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Molecular Identification and Characterization of Novel Members of the Human Organic Anion Transporter (OATP) Family

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We identified three novel transporters structurally belonging to the organic anion transporting polypeptide (OATP) family in humans. Since previously known rat oatp1 to 3 do not necessarily correspond to the human OATPs in terms of either tissue distribution or function, here we designate the newly identified human OATPs as OATP-B, -D and -E, and we rename the previously known human OATP as OATP-A. OATP-C proved to be identical with the recently reported LST1/OATP-2. Expression profiles of the five OATPs and the prostaglandin transporter PGT (a member of OATP family) in human tissues showed that OATP-C is exclusively localized in liver, OATP-A and PGT are expressed in restricted ranges of tissues, and OATP-B, -D and -E show broad expression profiles. OATP-B, -C, -D and -E exhibited transport activity for [^{3}H]estrone-3-sulfate as a common substrate. OATP-C has a high transport activity with broad substrate specificity.

Metabolic products and xenobiotics are mainly excreted via the kidney and liver, and the membrane transport processes in each tissue are important in determining the precise pathways. Various anionic compounds, including bile acids, bilirubin, anionic metabolites of endogenous compounds, and xenobiotics, are taken up by the liver via specialized mechanisms across the hepatic parenchymal cell membranes (1). We have previously studied renal and hepatic membrane transport mechanisms of anionic drugs (β-lactam antibiotics) in order to elucidate the determinant process for elimination of the antibiotics (2–5). The organic anion transporter molecules acting at the bile canalicular membrane include ATP-dependent MRP and BSEP (spgp) (1, 6). Hepatic basolateral membrane transporters include sodium-dependent ntcp and sodium-independent Npt1 and oatp (1, 7, 8). However, it is still not clear which transporter molecules operate in the biliary excretion of organic anions such as endogenous and xenobiotic organic anions, especially in humans.

Organic anion transporting polypeptide (oatp1) was first molecularly identified in rats as a multispecific and sodium ion-independent transporter for various organic anions, including bile acids, conjugated metabolites and xenobiotics (9). Subsequently, rat oatp2 and 3 were isolated as homologues of oatp1 and were shown to be present in various tissues and to transport anionic and neutral compounds (10, 11). As they are structurally very closely related, they are considered to form a single protein family—the OATP family. In humans, OATP was cloned from a human liver-derived cDNA library as a homologue of rat oatp1 (12). However, subsequent analysis showed that human OATP is localized mainly in the brain and not functional in the hepatic membranes (13, 14). Recently, a novel homologue of human OATP termed LST-1 was identified (14). LST-1 is exclusively expressed in liver and has a crucial role in the sinusoidal uptake of anionic drugs and metabolites. Subsequently, it was demonstrated that LST1 is localized at the basolateral membrane of hepatic parenchymal cells (15). In addition, a prostaglandin transporter PGT, which was cloned as the transporter for various prostaglandins in a sodium ion independent manner in rat, human and mouse, is also considered to belong to the OATP family (16–18). Previously observed sodium ion-independent transport process in various tissue cell membranes may be at least partially ascribed to these already identified

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OATP family members. However, for a clearer understanding of the membrane transport of endogenous and xenobiotic organic anions in humans, it is essential to identify all the members of the human OATP family.

In the present study, we report the molecular identification of three novel transporters termed OATP-B, -D and -E and we describe their characteristics in comparison with those of known members of the OATP family, here designated as OATP-A, -C and PGT.

MATERIALS AND METHODS

Materials. [^{14}C] Benzylpenicillin potassium (2.07 GBq/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, U.K.). [^{3}H]Estradiol-17β-D-glucuronide (2035 GBq/mmol), [^{3}H]estrone-3-sulfate, ammonium salt (1961 GBq/mmol) and [^{14}C]inulin (185 MBq/g) were from NEN Life Science Products, Inc. (Boston, MA). pcDNA3 vector was obtained from Invitrogen (Carlsbad, CA). All other enzymes and reagents for molecular biology and functional studies were purchased from Takara (Otsu, Japan), Toyobo (Osaka, Japan), Sigma (St. Louis, MO), Life Technologies Inc. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka).

