Developmental Changes in Hepatic Organic Cation Transporter OCT1 Protein Expression from Neonates to Children

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ABSTRACT

Organic cation transporter 1 (OCT1) plays an important role in the disposition of clinically important drugs, and the capacity of OCT1 activity is presumed to be proportional to the protein expression level in organ tissues. Knowledge of OCT1 protein expression in children, especially neonates and small infants, is currently very limited. Here, we report on the characterization of OCT1 protein expression in neonatal, infant, and pediatric liver samples performed using immunoblot analysis. OCT1 protein expression was detected in liver samples from neonates as early as postnatal days 1 and 2. This youngest group showed significantly lower OCT1 expression normalized by glyceraldehyde-6-phosphate dehydrogenase (values given as means ± S.D. in arbitrary units; 0.03 ± 0.02, n = 7) compared with samples from patients aged 3 to 4 weeks (0.08 ± 0.03, n = 5, P < 0.01), 3 to 6 months (0.23 ± 0.15, n = 7, P < 0.01), 11 months to 1 year (0.42 ± 0.32, n = 6, P < 0.01), and 8 to 12 years (1.00 ± 0.44, n = 7, P < 0.01). These data demonstrate an age-dependent increase in OCT1 expression from birth up to 8 to 12 years of age, and the findings of this study contribute to the understanding of OCT1 functional capacity and its effect upon the disposition of OCT1 substrates in neonates and small infants.

Introduction

Organic cation transporter 1 (OCT1, alternative gene name SLC22A1) is known to contribute to the disposition of clinically important drugs such as morphine (Tzvetkov et al., 2013), oxaliplatin (Zhang et al., 2006), and metformin (Umehara et al., 2007). Cellular expression of OCT1 is localized to sinusoidal membranes of hepatocytes (Koepsell et al., 2007) and likely also to basolateral membranes of kidney proximal tubule cells (Kimura et al., 2002; Mulgaonkar et al., 2013).

We recently demonstrated that OCT1 plays a key role in morphine clearance among pediatric patients aged older than 6 years (Fukuda et al., 2013). There are multiple pharmacokinetics studies of morphine in neonates and infants; however, the mechanistic understanding of what is driving maturation and variability in these populations remains to be addressed despite a high frequency of use in these developing patients, for whom organ size and function typically change rapidly. Contemporary studies relating to OCT1 expression generally report on levels of OCT1 mRNA transcripts and do not describe protein abundance, a deficit that was mentioned previously with some emphasis by the Pediatric Transporter Working Group (Brouwer et al., 2015). Given the linkage between OCT1 protein expression, hepatic function, and the capacity for morphine clearance, this knowledge gap prompted us to investigate the developmental changes of OCT1 protein expression in neonates and infants. Here, we report on the developmental trajectory of hepatic OCT1 protein expression in pediatric subjects, with emphasis given toward neonates and small infants. Importantly, the results of our study were of sufficient resolution to distinguish differences in OCT1 protein expression in neonatal subjects compared with older children.

Materials and Methods

Human Liver Samples. Liver tissue samples from pediatric patients, including neonates, were provided by the Better Outcomes for Children Biorepository, administered by the College of American Pathologists–accredited Cincinnati Biobank (Cincinnati, OH). Use of provided tissue and all experimental procedures were approved by the Cincinnati Biobank Tissue Use Committee. All samples provided were previously characterized by institutional pathologists as either healthy tissue or relatively healthy tissue adjacent to diseased or abnormal tissue and were deidentified prior to their release. All tissue samples were maintained at −80°C both before and after their release from the biorepository up until the time of their processing for assay. All tissue handling including weighing of tissue and subdividing of larger tissue samples was performed frozen over dry ice to prevent thawing prior to analysis.

Donor Information. The age of individual donors at the time of tissue collection was between 1 day postnatally up to 12 years (n = 32). Donor age groups were as follows: postnatal days 1 to 2 (n = 7 samples), 3 to 4 weeks (n = 5), 3 to 6 months (n = 7), 11 months to 1 year (n = 6), and 8 to 12 years (n = 7). These age ranges were used to bin expression data in the following analysis. Percentages of male and female donors were 53% and 47%, respectively. Percentages of donor race were as follows: white, 47%; black, 6%; other, 22%; and unknown, 25%.

