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Human UGT1A8 and UGT1A10 mRNA Are Expressed In Primary Human Hepatocytes

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Summary

It is widely believed that the UGT1A isoforms, UGT1A8 and –1A10, are expressed exclusively in extrahepatic tissues. In this work, human primary hepatocytes from six donors were analyzed for UGT1A8 and –1A10 mRNA expression by semi-quantitative RT-PCR. New primers to amplify UGT1A8 mRNA were designed and found to differ from those previously published. We demonstrated that UGT1A8 and –1A10 mRNA are expressed in hepatocytes. Although basal UGT mRNA levels were detected in untreated hepatocytes, significant up-regulation of the levels of mRNA for these isoforms were seen after treatment with 3-methylcholanthrene (3-MC) and rifampicin (Rif). RT-PCR products for all UGTs were sequenced and unambiguously identified as matching the corresponding cDNA. The discovery of these isoforms in hepatocytes is a novel discovery and will stimulate studies on the potential role for these isoforms in hepatic detoxification.

Keywords

UDP-glucuronosyltransferases; UGT1A8; UGT1A10; primary human hepatocytes; UGT induction; tissue specific expression

Introduction

UGTs are membrane-bound enzymes involved in the biotransformation of important endogenous compounds as well as drugs and xenobiotics.¹⁾ In humans, 17 functional UGTs have been identified by molecular cloning of individual UGT cDNA and analysis of the gene structure. UGTs have been classified into two gene families, UGT1A and UGT2B, on the basis of their amino acid sequence similarities.^{2–4)} The expression of UGTs from both families has been investigated in various human tissues, and the liver and gastrointestinal tract have been identified as the primary metabolic organs with the highest expression of UGT mRNA.

The tissue specificity of UGT1A isoform expression has been an area of intensive study, and it is well accepted that each isoform is expressed in a tissue specific manner. The most extensive studies of expression in humans have been carried out with hepatic tissue. UGT1A1, –1A3, –1A4, –1A6, and –1A9 cDNA has been cloned from the liver, and the expression of these isoforms has been confirmed by differential RT-PCR analysis.^{5–8)} Several UGTs have been identified as being expressed exclusively in extrahepatic tissue. UGT1A10 mRNA was found

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to be abundantly expressed in the GI tract⁹) but was not detected in the liver and studies have indicated that UGT1A8 is also strictly extrahepatic.^{10,11}) Based on these reports, the study of UGT1A8 and -1A10 has not been pursued in hepatic tissue.

Recently, several laboratories have begun to study regulation of UGTs, and these experiments have been carried out primarily in rat hepatocytes or Caco-2 and HepG2 cells.¹³⁻¹⁸) However, studies of UGTs in human hepatocytes are limited to only a few investigations. Assessments of UGT induction by several human UGT substrates has demonstrated that the primary human hepatocyte model is highly suitable for the investigation of UGT regulation.^{19,20}) Studies of UGT induction by prototypical cytochrome P450 inducers have shown an increase of UGT1A1 protein in hepatocytes exposed to 3-MC²¹) and, more recently, primary human hepatocytes have been effectively used to study the regulation of UGT2B4 expression by bile acids.²²) Our laboratory has also studied UGT mRNA induction by CYP450 inducers in primary human hepatocytes²³) (see footnote).

The present studies are a continuation of preliminary experiments carried out in our laboratory on the identification of mRNA expression of 3 UGTs (UGT1A5,²³) -1A8, and -1A10) in primary human hepatocytes. UGT1A8 and -1A10 have been postulated to be exclusively expressed in extrahepatic tissue,^{8,12,14,24-27}) and UGT1A5 was defined as the “missing UGT” since no expression has ever been demonstrated¹²); however, Finel *et al.* recently identified UGT1A5 mRNA expression in primary human hepatocytes.²³) Basal levels of mRNA for this isoform were low; however, significant up-regulation was observed after treatment with Rif and 3-MC. Expression of this isoform was also detected in various segments of the human intestine from different donors, with high individual variation and differing distribution patterns. This isoform was cloned and heterologously expressed as a His-tagged protein in baculovirus infected Sf9 insect cells and glucuronidation activity toward several different substrates was demonstrated.