cDNA cloning. A data base search with the TBLASTN program using the amino acid sequence of human OATP (12)/OATP-A as the query identified over 40 kinds of human ESTs (Expression Sequence Tags) and genomic sequences with significant similarities to the query sequence. From those sequences, the following primers were designed and used to clone cDNAs for members of the human OATP family, which we designated OATP-B, -C, -D, and -E in the present paper. Corresponding EST and PAC clone numbers are shown in parenthesis. OATP-B: A cDNA encoding a full open reading frame of OATP-B was amplified by polymerase chain reaction (PCR) from human adult brain poly A$^+$ RNA (Clontech, Palo Alto, CA)-derived cDNA using following primers: 5′-GATAAGCTTCTGTGTGGCCCAAGAAGAACTGAC-3′ (W19504) and 5′-GATAAGCTTTACTGCTGTGGCTGCTACTCTTGG-3′ (AI052501). OATP-C: The 5′ and 3′ ends of OATP-C were amplified by RACE (Rapid Amplification of cDNA Ends) from human fetal liver-derived Marathon-Ready-cDNA (Clonetech) using following primers: 5′-AAGCTTCCGTCAATAAAACC-AACA-3′ (H62893) and 5′-TTGGTGCTTTTACTTATGTCTTCA-3′ (T73863). The central region of OATP-C was amplified by PCR from human adult liver poly A$^+$ RNA (Clonetech)-derived cDNA using following primers: 5′-CTTCTC-TTGTTGGTTTTATTGACG-3′ (H62893) and 5′-TGTAAGTTATTCCATTGTTTCCAC-3′ (R29414). OATP-D: A cDNA fragment was amplified by PCR from human adult brain poly A$^+$ RNA-derived cDNA using following primers: 5′-CGCCCTCGTGGTTTTTGATGTAGC-3′ (AA280224) and 5′-GCGGTGCCTTACTCTTCTTCTCTT-3′ (pDJ430i19). Using this cDNA fragment as a probe, a human adult kidney-derived 5′-STRETCH PLUS cDNA library (Clonetech) was screened by a standard plaque hybridization method. Several positive clones were obtained and sequenced. The whole sequence of OATP-D was determined by assembling these sequences. OATP-E: A cDNA fragment was amplified by PCR from human adult lung poly A$^+$ RNA (Clonetech)-derived cDNA using following primers: 5′-TGTACAAGG-TGCTGGGCGTCCTCT-3′ (AI347130) and 5′-CGATCGGGTATAAAA-CACATTCTA-3′ (AI347130). By screening of a human adult kidney-derived cDNA library using this cDNA fragment as a probe, overlapping clones were obtained and the whole sequence of OATP-E was obtained as in the case of OATP-D. For construction of expression plasmid, cDNA fragments covering the full open reading frame of each OATP were reamplified by PCR and cloned into pcDNA3 vector (Invitrogen). Sequence-verified clones were selected and used for expression experiments. The regions where each cDNA fragments cover are as follows: OATP-B, nucleotide number 1-2354 of the sequence of GenBank Accession No. AB026256; OATP-C, 50-2215, AB026257; OATP-D, 184-2512, AB031050; OATP-E, 50-2320; AB031051. A direct sequencing of RT-PCR products of OATP-B and -C identified some mixed sequences which are considered to represent a single nucleotide polymorphism. The mixed sequences were confirmed to be not sequencing error by sequencing opposite strand of RT-PCR product.

Expression profile of human OATPs. The expression profiles of OATPs in multiple human tissues were examined by RT-PCR. Member-specific primers were designed as follows and used for PCR: OATP-A, OAA-1 primer, 5′-AAGAAGAGGTCAAGAAGGAAAAAT-3′; and OAA-2 primer, 5′-GGAGCATCAAGGAACAGTCAGGTC-3′; OATP-B, 4742-1 primer, 5′-CGTGCGGCCAAGTGTGTTCCATAA-3′; and 4742-2 primer, 5′-GAAGGAGTAGCCCCATAGCCAATC-3′; OATP-C: 9414-1 primer, 5′-TGTCATTGTCCTTTT-ACCTATTAT-3′; and 9414-2 primer, 5′-TGTAAGTTATTCCATTGTTTCCAC-3′; OATP-D: 0224-2 primer, 5′-CTCAAATCCTTCGCCTTCATCCTG-3′; and 0224-4 primer, 5′-AGGGTCAGAGTAGAGGCAAAGAAC-3′; OATP-E: 7130-2 primer, 5′-CACGGCGGGCACTCAGCATTTCCT-3′; and 7130-4 primer, 5′-CGATCGGGTATAAAACACATTCTA-3′; and PGT: HPGT-1 primer, 5′-CCATCTTATTTGCCATCTCTGTAT-3′; and HPGT-3 primer, 5′-GAGGCCAGCAATGACGGAGGAGAA-3′. For detection of the control gene G3PDH, upstream primer, 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′, and downstream primer, 5′-CATGTGGGCCATGAGGTCCACCAC-3′ were used. By using appropriate amounts of cDNA of a multiple tissue cDNA (MTC) panel (Clontech) derived from various human tissues and cancer cell lines as templates, each gene was amplified and the expression level was evaluated.

Transport experiments. To perform functional analysis, the constructs pcDNA3/OATP-B, C, D and E were used to transfect HEK293 according to the calcium phosphate precipitation method (19). HEK293 cells were routinely grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin, and streptomycin in a humidified incubator at 37°C and 5% CO_2. After 24 h cultivation of HEK293 cells in 10-cm dishes, each pcDNA3/OATP or pcDNA3 vector alone was transfected by adding 10 μg of the plasmid DNA per dish. At 48 h after transfection, the cells were harvested and suspended in transport medium containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 25 mM Hepes (pH 7.4). This suspension and a solution of a radiolabeled test compound in the transport medium were each incubated at 37°C for 10 min, then transport was initiated by mixing them. At appropriate times, 200 μL aliquots of the mixture were withdrawn and the cells were separated from the transport medium by centrifugal filtration through a layer of a mixture of silicon oil and liquid paraffin with a density of 1.03. Each cell pellet was solubilized in 3 N KOH, and neutralized with HCl, and the associated radioactivity was measured by means of a liquid scintillation counter. HEK293 cells transfected with pcDNA3 vector alone were used to obtain the background activity and are shown as Mock cells in the following. Correction for extracellular adhesion was done based on the apparent uptake of [^{14}C]inulin. Cellular protein content was determined according to the method of Bradford by using a BioRad (Hercules, CA) protein assay kit with bovine serum albumin as the standard (20).

Chromosomal mapping. By database searching, UniGene EST clusters corresponding to OATP-B, -C, -D and PGT were identified. These clusters have been mapped by The International RH Mapping Consortium by the radiation hybrid method and the locations ca be accessed at GeneMap '98 (http://www.ncbi.nlm.nih.gov/genemap98/page.cgi? F=Home.html). Chromosomal locations of OATP-A and -E, whose corresponding UniGene clusters have not been mapped, were determined by PCR analysis of the Genebridge 4 Radiation Hybrid panel (Research Genetics). The following gene-specific primers were used for PCR. OATP-A, OAA-6 primer, 5′-GGACATCAATAACAGCCCCCTAAA-3′; and OAA-8 primer, 5′-ATGCCTAGAATGAATAAGAAGAGT-3′; OATP-E, OAE-24 primer, 5′-TGA-TGCAATCA-

CACGGGAACTTCT-3′; and OAE-25 primer, 5′-GGATTCAC-AGCCCCT-GCGGAGTTT-3′.

