 mg/mL saline) was diluted to 2 mg/mL in saline and was injected as a single bolus into the tail vein of male mice (n = 3–4 for each group) at a dose of 10 mg/kg (5 μL/g b.wt). Animals were killed at indicated time points by cervical dislocation after methotrexate administration, and methotrexate was analyzed in plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis as described previously (van Tellingen et al., 1989).

Pharmacokinetic Calculations and Statistics. The two-sided unpaired Student’s t test was used to assess the statistical significance of differences between two sets of data. Results are presented as the means ± S.D. Differences were considered to be statistically significant when P < 0.05. Averaged
Results and Discussion

Transgenic Mice Show Liver-Specific and Stable Basolateral Expression of Human OATP1B1. Stable and specific expression of human OATP1B1 in the liver of transgenic mice was achieved by using an ApoE promoter-HCR1-driven expression cassette containing human SLCO1B1 cDNA (Fig. 1A). A similar expression cassette was used before to generate liver-specific CYP3A4 transgenic mice (van Herwaarden et al., 2005). Integration of the transgenic construct into the mouse genome was confirmed by PCR and Southern blot analysis (data not shown). Transgene transmission occurred at the expected Mendelian ratios, and two independent homozygous SLCO1B1 transgenic founder lines were generated. Homozygous SLCO1B1 transgenic mice were fertile and did not differ from wild-type mice in life span or body weights. Clinical chemical, hematological, and pathological analyses did not reveal any abnormalities. Crude membrane fractions of liver, small intestine, and kidney of these mice (Fig. 1B). Immunohistochemical staining confirmed basolateral (sinusoidal) localization of human OATP1B1 throughout the liver lobules of transgenic mice (Fig. 2), as was shown for OATP1B1 expression in human liver (König et al., 2000; Ho et al., 2006). This supports the physiological relevance of this model. However, for SLCO1B1 transgenic livers, immunohistochemical staining was strongest around the portal vein (perportal; Fig. 2B), whereas weaker staining was found toward the central vein (centrolobular; Fig. 2C). Expression of transgenic OATP1B1 did not influence hepatic expression levels of murine Slco1a1, -1a4, -1b2, and -2b1 as measured by RT-PCR analysis (data not shown). Transgenic OATP1B1 expression was monitored over approximately five generations and was found to be stable (data not shown).

Expression Levels of Endogenous Slo Genes in Human SLCO1B1 Transgenic Mice on Semisynthetic Diet. Pilot studies with high-dose MTX (50 mg/kg) in SLCO1B1 transgenic mice fed with a standard diet resulted in only minor differences between transgenic and wild-type mice [17% decrease in plasma AUC (6194 ± 220 versus 7506 ± 517 nmol · h L −1; P = 0.07) and maximally 2.0-fold increase in liver accumulation (P < 0.01) after intravenous administration; Supplemental Data 1]. These modest effects suggest that under standard conditions, the transgenic OATP1B1 activity does not go much beyond the endogenous murine Oatp activity. Therefore, we switched the mice from the standard diet to a semisynthetic diet, as we expected that this would result in down-regulation of some Oatps, because the semisynthetic diet contains less phytochemicals than the standard diet (composition of both diets are shown in Supplemental Data 2). Phytochemicals are well known inducers of detoxifying systems by activating pregnane X receptor, constitutive androstane receptor, and possibly other xenobiotic nuclear receptors (e.g., van Waterschoot et al., 2008a). RT-PCR analysis for a set of endogenous hepatic SLCO genes was performed to determine diet-dependent alterations in mRNA levels in the liver of
wild-type and SLCO1B1 transgenic mice. We found that mouse Slco1a1, Slco1a4, Slco1b2, and Slco2b1 were indeed (markedly) down-regulated in livers of both wild-type and SLCO1B1 transgenic mice on the semisynthetic diet. These observed decreases were of the same order of magnitude in the two strains, with 1.7- and 2.6-fold (Slco1a1), 3.9- and 6.3-fold (Slco1a4), 2.5- and 1.7-fold (Slco1b2), and 1.5- and 1.5-fold (Slco2b1) decreases in wild-type and SLCO1B1 transgenic mice on the semisynthetic diet, respectively (Fig. 3). Because mouse Oatp1a4 is also a MTX transporter (Sasaki et al., 2004), the marked down-regulation of Slco1a4 in mice fed with the semisynthetic diet might reduce background of murine Oatp-mediated MTX uptake in the liver. It is noteworthy that protein expression of the transgenic human OATP1B1 (which is controlled by the ApoE promoter) was not affected by the semisynthetic diet, as analyzed by Western blotting (data not shown).