In the current investigations, we have similarly re-examined the expression of UGT1A8 and -1A10 mRNA in human hepatocytes. Semi-quantitative RT-PCR analysis of UGT1A8 and -1A10 mRNA expression was carried out with untreated primary human hepatocytes and those exposed to Rif and 3-MC. We found that in these hepatocytes, mRNA for UGT1A8 and -1A10, was expressed at low levels and significantly induced by the addition of 3-MC or Rif to the medium.

Based on these data, we hypothesize that since the UGT1A locus is present in the liver, all UGT1A isoform transcripts are present in this organ, and consequently, all UGT1A proteins have the potential to be expressed in a liver-specific manner. Moreover, it is feasible that the activation of the nuclear receptor responsible for isoform regulation could depend on the availability of nuclear receptor ligands, which could be tissue specific. It is anticipated that this hypothesis will be further developed, and important new investigations of the regulation of these enzymes, which were previously defined as being exclusively extrahepatic, will be stimulated.

Methods

Primary human hepatocytes

Primary cultures of human hepatocytes were obtained through the Liver Tissue Procurement and Distribution System, University of Pittsburgh. Details on the individual donors are given in Table 1. Prior to use for studies, the overall metabolic function, synthetic capability (e.g., albumin synthesis and secretion) viability (approximately 75%), and morphological integrity (e.g., mitochondrion integrity, cell polarity) of each preparation of hepatocytes had been assessed.

Treatment of hepatocyte culture

Upon receipt of hepatocytes in 6-well culture plates, plating medium (Hepatocyte Maintenance Medium, HMM) was replaced with fresh HMM supplemented with 0.1 μ M insulin, 0.1 μ M dexamethasone, 50 μ g/mL gentamicin, and 0.25 μ g/mL amphotericin B (designed HMM⁺). After a 24 hr equilibration period at 37°C under an atmosphere of 95% air/5% CO₂, HMM⁺ was replaced with Dex-free medium for 48 hr before initiating the studies.

For the evaluation of the effects of inducers on the expression of UGT1A8 and -1A10 mRNA, hepatocytes were grown in culture at 60–80% confluence before treatment with 15 μ M Rif or 1 μ M 3-MC (in DMSO) for 24 hr, followed by measurement of UGT1A8 and -1A10 mRNA expression by semi-quantitative RT-PCR using GAPDH as an internal control.

Human intestinal tissue

Human gastrointestinal tissue was obtained from organ donors by transplant surgeons at the University of Arkansas for Medical Sciences under a protocol approved by the Human Research Advisory Committee. Working at 4°C, the tissue was divided into stomach (S), duodenum (D), 4 segments of the remaining small intestine (S1 to S4), and colon (C). Each segment was opened and cleaned, and mucosa was recovered by scraping with a glass slide. The mucosa from two donors (Donors 1 and 2) was used immediately for RNA isolation while the others were flash frozen in liquid N₂ and stored at -70°C.

RNA Isolation

Total RNA was isolated from cultured cells or mucosa using a phenol and guanidine isothiocyanate RNA extraction method (Trizol; Invitrogen, Carlsbad USA) following the instructions of the supplier. To avoid any contamination of the RNA by genomic DNA, DNAase treatment was performed using RQ1 RNase-Free DNase (Promega, Madison USA).

cDNA was synthesized by mixing 1 μ g of total RNA from each sample with 100 pmol random hexamers in 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 100 U M-MLV reverse transcriptase, 20 U RNase inhibitor, and 1 mM of each dNTP in a total volume of 20 μ L (Promega). The samples were incubated at 37°C for 60 min and heated at 95°C for 5 min to inactivate the reverse transcriptase. The reaction mixture was diluted to 100 μ L with sterile diethylpyrocarbonate-treated H₂O.

