POLYMORPHIC GENE REGULATION AND INTERINDIVIDUAL VARIATION OF UDP-GLUCURONOSYLTRANSFERASE ACTIVITY IN HUMAN SMALL INTESTINE

by

Christian P. Strassburg, Susanne Kneip, Juliane Topp, Petra Obermayer-Straub, Ayse Barut, Robert H. Tukey*, and Michael P. Manns

Department of Gastroenterology and Hepatology, Hannover Medical School, 30625 Hannover Germany and *Departments of Chemistry & Biochemistry and Pharmacology, University of California, San Diego, La Jolla, CA 92093

Running title: Differential expression of UGTs in small intestine.

Correspondence to:

Christian P. Strassburg, M.D.
Department of Gastroenterology and Hepatology
Hannover Medical School
Carl-Neuberg-Str. 1
30625 Hannover
Germany

Tel.: x49-511-532 2853
Fax.: x49-511-532 2093

email: strassburg.christian@mh-hannover.de
Abbreviations:

UGT, UDP-glucuronosyltransferase; DRT-PCR, duplex reverse transcription polymerase chain reaction; HDCA, hyodeoxycholic acid; 4-MU, 4-methylumbelliferone; PhIP, 2-amino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine

Acknowledgements:

This work was supported by Deutsche Forschungsgemeinschaft grant STR493/3-1 (to C.P.S.) and USPHS grant GM49135 (to R.H.T). The excellent technical assistance with the immunofluorescence analyses by Stephanie Loges and Eleonore Schmidt is gratefully acknowledged. Help in tissue sample procurement by Professor J. Klempnauer, M.D., Director Department of Abdominal and Transplant Surgery, Hannover Medical School is gratefully acknowledged.
SUMMARY:

UDP-glucuronosyltransferases (UGTs) convert dietary constituents, drugs and environmental mutagens to inactive hydrophilic glucuronides. Recent studies have shown that the expression of the UGT1 and UGT2 gene families is regulated in a tissue specific fashion. Human small intestine represents a major site of resorption of dietary constituents and orally administered drugs and plays an important role in extrahepatic UGT directed metabolism. Expression of 13 UGT1A and UGT2B genes coupled with functional and catalytic analysis were studied using 18 small intestinal and 16 hepatic human tissue samples. Hepatic expression of UGT gene transcripts was without interindividual variation. In contrast, a polymorphic expression pattern of all the UGT genes was demonstrated in duodenal, jejunal and ileal mucosa, with the exception of UGT1A10. To complement these studies, interindividual expression of UGT proteins and catalytic activities were also demonstrated. Hyodeoxycholic acid glucuronidation, catalyzed primarily by UGT2B4 and UGT2B7, showed a 7-fold interindividual variation in small intestinal duodenal samples, in contrast to limited variation in the presence of 4-methylumbelliferone, a substrate glucuronidated by most UGT1A and UGT2B gene products. Linkage of RNA expression patterns to protein abundance were also made with several mono-specific antibodies to the UGTs. These results are in contrast to a total absence of polymorphic variation in gene expression, protein abundance and catalytic activity in liver. In addition, the small intestine exhibits considerable catalytic activity towards most of the different classes of substrates accepted for glucuronidation by the UGTs, which is supported by immunofluorescence analysis of UGT1A protein to the mucosal cell layer of the small intestine. Thus, tissue specific and interindividual polymorphic regulation of UGT1A and UGT2B genes in small intestine is identified and implicated as molecular biological determinant contributing to interindividual prehepatic drug and xenobiotic metabolism in humans.
INTRODUCTION:

Glucuronidation is an important process of metabolism and detoxification performed by the UDP-glucuronosyltransferase (UGT) Supergene family (1). UGTs are resident in the endoplasmic reticulum and catalyze the conversion of hydrophobic substrates to usually inactive hydrophilic glucuronides, which subsequently undergo renal and biliary elimination. Compounds targeted for glucuronidation include dietary constituents, therapeutic drugs, endogenous metabolites, hormones, as well as environmental carcinogens. The human UGT genes are differentially regulated in a tissue specific fashion in hepatic and extrahepatic tissues of the gastrointestinal tract (2-4).

Human UGTs have been divided into the UGT1 and UGT2 multigene families (5). The human UGT1A gene locus is located on chromosome 2, which encodes at least 9 functional UGT1A proteins and 3 pseudogenes (6). Four exons are located at the 3' end of the UGT1A locus, which are combined with one of a consecutively numbered array of first exon cassettes towards the 5' end of the gene locus to form individual UGT gene products. Therefore, the amino terminal 280 amino acids of UGT1A proteins consist of unique exon 1 encoded sequences and the carboxy terminal 245 amino acids encoded by exons 2 - 5 are identical. The tissue specific expression of the UGT1A gene locus has been well characterized and has been suggested to define tissue specific glucuronidation activity in the human digestive system (2). An analysis of liver tissue led to the characterization of UGT1A1 (7), UGT1A3 (8), UGT1A4 (7), UGT1A6 (9), and UGT1A9 (10) cDNAs. Studies examining the human extrahepatic gastrointestinal tract have led to the identification of 3 extrahepatic UGT1A transcripts: UGT1A7 which is expressed in stomach and esophagus (3,4), UGT1A8 which is expressed in colon and esophagus (2,11,12), and UGT1A10 which is expressed in gastric, esophageal, biliary and colonic tissue (2,4,13,14).