RESULTS

Identification of Novel Members of the Human OATP Transporter Family

In order to identify unknown members belonging to the OATP transporter family in humans, we tried database searching with the TBLASTN program (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) using the amino acid sequence of previously identified human OATP (12) as the query. We searched the public database as well as our in-house constructed database (CMM seq), and found over 40 kinds of ESTs and some unannotated genomic sequences to encode amino acid sequences with significant similarity to OATP. Some of them turned out to be sequences apparently derived from PGT, which is been already known to belong structurally to the OATP family, or from OATP. However, around four-fifths of them did not correspond exactly to either OATP or PGT, suggesting that they were unidentified OATP-related gene(s)-derived sequences. We then tried to classify them into groups that represent distinct transcripts by clustering the sequences and examining whether each of the clusters was derived from the same transcript by RT-PCR bridging. Finally we concluded that those novel sequences came from four independent transcripts. Then, we cloned cDNAs with full open reading frames for all of them by PCR and conventional library screening. Since the entire deduced amino acid sequences of these four cDNAs showed significant similarity to OATP and PGT, they are considered to be members of the OATP family in humans. Thus we designated them as OATP-B, -C, -D, and -E, taking the previously known human OATP as OATP-A. The sequences of OATP-B, -C, -D and -E were deposited in GenBank with Accession Nos. of AB026256, AB026257, AB031050, and AB031051, respectively. During the preparation of this manuscript, cloning of a cDNA identical to OATP-C was reported by two groups, who designated it as LST-1 (14) or OATP-2 (21). OATP-B, -C, -D, -E consist of 709, 691, 710, and 722 amino acids, respectively. Multiple alignment of the amino acid sequences of the newly identified OATP members with previously known members is shown in Fig. 1a. The phylogenetic relationship among OATP family proteins was analyzed and it was suggested that there are three phylogenetic subgroups consisting of OATP-A/C, -B/PGT and -D/E, respectively (Fig. 1b). Based on hydropathy analysis according to TopPred II (22), it was predicted that all of the OATP members, including OATP-A and PGT, as previously suggested (10, 17), have twelve potential transmembrane regions (Fig. 1c). Several phosphorylation sites for protein kinases A and C, and *N*-glycosylation sites are also predicted. Chromosomal localization of all members determined by the radiation hybrid method is shown in Table 1.

In the course of cloning cDNAs, we found some single nucleotide polymorphisms (SNPs) in the normal population. In OATP-B one SNP (codon 486 tct/Ser and ttt/Phe) and in OATP-C two SNPs (codon 130 aat/Asn and gat/Asp, and codon 174 gtg/Val and gcg/Ala), which alter the coded amino acids, were detected, although their influence on the functional properties is not yet clear.

Expression Profiles of Human OATPs

The expression profiles of human OATPs in adult and fetal tissues as well as cancer cell lines were examined by RT-PCR. OATP-A, -C and PGT were found to be expressed in restricted ranges of tissues, whereas OATP-B, -D and -E showed much broader expression profiles (Figs. 2a and 2b). Strong expression of OATP-A was observed in both adult and fetal brain, with lower levels of expression in fetal lung and adult liver, testis and prostate. PGT was expressed in several tissues, including pancreas, lung, gut, and prostate in accordance with results obtained by Northern analysis (17). The tissue distribution of OATP-C was characteristic, in that it was exclusive to the liver (both fetal and adult) and is also consistent with a previous study on LST-1 expression in adult tissues examined by Northern analysis (14). Compared with OATP-A, -C and PGT, the expression profiles of OATP-B, -D, and E were much broader, with slight differences from each other. These three genes were expressed in all of the fetal tissues examined. In adults, OATP-B was expressed in a more restricted range of tissues compared with -D or -E, these include pancreas, liver, lung, gut, ovary, testes, and spleen. OATP-D and OATP-E were expressed rather ubiquitously with the exception that OATP-D was expressed strongly in leukocytes and spleen, but -E was not. In skeletal muscle, OATP-E was the only transporter detected, although the level was low. In cancer cell lines, OATP-D and -E were broadly expressed. OATP-C was not detected in any of the cell lines, suggesting liver specific expression. OATP-A and OATP-B were specifically detected in colon adenocarcinoma GI-112 and CX-1 cells, respectively. Accordingly, both in normal tissues and tumor cell lines, expressions of OATP-A, -B, -C and PGT were tissue-specific, while OATP-D and -E were distributed rather ubiquitously.

Transport Functions of Human OATPs Expressed in HEK293 Cells

By transfecting HEK293 cells with human OATP-B, -C, -D, or -E cDNAs subcloned into pcDNA3 vector, transport activity was studied by taking the activity of cells transfected with pcDNA3 vector alone (Mock) as the background. Figure 3 shows the time courses for