Crude Membrane Isolation. Membrane fractions were prepared from frozen pediatric liver tissue by previously reported methods, with some modifications (Ogihara et al., 1996). Tissue sample portions weighing between 20 and 50 mg were maintained at 0°C while being rapidly minced with a clean razor blade to particles of ≤1 mm³ in size. Minced tissue was immediately dispersed within a 15-fold volume (in microliters per milligram) of prechilled (0°C), filter-sterile homogenization buffer containing 250 mM sucrose, 6 mM HEPEs (pH 7.4), 0.4 mM sodium deoxycholate (Pierce, Rockford, IL), and freshly added 1× protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and 1 mM phenyl-methylsulfonyl fluoride (Amresco, Solon, OH). Disruption of minced liver tissue was performed by homogenization on ice using a Polytrom model PT 1300D homogenizer (Kinematica, Lucerne, Switzerland) equipped with the PT-DA
Immunoblot Analysis and Quantitation of OCT1 Protein in Human Liver Samples. Protein concentrations of liver membrane fractions were determined in triplicate using the Micro BCA Protein Assay Kit (Thermo Scientific, Rockford, IL) following the manufacturer’s protocol for the 96-well plate format. Immunoblot analysis for OCT1 (SLC22A1) and control protein glyceraldehyde-6-phosphate dehydrogenase (GAPDH) abundance in isolated membrane fractions was performed essentially as described previously (Hahn et al., 2014) with the following modifications. To preserve the integrity of the OCT1 protein, denaturation of membranes in Laemmli sample buffer (BioRad, Hercules, CA) was performed for 30 minutes at 37°C, and proteins resolved by PAGE electrophoresis were transferred to polyvinylidene fluoride membranes for downstream blotting. Immunoblot analysis was then performed using antibodies specific for OCT1 (SLC22A1 [2C5] mAb, item GTX80400, lot 821505071) and GAPDH (both from GenTex, Irvine, CA). The peptide sequence recognized by this OCT1 antibody showed 82% homology (including peptide tag as a part of recombinant antigen) for human OCT1 using the BLAST search.

**OCT1 Genotyping.** OCT1 genotyping was conducted according to the method reported by Fukuda et al. (2013). Briefly, DNA was prepared from liver samples using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Hilden, Germany). TaqMan probes were used for genotyping of single nucleotide polymorphisms in the genes of OCT1, including Arg61Cys (rs12208357), Gly401Ser (rs34130495), Gly465Arg (rs340599508), and the deletion of Met420 (rs73552763). In addition, the OCT1-Arg61Cys, single nucleotide polymorphism (rs622342) was also determined. The genotyping was performed using commercially available TaqMan assays (Applied Biosystems, Foster City, CA).

**Statistical Analysis.** Background-subtracted densitometry data were collected from 16-bit linear tagged image files of individual blot exposures using the Fiji image analysis platform for Imaged (National Institutes of Health, Bethesda, MD) (Schindelin et al., 2012) and initial data were composed in Microsoft Excel (Microsoft, Redmond, WA). Expression levels for all donor samples were calculated and expressed as an arbitrary unit (AU) based on GAPDH– and membrane protein amount–normalized OCT1 signal density and are expressed as a multiple of the mean ± S.D. value of the group aged 8 to 12 years (n = 7). Statistical differences in OCT1 expression were evaluated based on the non-parametric comparison of each pair by means of the Wilcoxon method using JMP statistical software (version 10; SAS Institute Inc., Cary, NC).

**Results and Discussion**

OCT1 protein expression was detectable in hepatocyte membranes of donors from all ages included in this study (between postnatal day 1 up to 12 years of age) (Fig. 1). OCT1 protein expression increased with donor age across this developmental time frame (Fig. 2). The observed change in OCT1 expression was nonlinear in relation to donor age, supporting the concept of rapid expression changes during development. The rate of OCT1 expression increase was reduced over time. A rapid increase of OCT1 protein was observed between samples from children aged 1 to 2 days and 3 to 4 weeks, although expression levels were lower for both groups compared with older children. OCT1 expression normalized by GAPDH in samples from the youngest subjects (aged 1 to 2 days; mean 0.03 ± 0.02 AU, n = 7) was significantly lower than all other age groups studied, inclusive of samples from children aged 3 to 4 weeks (0.08 ± 0.03 AU, n = 5, P < 0.01), 3 to 6 months (0.23 ± 0.15, n = 7, P < 0.01), 11 months to 1 year (0.42 ± 0.32 AU, n = 6, P < 0.01), and 8 to 12 years (1.00 ± 0.44 AU, n = 7, P < 0.01). The group aged 3 to 4 weeks also possessed significantly lower OCT1 protein compared with the groups aged 11 months to 1 year (P < 0.01) and 8 to 12 years (P < 0.01). The groups aged 3 to 6 months and 11 months to 1 year had significantly lower OCT1 protein expression compared with the group aged 8 to 12 years (P < 0.01 and P < 0.05, respectively). OCT1 expression normalized by membrane protein amount increased in a similar age-dependent manner.