Semi-Quantitative RT-PCR

Primers for GAPDH, UGT1A8, -1A9, and -1A10 and the conditions for PCR are described in Table 2. The specificity of all primer pairs was confirmed by sequencing of the PCR products.

PCR reactions were performed as follows: a 10 μ L cDNA aliquot was added to a reaction mixture containing 10 mM Tris-HCl buffer (pH 8), 20 mM KCl, 0.1% Triton X-100; 1.0 mM or 0.7 mM MgCl₂, 0.2 mM of each dNTP, 50 pmol of each primer and 2 units of Taq DNA polymerase (Promega), in a total volume of 50 μ L. Amplification of the ubiquitously expressed GAPDH cDNA was performed under the same conditions in separate experiments.

The mixture was subjected to 34 cycles (26 for GAPDH), each consisting of a 45 s denaturing step at 94°C, a 45 s annealing step at specified temperatures (see Table 2) and a 45 s elongation step at 72°C in a thermal cycler (PTC100, MJ Research, Reno, NV). Primer pairs were designed to specifically amplify across exon boundaries in mRNA from GAPDH, UGT1A8, -1A9 and -1A10 (Table 2). The PCR products were resolved by 2% agarose gel electrophoresis and detected by ethidium bromide. The bands were visualized under UV light and photographed with a computed-assisted camera. Quantification of each band was performed by densitometric analysis by using NIH Image software (NIH, Bethesda, MD).

cDNA sequencing

The RT-PCR products for UGT1A8 and -1A10 generated from mRNA from HH1117 were extracted from the gel and, after purification, were sequenced by the UAMS sequencing core. The sequences were then compared with the corresponding sequences from Genbank.

Results

Identification of UGT1A8 and -1A10 mRNA in primary human hepatocytes

Six primary human hepatocyte samples were available for the present studies and the details of each are given in Table 1. Primers were designed for the detection of UGT1A8, -1A9 and -1A10, and their sequences and annealing positions are presented in Table 2 and Fig. 1. The initial experiments with primary human hepatocytes (Fig. 2) evaluated the level of UGT1A8, -1A9 and -1A10 mRNA expression in samples from 6 donors using semi-quantitative RT-PCR. UGT1A9 has been recognized as a hepatic isoform, and its expression was consistently high in all six donors, in agreement with previously published data.⁸⁾ The levels of mRNA for UGT1A8 and -1A10 were highly variable. Interestingly, UGT1A8 and -1A10 mRNA was detectable in all hepatocyte samples with the exception of HH1122, which showed no UGT1A8 expression using these primers.

Induction of UGT1A8 and -1A10 expression in human hepatocytes from HH1148

It has been shown previously that UGT1A5 mRNA expression can be significantly induced in hepatocytes exposed to Dex, 3-MC, and Rif.²³⁾ Similar experiments using Rif and 3-MC were carried out in the current studies to measure the expression of UGT1A8 and -1A10 mRNA. Human hepatocytes from donor HH1148 were grown as described by Finel, *et al.*²³⁾ and the concentration and time dependent effects of 3-MC and Rif were evaluated (data not shown). Comparison of the induction of UGT1A8 and -1A10 is presented in Fig. 3. Significant induction of UGT1A8 and -1A10 was observed in hepatocytes (HH1148) when treated with Rif and 3-MC.

Comparison of UGT1A8 mRNA expression using two different primer sets

For the present studies, we designed original primers based on the published sequence for UGT1A8 wild type submitted to Genbank (AF462268). These primers (Table 2) were used to amplify UGT1A8 mRNA from primary human hepatocytes (Fig. 2). Comparison of these primers to those previously published for the amplification of UGT1A8^{8,24,25)} revealed a difference in the final nucleotide base (see Table 2).

