

Human Organic Anion Transporting Polypeptide 8 Polymorphisms: Identification and Characterization of a Novel hOATP8 Isoform

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Abstract

We have identified a novel hOATP8 isoform (hOATP8*2, GenBank Accession #: AY342017). Compared with the coding region of the previous cloned hOATP8 (hOATP8*1, König, et al., 2000), the novel isoform has two nonsynonymous polymorphisms: T334G (Ser112Ala) and G699A (Met233Ile); and one synonymous polymorphism: A1557G. A fragment (exon 5 to exon 8) of hOATP8 gene was amplified from human genomic DNA (n=11, Caucasians) by PCR, and the frequency of the nonsynonymous polymorphisms was determined by direct sequencing of the corresponding exons. Ten of the eleven (91%) tested samples were found to have both T334G and G699A polymorphisms. For function characterization, the novel hOATP8 isoform was cloned from human liver cDNA library by PCR using primers flanking the Open Reading Frame (ORF), and the isoform was expressed in *Xenopus laevis* oocytes. This novel hOATP8 mediated the transport of Digoxin, Taurocholate, Estrone-3-Sulfate, Prostaglandin E₂, and Estradiol-17 β -D-Glucuronide. BQ-123 had significant inhibition on the uptake of Estradiol-17 β -D-Glucuronide. The uptake of Estradiol-17 β -D-Glucuronide was saturable with K_m of 8.7 μ M. Our results demonstrated that a novel hOATP8 has been identified. This is the predominant isoform in Caucasians. Compared with the previously cloned hOATP8, the novel hOATP8 has similar substrate selectivity, similar inhibition profile, and similar substrate affinity, however, significant differences in transport rates of some substrates were observed.

Introduction

Human Organic Anion Transporting Polypeptide (hOATP8, *SLC21A8*, *SLCO1B3*) plays a key role in the transport of a variety of endogenous and xenobiotic compounds across the sinusoidal membrane of human hepatocytes. In this poster, we report the identification of a novel hOATP8 isoform, hOATP8*2. The DNA and protein sequences of hOATP8*2 have been submitted to GenBank with Accession Number AY342017. Compared with the coding region of the previous cloned hOATP8 (hOATP8*1, König, et al., 2000), the novel isoform has two nonsynonymous polymorphisms: T334G (Ser112Ala) and G699A (Met233Ile); and one synonymous polymorphism: A1557G. hOATP8 gene has 16 exons. Nonsynonymous polymorphisms T334G (Ser112Ala) and G699A (Met233Ile) occur at exon 5 and exon 8, respectively. Synonymous polymorphism A1557G occurs at exon 13. Genetic variations in exon 5 and exon 8 of eleven Caucasians were analyzed, and our results indicated that ten of the eleven (91%) tested samples were found to have both T334G and G699A polymorphisms. To determine if Ser112Ala and Met233Ile polymorphisms have effects on the function of hOATP8, the novel hOATP8 isoform was functionally characterized in *Xenopus laevis* oocytes. Compared with the previous identified hOATP8 (hOATP8*1), the novel hOATP8 has very similar substrate selectivity, similar inhibition profile, and similar substrate affinity. However, the transport rates of some substrates were significantly different. Further characterization on the effects of polymorphisms on drug transport activity is still in progress.

Methods

Polymorphisms Frequency Determination

Exon 5 to exon 8 fragment of hOATP8 gene was amplified from human genome DNA samples by PCR. Sequences of exon 5 and exon 8 were determined by direct sequencing at Tufts University.

cDNA Isolation

hOATP8 cDNA was cloned from human liver cDNA library (BD Bioscience Clontech) by PCR using primers flanking the Open Reading Frame (ORF). The primers were designed based on the published sequences (König, et al., 2000). All plasmid and DNA sequences were confirmed by restriction enzyme digestion analysis and by automated sequencing at Tufts University.

Expression and Transport Assay in *Xenopus laevis* Oocytes

Expression vector was engineered by inserting *Xenopus* β -globin 5' and 3' untranslated regions into pBluescript® II KS (+) (Stratagene) flanking the insert. cDNAs of the human transporters were subcloned into the vector with the 5' and 3' untranslated regions of the *Xenopus* β -globin gene flanking the insert. cRNAs were synthesized by using T3 polymerase (Stratagene). Oocytes were harvested and digested with collagenase D (Boehringer Mannheim). Fifty nanoliters of cRNA (~0.4 ng/nl) or water was injected individually into defolliculated oocytes. Oocytes were incubated at 16°C for five days and then uptake assays were performed at room temperature. After uptake, oocytes were washed with ice-cold transport buffer, lysed individually in 10% SDS, and the amount of radiolabeled compounds transported into each oocyte was determined by liquid scintillation counting.