In contrast to the UGT1A gene locus, the UGT2B and UGT2A genes have been mapped to chromosome 4 are individually encoded and comprise 6 exons (15-17). Transcripts have been identified for UGT2B4 (18), UGT2B7 (19), UGT2B10 (20), UGT2B11 (21), UGT2B15 (22), UGT2B17 (23,24) and UGT2A1 (17). Except for UGT2B17 and UGT2A1, hepatic expression was detected for all UGT2B transcripts. Extrahepatic UGT2B expression has been shown for UGT2B7 in intestine, kidney and brain (25,26), UGT2B10 and UGT2B15 in esophagus (3), as well as UGT2B10, UGT2B11, UGT2B15 and UGT2B17 in steroid sensitive tissues such as the mammary gland and the prostate (20-24). One report indicates that UGT2B4 is not expressed in the gastrointestinal tract (26).

The genetic multiplicity of the UGTs and their wide range of substrate specificities suggests that UGTs play an important role in human homeostasis and metabolism. Although hepatic glucuronidation is considered to play a central role in drug metabolism, direct contact with xenobiotic
compounds is first established in the gastrointestinal tract prior to resorption (27). The small intestine, which extends to a length of 300 to 400 cm in adults, forms the largest metabolically active external surface of the human digestive system and represents a significant localization for extrahepatic metabolism. The considerable degree of immediate xenobiotic contact in the small intestine including dietary components, drugs, and environmental mutagens would indicate that enzymes located in the mucosal layer are capable of influencing first pass metabolism and may function as a metabolic intestinal barrier. The presented study was undertaken to analyze the regulatory patterns of *UGT1A* and *UGT2B* genes in small intestine as a biochemical basis for defining human extrahepatic xenobiotic glucuronidation.
EXPERIMENTAL PROCEDURES:

Tissue samples:

Tissue samples were obtained from the Department of Abdominal and Transplant Surgery, Hannover Medical School, Hannover, Germany. Informed written consent was obtained and the project was approved by the ethics committee of Hannover Medical School. Macroscopically and histologically normal intestinal tissue was obtained from 18 German patients undergoing surgery for diagnoses summarized in Table 1. None of the patients received chemotherapy, steroids, diuretics or antibiotic therapy prior to sample collection. The records indicated the absence of smoking during 6 months prior to surgery. A high degree of sample normalization results from the additional fact that all patients were fasting at least 12 hours prior to the surgical procedures and tissue collection. The collected tissues showed no macroscopic signs of deterioration such as necrosis and were microscopically examined to document normal histology. One patient with ulcerative colitis received a colectomy and had no histological signs of ileal disease. Hepatic tissue RNA and microsomes used for comparisons have been described previously (27).

Intestinal mucosa was dissected immediately on ice after surgical removal, and specimens free of muscularis and most of the submucosa were used in all subsequent experiments except for indirect immunofluorescence. All tissue samples were frozen in liquid nitrogen within 10 min. of surgical removal and stored at -80°C until use.

Isolation of RNA and synthesis of complementary DNA

Tissue (approx. 200 mg) was pulverized under liquid nitrogen and immediately lysed in acidic phenol/guanidinium-isothiocyanate solution (Tripure, Boehringer Mannheim, Germany) as described previously (4). RNA Concentrations were determined by spectrophotometry at 260 nm and the purity verified by 260/280 nm ratios. Intact RNA was isolated from hepatic, duodenal, jejunal, and ileal tissue samples and stored in water at -80°C until further analysis.

cDNA synthesis: Three µg of total RNA were denatured for 10 min at 70°C in the presence of 0.5 µg oligo dT primer and quick chilled on ice. The volume of RNA was adjusted to 19 µl containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl2, 10 mM dithiothreitol, and 0.5 mM of each dNTP, incubated at 42°C for 5 min prior to the addition of 1 µl (200 U) of reverse transcriptase (SuperscriptII, Gibco BRL, Gaithersburg, MD). The final volume (20 µl) was incubated at 42°C for 50 min, 70°C for 15 min and chilled on ice. Contamination of total RNA with genomic DNA was excluded by RT-PCR using primers for human β-actin. The sense primer 5’-gcccggcacccacgtacct-3’ and the antisense primer 5’-aggggccggactcgtacact-3’ span the exon 4/intron 5/exon 5 junction of the β-actin gene. PCR with cDNA leads to a 202 bp product, but contamination with genomic DNA
template would lead to a 312 bp PCR product, which can be clearly distinguished from the 202 bp cDNA amplification product.

**Isolation of microsomal protein from intestinal tissue**

Approximately 300 mg of intestinal tissue was pulverized under liquid nitrogen, resuspended in 1 ml of 50 mM Tris-HCl (pH 7.4), 10 mM MgCl₂ and homogenized with a Potter-Elvehjem tissue grinder. The tissue homogenate was centrifuged at 10,000 x g for 5 min at 4°C in an Eppendorf (Hamburg, Germany) microcentrifuge and the supernatant collected. The pellet was resuspended in 0.5 ml of 50 mM Tris-HCl (pH 7.4) and 10 mM MgCl₂, centrifuged and the supernatant collected. The combined supernatants were centrifuged at 150,000 x g for 60 min at 4°C in a Beckman (Palo Alto, CA) TL100 ultracentrifuge and the pellet was resuspended in 0.2 ml of Tris-HCl (pH 7.4) and 10 mM MgCl₂. Protein concentration was determined by the method of Bradford. Microsomal protein was stored at -80°C.