According to the OCT1 genotype, pediatric donors were classified into three assumed phenotype groups: wild type (having two *1 genotypes, n = 19), heterozygotes (having one *1 genotype, n = 10), and homozygotes (n = 3) (Fig. 3). Nies et al. (2009) reported that the OCT1-Arg61Cys variant (rs12208357) strongly correlated with decreased OCT1 protein expression (P < 0.0001). In this study, we had four donors with this variant as heterozygotes, as shown in Fig. 3. Although the OCT1 expression level showed a decreasing trend according to the presence of the variant in the group aged 8 to 12 years, the sample size was too small to determine the genetic effect on OCT1 protein expression.

Our results are consistent with a previous report by Prasad et al. (2016), which showed significantly lower OCT1 protein levels in hepatic membranes of neonates (aged 0 to 28 days) and infants (aged 29 days to 1 year) compared with older children, adolescents, and adults. However, a limitation of the referenced work is its inability to discern age-dependent differences in OCT1 expression among donors aged younger than 1 year, owing to the low number of samples available (n = 4). Similarly, a limitation of our study was the lack of available adult tissue from which to compare OCT1 expression levels. Notably, however, Klaassen and Aleksunes (2010) reported previously that there is no significant difference in liver OCT1 mRNA expression levels between children aged 7 years and older and adults. In addition, Nies et al. (2009) reported a significant correlation between OCT1 protein and mRNA transcript levels in normal adult liver. It is therefore postulated that OCT1 protein expression may reach mature levels after 7 years of age. To capture the accurate ontogeny profile of hepatic OCT1 protein
humans, further accumulation of OCT1 protein expression data across the developmental age range is needed, in addition to consideration of the relationships of sex, race, and OCT1 genotypes.

In summary, the key findings of our study include the observations that OCT1 protein is expressed in the neonatal liver as early as postnatal day 1 and that hepatic OCT1 protein expression increased rapidly from birth through early infancy and up to 8 years of age. This observation suggests that the contribution of OCT1 transporter function to drug disposition may likewise increase rapidly in the neonatal period in conjunction with OCT1 protein expression. This knowledge will be used as an age-dependent system physiologic parameter for physiologically based pharmacokinetics modeling to simulate the pharmacokinetic profiles of OCT1 substrates in neonates.

**Fig. 2.** Comparative analysis of hepatic membrane OCT1 protein levels by donor age. Relative levels of OCT1 protein were quantitated by immunoblot of isolated membrane fractions from liver tissues collected from donors aged between postnatal day 1 and 12 years. Data are binned by donor age into five discrete age groups as described in the Materials and Methods. OCT1 levels were calculated from membrane GAPDH– (A and C) and membrane protein–normalized (B and D) OCT1 signal density from a minimum of three immunoblots per sample. Final OCT1 values were normalized to the mean protein level for subjects aged 8 to 12 years and are expressed in AU. (A and B) Relative OCT1 protein levels for individual samples in each age group. Each symbol represents individual data and bars represent the group mean value ± S.D. (C and D) Statistical analysis of differential OCT1 protein expression by age group. Statistical differences in OCT1 expression were evaluated based on the nonparametric comparison of each pair by means of the Wilcoxon method using JMP statistical software (version 10; SAS Institute Inc.). Significant differences are reported as P values; where none are reported, associated groups were not significantly different in OCT1 protein concentration.

**Fig. 3.** Relative levels of hepatic membrane OCT1 protein levels by assumed phenotype groups. Pediatric donors were classified into three assumed phenotype groups: (A) wild type (having two *1 genotypes, n = 19), (B) heterozygotes (having one *1 genotype, n = 10), and (C) homozygotes (n = 3). Relative OCT1 protein levels, which were calculated from membrane GAPDH-normalized OCT1 signal density, are presented for individual samples at each age group. Each symbol represents individual data. Asterisks indicate individual donors with the OCT1-Arg61Cys variant (rs12208357).
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References


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