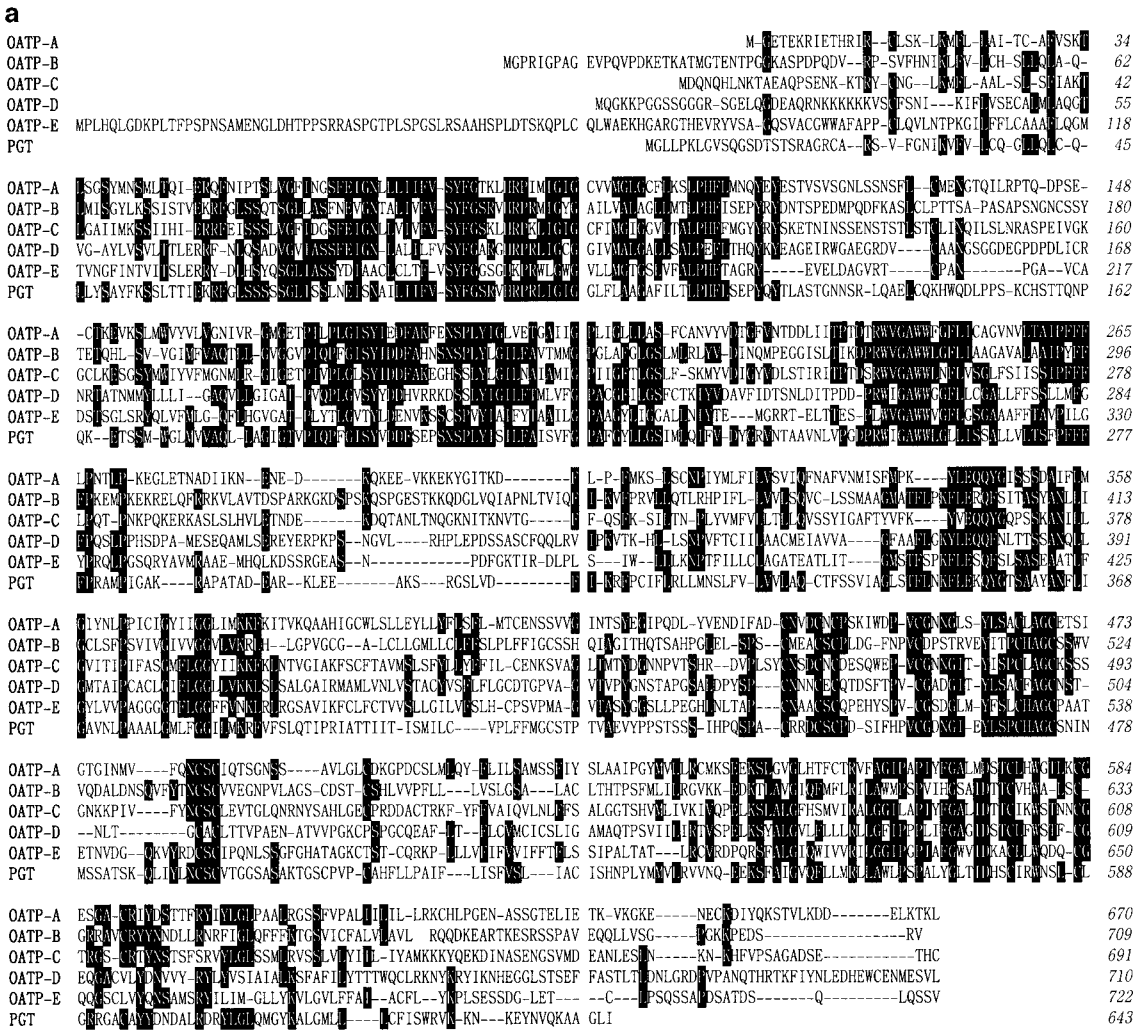

FIG. 1. (a) Multiple alignment of amino acid sequences of human OATP-A, -B, -C, -D, -E and PGT. Identical amino acids residues are highlighted. Gaps are introduced to optimize the alignment. (b) Phylogenetic relationships among human members of the OATP family. The evolutionery tree was calculated by the Neighbor-Joining Method using GENETYX-WIN Version 4.0.2 (Software Development Co., Ltd.). The phylogenetic distances are indicated by the numbers written in italic. (c) Hydrophobicity plot of human OATPs. Transmembrane domains predicted by TopPred II are indicated by numbers based on the method of Kyte and Doolittle. Potential phosphorylation sites and *N*-glycosylation sites are indicated by asterisks and triangles, respectively.

the uptake of [^{3}H]estrone-3-sulfate, which is a good substrate for rat and human organic anion transporting polypeptides, oatps, and OATP or LST-1, at the concentration of 5 nM. All of OATP-B, -C, -D, and -E showed transport activity for estrone-3-sulfate. High uptake activities were observed with OATP-B and -C and low activities with OATP-D and -E. Thus, although there are differences in the uptake activities, human OATP-B, -C, -D, and -E all exhibited transport activity for estrone-3-sulfate as a common substrate. In addition, the transport activity of OATP-B, -C, -D, and -E for prostaglandin E_2 and another steroid hormone-metabolite, estradiol-17β-glucuronide as endogenous compounds and benzylpenicillin as a xenobiotic, was examined (Fig. 4). With estradiol-17β-glucuronide, and benzylpenicillin, OATP-C exhibited very clear transport activity, while the other members showed minimal activity. In the case of prostaglandin E_2, all of OATPs showed slight but significant transport activity. These results show that OATP-C accepts various compounds. Further studies are needed to establish the patterns of specificity with a broader range of substrates.

DISCUSSION

In the present study, we have isolated novel members of the human OATP transporter family, and analyzed their expression profiles and transport functions in comparison with those previously identified members.