**Catalytic activity assay of human intestinal and liver microsomes:**

Glucuronidation substrates (all 18 tested substrates are listed in the legend to Figure 3) were solubilized in methanol with the exception of 7-hydroxy-benzo(α)pyrene, which was resuspended in acetone. 7-hydroxy-benzo(α)pyrene, 3-hydroxy-acetylaminofluorene, and 2-amino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (PhIP) were obtained from the National Cancer Institute Chemical Carcinogen repository, Midwest Research Institute, Kansas City, MO, 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (N-hydroxy-PhIP) was purchased from Toronto Research Chemicals Inc., Toronto, Canada, and all other substrates were from Sigma-Aldrich, St. Louis, MO. Catalytic activities of 25 µg of microsomal protein isolated from intestinal or hepatic tissue were assayed in duplicate as previously described in detail (3). Protein was precipitated, supernatants lyophilized and resuspended in methanol prior to separation by thin-layer liquid chromatography in n-butanol/acetone/acetic acid/water (35:35:10:20%). The production of 14C-labeled glucuronides was detected by autoradiography. To determine specific catalytic activities the 14C-labeled glucuronides were quantitated using a FujiFilm BAS-1000 phosphorimager (Raytest GmbH, Straubenhardt, Germany) and TINA 2.0 software (Raytest GmbH, Straubenhardt, Germany) and expressed as pmol glucuronide formed/min/mg of microsomal or recombinant protein. As a control, autoradiography hard copies were additionally analyzed with a GS-710 calibrated imaging densitometer (BioRad Laboratories, Hercules, CA) using the Quantity One software package.
Duplex reverse transcription polymerase chain reaction for UGT1A and UGT2B transcripts:

The presence of UGT1A and UGT2B transcripts in total tissue RNA was analyzed by PCR amplification performed as a duplex RT-PCR coamplification with β-actin cDNA as a control, as outlined below.

**UGT1A DRT-PCR**: The UGT1A locus predicts the existence of 9 proteins termed UGT1A1 and UGT1A3-1A10. UGT1A2, UGT1A11 and UGT1A12 lack an uninterrupted open reading frame and have therefore been identified to be pseudogenes. DRT-PCR detection of all 9 UGT1A transcripts predicted by the human UGT1A locus was performed using 9 exon 1 specific sense primers and 2 antisense primers located within exons 2-5 or within a common portion of the 3’ end of the first exons (4). As already reported elsewhere (4) exon specific primers were generated which lead to RT-PCR products of distinct molecular sizes: UGT1A1 - 644 bp, UGT1A3 - 483 bp, UGT1A4 - 572 bp, UGT1A5 - 659 bp, UGT1A6 - 562 bp, UGT1A7 - 754 bp, UGT1A8 - 514 bp, UGT1A9 - 392 bp, and UGT1A10 - 478 bp. Coamplification of UGT1A first exon and β-actin sequences was performed using three cycling protocols: UGT1A1 and UGT1A6 - 94°C (1 min), 59°C (1 min), 72°C (1 min); UGT1A3, UGT1A4, UGT1A5 - 94°C (1 min), 56°C (1 min), 72°C (1 min); UGT1A7, UGT1A8, UGT1A9, UGT1A10 - 94°C (1 min), 64°C (1 min), 72°C (1 min). Each protocol was preceded by a 3 min incubation of the reaction mixture at 94°C and followed by a 7 min elongation at 72°C. The specificity and kinetics of this assay have previously been documented in detail (4). Experiments were performed in duplicate and controls without cDNA, primers or therophilic polymerase included.

**UGT2B DRT-PCR**: Specific primer pairs were generated for the amplification of UGT2B4, UGT2B7, UGT2B10 and UGT2B15 sequences, respectively as recently reported elsewhere (3). Cross reactivity was excluded using sequence alignments and PCGene (Oxford Molecular, Campbell, CA) software, as well as a computerized databank search using the blastn software (GenBank). UGT2B cDNA was coamplified with β-actin cDNA in a starting volume of 92 µl containing 10 mM KCl, 20 mM Tris-HCl (pH 8.8), 10 mM ammonium sulfate, 2 mM magnesium sulfate, 1% Triton X-100, 0.2 mM each dNTP and 2 µM of UGT2B primers and VENT (exo-) DNA polymerase (NEB, Beverly, MA). After a hot start at 94°C for 3 min 6 cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 30 sec were run on a Perkin Elmer GeneAmp PCR 2400 system. The same β-actin primers used for UGT1A DRT-PCR were added to 0.4 µM each and cycling was resumed for a total of 32 cycles. Specificity of this assay was determined by PCR using all four primer pairs on each cloned UGT2B4, UGT2B7, UGT2B10 and UGT2B15 template cDNA to exclude cross reactivities. PCR products of the expected sizes were generated: UGT2B4 - 281 bp, UGT2B7 - 407 bp, UGT2B10 - 388 bp, UGT2B15 - 330bp.

To confirm the detection of specific UGT1A and UGT2B cDNAs using this assay the PCR
products were partially sequenced to document the identity of the specific gene product.

**Western blot analysis:**

Twenty µg of microsomal protein from 5 human duodenal and 5 human hepatic tissues samples was boiled for 90 seconds in loading buffer (2% sodium dodecyl sulfate, 62.5 mmol/L Tris-HCl (pH 6.8), 10% glycerol, and 0.001% bromphenol blue) with β-mercaptoethanol and resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis prior to electrotransfer onto nitrocellulose membrane. As controls, a 5 µg sample of total Sf9 cell lysate expressing recombinant UGT1A1 and UGT2B7 protein as well as Sf9 cells expressiong no recombinant UGT protein were included (3). Immunodetection was performed following published protocols (28). UGT1A1 and UGT2B7 protein was detected using a monospecific rabbit anti human UGT1A1 and rabbit anti human UGT2B7 antibody purchased from NatuTec/Gentest (Frankfurt, Germany) at a dilution of 1:1500. Visualization was achieved with an alkaline phosphatase conjugated goat anti rabbit IgG (Sigma, Deisenhofen, Germany) diluted 1:4000.