As a next step, we used the previously described primers (1A8-G) to analyze the expression of UGT1A8 mRNA in the same human hepatocyte samples that we had previously analyzed with our primers (1A8-C). The comparative expression of UGT1A8 mRNA generated using these two primer sets is shown in Fig. 4. Interestingly, in HH1122, we observed amplification of this isoform with 1A8-G but not 1A8-C. This indicates that 1A8-G primers, which had been previously used to establish that the expression of UGT1A8 was exclusively extrahepatic, were also able to identify UGT1A8 expression in hepatocytes.

Sequencing of products amplified by RT-PCR

To confirm the identity of all the products amplified by RT-PCR, all PCR products were sequenced and compared to the specific sequences for each isoform from Genbank. These comparisons confirmed that each product was correctly identified. The partial sequence of the products produced using 1A8-C and 1A8-G primers and alignment of this with the known sequences of wild type UGT1A8 (1A8*1) and its polymorphic forms (1A8*2 and -1A8*3) are shown in Fig. 5. Comparison of these sequences shows that the 1A8-C primers amplify

UGT1A8*1 (and potentially UGT1A8*3), whereas, the 1A8-G primers only amplify UGT1A8*2.

Expression of UGT1A8 and –1A10 mRNA in segments from the human GI tract

Mucosa from five intestinal segments from 2 different donors was used for analysis of the expression of UGT1A8 and –1A10 mRNA (Fig. 6). However, the patterns and levels of expression differed greatly between both donors and different segments from the same donor. A random distribution and discontinuous expression was observed, which is in agreement with previous reported studies from Basu *et al.*²⁸) The discontinuous expression in these intestinal segments can be explained by intestinal segment-specific regulation of gene expression. The expression of UGT1A8 measured with 1A8-C and 1A8-G primers were very similar.

Discussion

In this report, we have extended our preliminary studies and those of Finel, *et al.*,²³) which analyzed the mRNA expression of UGT1A isoforms in hepatic cells and tissues. Here, we have re-examined the hepatic expression of UGT1A8 and –1A10, and we present the first evidence that UGT1A8 and –1A10 mRNA is expressed in primary human hepatocytes at low levels (Fig. 2), and that significant induction of UGT1A8 and –1A10 mRNA expression is seen in hepatocytes treated with Rif and 3-MC (Fig. 3). In addition to these findings, we have also begun to investigate the hepatic expression of UGT1A7. We have found that its mRNA is not detected in untreated hepatocytes; however, significant induction by Rif and 3-MC in a similar manor as was seen with UGT1A8 and –1A10 was observed (data not shown). Although only preliminary data is available at this time, further characterization of hepatic UGT1A7 mRNA expression is under investigation.

It is widely recognized that all human UGT1A isoforms are encoded by a single locus on chromosome 2.^{2,4}) Each of the UGT1A genes contains 5 identical exons and 1 divergent first exon. The transcripts of individual first exon cassettes located at the 5' end of the human UGT1A locus are spliced onto exons 2–5 leading to the expression of at least nine different UGT genes. Studies investigating the UGT1A locus show that the first exon of each isoform is preceded by a unique promoter region. This region is recognized as being in control of the induction and suppression of a given isoform. Our analysis of the proximal promoters for UGT1A8 and –1A10 reveals over 90% homology, whereas comparing the full-length promoters (4589 bp and 4200 bp respectively) reveals 83% homology (data not shown). Therefore, it is very probable that the pattern of expression of these two isoforms in specific tissues and their mechanisms of gene regulation may be very similar.

During the past decade, studies have been carried out that promote the idea of tissue-specific expression of the UGT1A locus by demonstrating the identification of UGT1A8 and –1A10 mRNA only in extrahepatic tissue.⁸) Several other laboratories simultaneously carried out studies on the intestinal expression of UGT1A8 and –1A10.^{10,14,26,29}) The general conclusions from the studies were that UGT1A8 and –1A10 were not expressed in hepatic tissue. These conclusions were taken as evidence of tissue-specific expression and regulation of the human UGT1A locus.

