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Identification, Expression, and Functional Characterization of Full-Length and Splice Variants of Murine Organic Anion Transporting Polypeptide 1b2

Henriette E. Meyer zu Schwabedissen,[†] Joseph A. Ware,[‡,§] Rommel G. Tirona,[†,||] and Richard B. Kim*[,†,||]

Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, London, Ontario, Canada, Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada, Pfizer Global Research and Development, Ann Arbor, Michigan, and Genentech Inc., San Francisco, California

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Abstract: The family of organic anion transporting polypeptides (OATPs) plays an important role in mediating the cellular uptake of numerous endogenous and exogenous compounds. Members of this family include human OATP1B1 and OATP1B3, which have been widely studied and shown to be involved in the hepatic uptake of hormones, bile acids, and many clinically used drugs. However, little is known about the murine orthologue, Oatp1b2. We determined expression of mouse oatp1b2 mRNA and protein using real-time PCR and Western blot analysis. Interestingly, mRNA transcripts and protein were detectable in a number of tissues including kidney and stomach, and, not surprisingly, the highest expression was noted in liver. Cloning of the full coding region of oatp1b2 revealed the presence of two novel splice variants. Interestingly, these splice variants were significantly expressed in organs such as the kidney, but much less in liver. Heterologous expression of the full-length Oatp1b2 cDNA revealed that taurocholic acid, estrone 3-sulfate, estradiol 17β-glucuronide and pravastatin are substrates of this transporter. The newly identified splice variants were unable to significantly transport substrate compounds due to defects in cell surface trafficking. Our findings of murine Oatp1b2 expression and function will likely aid in better defining species related differences in OATP transporter function and the use of mouse models to predict hepatic drug disposition in humans.

Keywords: Organic anion transporting polypeptide; Oatp1b2; mouse; uptake transporter; liver

Introduction

The liver plays an important role in the elimination of a wide variety of endogenous and exogenous compounds. The first step leading to the hepatic elimination process is the cellular uptake from the portal blood across the sinusoidal membrane of hepatocytes. Members of the organic anion transporting polypeptides family (OATPs) including OATP1B1 (OATP-C, LST-1, *SLCO1B1*) and OATP1B3 (OATP-8, LST-2, *SLCO1B3*) are known to exhibit a liver-enriched pattern of expression and mediate the sodium independent uptake of several compounds including bile salts, organic anions, steroid hormones and their conjugates, and a variety of clinically used drugs including HMG-CoA inhibitors,[1]

* Corresponding author: Richard B. Kim, M.D., ALL-152 LHSC—University Hospital, 339 Windermere Road, London, Ontario, N6A 5A5, Canada. Tel: 519 663 3553. Fax: 519 663 3232. E-mail: richard.kim@lhsc.on.ca.

[†] Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario.

[‡] Pfizer Global Research and Development.

[§] Genentech Inc.

[||] Department of Physiology and Pharmacology, University of Western Ontario.

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angiotensin II receptor antagonists,[2,3] and the antituberculosis agents rifampin and rifamycin SV.[4,5]

Oatp1b2 is the murine orthologue of the human OATP1B1 (OATP-C) and OATP1B3 (OATP-8) and shares 65% sequence identity with the human OATP1B transporters, whereas the OATP1B1 and -1B3 share 80% sequence homology.[6] The murine Oatp1b2 shares 54% and 44% sequence homology with mOatp1a1 and mOatp2b1, respectively. Interestingly, while there is a significant amount of published data regarding rat Oatps, not much is known about murine Oatps, particularly in relation to substrate specificity. Although there is the implicit assumption that Oatp1b2 has tissue expression and substrate specificity similar to that of OATP1B1 and -1B3, at the present time there is little known regarding this transporter. What we do know is, that Oatp1b2 has been shown to be expressed exclusively in mouse liver as detected by Northern blot analysis.[7] However additional expression in the ciliary body epithelium of the eye has been reported previously.[8] Recently, our group has reported that Oatp1b2 is a major determinant of hepatocellular uptake of rifampin and pravastatin *in vivo* using a *Slco1b2*$^{-/-}$ mouse model.[11] Another study revealed that effects of the hepatotoxins microcrystin-LR and phalloidin, known substrates of members of the human OATP1B transporters, are markedly attenuated in the absence of this transporter in mice.[9]

However, it is clear that additional *in vitro* data regarding expression and function of murine Oatp1b2 are needed if extrapolation to the human OATP1B transporters is to be made based on Oatp1b2 mouse models. We now provide new data that show that murine Oatp1b2 is able to transport a spectrum of OATP1B substrates and that a number of splice variants exists. Importantly these splice variants appear to be more readily expressed in nonhepatic tissues but unable to appropriately traffic to the cell surface. Our findings may be of particular relevance in the use of murine models to predict human OATP drug transporter function.