**Indirect immunofluorescence analysis:**

Fresh intestinal resection specimens were subjected to cryostat sectioning following previously published methods (29). Tissue sections were used for indirect immunofluorescence using a previously described rabbit anti-peptide (SSLHKDRPVEPLDLA) anti human UGT1A antibody, which was generated using branched lysine multiple antigen peptide (MAP) technology (2). Antibody was diluted 1:20, 1:40, 1:80 and 1:160 in phosphate buffered saline without magnesium or calcium (PBS), and immobilized tissue slices were incubated at room temperature in a humidified chamber for 60 min. Incubation with a normal rabbit serum was included as a control. Following two wash steps with PBS, the slides were incubated at room temperature in a humidified, dark chamber for 60 min with fluorescein (dichlorotriazinyl-aminofluoresceine)-conjugated affinity purified goat anti-rabbit IgG (H+L) (Dianova, Hamburg, Germany) diluted 1:100 in PBS. Following two wash steps with PBS, tissue slices were covered with glycerol containing 10% PBS and were immediately analyzed using an Olympus IMT 2 immunofluorescence microscope (Tokyo, Japan).
RESULTS:

**Polymorphic regulation of UGT1A and UGT2B gene transcripts in human duodenum, jejunum and ileum.**

UGT mRNA expression was analyzed by isoform specific DRT-PCR (Figure 1 and Table 2 right column). The liver was characterized by the expression of UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, and UGT2B15 mRNA, as previously demonstrated (3,26). In the 16 different liver samples, there was little variation in abundance of the RNA transcripts when each was compared to the levels of expression of actin (not shown but previously demonstrated in (14)). This is in sharp contrast to intestinal tissue, which exhibited dramatic differences in UGT gene transcript expression. Intestinal expression was characterized by the presence or absence of UGT1A and UGT2B transcripts in the different samples of intestinal tissue.

Analyses of 13 different UGT transcripts demonstrated that UGT1A10 mRNA was expressed in each sample of duodenal, jejunal and ileal mucosa, while UGT2B15 was absent in only two of the duodenal samples. UGT1A3 and UGT1A4 were found to be expressed in the majority of duodenal, jejunal and ileal mucosa samples. In contrast, UGT1A5, UGT1A7, UGT1A8 and UGT1A9 transcripts were not detected, and UGT2B10 was found in only one of the ileum preparations. It is interesting to note that in the ileum sample in which UGT2B10 RNA was expressed, all of the other UGT2B gene products were also detected (Figure 1, bottom panel).

The appearance of UGT1A1, UGT1A6, UGT2B4 and UGT2B7 mRNA showed the most dramatic variability between the different intestinal samples. In the duodenum, UGT1A1 was detected in 3 of the 5 samples examined, UGT1A6 in 4 of the 5 samples, UGT2B4 in 1 of the 5 samples and UGT2B7 in 2 of the 5 samples. The ratios of these gene products were found to be similar in the jejunum, although UGT1A6 mRNA was only detected in 1 sample. In the ileum, UGT2B4 was detected in a greater number of samples than found in the duodenum and jejunum, demonstrating that UGT2B4 is not liver specific as previously indicated (26). Combined, analysis of UGT gene expression as presented by sensitive RT-PCR analysis clearly demonstrate that the expression of RNAs encoding UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT2B4, UGT2B7, UGT2B10 and UGT2B15 are not coordinately regulated in the different tissues of the small intestine. The only exception appears to be UGT1A10, which is expressed in all portions of the small intestine, as well as all other tissues of the GI tract including the colon, the esophagus, the stomach, and the biliary tract (2-4,26,27).

**Interindividual differences in UGT activities in small intestine.**
Most of the UGTs possess the ability to glucuronidate many of the same substrates, making it a challenge to use functional studies to follow the expression patterns of any single UGT (5). However, several substrates can be employed to monitor the catalytic activities of a limited number of the UGTs. For example, HDCA has been identified to be glucuronidated primarily by UGT2B7 (30), with detectable activity observed with expressed UGT2B4 (31) and UGT1A3 (5,32). Since the gene expression pattern demonstrated considerable interindividual differences in UGT2B4 and UGT2B7 expression, experiments were undertaken to examine the functional properties of these proteins in microsomal preparations from small intestinal tissue samples using HDCA as a substrate. In contrast, 4-methylumbelliferone (4-MU) was chosen as a more general substrate since it is glucuronidated by most of the UGT1A as well as some of the UGT2 proteins (34). Using five duodenal, jejunal, and hepatic microsomal preparations, UGT activities confirmed there existed considerable interindividual variation of HDCA glucuronidation in the small intestinal samples when compared to liver microsomal preparations. In jejunum tissues, this variation was seen to be 7-fold. It is therefore likely that the differences in HDCA UGT activity reflect the variation observed in UGT2B4 and UGT2B7 RNA transcript expression in these samples. In addition, up to a 2.3-fold variation of 4-MU glucuronidation (Figure 2A, top panel) was observed with both intestinal tissues, which might be reflected in the differences seen with UGT1A1, UGT1A3, UGT1A4 and UGT1A6 RNA expression (Table 2). As predicted from the mRNA expression data, there was very little variation of HDCA and 4-MU glucuronidation activity in the different liver samples. Although the duodenum, jejunum and liver tissue samples were taken during surgery from different individuals, the differences observed in catalytic activity in the intestinal tissue strongly implicates that the polymorphic regulation of UGT mRNA leads to interindividual variation in UGT expression and activity.