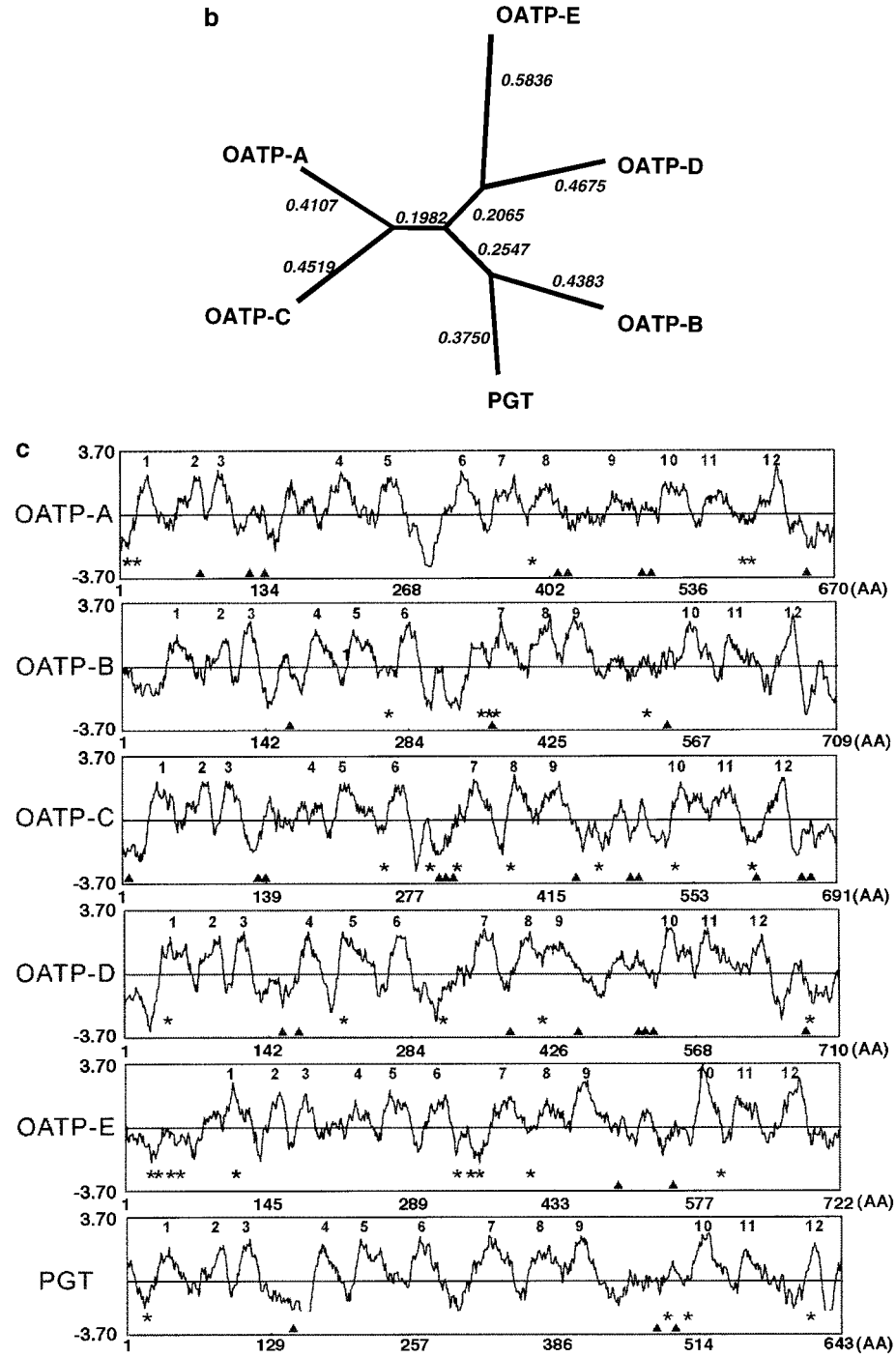

FIG. 1—*Continued*

All of the transporters, including OATP-A and PGT are structurally related to each other. This suggests that these six proteins are all bonafide members of the OATP transporter protein family. From hydropathy analysis, all of these proteins are predicted to have a twelve-transmembrane domain structure, which is commonly observed in transporter proteins. As shown in Table 1, chromosomal localization for all the genes have been assigned. The localizations of OATP-C and PGT were consistent with those assigned on the basis of cytogenetic studies, 12p12 and 3q21, respectively (23, 24). In the present study the localizations were defined more precisely with the radiation hybrid method, and this will be useful for future studies of their involvement in human inherited diseases. OATP-C, which is identical to the recently identified

TABLE 1

Chromosomal Localization of Human Members of the OATP Family

Gene	Chromosome	RH map (interval)	UniGene*	Gene symbol
OATP-A	12	WI-9881–WI-3881		SLC21A3
OATP-B	11	D11S916–D11S911	Hs. 7884	OATP-B
OATP-C	12	D12S358–D12S1596	Hs. 137425	SLC21A6
OATP-D	15	D15S202–D15S157	Hs. 14805	N.D.
OATP-E	20	D20S173–qTelomere		N.D.
PGT	3	D3S3606–D3S3554	Hs. 83974	SLC21A2

Note. Chromosomal mapping of OATP-A to -E was performed by the radiation hybrid (RH) method using the Genebridge 4 Radiation Hybrid panel (Research Genetics, Inc.). The UniGene clusters belonging to other members were mapped by The International RH Mapping Consortium and the localizations are accessible in GeneMap 98. * Mapped UniGene cluster numbers are shown. N.D., not designated.

LST-1/OATP-2 is considered to be a physiologically and pharmacologically important hepatic transporter in humans (14, 21). We have found SNPs which alter coded amino acids in OATP-B and -C. Although their effect on the transport properties is not presently clear, it would be of interest to explore whether these polymorphisms contribute to the differences in hepatic excretion ability of drugs observed among individuals.

Expression profiles were assessed for all members of the OATP family by RT-PCR. Although the tissue distributions of some members have been studied by Northern analysis (14), the number of tissues examined was limited and the signals were not clear, probably due to the problem of cross hybridization with related genes. Here we used gene-specific primers, which allowed precise determination of the expression level for each gene. In addition, we examined a large variety of tissues, including fetal tissues, and cancer cell lines. As mentioned above, it was clearly demonstrated that OATP-A, -C, and PGT are expressed in a restricted range of tissues. These expression patterns provide useful clues to the physiological roles *in vivo.* In particular, OATP-C is strongly and exclusively expressed in liver, suggesting a crucial role in this tissue. In contrast, OATP-B, -D, and -E were expressed at significant levels in a wide range of tissues. They were expressed ubiquitously in fetal tissues, whereas the expression in adult tissues was variable. This may indicate that they have distinct roles from OATP-A or -C, transporting compounds essential for development and/or differentiation in the fetus. Among cancer cell lines, OATP-C was not found in any of those examined. Similarly, the expression of OATP-A, B and PGT was very limited, which may reflect the restricted distributions of these genes in adult tissues. In contrast, OATP-D and -E were expressed in most cell lines. Interestingly, OATP-E was found in all the cancer cell lines examined, but not in normal blood cells. Such an expression profile might be useful for transporter-mediated delivery of anticancer agents to tumors without accumulation in blood cells, which often causes severe side effects.