Experimental Section

Chemicals. The following radiolabeled compounds used for transport experiments were obtained from GE Healthcare (Baie d'Urfe, Quebec, Canada): [^{3}H]-cortisol, [^{3}H]-CCK8, and [^{3}H]-fexofenadine. [^{3}H]-Folic acid was purchased from Moravek Biochemicals (Brea, CA), and [^{3}H]-atorvastatin, [^{3}H]-tetracycline, [^{3}H]-ezetimibe and [^{3}H]-verapamil were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). [^{3}H]-Digoxin, [^{3}H]-estrone 3-sulfate, [^{3}H]-estradiol 17β-glucuronide, [^{3}H]-taurocholate, [^{3}H]-cholic acid and ^{14}C-erythromycin were obtained from PerkinElmer (Waltham, MA). [^{3}H]-Pravastatin was kindly provided by Dr. Todd Kirchgessner (Bristol-Myers Squibb). Unlabeled rifampin, folic acid, tetracyclin, verapamil, digoxin, estrone 3-sulfate, estradiol-17β glucuronide, taurocholate, pravastatin and erythromycin were obtained from Sigma Aldrich (St. Louis, MO).

Tissue Samples. Mouse tissue samples were obtained from two DBA1/lacJ mice. After harvesting, the tissue samples were snap frozen in liquid nitrogen and stored at −80 °C until further use.

RNA Isolation from Mouse Tissue. RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). After mechanical homogenization of the tissue in RLT-buffer supplemented with β-mercaptoethanol, the homogenate was thawed and centrifuged through a QiaShredder column. Subsequently the eluted solution was supplemented with an equal volume of isopropanol and loaded on the purification columns. The following RNA isolation was performed as described by the manufacturer. The integrity and content of the RNA was determined using the Agilent Bioanalyzer (Agilent, Santa Clara, CA). RNA samples were stored at −80 °C.

Real-Time PCR. Total RNA was reverse transcribed in a 50 μL reaction volume containing 1500 ng of total RNA using the TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, CA) supplied with random hexamers. Subsequently the expression of oatp1b2 and 18S were measured using quantitative real-time PCR Assays from Applied Biosystems Mm00451513_m1 and Hs99999901_s1. Real-time PCR was performed using the ABI Prism 7700 sequence detection system (Applied Biosystems). The quantitative PCR was carried out in a 25 μL reaction volume containing 1×Primer and Probe Mix, 1×PCR Master Mix

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and 40 ng of the reverse transcribed cDNA. The amount of the transporter was normalized to 18S rRNA.

Enrichment of Membrane Proteins from Mouse Tissue. Protein isolation was performed as described elsewhere.[10] Briefly, after homogenization of the tissue in 5 mM Tris-HCl pH 7.4 containing protease-inhibitors (Sigma-Aldrich) using a Potter Elvejhem, the homogenate was incubated 1.5 h under constant stirring on ice and then was centrifuged for 20 min at 4 °C and 9000*g*. Subsequently, the supernatant was transferred to an ultracentrifugation tube and centrifuged for 45 min at 4 °C and 100000*g* to enrich the crude membrane fraction of each sample. After that the supernatant which contains the majority of the intracellular proteins was transferred to a new tube and stored at −80 °C. The remaining pellet containing the crude enriched membrane fraction of the homogenized tissue was resuspended in 100 μL of 5 mM Tris-HCl supplemented with protease-inhibitors and stored at −80 °C. Protein content was determined using a BCA-Assay System (Pierce, Rockford, IL).

Western Blot Analysis Using Crude Enriched Membrane Fraction. The enriched membrane fraction of mouse tissue was separated using a 4−10% NUPAGE SDS−PAGE gradient gel (Invitrogen, Carlsbad, CA). After separation, proteins were electrotransferred to a nitrocellulose membrane. Equal loading and separation capacity was controlled using Ponceau S solution (Sigma Aldrich). After blocking with 5% FBS in TBST, the expression of oatp1b2 was detected using a polyclonal anti-Oatp1b2 antibody (1:1000) raised against the sequence specific for mouse Oatp1b2 as described elsewhere.[11] The amount of immobilized antibody was detected using a HRP-conjugated secondary antibody (1:200) (BioRad, Hercules, CA), and visualized using the ECL Plus Detection System (GE Healthcare) and the KODAK Image-Station 4000MM (Mandel, Guelph, ON, Canada).