Additional support to the findings observed with RNA and as well as functional analysis that the UGTs are differentially expressed in intestinal tissue could be verified by Western blot analysis. In Figure 2B the analysis of UGT1A1 and UGT2B7 protein expression in 5 samples of human duodenum and liver are shown and correlated with the detection of transcripts of these genes. The expression of UGT1A1 mRNA in 3 of the 5 samples and UGT2B7 mRNA in 2 of the 5 samples (Table 2) is confirmed at the protein level by Western blot analysis which detected UGT1A1 and UGT2B7 in the same samples. Duodenal sample 5 in Figure 2B was used in the RT-PCR analysis and is shown in Figure 1, demonstrating the expression of UGT1A1 RNA but not of UGT2B7 RNA. This finding convincingly links the expression of RNA to protein. Interestingly, this same sample does not express UGT2B4 RNA while UGT1A3 RNA is barely detectable. UGT2B4, UGT2B7 and UGT1A3 are capable of catalyzing the glucuronidation of HDCA and this sample of duodenum elicited the lowest HDCA UGT glucuronidation activity of the small intestinal samples that were collected. Combined,
these findings demonstrate that the polymorphic interindividual regulation of UGT1A1 and UGT2B7 gene expression results in the detectable presence or absence of these specific UGT proteins.

Thus, polymorphic regulation of UGT genes in small intestine leads to variations of catalytically active UGT which determine microsomal glucuronidation activity between individuals.

**Differences of hepatic and small intestinal glucuronidation.**

A panel of 18 substrates was used to characterize the UGT activity profile of the small intestine and liver. The consistent expression of UGT1A3, UGT1A4, UGT1A10 and different UGT2B forms would suggest activities towards steroid hormone and tertiary amine substrates in addition to phenolics. The putative tobacco carcinogens PhIP, N-hydroxy-PhIP and 7-hydroxy benzo(α)pyrene, as well as 3-hydroxy-acetylaminofluorene were also included. Microsomal protein from 2 samples each of duodenal, jejunal and ileal mucosa, were analyzed in the presence of the substrates and their activities shown in Figure 3. The specific activity towards 1-naphthol (1-naph), 4-methylumbelliferone (4-MU), 4-nitrophenol (p-nitrophenol), and hyodeoxycholic acid (HDCA) was greater in liver than in the individual intestinal samples. Yet, there were a greater number of compounds glucuronidated at higher rates in small intestine. This is best demonstrated by examining the glucuronidation of steroids such as 2-hydroxy estrone (2-OH-estrone), β-estradiol, estrone, but also of the carcinogens 2-amino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (PhIP), 7-hydroxy benzo(α)pyrene (7-OH-BAP), and 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (Nitro-PhIP), in addition to the tertiary amine antidepressant drugs imipramine and amitriptyline.

This experiment also demonstrates that the catalytic activities were found to be greater in the jejunum than in the proximally located duodenum or the distally located ileum. In addition, the finding that the catalytic activities in the jejunum are universally greater than those found in liver would suggest that this tissue plays an important role in the metabolism of dietary and xenobiotic material.

**Detection and localization of UGT protein in human intestine**

To confirm the mucosal distribution of UGT protein an indirect immunofluorescence analysis was performed using a rabbit anti-human UGT1A antibody directed against all UGT1A protein species (Figure 4). Staining was exclusively localized to the epithelial cell layer of the intestinal mucosa (Figure 4 A and B). No staining was observed in the submucosa or muscularis as well as with a normal rabbit serum (not shown). UGT1A protein expression was found only in the epithelial cell layer of the crypt (Figure 4 C) as well as the vili (Figure 4 B).
DISCUSSION:

Human UDP-glucuronosyltransferases are expressed in a tissue specific fashion, which defines tissue specific glucuronidation activities in metabolically active organs including the liver and the extrahepatic gastrointestinal tract (2-4,11,27). Microsomal glucuronidation and UGT mRNA expression have been analyzed in human esophagus, stomach and colon, establishing the role of these external surface tissues in extrahepatic glucuronidation. Although intestinal glucuronidation has been documented (26,33-35), UGT1A and UGT2B gene regulation and biological function have not been correlated.

Using DRT-PCR, the regulation of the UGT1A locus as well as the UGT2B4, UGT2B7, UGT2B10, and UGT2B15 genes was analyzed in 18 tissue samples from duodenum, jejunum and ileum. A pattern of tissue specific gene expression was observed in small intestine, which exhibited considerable differences from that found in liver and colon (Table 2, Figure 1). Liver (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9) and colon (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10) tissue have been characterized to express specific UGT1A transcript patterns without variation (4,27,36). In intestine, gene expression included UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A10, UGT2B4, UGT2B7, and UGT2B15 transcripts, and the absence of UGT1A5, UGT1A7, UGT1A8, UGT1A9, and most UGT2B10 transcripts. However, the regulation of all UGT1A and UGT2B transcripts with the exception of UGT1A10 was polymorphic with variations between individuals and between the proximal and distal sections of the small intestine. As an example, the UGT1A6 gene was expressed in fewer jejunum samples, UGT2B4 mRNA was expressed more often in the ileum and UGT2B7 transcripts were expressed more often in the jejunum.