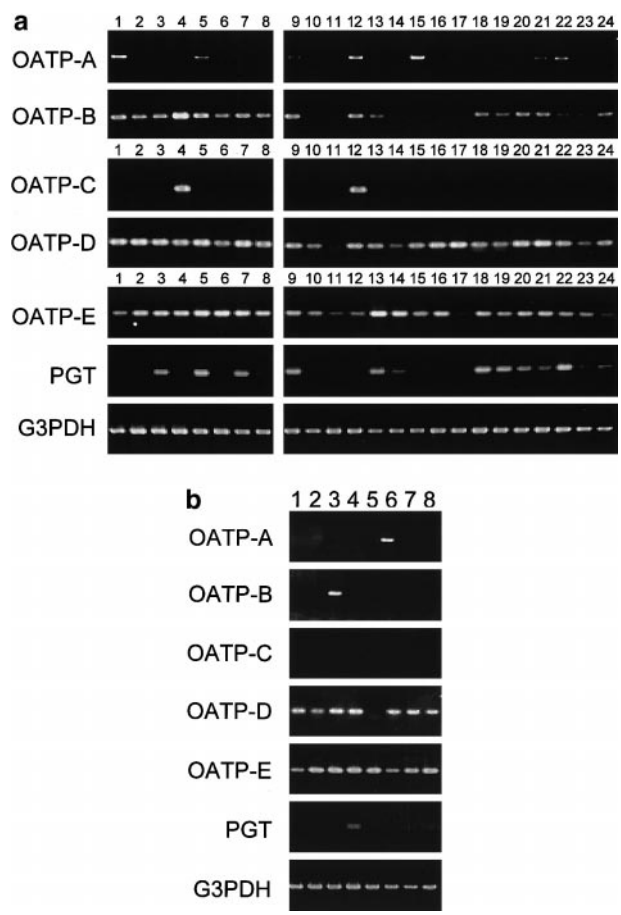

FIG. 2. The expression profiles of human members of the OATP family analyzed by RT-PCR. Member-specific primers were used to detect each gene as described under Materials and Methods. (a) Multiple human normal adult and fetal tissues. 1, fetal brain; 2, fetal heart; 3, fetal kidney; 4, fetal liver; 5, fetal lung; 6, fetal skeletal muscle; 7, fetal spleen; 8, fetal thymus; 9, pancreas; 10, kidney; 11, skeletal muscle; 12, liver; 13, lung; 14, placenta; 15, brain; 16, heart; 17, peripheral blood leukocyte; 18, colon; 19, small intestine; 20, ovary; 21, testis; 22, prostate; 23, thymus; 24, spleen. (b) Human cancer cell lines. 1, breast carcinoma (GI-101); 2, lung carcinoma (LX-1); 3, colon adenocarcinoma (CX-1); 4, lung carcinoma (GI-117); 5, prostatic adenocarcinoma (PC3); 6, colon adenocarcinoma (GI-112); 7, ovarian carcinoma (GI-102); and 8, pancreatic adenocarcinoma (GI-103).