Incidentally, our results show that expression of some Slco1 and Slco2 genes can be markedly affected by dietary conditions. Given the impact of OATP on drug disposition (see also below), it will be interesting to investigate whether this also applies in humans.

SLCO1B1 Transgenic Animals Show Increased Hepatic Uptake of MTX and Lower Plasma Concentrations. To test the in vivo functionality of the transgene, we evaluated MTX disposition in SLCO1B1 transgenic versus wild-type mice fed with the semisynthetic diet. At various time points after intravenous administration of 10 mg/kg MTX, blood samples were taken and livers were isolated. The amounts of MTX, and its main metabolite 7-OH-MTX, were determined by HPLC analysis. Plasma AUC for MTX in SLCO1B1 transgenic mice was 1.5-fold decreased compared with wild-type mice (1261 ± 30.3 versus 1857 ± 112 nmol · h⁻¹; P < 0.05; Fig. 4A). The inset in Fig. 4 illustrates that the terminal elimination of plasma MTX is somewhat faster in SLCO1B1 transgenic mice compared with wild-type mice (semilog scale). This supports a role of transgenic OATP1B1 not just in short-term liver accumulation, but also in longer-term plasma clearance. Furthermore, the amount of MTX in the liver was markedly increased (~2-fold) at all time points in the SLCO1B1 transgenic mice compared with wild-type mice (Fig. 4B). Liver to plasma ratios of MTX showed 2.2-, 2.6-, and 4.2-fold increases in mice expressing human OATP1B1 compared with wild-type mice at 15, 30, and 60 min after injection, respectively (P < 0.001; Fig. 4C). Plasma concentrations of 7-OH-MTX, which is primarily formed by aldehyde oxidase in the liver, were low and only significantly decreased in SLCO1B1 transgenic mice compared with wild-type mice 30 min after MTX administration (93.3 ± 24.3 versus 192.7 ± 67.8 nM; P < 0.05). 7-OH-MTX amount in the liver did not differ between transgenic and wild-type mice (data not shown).

MTX was earlier identified as a substrate for human OATP1B1 in vitro (Abe et al., 2001; Sasaki et al., 2004). To the best of our knowledge, this is the first study that shows that OATP1B1 is an
important hepatic uptake transporter for MTX in vivo, with a rate-limiting role in MTX plasma elimination. Interindividual variation in MTX efficacy and toxicity correlating with plasma levels (Gorlick and Bertino, 1999) is a well recognized obstacle in the treatment of patients with cancer and rheumatoid arthritis. Because many genetic and functional variants in SLCO1B1 have been identified (Tirona et al., 2001; Nozawa et al., 2002; Nishizato et al., 2003; Niemi et al., 2004; Chung et al., 2005), the results of this study imply that variations in OATP1B1 activity, due to genetic polymorphism, dietary conditions, and perhaps drug-drug interactions, can have profound effects on plasma pharmacokinetics of MTX in patients and therefore partly explain interindividual variation. Thus, OATP1B1, in addition to other hepatic transporters like multidrug resistance protein 2 (Vlaming et al., 2006), may play an important role in MTX-related hepatic and/or plasma exposure-dependent toxicity. For example, MTX treatment is associated with acute and chronic liver damage (Hirvikoski et al., 1997; van Outryve et al., 2002). It would be interesting to see whether the observed toxicity could be correlated with genetic polymorphisms in SLCO1B1. At this moment, we can only speculate about this, and further research needs to investigate the clinical implications of MTX as an OATP1B1 substrate.

In conclusion, this study describes a novel liver-specific SLCO1B1 transgenic mouse model that provides an appropriate tool to study the role of human OATP1B1 in drug pharmacokinetics in vivo. In recent studies, Slc1b2 knockout mice have been described previously (Lu et al., 2008; Zaher et al., 2008). Because mouse Slc1b2 is orthologous to human SLCO1B1 and SLCO1B3 (Hagenbuch and Meier, 2004), Slc1b2 knockout mice might be a useful model to combine with our SLCO1B1 transgenic mice to generate a humanized model for analysis of human OATP1B1 function in vivo.

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References


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