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Human UDP-glucuronosyltransferase 1A5: identification, expression and activity*

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d. Abbreviations: PXR: pregnane X receptor; UGT: UDP-glucuronosyltransferase; Dex: dexamethasone; Rif: rifampicin; 3-MC: 3-methylcholanthrene, 4-ABP: 4-aminobiphenyl, 4-MU: 4-methylumbelliferone; 1-HP: 1-hydroxypyrene.

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Abstract

The human UDP-glucuronosyltransferase (UGT) subfamily 1A includes 9 genes. The expression of all the UGT1A isoforms, apart from UGT1A5, has been reported previously. We have now detected a low basal level of UGT1A5 expression in cultured human hepatocytes and treatment with rifampicin or 3-methylcholanthrene increased the level of UGT1A5 mRNA. Low level UGT1A5 expression was also found in HepG2 and Caco-2 cells, as well as human liver. Furthermore, UGT1A5 expression has been detected in various segments of the intestine from human donors, revealing high interindividual variability in its level and distribution along the intestine. Full-length UGT1A5 cDNA was isolated from Caco-2 cells that had been transfected with the pregnane X receptor and treated with rifampicin. Recombinant UGT1A5, expressed in baculovirus-infected insect cells, exhibited very low rates of 4-methylumbelliferone and scopoletin glucuronidation, while 1-hydroxypyrene was a much better substrate. UGT1A5 did not glucuronidate 4-aminobiphenyl, a good substrate for the highly homologous enzymes UGT1A4 and UGT1A3. Replacing the first 110 amino acids of UGT1A5, a region that may be involved in substrate binding, with the counterpart segment from UGT1A4 did not increase the 4-aminobiphenyl glucuronidation activity, however. Collectively, this work demonstrates for the first time that the human UGT1A5 is expressed in several tissues, is inducible, and is catalytically active.

Introduction

The UDP-glucuronosyltransferases (UGTs) are membrane-bound enzymes of the endoplasmic reticulum and nuclear envelope that catalyze the glucuronidation of many endogenous and xenobiotic compounds (Radominska-Pandya et al., 1999; Ouzzine et al., 2003; Wells et al., 2004). The human genome contains many UGT-encoding genes, which have been divided into two subfamilies, UGT1 and UGT2. The UGT1A isoforms are encoded by a single large gene cluster on chromosome 2 and the expression of individual UGTs of this subfamily is governed by exon sharing (Ritter et al., 1992; Mackenzie et al., 1997). Hence, exon 1, encoding the N-terminal portion of each UGT1A, is unique, whereas exons 2-5, that together encode the C-terminal portion of these proteins, are shared by all the UGT1A isoforms (Ritter et al., 1992).

The expression of the UGTs is tissue specific and there are large differences in their expression levels in different tissues. Several UGT1A isoforms, including 1A1, 1A3, 1A4, 1A6 and 1A9, are rather highly expressed in the liver, whereas UGT1A7, 1A8 and 1A10 are mainly expressed in extra-hepatic tissues, particularly the intestine (Strassburg et al., 1997; Tukey and Strassburg, 2001; Mackenzie et al., 2003; Gregory et al., 2004). The tissue specific expression of UGT1As has been studied extensively but, thus far, there are no reports about the expression of human UGT1A5 in any tissue. Therefore, there was the question of whether or not the gene encoding this “missing UGT” was functional (see legend to Fig. 5 of Tukey and Strassburg, 2001). Recombinant human UGT1A5 was previously synthesized and assayed for activity towards several different substrates (Ciotti et al., 1999; Basu et al., 2004). The only detectable activity of UGT1A5 reported in these studies was glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38) at very low rates (Ciotti et al., 1999). In the absence of more significant activity, one might wonder about the function of UGT1A5, even if it is shown to be expressed. However, the expression of xenobiotic

metabolizing enzymes, including the UGTs, is often induced by different compounds via nuclear receptors and promoter elements that are specific for certain xenobiotics (Mackenzie et al., 2003; Bock and Kohle, 2004). Hence, the absence of detectable expression of a given UGT in human tissues may not be sufficient to indicate that the gene in question is not functional.