Immunofluorescence Microscopy. Protein localization of Oatp1b2 was investigated by immunofluorescence microscopy. Briefly, the tissue slides were boiled in citrate buffer (10 mM, pH 6.0) for 20 min. After washing twice for 10 min in ice-cold PBS, endogenous peroxidase activity was reduced in tissue slides for immunofluorescence by incubation in 0.03% H_2O_2 diluted in methanol for 20 min and washed again 2 × 5 min in PBS. Subsequently the slides were blocked for 30 min at room temperature with 5% FBS which has been diluted in PBS. Thereafter the slides were incubated with diluted anti-Oatp1b2-antibody (1:50) in a humidified atmosphere at 4 °C overnight. The unbound antibodies were removed by several washing steps with PBS. Then the sections were incubated with the fluorescent-labeled secondary antibody (Alexa Fluor 488 antirabbit) (Invitrogen) or peroxidase labeled secondary antibody (Vectorstain ABC-Kit, Vector Laboratories, Burlingame, CA) for 1.5 h at room temperature. After the slides were washed with PBS several times, tissue slides for immunofluorescence were mounted in antifading mounting medium containing DAPI (Vector Laboratories). Images were obtained by fluorescence microscopy.

Cloning of the Oatp1b2 Coding Sequence. For heterologous expression experiments using the T7-promoter driven vaccinia expression system, the oatp1b2 coding sequence was amplified using the AmpliTaq Gold PCR system (Applied Biosystems) and the following primers: oatp1b2-for 5′-TCAATCATGGACCAAACTCAGCATCCA-3′ and oatp1b2-rev 5′-TCTCTTCCCTTAAAGAGGT-GTTTCACT-3′. The PCR product was purified and ligated into pCR2.1-TOPO (Invitrogen), and after that the oatp1b2 sequence was transferred into pcDNA3.1-Hygro(−) (Invitrogen) using *Hin*dIII and *Xba*I. The sequence of the obtained oatp1b2-coding sequence clones was verified by sequencing using a T7 primer and the following sequencing primer: oatp1b2-seq1 5′-ATGTACTTAGGTACTCTGCAT-3′ and oatp1b2-seq2 5′-ATGGCCTATGTGTTCTAC-3′. The creation of the cDNA constructs for rat Oatp1b2 and human OATP1B1 and OATP1B3 is described in detail in our previous study.[5]

Heterologous Expression of Oatp1b2 in HeLa Cells. Transport studies using heterologous expression of oatp1b2 were performed as described elsewhere.[12] Briefly, HeLa cells grown in 12-well plates (~0.8×10^6 cells/well) were infected with vTF7-3 (ATCC# VR-2153) virus. After 30 min incubation at 37 °C, the cells in each well were transfected with 1 μg of vector control or variants of oatp1b2 packaged into pcDNA3.1-Hygro(−) (Invitrogen) along with Lipofectin (Invitrogen) and incubated at 37 °C for 16 h. The parental plasmid lacking any insert was used as control. Afterwards the cells were briefly washed with OptiMEM and then incubated with the radiolabeled compounds. Uptake of [^{3}H]-estradiol 17β-glucuronide, [^{3}H]-estrone 3-sulfate, [^{3}H]-pravastatin [^{3}H]-digoxin, [^{3}H]-fexofenadine and [^{3}H]-folate was assessed at 1 μM concentration, whereas [H]^3-cortisol, [^{3}H]-cholic acid, [^{3}H]-atorvastatin, [^{3}H]-verapamil and [^{3}H]-erythromycin were studied at 0.5 μM concentration. [^{3}H]-Taurocholate was assessed at 0.4 μM, while [^{3}H]-CCK8 and [^{3}H]-ezetimibe uptake was performed at tracer only concentrations of approximately 0.001 μM and 0.005 μM, respectively. After 10 min incubation at 37 °C, the cells were washed twice with ice cold PBS and lysed in the presence of 1% SDS. The cellular uptake was determined using Ultima Gold scintillation liquid and a liquid scintillation counter (Liquid Scintillation Counter, Tri-Carb 2900TR,

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Perkin-Elmer Life Sciences). All experiments were carried out in duplicate on at least 3 experimental days. The uptake is expressed as % of pcDNA3.1-Hygro(−) vector control.