Statistics and Data Analysis

Groups of eight cRNA-injected or un-injected oocytes were used for each experiment. Uptake values are expressed in mean \pm S.E. For kinetic studies, uptake rates (V) determined at different substrate concentrations (S) were fit to the Michaelis-Menten equation: $V = V_{max} \times S / (K_m + S)$, where V_{max} is the maximal uptake rate and apparent K_m is the Michaelis-Menten constant. For uptake and inhibition studies, statistical analysis was carried out by comparing the results obtained from the same experiments using a two-tailed, two-sample equal variance t test. Results with the probability of p<0.05 were considered significantly different.

Results

1 Genomic DNA Sequencing Results

| Donor | Age | Gender | DNA (334) | AA (112) | DNA (699) | AA (233) |
|-------|-----|--------|-----------|----------|-----------|----------|
| HH25 | 66 | F | G | Ala | A | Ile |
| HH26 | 41 | M | G | Ala | A | Ile |
| HH27 | 66 | F | G | Ala | A | Ile |
| HH28 | 57 | M | G | Ala | A | Ile |
| HH29 | 53 | M | G | Ala | A | Ile |
| HH37 | 54 | M | G | Ala | A | Ile |
| HH39 | 26 | M | G | Ala | A | Ile |
| HH40 | 66 | M | T | Ser | G | Met |
| HH41 | 58 | M | G | Ala | A | Ile |
| HH42 | 57 | F | G | Ala | A | Ile |
| HH43 | 19 | M | G | Ala | A | Ile |

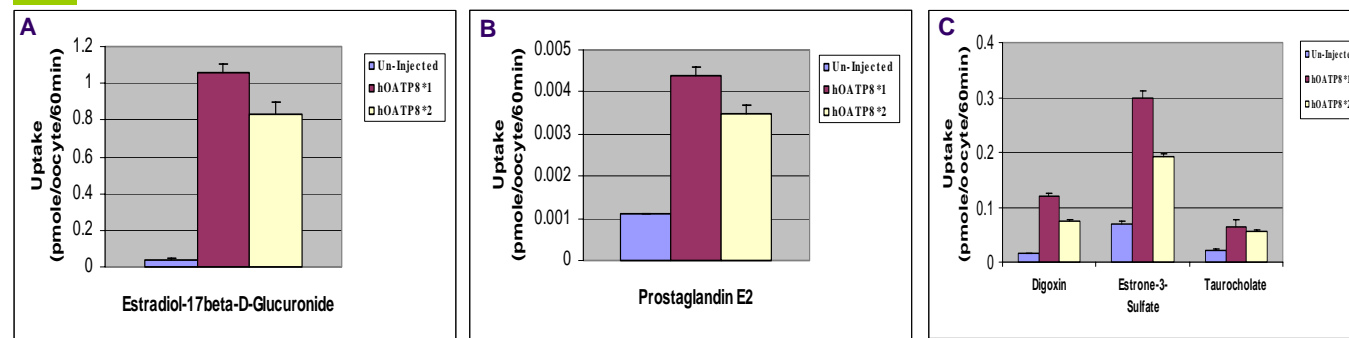
Note:
1. hOATP8 gene has 16 exons.
2. DNA position starts from ATG in the ORF. Nucleotides 334 and 699 are in exon 5 and exon 8, respectively.

2 Protein Sequence Alignment

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hOATP8*1 1 60
hOATP8*2 1 60
hOATP8*1 61 120
hOATP8*2 61 120
hOATP8*1 121 180
hOATP8*2 121 180
hOATP8*1 181 240
hOATP8*2 181 240
hOATP8*1 241 300
hOATP8*2 241 300
hOATP8*1 301 360
hOATP8*2 301 360
hOATP8*1 361 420
hOATP8*2 361 420
hOATP8*1 421 480
hOATP8*2 421 480
hOATP8*1 481 540
hOATP8*2 481 540
hOATP8*1 541 600
hOATP8*2 541 600
hOATP8*1 601 660
hOATP8*2 601 660
hOATP8*1 661 720
hOATP8*2 661 720
hOATP8*1 721 780
hOATP8*2 721 780
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Note:
1. Transmembrane domain (TMD) and topology of hOATP8 was predicted using HMMTOP, Version 1.
2. hOATP8 has 12 TMDs. The N-terminal is in the cytosol. AA112 is in TMD 3. AA233 is in the extracellular loop.