Most human UGTs glucuronidate multiple substrates and there are extensive overlaps in their substrate specificities. Little is currently know about the structural elements that determine substrate binding to UGTs, or the detailed location of the substrate binding site(s) within these enzymes. The most studied enzyme in this respect is UGT2B7, for which the interaction of morphine with several different peptides from the N-terminal region of the enzyme has been studied by nuclear magnetic resonance (Coffman et al., 2001 and 2003). These experiments suggested that the binding site for the opioid is between residues 84-118 of UGT2B7, within which residues 96-101 are directly involved in substrate binding (Coffman et al., 2003). Human UGT1A3-1A5 are highly homologous to each other and sequence alignment of UGT1A4 and 1A5 (Fig. 1) reveals that the majority of the 35 non-identical residues in these isoforms are located among the N-terminal 110 residues. This observation, taken together with the above results from UGT2B7, suggests that any differences in activity between UGT1A5 to UGT1A4 could originate from one or more of these non-identical amino acids within this N-terminal region.

In the present study, we have investigated both the mRNA expression and the activity of human recombinant UGT1A5. The results indicate that this is a functional member of the UGT1A subfamily, and its close examination could yield important new findings about both the regulation of and structure-function relationships within the UGTs.

Methods

Cell Culture. The human hepatocellular carcinoma cell line, HepG2 (ATCC HB-8065) and human adenocarcinoma cell line, Caco-2, (ATCC HTB-37), were obtained from the American Type Culture Collection (Manassas, VA). Both cell lines were maintained at 37ºC, 5% CO_2 in high glucose Dulbecco's Modified Eagle Medium (DMEM) with Earle's salts and L-glutamine (Invitrogen, Carlsbad, CA), supplemented with 1% non-essential amino acids, 1 mM sodium pyruvate and 10% fetal bovine serum (Invitrogen or Trace Biosciences, Australia). The culture medium was changed twice weekly during maintenance. Untransfected cells used for RNA isolation were harvested when they neared confluence.

Human primary hepatocyte cultures and treatment of cells. 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 the legends to the appropriate figures. 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. Primary cultures of human hepatocytes were maintained in culture in plating medium [Hepatocyte Maintenance Medium (HMM), Cambrex, E. Rutherford, NJ] containing 0.1 μM dexamethasone (Dex) for 24 h after receipt, and this medium was replaced with Dex-free medium 48 hr prior to initiating studies. Cells were grown in culture to 60-80% confluence before treatment with Dex, rifampicin (Rif) and/or 3-methylcholanthrene (3-MC) dissolved in dimethylsulfoxide (DMSO). For evaluation the effects of Dex, cells were grown in 0.1 μM Dex. The effects of 15 μM Rif and 1 μM 3-MC on UGT1A5 mRNA expression were measured after 24 h by semi-quantitative RT-PCR, using GAPDH as an internal control.

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Human intestinal tissue. Human gastrointestinal tissue was obtained from organ donors (details are given in the appropriate figure legend) 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 segments, stomach (ST), duodenum (D), 4 segments of the remaining small intestine (S-1 to S-4) and colon (C). Each segment was opened and cleaned and mucosa was recovered by scraping with a glass slide. RNA from H1 and H2 was prepared from mucosa that had been stored at –80°C, whereas the mucosa from H3 and H4 was collected and used immediately for RNA isolation, as described below.

Human hepatic tissue. The two human livers used in these studies were also obtained from the transplantation program at the University of Arkansas for Medical Sciences. Donor information is given in the legend to Fig. 2.

RNA Isolation and cDNA synthesis. Total RNA was isolated from cultured cells or mucosa using a phenol and guanidine isothiocyanate RNA extraction method following the instructions of the supplier (Trizol; Invitrogen). To avoid any contamination of the RNA by genomic DNA, DNase treatment was performed using RQ1 RNase-Free DNase (Promega, Madison, WI). cDNA was synthesized by mixing 1 μg 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 each dNTP (all from Promega) in a total volume of 20 μl. 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 μl with sterile diethylpyrocarbonate-treated H_2O.

Semi-Quantitative RT-PCR. The primers for GAPDH and UGT1A5 are described in Table 2. The specificity of all primer pairs was confirmed through sequencing or restriction analysis of the PCR products. The PCR reactions were performed as follows: a 10 μl cDNA

aliquot was added to a reaction mixture containing 10 mM Tris-HCl buffer (pH 8), 20 mM KCl, 0.1% Triton X-100; 1.5 mM $MgCl_2$, 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 µl. 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 59 °C and a 45 s elongation step at 72 °C in a thermal cycler (PTC100, MJ Research, Reno, NV). Amplification of the ubiquitously expressed GAPDH cDNA was performed under the same conditions in separate experiments. Primer pairs were designed to specifically amplify across exon boundaries in mRNA from UGT1A5 and GAPDH and Table 1 details the annealing temperature and cycle number required for each template. For each primer pair, PCR was performed with different cycle numbers and this data was plotted to form a standard curve. The cycle that was found to be within the non-saturable range of amplification was chosen for use in further experiments. All other conditions were kept consistent unless significant changes in mRNA level were observed. The PCR products were resolved by electrophoresis on 2% agarose gels 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). The RT-PCR products for UGT1A5 generated from mRNA from hepatocyte donor HH1117 were extracted from the gel and, after purification, were sequenced by the UAMS sequencing core. The sequences were then compared with corresponding known sequences from Genebank.