Expression Levels of Oatp1b2 Isoforms. Expressed level of the oatp1b2 isoforms was detected using the following primers specific for the isoforms 2 and 3 as the primer binding sites are located in the area of splicing as determined by sequencing of the cloned PCR fragments: oatp1b2-isoform2-for 5′-TCCAAAGAGATCACAGAAGG-3′ and oatp1b2-isoform2-rev 5′-GACAATCCTCAAGTGGAACA-GACCGTC-3′ resulting in a 311bp PCR fragment; and oatp1b2-isoform3-for 5′-TCAGCTACATATGCAAGGCA-3′ and oatp1b2-isoform3-rev 5′-CTAATATCTTGTTAGT-TGCATACCTG-3′ resulting in a 282bp PCR fragment. For detecting the overall oatp1b2 expression in tissue the following primer pair was used: oatp1b2- (isoform3) for 5′-TCAGCTA-CATATGCAAGGCA-3′ and oatp1b2-consensus-rev 5′-GAAGCAACAACAGTTCCAATCA-3′ resulting in a 280bp PCR fragment. The amplification was performed in a 25 μL reaction volume containing 20 ng of cDNA, 1×PCR Buffer II, 1 mM $MgCl_2$, 300 nM of each primer and 0.5 U AmpliTaq Gold (Applied Biosystems). The PCR was carried out with initial denaturation at 95 °C for 1 min followed by 25 cycles with 30 s at 95 °C denaturation, 30 s at 54 °C annealing and a 30 s extension at 72 °C. The PCR product was separated on a 1.2% agarose gel containing ethidium bromide and imaged using a KODAK ImageStation 4000MM (Mandel, Guelph, ON, Canada). The band intensity was determined using the KODAK MI software.

Statistical Analysis. GraphPad Prism software was used for statistical analysis. The differences in tissue expression and cellular accumulation were evaluated using the unpaired *t* test, *p*-values <0.05 were considered statistically significant.

Results

Oatp1b2 Tissue Distribution. Oatp1b2 mRNA and 18Sr-RNA (housekeeping gene) were quantified using real-time PCR. Oatp1b2 was detectable in liver, stomach and kidney. The highest expression of Oatp1b2 was noted for liver, but measurable levels of mRNA transcripts were also seen in kidney and stomach (Figure 1A). Similar results were obtained for protein expression when assessed using Western blot analysis of crude enriched membrane fraction of liver, stomach, and kidney (Figure 1B) using a specific antibody. It is noteworthy that only 5 μg of protein was loaded of liver, whereas 50 μg protein of the other tissues was loaded when performing the Western blot analysis (Figure 1B).

Heterologous Expression of Oatp1b2. Transport activity of Oatp1b2 was determined using a heterologous transporter expression system. As shown in Figure 2 significant uptake after 10 min incubation was detected for bile acid and hormone conjugates such as ^{3}H-taurocholate (mean uptake % of vector control ± SD; 217.8 ± 23.7; $p < 0.05$), ^{3}H-estrone-3-sulfate (E_1S) (199.5 ± 8.2; $p < 0.05$) and ^{3}H-estradiol-17β-glucuronide (E_2G) (185.2 ± 19.8; $p < 0.05$). Significant uptake was also noted for drugs such as pravastatin (mean uptake % of vector control ± SD 140.3 ± 16.3, $p < 0.05$) and atorvastatin (125.6 ± 15.2; $p = 0.053$) (Figure 2A). Uptake of cortisol, CCK8, cholic acid, ezetimibe, digoxin, fexofenadine, folate, and erythromycin did not significantly differ from cells transfected with only the parental plasmid lacking Oatp1b2 (Figure 2A). Time dependency of the uptake of TCA uptake is shown in Figure 2B.

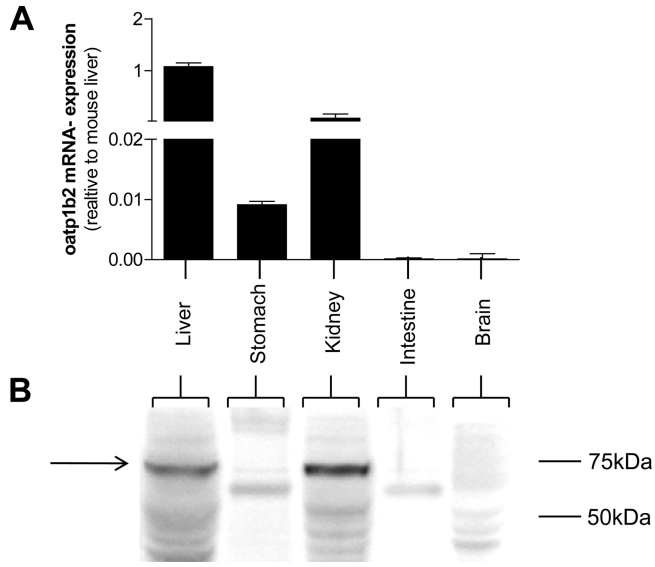

Figure 1. Tissue distribution of Oatp1b2. Expression of oatp1b2 was assessed using real-time PCR (A) and Western blot analysis (B). Expression of oatp1b2 was detected in the stomach and kidney with highest abundance in liver. Note that 5 μg of protein was loaded for liver, whereas 50 μg protein of the other tissues was loaded for the Western blot analysis (panel B) due to markedly greater band intensity of the liver sample. An arrow indicates the expected protein size. Data are expressed as mean ± SD.