Our rationale for the re-examination of the expression of UGT1A isoforms previously defined as exclusively extrahepatic was based on the assumption that if the UGT1A locus is present in the tissue then all the UGT1A transcripts are present. Therefore, we hypothesize that if this locus is present in a given tissue, there is potential for all the UGT1A isoforms to be expressed provided the necessary tissue-specific regulatory and inductive factors are present.

In the present studies using new primers and RT-PCR conditions that differ from those published previously (Table 2), we have demonstrated expression of UGT1A8 and –1A10 mRNA in human hepatocytes. The identification of the hepatic expression of UGT1A8 in these studies was most probably due to the fact that the set of primers generated were designed based on the published sequence for UGT1A8 wild type (UGT1A8*1, AF462268). These primers differed from the previously published primers^{8,24,25}) by a single 3'-terminal nucleotide base. A thorough review of the literature showed that this latter primer sequence has been used in all previously published investigations of UGT1A8 expression in various tissues, with the exception of those experiments done in Peter Mackenzie's laboratory.^{26,30}) Using the previously published primers (UGT1A8-G), no hepatic expression of UGT1A8 was found prior to our investigations.^{8,24,25})

Our side-by-side comparison using both UGT1A8-C and –1A8-G primers to analyze the expression of UGT1A8 mRNA in the human hepatocytes resulted in the identification of UGT1A8 expression in all the hepatocytes analyzed. In five of the six donors investigated, the mRNA product was only generated using the –1A8-C primers. In only one sample were we able to observe amplification of this isoform with the –1A8-G primers. This indicates that the –1A8-G primers, which had been previously used to establish that the expression of UGT1A8 was exclusively extrahepatic, were actually specific for the polymorphic variant, –1A8*2. This could explain why this isoform was not found previously in the liver. More significantly, we have documented here that, when using the –1A8-C primers, the expression of UGT1A8 mRNA in hepatocytes commonly occurs.

In order to confirm the identity of the products amplified by PCR, we carried out sequence analysis of each amplified PCR product generated with both –1A8-C and –1A8-G primers and compared these to the sequences of three polymorphic UGT1A8 isoforms. The results showed that the –1A8-C primers amplify UGT1A8*1 (and potentially –1A8*3), whereas the –1A8-G primers amplify only UGT1A8*2. The allelic frequencies of UGT1A8*1, UGT1A8*1a, UGT1A8*2 and UGT1A8*3 are 0.551, 0.282, 0.145 and 0.022, respectively.³¹)

Our current and previously reported studies on the hepatic expression of UGT1A10 are supported by the recent characterization of UGT1A-humanized mice.³²) Transgenic UGT1A (Tg-UGT1A) mice have been generated expressing the entire UGT1A locus, encoding all of the functional exon 1 sequences (–1A1 through –1A10), as well as the conserved exons 2–5.³²) Two interesting observations were made in relation to the hepatic expression of UGT1A10. The pattern of UGT1A isoform mRNA expression in Tg-*UGT1* transgenic mouse tissue demonstrated a low level of expression of UGT1A10; however, UGT1A5 and –1A8 were not detected. When these transgenic mice were exposed to ligands for the nuclear receptors AhR and PXR, such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and pregnenolone 16 α -carbonitrile (PCN), the analysis of UGT1A gene transcripts showed significant induction of UGT1A10. This directly confirms our findings in human hepatocytes (Fig. 3) where UGT1A10 was highly inducible after exposure to AhR and PXR ligands, 3-MC and Rif. However, in the Tg-*UGT1* transgenic mice, no induction of UGT1A5 or –1A8 was demonstrated. These results differ from our current and previously published²³) hepatocyte studies, which show that treatment with AhR and PXR inducers results in induction of all three isoforms. It would be interesting to investigate the expression and induction of UGT1A8 mRNA in these transgenic animals with the 1A8-C primers.