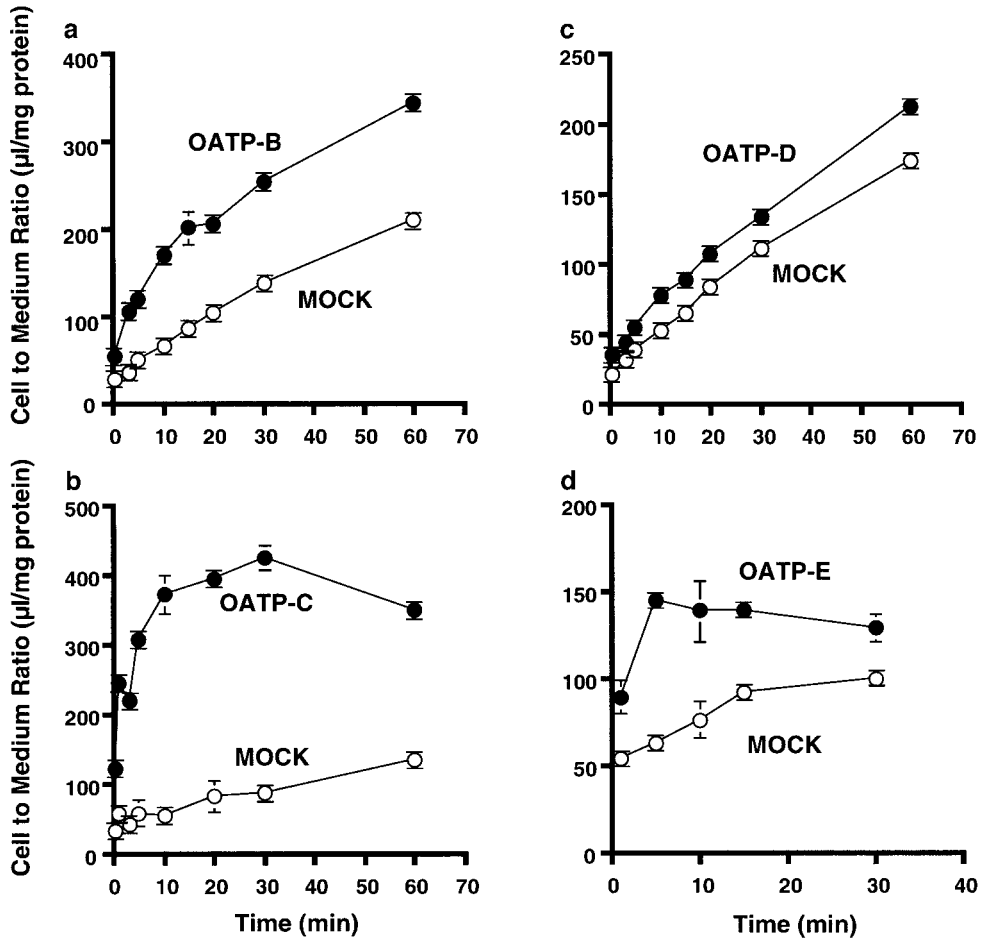

FIG. 3. Time course of uptake of estrone-3-sulfate by OATP-B (a), -C (b), -D (c) or -E (d). Uptake of [^{3}H]estrone-3-sulfate by HEK293 cells expressing each OATP (closed symbols) and Mock cells transfected with pcDNA3 vector alone (open symbols) was measured at the concentration of 5 nM and at 37°C, and the uptake was expressed as cell-to-medium ratio, which was obtained by dividing the amount taken up in the cells by the substrate concentration in the uptake medium. Each result is the mean and SEM (n = 3 or 4).

For the study of functionality, we used transient expression of the genes in the mammalian cell line HEK293. A recent study on OATP-C (OATP-2) demonstrated that the transporter protein is sorted into plasma membrane in the glycosylated form, and no correspondingly sized protein was detected in the parental cells by immunocytochemical studies (15). So, the cell line used in the present study should be adequate to examine the transport function of OATP. OATP-A and -C have already been reported to accept various organic anions, including conjugated metabolites of steroid hormones, prostaglandins, bile acids, and anionic drugs. We chose estrone-3-sulfate, estradiol-17β-glucuronide, prostaglandin E_2 and benzylpenicillin as test compounds for OATP-B, -C, -D, and -E. Although the extent of expressed activity of the four members was variable, they all transported the conjugated steroid hormone, estrone-3-sulfate. Apparently higher activity was observed with OATP-B and -C, intermediate activity with OATP-E and low activity with OATP-D. The values of uptake of estrone-3-sulfate at 30 min, which is nearly at the steady state, were more than 300 and 100 μL/mg protein for OATP-C and OATP-B, respectively. Since the water-space of HEK293 cells examined in our laboratory was about 10 μL/mg protein (19), estrone-3-sulfate was apparently accumulated against a concentration gradient in the cells expressing OATPs, especially high accumulation by the cells expressing OATP-B and -C. The results may suggest that OATPs are active transporters, although intracellular binding and/or metabolism may play a role and should be studied. We examined preliminary the effects of sodium and chloride ions on OATP-C transport activity by replacing them with *N*-methylglucamine and gluconate ions, respectively; however, no significant alteration in the uptake activity was observed (data not shown). When OATP-C (LST-1) was expressed in *Xenopus laevis* oocytes, no sodium ion dependence was observed in the uptakes of thyroxine and triiodothyronine (14). In addition, when it was expressed in HEK293 cells, the uptake of