Transport Activity Comparing Rodent and Human Orthologues. Uptake of endogenous compounds including ^{3}H-TCA, ^{3}H-E_1S and ^{3}H-E_2G was compared in cells transfected with mouse (moatp1b2), rat (roatp1b2), and human (OATP1B1 and OATP1B3) OATP1B orthologues. The uptake was determined after 10 min incubation with the radiolabel. As shown in Figure 3A differences in uptake of taurocholate is evident (mean uptake % of vector control ± SD; OATP1B1, 211.8 ± 107.6; OATP1B3, 140.3 ± 15.1; rat oatp1b2, 395.7 ± 23.6; moatp1b2, 195.9 ± 17.8) with highest uptake in cells transfected with rat Oatp1b2. Uptake of estrone-3-sulfate was comparable in each transfected cell line (mean uptake % of vector control ± SD; OATP1B1, 294.4 ± 34.1; OATP1B3, 219.9 ± 4.0; roatp1b2, 246.5 ± 10.7; moatp1b2, 219.0 ± 55.6) (Figure 3B). Similar to taurocholate, uptake of ^{3}H-estradiol 17β-glucuronide was seen for all the tested transporters, but far greater for rat Oatp1b2 (mean uptake % of vector control ± SD; OATP1B1, 868.9 ± 73.5; OATP1B3, 286.7 ± 66.9; roatp1b2, 2290.1 ± 43.1; moatp1b2: 233.6 ± 102.1) (Figure 3C). Note that the uptake data as presented should not be taken to mean differences in relative substrate affinity between the ortho-

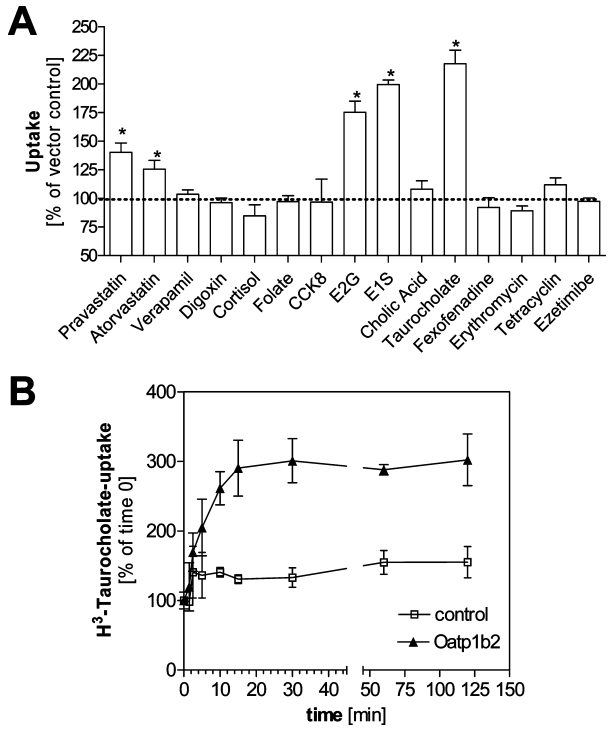

Figure 2. Oatp1b2 transport activity. Oatp1b2 was expressed using a recombinant vaccinia-based heterologous expression system. The uptake of a number of endogenous and exogenous compounds was assessed in HeLa cells (A). Cells transfected with the plasmid vector lacking any transporter cDNA were used as control. Uptake over time for taurocholic acid is shown in panel B.

logues. Also note that transcriptional activation is driven by an identical T7 promoter for all of the transporters, so in principle, transcription efficiency should be the same for all of the transfected cDNAs. However, it is not possible to cross compare different target protein levels for the various transporters.

Splice Variants of Oatp1b2. Sequencing of several cloned cDNAs of oatp1b2 amplified from Marathon mouse liver cDNA (Promega) revealed two new splice variants of the murine transporter. Additional splice donor sites in the *Slco1b2* gene encoding oatp1b2 on chromosome 6 resulted in truncated proteins. The splice variant 2 of Oatp1b2 (oatp1b2_v2) is missing exon 11 and parts of exon 12 of the coding sequence of oatp1b2 and the splice variant 3 (oatp1b2_v3) lacks exon 5 to 15 (Figure 4A,B). We sequenced a number of clones, and it appeared that these are common splice variants (10% and 20% of the clones sequenced, respectively). We then assessed for expression of these variants in different tissues as shown in Figure 4C. First the overall expression of full-length Oatp1b2 was determined by semiquantitative PCR which showed highest expression of the transporter in liver and much lower expression in kidney, confirming the real-time PCR results. Expression of oatp1b2_v2 and oatp1b2_v3 splice variants was determined using splice variant specific PCR primer