Cloning. The preparation of cDNA from Caco-2 cells following transfection with PXR and treatment by Rif has been described recently elsewhere (Gardner-Stephen et al., 2004). The coding region of the human UGT1A5 was amplified from such cDNA by nested PCR. The initial PCR reaction consisted of 4 µl of Caco-2 cDNA, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 2 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.1 mg/ml BSA, 0.2 mM

dNTPs, 2.5 ng/μl each of the oligonucleotide primers, UGT1A 3'Spe1 and 1A5cDNAUTR (Table 1), and 2.5 units Pfu Turbo DNA polymerase (Stratagene, La Jolla, CA) in 50 μl. The PCR cycling conditions were as follows: 4 min denaturation at 95°C followed by 30 cycles of: 30 sec at 95°C, 30 sec at 50°C and 5 min at 72°C, and a final step of 72°C for 5 minutes. The product from the first PCR, 1 μl, was used as a template in the second PCR, which was done similarly except that the primers were UGT1A3'Spe1 and 1A5cDNAATG, the annealing temperature was 55°C and the reaction was extended to include an extra 10 PCR cycles. The resulting PCR product was subjected to restriction digestion and cloned into the XhoI and SpeI sites of pBluescript II SK (Stratagene), facilitated by sites engineered into the 1A5cDNAATG and UGT1A3'Spe1 oligonucleotides, respectively. The cloned UGT1A5 was sequenced and found to have a single silent nucleotide difference (T792C) with respect to the published genomic sequence (AF297093).

Production of recombinant UGT1A5 and enzymatic assays. The cloned UGT1A5 was transferred in two steps from the original pBluescript into pFBXHC, a derivative of pFastBac1 (Invitrogen) designed to add a C-terminal enterokinase cleavage site followed by a His-tag (Kurkela et al., 2003). The Xho1-Spe1 fragment from the pBluescript construct was first subcloned into Litmus 29 (New England Biolabs, Beverly, MA) that was digested with the same enzymes. Subsequently, the BssH2-BstE2 fragment from the latter construct was subcloned, together with the BstE2-Hind3 fragment from the previously-cloned UGT1A6 in pFB-XHC (Kurkela et al., 2003) were subcloned into pFastBac1 that was digested with BssH2 and Hind3. The preparation of recombinant baculoviruses, insect cell infection and membrane isolation were done as described previously (Kurkela et al., 2003). The glucuronidation activity of the recombinant UGTs was assayed in the presence of 5 mM UDP-glucuronic acid, 0.5 mM of the appropriate substrate and 15 μg/ml membrane protein. The samples were incubated at 37°C for 60 min in the cases of scopoletin, 4-

methylumbelliferon (4-MU) and 4-aminobiphenyl (4-ABP), or 45 min when 1-hydroxypyrene (1-HP) glucuronidation was assayed. These incubation conditions were within the linear range of the reactions with respect to both time of incubation and protein concentration. The glucuronides were analyzed and quantified by HPLC with sensitive fluorescence detection, using authenticated (scopoletin, 1-HP, 4-MU) or radio-labeled (4-ABP) standards, as detailed elsewhere (Luukkanen et al., 2005).

Preparation of the chimeric enzymes, UGT1A4/5 and UGT1A5/4. The pFastbac derivatives containing the subcloned UGTs 1A4 and 1A5 were each digested with the restriction enzymes Xba1 and Hind3. The fragment from the digestion of UGT1A5 containing the gene segment from the internal Xba1 site to the Hind3 site immediately downstream of the stop codon, was subsequently ligated into the digested vector from UGT1A4 (that also contained the 5' segment of UGT1A4, from the first ATG to the internal Xba1 site) giving rise to 1A4/5. The construction of 1A5/4 was performed in a similar manner, except that the Xba1-Hind3 fragment was from 1A4 and the digested vector from 1A5. Expression in baculovirus-infected insect cells and the preparation of membranes were done as for UGT1A5.

Protein concentrations were measured by the bicinchoninic acid (BCA) system (Pierce Chemical, Rockford, IL, USA) using bovine serum albumin as a standard.