At present, we can only speculate on the possible mechanisms, which could explain the discrepancies observed in patterns of mRNA expression between primary human hepatocytes and human hepatic tissue. A set of interesting experiments were published on the identification of an additional form of cytochrome P450, which was identified only in primary cultures of rat hepatocytes.³³) These studies were followed by the identification of a novel rat UGT1A2

isoforms, which was not significantly expressed in rat liver, but which was found to be highly expressed in corresponding primary cultures of rat hepatocytes not exposed to chemical inducers.³⁴) It was postulated that the presence of a 66-nucleotide region designated as the culture-associated expression responsive enhancer module (CEREM), which interacts with a specific nuclear protein, is involved in the enhancement of UGT1A2 mRNA expression in cultured hepatocytes. In follow-up studies it was demonstrated that the transcriptional enhancement of UGT1A2 was mediated by Nuclear Factor I-A, which is present in rat hepatocytes.³⁵) It is possible that a similar mechanism can be responsible for the elevated basal expression levels of UGT1A8 and -1A10 in our human hepatocyte studies. Preliminary analysis of these isoforms' 5'-promoter regions identified several potential core binding motifs of a CEREM (data not shown). However, the phenomenon observed in our experiments differs significantly from the experiments in rat hepatocytes because these basal mRNA levels are inducible in response to specific chemical inducers, and this was not true for rats. It is possible that a dual phenomenon is involved in our observed expression and induction of UGT1A8 and -1A10 in human hepatocytes.

In the final experiments, we investigated the expression of UGT1A8 and -1A10 in human intestinal tissue available in our laboratory. In these experiments, mRNA was isolated from mucosa taken from fresh tissue. As shown in Fig. 6, both UGT1A8 and -1A10 are expressed in different segments of the intestine. Significant, discontinuous expression was noted, with the colon being the site of highest expression. Expression of UGT1A8 was examined using both the 1A8-C and 1A8-G primers. The similar expression pattern found using these different primers for all intestinal segments with the exception of the colon of Donor 1 suggests that, for intestinal mucosa, the one base difference in the sequence of these two primers does not seem to affect their ability to detect 1A8 mRNA. This could explain why primers for the polymorphic form of this isoforms have been used in the past without this being noticed.

It is anticipated that these observations will stimulate further new investigations of the hepatic regulation of these enzymes, which were previously defined as being exclusively extrahepatic.

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Fig. 1. Localization of primers and RT-PCR products

Isoform-specific sense primers are located within the first exon. The different anti-sense primers used are located within the common 3' sequence (exons 2–5) so as to amplify across the exon boundaries in mRNA from the target genes.

Fig. 2. UGT1A8, -1A9, and -1A10 mRNA levels in primary human hepatocytes from 6 donors
 Upon receipt of hepatocytes in 6-well culture plates, plating medium was replaced with fresh HMM supplemented with 0.1 μ M insulin, 0.1 μ M dexamethasone, 50 μ g/mL gentamicin, and 0.25 μ g/mL amphotericin B (designed HMM⁺). After an overnight equilibration period at 37°C under an atmosphere of 95% air/5% CO₂, hepatocytes were harvested and total RNA was prepared with Trizol. UGT1A8, UGT1A9 and UGT1A10 mRNA levels expression was analyzed by semi-quantitative RT-PCR (described under “Experimental Procedures”). Data are the mean $\pm$ SD of four data points.

Fig. 3. 3-MC and Rif increase UGT1A8 and -1A10 levels in primary human hepatocytes from donor HH1117

Upon receipt of hepatocytes in 6-well culture plates, plating medium was replaced with fresh HMM⁺. After an overnight equilibration period at 37°C under an atmosphere of 95% air/5% CO₂, hepatocytes were treated with 3-MC (1 μ M) or Rif (15 μ M) for 24 hr. UGT1A8 and -1A10 mRNA expression was analyzed by semi-quantitative RT-PCR. Results are normalized to fold of control (treated cells versus vehicle), and data are the mean $\pm$ SD of four data points.

Fig. 4. Comparison of RT-PCR products generated using mRNA from 6 sets of hepatocytes and the UGT1A8-G and UGT1A8-C primers. PCR and incubation conditions are as in Figs. 2 and 3. Expression of GAPDH is included as a control.