To confirm the data observed at the gene transcript level, immunodetection of the polymorphically regulated UGT1A1 and UGT2B7 gene products was analyzed with monospecific antisera. This analysis confirmed the presence of UGT1A1 protein in 3 of the 5 samples and of UGT2B7 protein in 2 of the 5 samples (Figure 2B) as detected by DRT-PCR at the mRNA level (Table 2). To assess the biological effect of the identified polymorphic regulation in small intestine catalytic UGT activity assays using hyodeoxycholic acid (HDCA) and 4-methylumbelliferone (4-MU) and endoplasmic reticulum protein prepared from the mucosa of small intestine and from liver tissue were performed. HDCA glucuronidation has been identified for UGT2B4, UGT2B7 (30,31) and UGT1A3 (32), whereas 4-MU glucuronidation can be catalyzed by most UGT1A proteins (5). As predicted from the polymorphic expression of UGT2B4 and UGT2B7 transcripts in the duodenum and the jejunum, HDCA glucuronidation varied 7-fold between individuals whereas 4-MU glucuronidation in the same samples varied only little but clearly more than the absence of interindividual variation seen in liver tissue. This finding is explained by a greater redundancy of UGT
proteins active with the substrate 4-MU than with the substrate HDCA. Importantly, both 4-MU and HDCA glucuronidation showed no significant variation between individual hepatic samples, a finding which reflects the absence of polymorphic UGT transcript regulation in human liver. The biological effect is best demonstrated by the analysis of the sample shown in Figure 2A, lane 4 (duodenum). In this individual, neither UGT2B7 nor UGT2B4 are expressed while UGT1A3 transcripts are merely present at low levels (Figure 1 top panel). As a result of the absence or reduced levels of the UGTs with specificity for HDCA, this duodenum sample was found to have dramatically reduced HDCA glucuronidation activity. In combination, the mRNA, Western blot and catalytic activity data provide evidence for the finding that the polymorphic regulation of UGT genes in the small intestine represents a molecular biological basis of interindividual variations of mucosal glucuronidation activity.

While UGT activity located in the mucosa of the small intestine is characterized by interindividual variation caused by polymorphic gene regulation, control of hepatic glucuronidation remains constant. The biological significance of this finding may be reflected in the rate of metabolism in these tissues. As a consequence the therapeutic efficacy or toxicity of pharmaceutical compounds could be influenced directly by extrahepatic glucuronidation during or prior to resorption from the substantial surface of the small intestine in humans. Analysis of gene expression provides evidence for the strictly individual regulation of UGT1A genes, which share common exons 2-5 in the UGT1A gene complex, and of the UGT2B genes in humans. Polymorphic regulation of human UGT2B transcripts represents the second example of differential expression of the human UGTs identified to date. In human gastric epithelium, the polymorphic regulation of UGT1A3, UGT1A4 and UGT1A6 transcripts in contrast to a constitutive expression of UGT1A7 and UGT1A10 mRNA was recently reported (14). The polymorphic expression of UGT genes in the gastrointestinal tract indicates that these enzymes may be regulated by a general biochemical mechanism contributing to interindividual differences in drug and xenobiotic metabolism (14). Importantly, this finding differs from the principle of bimodally distributed genetic polymorphisms reported for other drug metabolizing enzymes (37,38).

In a recent study, the expression of UGT2B7 but not of UGT1A6 and UGT2B4 were reported in intestinal tissue by RT-PCR (26). In our analysis, UGT2B4 transcripts were detectable in 8 out of the 18 small intestinal tissue samples. When the analysis is subdivided into the different segments of the small intestine the UGT2B4 gene was expressed in 1 out of the 5 duodenal samples. Similarly UGT1A6 transcripts were identified in 11 out of the 18 tissue samples, but in jejunum UGT1A6 mRNA was only present in 1 out of the 5 samples. Both genes were expressed more frequently at other levels of the small intestine. The data presented in this manuscript provide evidence for the expression of the UGT1A6 and UGT2B4 genes in human small intestine. In light of the identified polymorphic
regulation of both genes, sample number as well as the biopsy position in the small intestine are likely to influence the detection of individual UGT transcripts and represent a likely explanation for the contrasting findings.

The detection of UGT1A8 mRNA in jejunum, ileum and colon was recently reported (11). In experiments presented here, UGT1A8 transcripts were not detectable in any of the 17 intestinal specimens. In samples removed near the ileo-cecal valve, we were able to detect UGT1A8 transcripts in the cecal portions of the mucosa but not in the terminal ileum (data not shown). Our data suggest, that the UGT1A8 transcripts are expressed in esophagus (3) and colon (2) but are not expressed in small intestine. However, genetic or evolutionary differences of patient cohorts of different geographic origin may account for differences in UGT1A8 gene expression. Specimen sampling in the area of the ileocecal valve may additionally influence the detection of UGT1A8 mRNA.