Results

Identification of UGT1A5 mRNA in human liver, primary hepatocytes and cell lines. The estimation of UGT1A5 mRNA levels in human liver, hepatocytes, and two human cell lines, HepG2 and Caco-2, was carried out by semi-quantitative RT-PCR. The expected PCR products for UGT1A5 were detected in representative normal human livers from two donors, human hepatocytes from one donor, and HepG2, and Caco-2 cells (Fig. 2). The highest level of mRNA expression was in the primary human hepatocytes and similar levels were found in HepG2 cells. The mRNA levels measured in both human livers and in Caco-2 cells were very low and comparable to each other. The mRNA levels of UGT1A5 in human hepatocyte cultures from six donors were examined and the results revealed very high interindividual variation (up to nine fold) in mRNA expression levels among the donors (Fig. 3).

Induction of UGT1A5 expression in human hepatocytes by 3-MC and Rif. Given that human hepatocytes are frequently cultured in the presence of Dex, the effects of Dex, and two prototypic drug metabolizing enzyme inducers, 3-MC and Rif, on the level of UGT1A5 mRNA were measured. Human hepatocytes from one donor cultured in Dex-free medium for 24 hours, followed by addition of 0.1 μM Dex to one group of cells while a second group of cells remained Dex-free as a control. After an additional 24 hours, both cultures were exposed to 15 μM Rif, a potent PXR activator, or 1 μM 3-MC, a ligand for the aryl hydrocarbon receptor, for 24 hours, after which the levels of UGT1A5 mRNA were measured by RT-PCR. The pattern of induction was essentially the same in the presence and absence of Dex (data not shown). In each of the treatment groups, both inducers increased the mRNA levels of UGT1A5 (Fig. 4). The induction by Rif, 3.5 fold, was more pronounced than the induction by 3-MC, 2.5 fold (Fig. 4. results from Dex-treated cells).

In addition to the induction of UGT1A5 mRNA levels in hepatocytes in response to inducers, the levels of mRNA for other UGT1A isoforms were also elevated (data not shown). It would be impossible to demonstrate an increase in UGT1A5 protein level by Western blot or an increase in UGT1A5 specific catalytic activity due to the fact that an anti-UGT1A5 specific antibody is not available and specific UGT1A5 substrates have not been identified.

Expression of UGT1A5 mRNA in the GI tract. mRNA was isolated from the mucosa of the available segments of the GI tracts of four human donors. The expression level of UGT1A5 mRNA in each segment from each donor was determined by parallel RT-PCR, using the ubiquitous GAPDH mRNA for normalization (Fig. 5). The expected product for UGT1A5 was identified in all four donors; however, expression levels varied widely along the length of the GI tract and between donors. Mucosa from some segments contained no measurable UGT1A5 mRNA while others contained relatively high levels of mRNA for this isoform (Fig. 5).

Enzymatic activities of recombinant UGT1A5. The enzymatic activity of the recombinant UGTs 1A4 and 1A5 toward several substrates was measured in the membrane fractions of insect cells expressing each UGT. The results of the first assays revealed very low glucuronidation activity towards scopoletin and 4-methylumbelliferone (4-MU, Table 2). Low 4-MU glucuronidation activity was also observed in HEK293 cells expressing native UGT1A5 without the C-terminal His tag (Mackenzie and Gardner-Stephen, results not shown). We have recently demonstrated that 1-hydroxypyrene (1-HP) is a good substrate for many UGTs (Luukkanen et al., 2005) and, therefore, examined its suitability as a substrate for recombinant UGT1A5. The results indicated that 1-HP was a much better substrate for UGT1A5 than any of the other substrates reported thus far. Glucuronidation of

1-HP by recombinant UGT1A5 was about 100 fold higher than its activity towards either 4-MU or scopoletin (Table 2).

The chimeric UGTs 1A4/5 and 1A5/4. At the level of amino acid sequence, UGT1A5 is highly homologous to UGT1A4 (and UGT1A3) (Fig. 1). Due to this similarity and the efficiency of both UGT1A4 and 1A3 in N-glucuronidation of 4-ABP (Green et al., 1998), it was anticipated that UGT1A5 would also catalyze this activity. The results, however, showed that recombinant UGT1A5 did not glucuronidate 4-ABP (Table 2). Consequently, two chimeric enzymes, UGT1A4/5 and UGT1A5/4 were prepared, taking advantage of a suitably-located internal Xba1 restriction site, and expressed in baculovirus-infected insect cells. The activities of the chimeric enzymes were determined and the results were somewhat surprising (Table 2). UGT1A4/5, like UGT1A5, did not glucuronidate 4-ABP, whereas UGT1A5/4 did exhibit low levels of activity toward this substrate. Although the rate of 4-ABP glucuronidation by UGT1A5/4 was very low in comparison to UGT1A4 (Table 2), it is significant that this activity was detectable in only UGT1A5/4 but not UGT1A5 or UGT1A4/5.