UGT1A8-G primer		-----GGTCTTCGCCAGGGGAATAGG-----	
1A8-G PCR product		-----NNNNNTGGTCTTCGCCAGGGGAATAGGTTGCCACTATCTTGAAGAAGGT	
UGT1A8-C primer		-----GGTCTTCGCCAGGGGAATAGC-----	
1A8-C PCR Product		-----NNNNNTGGTCTTCGCCAGGGGAATAGCTTGCCACTATCTTGAAGAAGGT	
1A8*1	544	TTCTCCCTCCCTCTGTGGTCTTCGCCAGGGGAATAGCTTGCCACTATCTTGAAGAAGGT	603
1A8*2	544	TTCTCCCTCCCTCTGTGGTCTTCGCCAGGGGAATAGGTTGCCACTATCTTGAAGAAGGT	603
1A8*3	544	TTCTCCCTCCCTCTGTGGTCTTCGCCAGGGGAATAGCTTGCCACTATCTTGAAGAAGGT	603
UGT1A8-G primer		-----	
1A8-G PCR Product		TTTtagTGCCCTGCTCCTCTTTCTATGTCCCAGAATTCTCTTAGGGTTCTCAGATGCC	
UGT1A8-C primer		-----	
1A8-C PCR Product		GCACAGTGCCCTGCTCCTCTTTCTATGTCCCAGAATTCTCTTAGGGTTCTCAGATGCC	
1A8*1	604	GCACAGTGCCCTGCTCCTCTTTCTATGTCCCAGAATTCTCTTAGGGTTCTCAGATGCC	663
1A8*2	604	GCACAGTGCCCTGCTCCTCTTTCTATGTCCCAGAATTCTCTTAGGGTTCTCAGATGCC	663
1A8*3	604	GCACAGTGCCCTGCTCCTCTTTCTATGTCCCAGAATTCTCTTAGGGTTCTCAGATGCC	663
UGT1A8-G primer		-----	
1A8-G PCR Product		ATGA A CTTTCAAGGAGAGAGTACGGAACACATCATGCACTTGGAGGAACATTTATTTTGC	
UGT1A8-C primer		-----	
1A8-C PCR Product		ATGA A CTTTCAAGGAGAGAGTACGGAACACATCATGCACTTGGAGGAACATTTATTTTGC	
1A8*1	664	ATGA A CTTTCAAGGAGAGAGTACGGAACACATCATGCACTTGGAGGAACATTTATTTTGC	723
1A8*2	664	ATGA A CTTTCAAGGAGAGAGTACGGAACACATCATGCACTTGGAGGAACATTTATTTTGC	723
1A8*3	664	ATGGCTTTCAAGGAGAGAGTACGGAACACATCTTGCACTTGGAGGAACATTTATTTTGC	723

Fig. 5.

Alignment of RT-PCR products generated by UGT1A8G and -1A8C primers with UGT1A8*1 (A¹⁷³C²⁷⁷) (AF462268), -1A8*2 (G¹⁷³C²⁷⁷) (AF462267), and -1A8*3 (A¹⁷³Y²⁷⁷) (AF465200).

Fig. 6. RT-PCR analysis of mRNA expression of UGT1A10, and -1A8 in the human GI tract
 RT-PCR was carried out as described in the text using the UGT1A8-G and -1A8-C primers and freshly prepared human intestinal mucosa (S1–S4, segments of remaining small intestine; C: colon) from Donor 1 (43 year-old male; cause of death: overdose of Atavan and valproic acid; history: valproic acid, Zyprexa, Haladol, Atavan) and Donor 2 (39 year-old male; cause of death: motor vehicle accident). The bar graphs show the results of quantitation by densitometry of ethidium bromide gel electrophoresis of the RT-PCR amplicons obtained using UGT1A10, -1A8-C, 1A8-G, and GAPDH (shown below each graph) primers. The results for each amplicon have been normalized to the recovery of GAPDH mRNA.