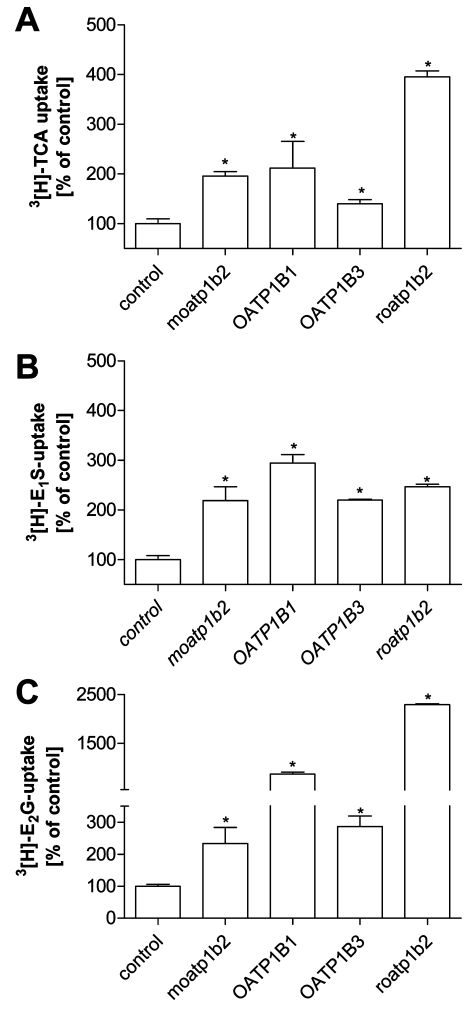

Figure 3. Comparison of transport activity for OATP1B orthologues. Uptake of endogenous compounds including taurocholic acid (TCA) (A), estrone 3-sulfate (E1S) (B) and estradiol-17β-glucuronide (E2S) (C) was measured using a recombinant vaccinia-based heterologous expression system. HeLa cells were transfected with human OATP1B1 human OATP1B3, rat Oatp1b2 and mouse Oatp1b2 and the uptake of labeled compounds were assessed after 10 min of incubation. Data are expressed as mean $\pm$ SD, $^*p < 0.05$ Students's *t* test.

pairs. As shown in Figure 4C and summarized in Table 1, the splice variants oatp1b2_v2 and oatp1b2_v3 are highly expressed in kidney, whereas very low expression of this variant was seen in mouse liver. In addition, oatp1b2_v2 was detectable in stomach.

Subcellular Expression and Transport Activity of Oatp1b2 Splice Variants. Transport of the prototypical substrates [^{3}H]-estrone 3-sulfate (Figure 5A), [^{3}H]-estradiol 17β-glucuronide (Figure 5B) and [^{3}H]-taurocholic acid (Figure 5C) was assessed using HeLa cells transfected with full-length and splice variants of Oatp1b2. As shown in Figure 5 Oatp1b2_v3 shows no significant transport activity toward the tested substrates. However, statistically significant but modest uptake of taurocholic acid was detected in cells expressing the splice variant 2 (% uptake of vector control