Discussion

The human UGT1A5 has not previously been found to be expressed in any human tissue (Tukey and Strassburg, 2001). Nonetheless, we have investigated its expression in induced Caco-2 cells, since it appeared feasible that this gene, like several other xenobiotic metabolizing enzymes, would be induced by certain treatments. Once the cDNA for UGT1A5 had been detected in induced Caco-2 cells, we have examined the expression of this gene in several different tissues and cultured cell systems. The detection of UGT1A5 mRNA in human hepatocytes and HepG2 cells (Figs. 2-4) indicated that its expression was not restricted to induced Caco-2 cells. Moreover, the effects of 3-MC and Rif on the expression level in hepatocytes (Fig. 4) suggests that UGT1A5 expression is regulated, at least in part, through PXR.

The RT-PCR experiments with intestinal segments from four donors (Fig. 5) yielded two interesting results. The first is the clear demonstration that this gene is expressed in the human intestine. The second result is that, as has been shown for UGT protein expression and enzymatic activity (Radominska-Pandya et al., 1998; Little et al., 1999; Czernik et al., 2000), there is an extensive interindividual variation in the expression level of UGT1A5 among donors and along the length of the intestine (Fig. 5). For example, in donors 1 and 2, the level of UGT1A5 mRNA in the duodenum was very close to or even below the detection limit, whereas, in donors 3 and 4, it was clearly detectable in this intestinal segment. These findings might partly explain why, in some previous studies, no expression of UGT1A5 in the duodenum was detected (Strassburg et al., 2000), while, in another case, it appeared to be expressed (see Fig. 7 of Tukey and Strassburg, 2001). It may be added here that a very recent abstract has described the identification of UGT1A5 mRNA in small intestine and colon of a transgenic mice that express the human UGT1A locus (Buser et al., 2005).

Having demonstrated that UGT1A5 is expressed in certain human tissues and cell cultures, we have investigated the physiological significance of this expression by examining the activity of the recombinant protein. UGT1A5 has been reported previously to glucuronidate 7-ethyl-10-hydroxycamptothecin (SN-38) at a very low rate (Ciotti et al., 1999). However, unlike results with all other UGT1A isoforms, we found no report of high glucuronidation activity of UGT1A5 towards any substrate. This corresponded to the results of the initial activity assays in our laboratory. Using sensitive HPLC methods and employing 4-MU and scopoletin, substrates that can be glucuronidated by many human UGTs, we have shown that recombinant UGT1A5 could glucuronidate these substrates, although at very low levels, in the range of 1 pmol per min per mg of membrane protein (Table 2). However, the activity rate was about 100 time higher when the recombinant UGT1A5 was assayed for 1-HP glucuronidation (Table 2). The latter results with 1-HP, a compound that was recently shown to be a very good substrate for other UGT1A isoforms (Luukkanen et al., 2005 and unpublished results), have demonstrated that UGT1A5 is a fully functional enzyme (Table 2).

It has been reported previously that the morphine binding site of UGT2B7 is within the N-terminal 120 residues, and that amino acids 96-101 are probably directly involved in the binding (Coffman et al., 2003). Inspection of the alignment of UGT1A4 and UGT1A5 (Fig. 1) indicates that the few differences in amino acids between them are concentrated within the first 120 residues. Therefore, it was possible that replacing the N-terminal region of UGT1A5 with the equivalent segment from UGT1A4 would yield a chimeric enzyme with activity toward 4-ABP, an activity that the recombinant UGT1A5 failed to catalyze (Table 2). The presence of an internal Xba1 restriction site at a suitable location in both UGT1A4 and 1A5 (Fig. 1) prompted the selection of UGT1A4 rather than UGT1A3 for the first round of chimera construction, as well as the position in which to exchange the segments between

them. It was anticipated that UGT1A4/5, the hybrid containing residues 1-110 from 1A4, would be more active in 4-ABP glucuronidation than UGT1A5. The results, however, showed that only UGT1A5/4, the hybrid that carried residues 1-110 from UGT1A5, exhibited any 4-ABP glucuronidation activity (Table 2). Nevertheless, since the activity of UGT1A5/4 towards 4-ABP was very low, it may be premature to conclude from these results that residues 29-110 (the first 28 residues at the N-terminal are a signal sequence) do not participate in substrate binding. Further experiments to determine substrate binding directly are needed to answer this question, an undertaking that is beyond the scope of this study.