Table 1
Descriptions of hepatocyte donors. OR/ER: drugs administered in the operating room or emergency room

Sample	Sex	Age	Race	Cause of Death	Drug History
HH1117	Female	68	Caucasian	Subarachnoid hemorrhage	History: Labetalol, Plavix, Diallyl, Verapamil, Clonidine, Zocor, Reglan OR/ER: Solumedrol, insulin Levothyroxine
HH1121	Female	65	Caucasian	Subarachnoid hemorrhage	History: Smoker, Scription OR/ER: Dopamine(13–20), neosynephrine, insulin, thyroxine, and dexmethasone
HH1122	Female	46	Caucasian	Critical head trauma	History: Smoker Trianaterene, Propanolol, levothyroxin, Syntest, verapamil, Alprazolam, Provigil, Copaxone OR/ER: Dopamine(7–13), Ancef, T-4, Lobetolol
HH1139	Male	3	Caucasian	Motor vehicle accident critical head trauma	OR/ER: dopamine(15), Etomidate, succinate, Vecuronium, Versed, Benadryl, epinephrine, mannitol, insulin, vasopressin, Lasix, thyroxine
HH1142	Male	19	Caucasian	Motor vehicle accident critical head trauma	History: Smoker
HH1148	Male	60	Caucasian	Motor vehicle Accident critical head trauma	History: Smoker, drugs for hypertension, hyperlipidemia, and heart condition OR/ER: Dopamine(5), neosynephrine, and thyroxine

Table 2

Primer sequences and PCR conditions used. Differences in primer sequences are indicated by underlining. The modified 1A8 sense primer ends in a C and has been called 1A8-C. The published 1A8 sense primer ends in a G and has been called 1A8-G. S: sense primer; AS: antisense primer

	Primer name	Primer Sequences	RT-PCR Conditions
Modified primers Primer Added: 0.5 μ mol	UGT1A10 S	5'-CCTCTTTCCTATGTCCCAATGA-3'	Annealing Temperature: 57.6°C Number of Cycles: 34; [MgCl ₂]: 1.0 mM
	UGT1A10 AS	5'-CCTTAGTCTCCATGCGCTTTGC-3'	
	UGT1A8 S	5'-GGTCTTCGCCAGGGGAATAGC-3'	Annealing Temperature: 56.9°C Number of Cycles: 34; [MgCl ₂]: 0.7 mM
	UGT1A8 AS	5'-GTTCGCAAGATTCGATGGTCG-3'	
	UGT1A9 S	5'-GGAGGAACATTATTATGCCACCG-3'	Annealing Temperature: 57.1°C Number of Cycles: 34; [MgCl ₂]: 1.0 mM
	UGT1A9 AS	5'-GTTCGCAAGATTCGATGGTCG-3'	
Published primers Primer Added: 2 μ mol	UGT1A10 S	5'-CCTCTTTCCTATGTCCCAATGA-3'	Annealing Temperature: 64°C Number of Cycles: 34; [MgCl ₂]: 2.38 mM
	UGT1A10 AS	5'-GTTCGCAAGATTCGATGGTCG-3'	
	UGT1A8 S	5'-GGTCTTCGCCAGGGGAATAGG-3'	Annealing Temperature: 64°C Number of Cycles: 34; [MgCl ₂]: 2.38 mM
	UGT1A8 AS	5'-GTTCGCAAGATTCGATGGTCG-3'	
	UGT1A9 S	5'-GGAGGAACATTATTATGCCACCG-3'	Annealing Temperature: 64°C Number of Cycles: 34; [MgCl ₂]: 2.38 mM
	UGT1A9 AS	5'-GTTCGCAAGATTCGATGGTCG-3'	
Control	GAPDH S	5'-ACCCACTCCTCCACCTTTG-3'	Annealing Temperature: 57.6°C Number of Cycles: 34; [MgCl ₂]: 1.0 mM
	GAPDH AS	5'-CTCTTGCTCTTGTCTGGG-3'	