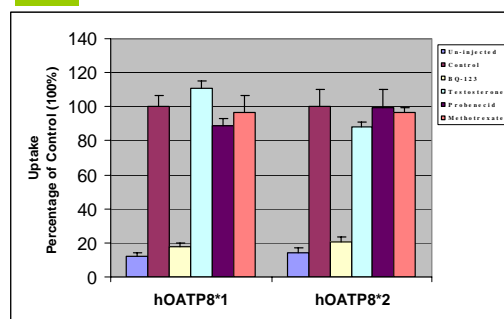
3 Uptake Results



Uptake of ³H-Estradiol-17 β -D-Glucuronide (5 μ M) (A), ³H-Prostaglandin E₂ (20 nM) (B), ³H-Digoxin (1 μ M), ³H-Estrone-3-Sulfate (2 μ M), and ³H-Taurocholate (2 μ M) (C) mediated by hOATP8*1 and hOATP8*2. Five days after expression, uptake was carried out by incubating the hOATP8 cRNA-injected or un-injected control oocytes with the tested substrates in transport buffer at room temperature for 60 min. Values are expressed as mean \pm S.E. (n = 8).

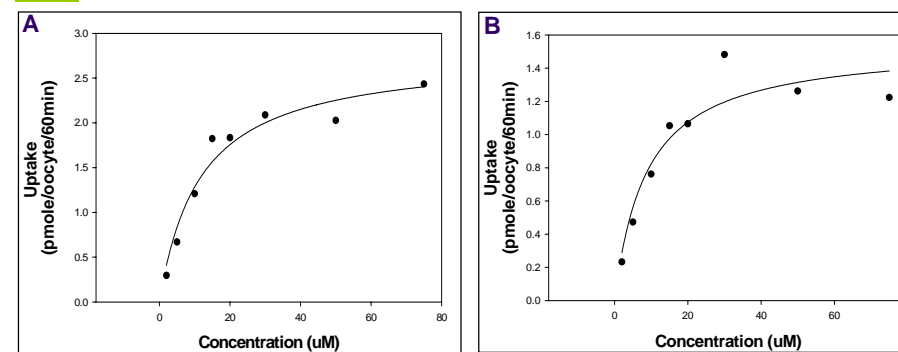
T test results between hOATP8*1 and hOATP8*2: Estradiol-17 β -D-Glucuronide: p=0.011, Prostaglandin E₂: p=0.008, Digoxin: p<0.001, Estrone-3-Sulfate: p<0.001, Taurocholate: p=0.3

4 Inhibition Results



Inhibition on the uptake of Estradiol-17 β -D-Glucuronide mediated by hOATP8*1 and hOATP8*2. Five days after expression, uptakes of ³H-Estradiol-17 β -D-Glucuronide (5 μ M) were carried out into un-injected or cRNA-injected in the absence (control) or in the presence of the tested compounds at room temperature for 60 minutes. Values are expressed as mean \pm S.E. (n = 8). The final inhibitor concentration of the tested compounds was 200 μ M. BQ-123 had significant inhibition on the uptake (p<0.05).

5 Kinetic Results



Uptake of ³H-Estradiol-17 β -D-Glucuronide mediated by hOATP8*1 (A), and hOATP8*2 (B) as the function of substrate concentration. Five days after expression, uptake was measured by incubating the cRNA-injected or un-injected control oocytes with Estradiol-17 β -D-Glucuronide of different concentrations for 60 minutes at room temperature. Uptake was calculated after subtracting non-specific uptake by un-injected control oocytes. Values are expressed as mean \pm S.E. (n = 8). Apparent K_m: 11.6 \pm 2.7 mM (A), 8.7 \pm 3.1 μ M (B).

Conclusions

1. hOATP8*2 is the predominant isoform in Caucasians
2. Both hOATP8*1 and hOATP8*2 mediated the transport of Estradiol-17 β -D-Glucuronide, Digoxin, Prostaglandin E₂, Estrone-3-Sulfate, and Taurocholate
3. Significant differences were observed between hOATP8*1 and hOATP8*2 mediated transport of Estradiol-17 β -D-Glucuronide, Digoxin, Prostaglandin E₂, and Estrone-3-Sulfate, but not Taurocholate
4. Both hOATP8*1 and hOATP8*2 had similar inhibition profile. Of the tested compounds, only BQ-123 had significant inhibition on hOATP8 mediated uptake of Estradiol-17 β -D-Glucuronide, while Testosterone, Probenecid, and Methotrexate had no significant effects on the uptake.
5. hOATP8*1 and hOATP8*2 had similar affinity to Estradiol-17 β -D-Glucuronide.