In summary, the results of this work show that UGT1A5 is a functional enzyme that is expressed, at relatively low levels and high interindividual variability, in human liver and intestine.

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Footnote:

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Legends to figures

Figure 1. **Sequence similarity between the human UGTs 1A5 and 1A4.** UGTs 1A5 and 1A4 are 93.4% identical at the amino acid level and in this alignment only the 35 nonidentical residues are shown along the 1A4 sequence. The arrow indicates the start of the mature proteins. Ser 111 and Arg 112 are highlighted in bold phase and underlined to indicate the position of the Xba1 restriction site that was employed for the generation of the chimeric enzymes 1A45 and 1A54 (see text).

Figure 2. **RT-PCR analysis of mRNA expression of UGT1A5 in human liver, human hepatocytes, HepG2 cells and Caco-2 cells.** RT-PCR was carried out as described in the text using the primers listed in Table 2. cDNA was synthesized from RNA extracted from human livers, human hepatocytes, and HepG2 and Caco-2 cells. The bar graphs show the results of quantitation by densitometry of the ethidium bromide gel electrophoresis of the RT-PCR amplicons obtained using the primers for both UGTs and GAPDH (shown below each graph). The results for each amplicon have been normalized to the recovery of GAPDH mRNA and are expressed as relative expression level (amplicon expression/GAPDH expression). Data are the mean ± SD of two experiments. Donor details: HL1: 67 year old Caucasian female, normal liver sample; HL2: 45 year old Caucasian female, normal liver sample; HH3: 46 year old Caucasian female who died from critical head trauma. Smoker, no medication reported.

Figure 3. **UGT1A5 mRNA levels in primary human hepatocytes from 6 donors.** On receipt of hepatocytes in 6-well culture plates, medium was replaced with fresh HMM supplemented with 0.1μM insulin, 0.1 μM Dex, 50 μg/ml gentamycin, and 0.25 μg/ml

amphotericin B (HMM^+). After overnight equilibration at 37°C in an atmosphere of 95% air/5% CO2, hepatocytes were harvested and total RNA was prepared as described in the text. UGT1A5 mRNA expression level was analyzed by semi-quantitative RT-PCR (as described under Methods). The results for each amplicon have been normalized to the recovery of GAPDH mRNA and are expressed as relative expression level (Amplicon expression/GAPDH expression). Data are the mean ± SD of two experiments. Hepatocyte donor details: HH1: 68 year old female who died of a subarchnoid hemorrhage. Medications included labatolol, clopidogrel bisulfate (Plavix), verapamil, clonodine, simvastatin (Zocor) medoclopramide (Reglan); HH2: 65 year old female who died from a subarchnoid hemorrhage. Smoker, medications included HH3: 46 year old Caucasian female who died from critical head trauma. Smoker, no reported medications; HH4: 3 year old Caucasian male who died of critical head trauma. No reported medications. HH5: 19 year old Caucasian male who died fromcritical head trauma. Smoker, no medications reported; HH6: 60 year old Caucasian male who died from critical head trauma. Smoker, taking unnamed medications for hypetension, hyperlipidemia and heart condition.

Figure 4. **Induction of UGT1A5 mRNA expression by Dex, 3-MC and Rif in primary human hepatocytes.** Hepatocytes, from donor HH3, were firstly incubated with HMM^+ overnight at 37°C in an atmosphere of 95% air:5% CO2. The medium was then replaced with HMM^+ containing 1 μM 3-MC or 15 μM Rif. The UGT1A5 mRNA expression level was analyzed by semi-quantitative RT-PCR. The results were normalized to fold of control (3-MC or Rif versus vehicle). The data are the mean ± SD of three experiments.

Figure 5. **RT-PCR analyses of UGT1A5 expression in the human GI trac**t. RNA was extracted from human intestine (HI1, HI2, HI3, HI4) as described in Methods. The tissue was divided into segments, stomach (ST), duodenum (D), 4 segments of the remaining small intestine (S-1 to S-4) and colon (C). The bar graphs show the results of quantitation by densitometry of the ethidium bromide gel electrophoresis of the RT-PCR amplicons obtained using the primers shown in Table 2 (indicated below each graph). The results for each amplicon have been normalized to the recovery of GAPDH mRNA and are expressed as relative expression level (Amplicon expression/GAPDH expression). Intestine donor details: HI1: 63 year old Caucasian male who died from a stroke. Hypertensive, taking atorvastatin calcium (Lipitor); HI2: 25 year old male who died ofroma motor vehicle accident. Beer drinker, no medications reported; HI3: 66 year old male who died from a stroke. Hypertensive, diabetic. Medications included amlodipine and benzazepril and oral medication for diabetes; HI4: 45 year old male who died from a stroke. Smoker, hypertensive, diabetic. Medications included rosiglitazone (Avandia), clarithromycin (Biaxin), hydrochlorothiazide, esimeprezole (Nexium), atenolol, metformin, nabumeton (Relafen), nitroglycerin, albuterol inhaler, fluticasone (Advair), clopidogrel (Plavix), aspirin.