Although the human UGT proteins exhibit a considerable overlap of substrate specificity, the regulation of individual UGT genes in a tissue allows for a prediction of overall catalytic UGT activity towards different substrates. In additional experiments, 18 specific UGT activities in small intestine were determined to demonstrate that extrahepatic glucuronidation in small intestine can function to complement hepatic glucuronidation, which would represent an important consequence in light of the discovered polymorphic regulation of UGT genes. Specific activities were predicted based on the gene expression, since UGT1A3 and UGT1A10 display catalytic activity towards steroid hormones (2,8), UGT1A4 catalyzes the glucuronidation of tertiary amine substrates such as antidepressants (39), and UGT1A10 exhibits UGT activity with putative tobacco carcinogens (3). The hepatic UGT activity profile favored steroids such as 4-hydroxy-estrone and phenolics such as 1-naphthol, 4-methylumbelliferone and 4-nitrophenol. Interestingly, specific activities in the small intestine towards commonly used drugs such as imipramine and amitriptyline, as well as steroids such as estrone and putative tobacco carcinogen metabolites such as 7-hydroxybenz(α)pyrene and 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (N-hydroxy-PhIP) exceeded the UGT activities measured in liver. Specific UGT activities followed a gradient which peaked in the jejunum (Figure 3) and demonstrates that the specific activity of steroid hormone and putative tobacco carcinogen metabolite glucuronidation is highest in the jejunum, where it exceeds the specific UGT activities of the liver. In comparison to other extrahepatic tissues such as the esophagus, the stomach and the colon, jejunal UGT activity is identified to represent some of the highest specific glucuronidation activities in the gastrointestinal tract (3,14,27). Polymorphic gene regulation in this tissue may therefore, more than in other epithelia, have a significant impact on human xenobiotic metabolism. Intestinal glucuronidation is capable of determining the extrahepatic metabolism of pharmacologically active drugs and may also serve as metabolic barrier for mutagen associated genotoxicity and cytotoxicity,
which is implicated by the presented characterization of specific UGT activities towards tobacco
carcinogen metabolites in small intestine. The cellular and subcellular localization of UGT protein
exclusively in intestinal villi and crypts forming this putative barrier was demonstrated by indirect
immunofluorescence analysis and is in agreement with the data obtained at the transcript and
functional levels (Figure 4).
References:
1. Dutton, G. J. (1980) *Glucuronidation of drugs and other compounds*, CRC Boca Raton Press, Boca Raton, FL
2. Strassburg, C. P., Manns, M. P., and Tukey, R. H. (1998) *J Biol Chem* 273(15), 8719-26
3. Strassburg, C. P., Strassburg, A., Nguyen, N., Li, Q., Manns, M. P., and Tukey, R. H. (1999) *Biochem J* 338(Pt 2), 489-498
4. Strassburg, C. P., Oldhafer, K., Manns, M. P., and Tukey, R. H. (1997) *Mol Pharmacol* 52(2), 212-20
5. Tukey, R. H., and Strassburg, C. P. (2000) *Annu Rev Pharmacol Toxicol* 40, 581-616
6. Burchell, B., Brierley, C. H., Monaghan, G., and Clarke, D. J. (1998) *Adv Pharmacol* 42, 335-8
7. Ritter, J. K., Crawford, J. M., and Owens, I. S. (1991) *J Biol Chem* 266(2), 1043-7
8. Mojarrabi, B., Butler, R., and Mackenzie, P. I. (1996) *Biochem Biophys Res Commun* 225(3), 785-90
9. Harding, D., Fournel-Gigleux, S., Jackson, M. R., and Burchell, B. (1988) *Proc Natl Acad Sci USA* 85(22), 8381-5
10. Wooster, R., Sutherland, L., Ebner, T., Clarke, D., Da Cruz e Silva, O., and Burchell, B. (1991) *Biochem J* 278(Pt 2), 465-9
11. Cheng, Z., Radominska-Pandya, A., and Tephly, T. R. (1998) *Arch Biochem Biophys* 356(2), 301-5
12. Mojarrabi, B., and Mackenzie, P. I. (1998) *Biochem Biophys Res Commun* 247(3), 704-9
13. Mojarrabi, B., and Mackenzie, P. I. (1997) *Biochem Biophys Res Commun* 238(3), 775-8
14. Strassburg, C. P., Nguyen, N., Manns, M. P., and Tukey, R. H. (1998) *Mol Pharmacol* 54(4), 647-54
15. Monaghan, G., Clarke, D. J., Povey, S., See, C. G., Boxer, M., and Burchell, B. (1994) *Genomics* 23(2), 496-9
16. Beaulieu, M., Levesque, E., Tchernof, A., Beatty, B. G., Belanger, A., and Hum, D. W. (1997) *DNA Cell Biol* 16(10), 1143-54
17. Jedlitschky, G., Cassidy, A. J., Sales, M., Pratt, N., and Burchell, B. (1999) *Biochem J* 340(Pt 3), 837-43
18. Jackson, M. R., McCarthy, L. R., Harding, D., Wilson, S., Coughtrie, M. W., and Burchell, B. (1987) *Biochem J* 242(2), 581-8
19. Ritter, J. K., Sheen, Y. Y., and Owens, I. S. (1990) *J Biol Chem* 265(14), 7900-6
20. Jin, C. J., Miners, J. O., Lillywhite, K. J., and Mackenzie, P. I. (1993) *Biochem Biophys Res Commun* 194(1), 496-503
21. Beaulieu, M., Levesque, E., Hum, D. W., and Belanger, A. (1998) *Biochem Biophys Res Commun* 248(1), 44-50
22. Chen, F., Ritter, J. K., Wang, M. G., McBride, O. W., Lubet, R. A., and Owens, I. S. (1993) *Biochemistry* 32(40), 10648-57
23. Beaulieu, M., Levesque, E., Hum, D. W., and Belanger, A. (1996) *J Biol Chem* 271(37), 22855-62
24. Belanger, A., Hum, D. W., Beaulieu, M., Levesque, E., Guillemette, C., Tchernof, A., Belanger, G., Turgeon, D., and Dubois, S. (1998) *J Steroid Biochem Mol Biol* 65(1-6), 301-10
25. King, C. D., Rios, G. R., Assouline, J. A., and Tephly, T. R. (1999) *Arch Biochem Biophys* 365(1), 156-62
26. Radominska-Pandya, A., Little, J. M., Pandya, J. T., Tephly, T. R., King, C. D., Barone, G. W., and Raufman, J. P. (1998) *Biochim Biophys Acta* 1394(2-3), 199-208
27. Strassburg, C. P., Nguyen, N., Manns, M. P., and Tukey, R. H. (1999) *Gastroenterology* 116(1), 149-60
28. Strassburg, C. P., Kasai, Y., Seng, B. A., Miniou, P., Zaloudik, J., Herlyn, D., Koprowski, H., and Linnenbach, A. J. (1992) *Cancer Res* 52(4), 815-21
29. Strassburg, C. P., Alex, B., Zindy, F., Gerken, G., Luttig, B., Meyer zum Buschenfelde, K. H.,
Brechot, C., and Manns, M. P. (1996) J Hepatol 25(6), 859-66
30. Ritter, J. K., Chen, F., Sheen, Y. Y., Lubet, R. A., and Owens, I. S. (1992) Biochemistry 31(13), 3409-14
31. Fournel-Gigleux, S., Jackson, M. R., Wooster, R., and Burchell, B. (1989) FEBS Lett 243(2), 119-22
32. Gall, W. E., Zawada, G., Mojarrabi, B., Tephly, T. R., Green, M. D., Coffman, B. L., Mackenzie, P. I., and Radominska-Pandya, A. (1999) J Steroid Biochem Mol Biol 70(1-3), 101-8
33. Hartmann, F., and Bissell, D. M. (1982) J Clin Invest 70(1), 23-9
34. Matern, S., Matern, H., Farthmann, E. H., and Gerok, W. (1984) J Clin Invest 74(2), 402-10
35. Parquet, M., Pessah, M., Sacquet, E., Salvat, C., Raizman, A., and Infante, R. (1985) FEBS Lett 189(2), 183-7
36. Strassburg, C. P., Manns, M. P., and Tukey, R. H. (1997) Cancer Res 57(14), 2979-85
37. Lampe, J. W., Bigler, J., Horner, N. K., and Potter, J. D. (1999) Pharmacogenetics 9(3), 341-9
38. Meyer, U. A., and Zanger, U. M. (1997) Annu Rev Pharmacol Toxicol 37, 269-96
39. Green, M. D., and Tephly, T. R. (1998) Drug Metab Dispos 26(9), 860-7
Figure legends