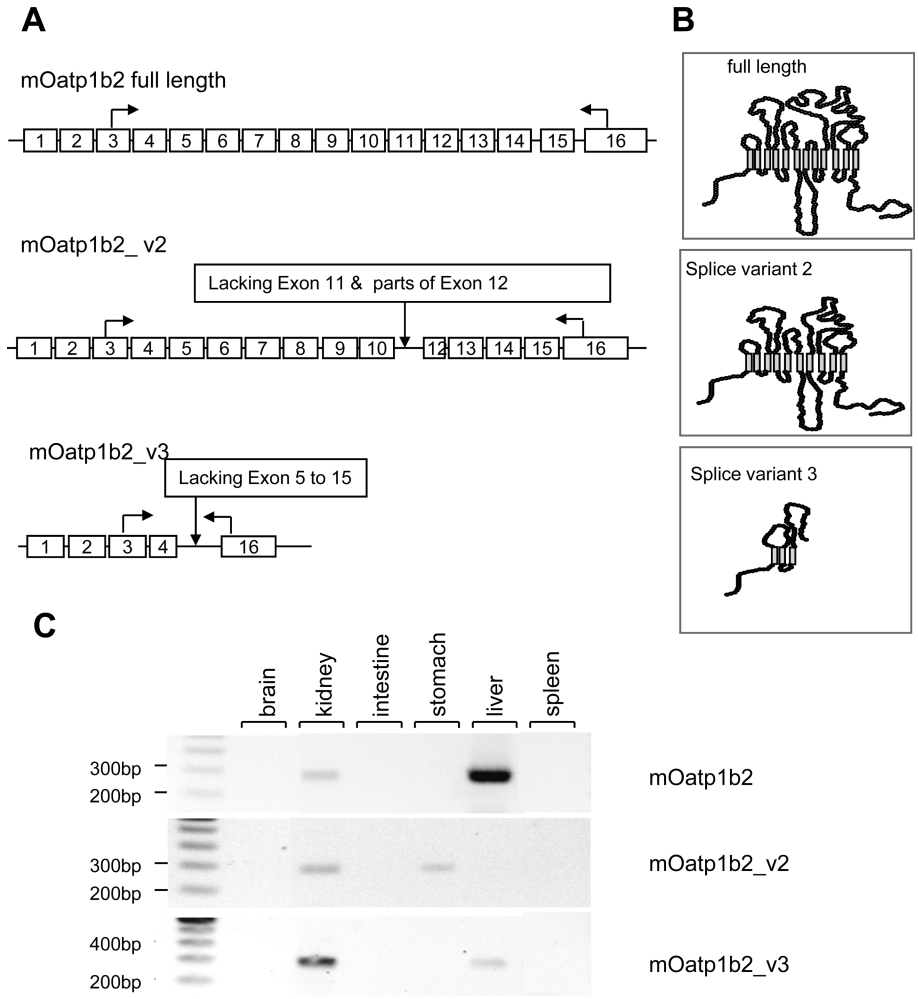

Figure 4. Genomic structure and expression of Oatp1b2 variants. The genomic structure of the full-length and the newly identified splice variants of Oatp1b2 are shown (A). The structure of the translated proteins was predicted using the TMpredict-algorithm (B). Expression of the identified splice variants was determined in multiple tissues using splice variant specific primers (C).

Table 1. Relative Band Intensity of Oatp1b2 Variants Detected Using Semiquantitative PCR[a]

	brain	kidney	intestine	stomach	liver	spleen
Oatp1b2	nd	1.00	nd	nd	18.93	nd
Oatp1b2 variant 2	nd	1.00	nd	0.91	nd	nd
Oatp1b2 variant 3	nd	1.00	nd	nd	0.58	nd

[a] The expression of Oatp1b2 variants was determined by semiquantitative PCR followed by agarose gel electrophoresis. Band intensity was determined using the KODAK image station. nd (not detected).

± SD, Oatp1b2 215.53 ± 24.75; Oatp1b2_v2 127.77 ± 10.74, $p < 0.05$). Expression of Oatp1b2 and Oatp1b2_v2 in HeLa cells was confirmed by immunofluorescence showing lower sorting to the membrane in cells transfected with the splice variant 2 (Figure 5D). However, Oatp1b2_v3 in HeLa cells was not detectable when assessed using the same conditions. It is possible that this is due to the absence of the epitope in exon 13 that is recognized by the Oatp1b2 antibody or that the protein is not synthesized.

Discussion

We know members of the human OATP1B subfamily are involved in mediating the uptake of many drugs used to treat diabetes, lipid disorders, and cancer. Knockout-mouse models are widely used to elucidate the function of specific drug transporters in drug disposition and elimination and have proven to be remarkably helpful to understanding drug disposition. Recent findings from our group and others show that deletion of *Slco1b2* results in reduced hepatic uptake and increased plasma levels of the known OATP1B substrates pravastatin and rifampin,[11] while protecting the mice from the hepatotoxic effect of micocrystin-LR and phalloidin,[13] also known substrates of the human OATP1B subfamily.[14,15]

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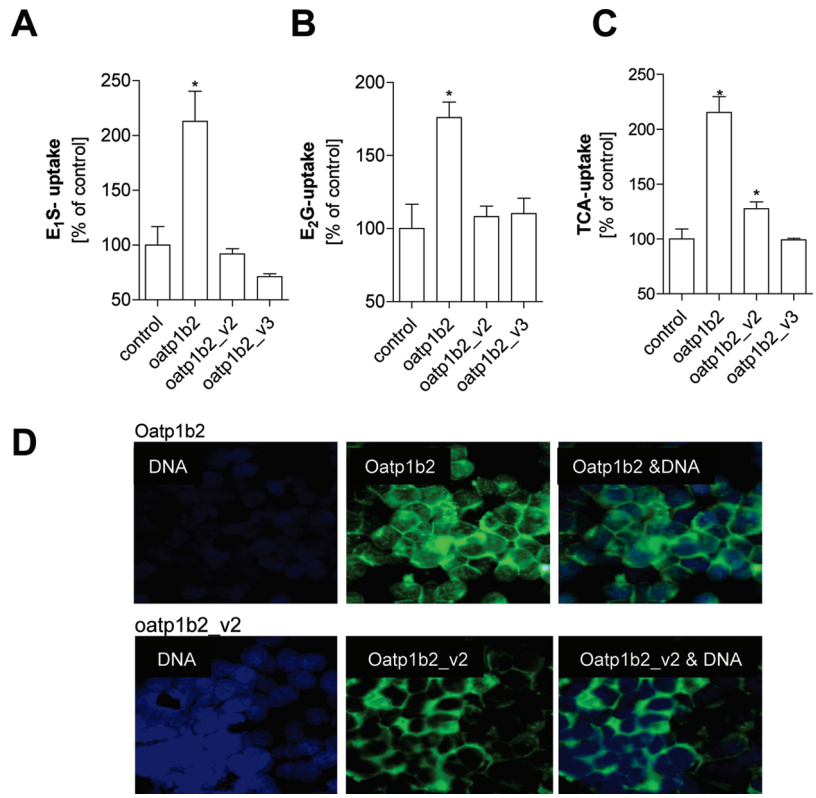