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Table 1. Oligonucleotide primers and PCR conditions

Primer	Primer Sequences	Comments
UGT1A5 S	5'- TGGCAATTATGAACAATATGTCT-3'	Annealing Temp. 56.1°C
UGT1A5 AS	5'- GATGCATGGCTGACAAGAT-3'	30 cycles, 1.5 mM $MgCl_2$
GAPDH S	5'-ACCCACTCCTCCACCTTTG-3'	Annealing Temp. 57.6°C
GAPDH AS	5'-CTCTTGTGCTCTTGCTGGG-3'	34 cycles, 1.0 mM $MgCl_2$
UGT1A 3' Spe1	5'-AGCCATACTAGTTCAATGGGTCTTGGATTTGT-3'	See Methods
1A5cDNAUTR	5'- TTAAGACGAAGGAAACAATTC-3'	
1A5cDNAATG	5'- AGCCATCTCGAGATGGCCACAGGACTCCAGGTTC CCCTGCCGCA-3'	

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Table 2. Glucuronidation activities of the recombinant UGTs 1A5 and 1A4, as well as the chimeric enzymes 1A4/5 and 1A5/4. The units are pmol x min^{-1} x mg^{-1} and the results are the mean ± SD (n=3).

	4-methylumbelliferone	Scopoletin	4-aminobiphenyl	1-hydroxypyrene
UGT1A5	0.9 ± 0.1	1.1 ± 0.1	ND*	96.8 ± 5.3
UGT1A4	1.1 ± 0.1	1.4 ± 0.1	945.6 ± 32.6	103.8 ± 7.2
UGT1A4/5	1.3 ± 0.1	3.0 ± 0.1	ND*	59.5 ± 1.8
UGT1A5/4	2.3 ± 0.1	6.0 ± 0.3	0.6 ± 0.1	46.4 ± 4.9