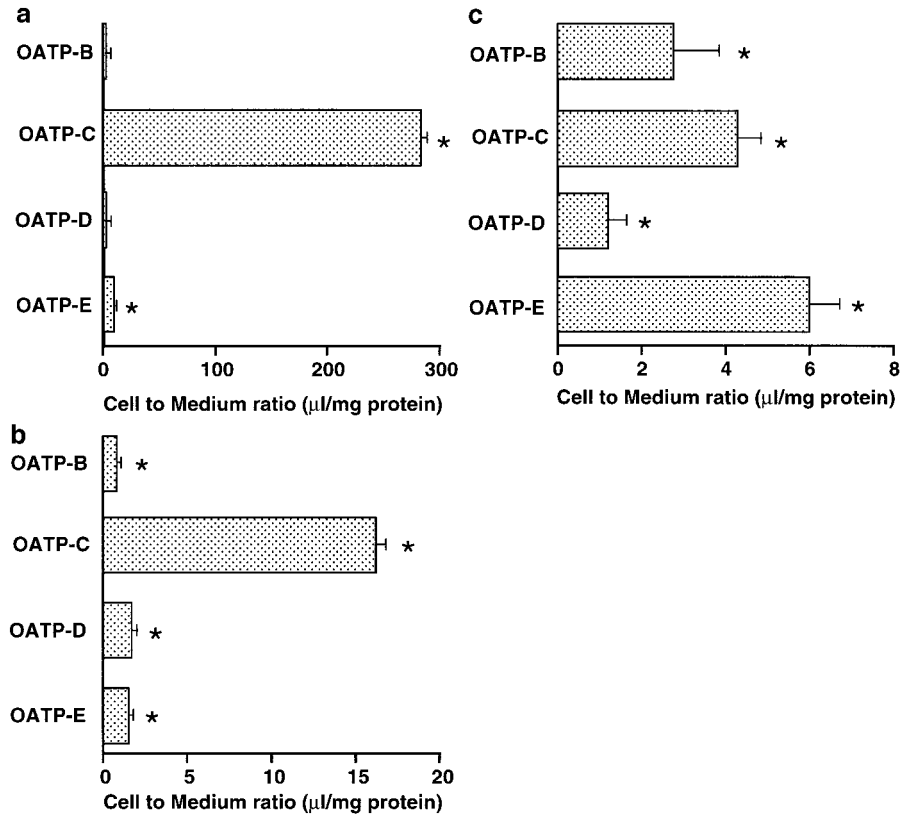

FIG. 4. Uptakes of estradiol-17β-glucuronide (a), benzylpenicillin (b) and prostaglandin E_2 (c) by HEK293 cells expressing OATP-B, -C, -D, or -E. Uptakes of [^{3}H]estradiol-17β-glucuronide, [^{14}C]benzylpenicillin and [^{3}H]prostaglandin E_2 were measured at the concentrations of 4.5 nM, 4.5 μM, and 1.3 μM for 30 min, respectively, after subtracting the uptake by Mock cells obtained by using HEK293 cells transfected with pcDNA3 vector alone. The uptake values by Mock cells were 27.1 $\pm$ 2.62, 17.9 $\pm$ 1.22 and 25.7 $\pm$ 2.45 μl/mg protein (mean $\pm$ S.E.M.) for estradiol-17β-glucuronide, benzylpenicillin and prostaglandin E_2, respectively. The uptake was expressed as cell-to-medium ratio, which was obtained by dividing the amount taken up in the cells by the substrate concentration in the uptake medium. Each result is the mean and SEM (n = 3 or 4). Significantly increased uptake compared with that by Mock cells was observed (*) by t-test ($P < 0.05$).

estradiol-17β-glucuronide was not affected by sodium ion (15). On the other hand, the prostaglandin transporter PGT1 may exhibit active transport via anion exchange (25). In rat oatp1, anion exchange and/or exchange with glutathione and organic anions were suggested as mechanisms of active transport (26, 27). At present, the reason for the high accumulation of estrone-3-sulfate via OATP-B and -C has not been clarified. There may be differences in the driving forces among OATP members, which may be ascribed to different physiological roles of OATP transporters. Since OATP-B and -C showed high transport activities for estrone-3-sulfate, we studied the difference between them by comparing apparent affinity for estrone-3-sulfate. The affinity of OATP-C for estrone-3-sulfate was higher, with apparent Km values about 0.2 and 5 μM for OATP-C and OATP-B, respectively (data not shown). OATP-A has already been shown to have much lower affinity (59 μM) to estron-3-sulfate (8), while rat oatp1 (4.5 μM) and oatp2 (11 μM) showed comparable affinity to OATP-B (8). So, at least OATP-A, -B and -C seem to have differential affinity to transport estrone-3-sulfate. Another steroid conjugate, estradiol-17β-glucuronide, was transported with high activity by OATP-C, while other members showed negligible activity. Since OATP-B showed the highest homology with PGT, we expected that OATP-B transports prostaglandins as good substrates. However, it did not show high activity for the transport of prostaglandin E_2.

As a xenobiotic, the β-lactam antibiotic benzylpenicillin was studied. OATP-C clearly transported [^{14}C]benzylpenicillin, whereas the uptake of [^{14}C]benzylpenicillin by the other members was negligible. Previously, to determine the transporter responsible for biliary excretion of β-lactam antibiotics, we used benzylpenicillin and related derivatives and found contributions of sodium ion-independent and membrane potential-dependent transporters across the hepatic basolateral and bile canalicular membranes, respectively, in rats (2–5). Basolateral membrane transporters for the antibiotics also transported organic anions such as bromosulfophthalein (2, 3), and later MRP

(cMOAT) was demonstrated to contribute to canalicular membrane transport of some β-lactam antibiotics (28). Since OATP-C is located at the hepatic basolateral membrane exclusively (15), it may determine the biliary excretion of the antibiotics. β-Lactam antibiotics are classified to two types in terms of elimination pathways, namely urinary and biliary excretion types (28, 29), and it has not been established what is the determining factor, we suggest that differences of affinity for OATP-C may determine, at least in part, the level of biliary excretion. More precise studies on the transport of β-lactam antibiotics via OATP-C should help to clarify the pharmacological relevance of OATP transporters.

The first identified member of the OATP family was oatp1, which is present at the sinusoidal membrane of the liver in rat (9). Subsequently, oatp2 (brain-specific transporter), oatp3, oat-K1 and oat-K2 were isolated, also from rat (10, 11, 30, 31). PGT was found to be responsible for physiological prostaglandin transport for clearance, and structurally belongs to the same family. Oatp1, 2, 3 and oat-K1 and K2 are closely related to each other, constituting a subgroup in the family. In humans, OATP was first cloned from human liver as a homologue of rat oatp1 (12). Although there are many functional similarities between rat oatp1 and human OATP, it was suggested that human OATP is not a functional counterpart of rat oatp1 due to obvious differences in substrate specificity, affinity and tissue distribution (13, 32). Human OATP also shows high similarity to rat oatp2, oatp3, oat-K1, and oat-K2, but it is not clear whether it is the human counterpart of any of these. Although all the human proteins identified in this study have sufficiently high similarity to be considered members of the OATP family, their similarities are not high enough to judge them as counterparts of the rat oatp1, 2, 3, oat-K1 or oat-K2 grouping.

All human ESTs and genomic sequences identified by our database search were found to be derived from OATP genes known at present, including those identified here. Furthermore, no related genes cross-hybridized during several rounds of cDNA library screening and PCR-based cloning. Whilst the imminent completion of the human genome sequencing program will provide the final answer, it seems very unlikely that any further members of the human OATP family remain to be found, particularly counterparts to rat oatp1, 2, 3, oat-K1 and oat-K2. Thus, we speculate that the composition of the OATP family in humans is different from that of rat and, probably, mouse. Consequently, the one-to-one correspondence generally found between rat and human gene counterparts is not evident in the OATP family. For this reason, and also in order to avoid unnecessary confusion, we propose to apply novel nomenclature as used in this paper for human OATPs independently of the rat nomenclature. That is, OATP is renamed OATP-A, and the newly identified members are called OATP-B, -C, -D, and -E. Further functional analysis to clarify transport properties such as substrate preference and kinetics will be necessary to understand how these proteins share roles in different species.

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