Figure 5. Functional activity of Oatp1b2 variants. The functional activity of Oatp1b2 splice variants 2 (Oatp1b2_v2) and 3 (Oatp1b2_v3) was assessed using the Oatp1b2 substrates estrone 3-sulfate (E1S) (A), estradiol 17β-glucuronide (E2G) (B), and taurocholate (TCA) (C). Oatp1b2 expression (green) was verified by immunfluorescent staining of transfected cells, where distinct membrane staining in cells transfected with Oatp1b2 variant 1 and to a lower extent in cells transfected with variant 2 were noted (D). DNA was counterstained with DAPI (blue). Data are expressed as mean $\pm$ SD.

However to fully understand the impact of human OATP1B transporters, additional insights relating to the expression and function of murine Oatp1b2 are needed. Expression of Oatp1b2 had been shown to be exclusive to liver.[16] However, we now note expression of Oatp1b2 mRNA and protein in other nonhepatic tissues including stomach and kidney (Figure 1A,B). Nevertheless Oatp1b2 is highly expressed in liver. In addition, murine Oatp1b2 is able to mediate the cellular uptake of known OATP1B substrates such as pravastatin, taurocholic acid, estrone 3-sulfate and estradiol 17β-glucuronide.[17] It should be noted that although much more data are available in relation to rat Oatps, not much is known regarding mouse Oatps, particularly in terms of substrate specificity or overlap.

We do note that much of the nonhepatic expression of Oatp1b2 appears to be due to novel splice variants of the transporter that result in the expression of truncated proteins. Oatp1b2-variant 2 (Oatp1b2_v2) and Oatp1b2-variant 3 (Oatp1b2_v3) are generated by the use of alternate splice donor sites in exon 10 and exon 4, respectively. Therefore simple mRNA measurements for Oatp1b2 in multiple tissues may result in misleading information regarding the true extent of expression of the fully functional and full-length Oatp1b2. Alternative splicing is a known source of diversity in the proteome of the cell and is recognized as a ubiquitous mechanism for controlling gene expression in a tissue specific manner.[18,19] Alternate splicing of pre-mRNA has been shown for a number of human transporters including the organic anion transporters OAT1 and

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OAT2,[20,21] and OATP3A1 (OATP-D, *SLCO3A1*). This transporter has been implicated in prostaglandin (PG) transport.[22,23] The unique feature of this transporter is its high degree of sequence similarity (97%) with the rodent counterpart, suggesting that the SLCO3A1 gene product likely serves an important physiological function in mammals. Two splice variants of OATP3A1 were isolated from human brain and distinct patterns of expression have been shown for both variants. The OATP3A1 variant 2, which is shorter than the consensus sequence, exhibits a more restricted expression with predominant expression in brain and testes, and the subcellular localization of the transporter suggests an apical and/or subapical localization in the epithelial cells of the choroid plexus, whereas the OATP3A1 variant 1 is more broadly expressed and exhibits a basolateral expression pattern in the endothelial cells of the choroid plexus.[24] In the case of the Oatp1b2, organ-dependent differences in expression and possibly processing of the splice variants appear to exist (Figures 1 and 4). However, it should be emphasized that Oatp1b2_v3 had no detectable transport activity, while Oatp1b2_v2 only had very modest activity toward taurocholate.

In conclusion, we show that murine Oatp1b2 is highly expressed in liver and that alternative splicing occurs. Importantly, Oatp1b2 is able to mediate the cellular uptake of a number of known human OATP1B substrate compounds. However, the splice variants appeared to lack significant transport capability, although they are more likely to be expressed in nonhepatic tissues. Importantly, our findings suggest that caution is warranted when interpreting mRNA tissue expression patterns for transporters such as Oatp1b2 since expressed forms may not be the fully functional transporter and may result in inaccurate assumptions regarding nonhepatic function of this transporter.

Abbreviations Used

OATP, organic anion transporting polypeptide; oatp1b2, organic anion transporting polypeptide 1b2; E_1S, estrone 3-sulfate; E_2G, estradiol 17 β-glucuronide; TCA, taurocholic acid.

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