*ND, no detectable activity

Fig. 1

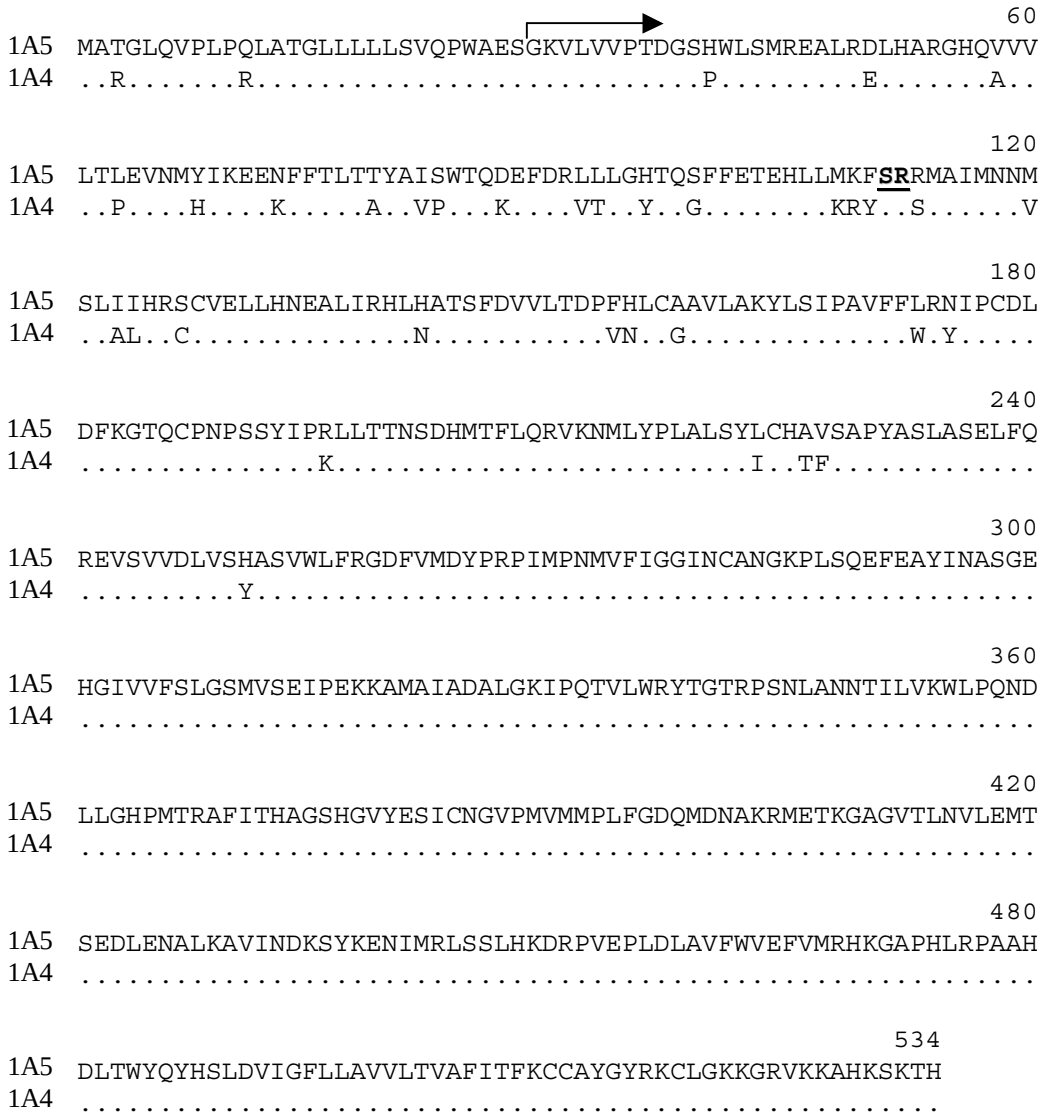

Fig. 2

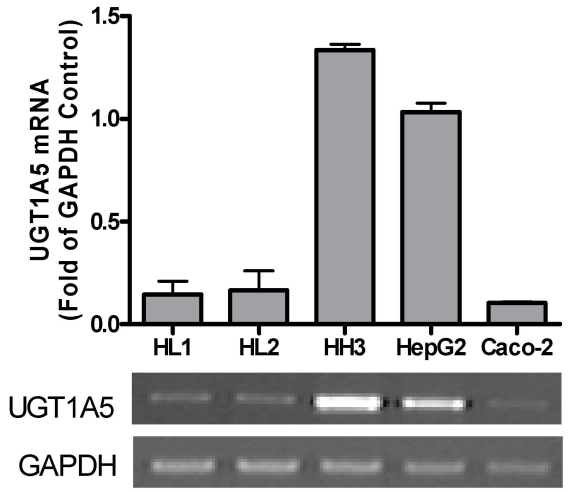

Fig. 3

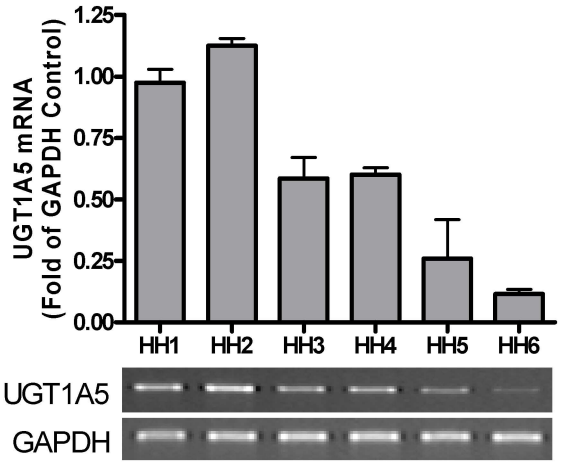

Fig. 4

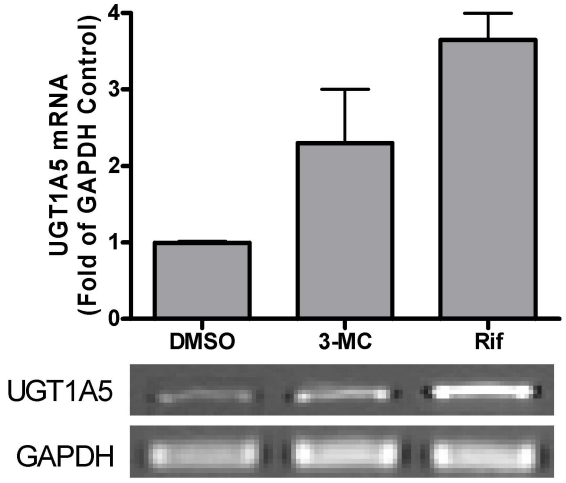

Fig. 5