Figure 1: Polymorphic regulation of the UGT1A gene locus and UGT2B genes in human duodenum, jejunum, ileum and liver. UGT gene expression in duodenal, jejunal, ileal and hepatic epithelium was detected by UGT isoform specific duplex reverse transcription polymerase chain reaction analysis (DRT-PCR). The ethidium bromide stained gels show isoform specific DRT-PCR products coamplified in the presence of β-actin primers as a control. Examples of a single patient are given for each tissue source. In the duodenal example UGT1A3 mRNA is low and none of the UGT2B transcripts are detectable. The jejunal sample shows a typical intestinal pattern with the absence of UGT1A5, UGT1A7, UGT1A8, UGT1A9, UGT2B4, UGT2B7, and UGT2B10. The lower panel shows the previously reported hepatic UGT expression profile found in all liver samples examined (4).

Figure 2: Interindividual variation of specific UGT activity in small intestine but not in liver.

Figure 2A. Five preparations of endoplasmic reticulum protein from duodenum, jejunum and liver were analyzed for catalytic activity towards hyodeoxycholic acid (HDCA) and 4-methylumbelliferone (4-MU) (top panel). Duodenal sample 4 is the same as that shown in Figure 1, top panel. In the duodenal sample 4, HDCA glucuronidation was found to be lowest. This tissue sample lacks both UGT2B4 and UGT2B7 expression and has low levels of UGT1A3 expression (compare Figure 1, top). In the other duodenal samples there is either expression of UGT2B4 (sample 3), UGT2B7 (sample 1 and 5) or UGT1A3 (sample 2, 4 and 5), which would lead to the observed HDCA glucuronide formation (compare Table 2). The bottom panel demonstrates a graphic representation of interindividual variations of the measured UGT activities.

Figure 2B. Western blot analysis using 20 µg of microsomal protein from 5 duodenal and 5 liver tissue samples. The detection of UGT1A1 (top panels) and UGT2B7 (bottom panels) protein was performed using monospecific rabbit anti human UGT1A1 and rabbit anti human UGT2B7 antisera as described in materials and methods. The duodenal sample shown in lane 5 (expression of UGT1A1 but not of UGT2B7) is demonstrated in the top panel of Figure 1, confirming the expression pattern found at the transcript level. The (-) denotes a negative control using Sf9 cell extracts not expressing UGT protein; (+) represents Sf9 cells expressing UGT1A1 or UGT2B7, respectively. The + and - marks below the Western analysis indicates the presence or absence of UGT1A1 or UGT2B7 mRNA detectable by DRT-PCR.

Figure 3: Catalytic UGT activities in small intestine and liver. Graphic representation of the average (n=2) specific UGT activities in duodenum, jejunum, ileum and liver using 18 substrates as described in materials and methods. Abbreviations: 1-naphth, 1-naphthol; 4-OH biphen, 4-hydroxybiphenyl;
2-OH-estriol, 2-hydroxyestriol; 4-OH-estrone, 4-hydroxy estrone; HDCA, hyodeoxycholic acid; 4-MU, 4-methylumbelliferone; P-nitrophenol, 4-nitrophenol; PhIP, 2-amino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine; 7-OH-BAP, 7-hydroxy benzo(α)pyrene; 3-OH-AAF, 3-hydroxy acetylaminofluorene; 4-tert-butylph, 4-tert-butylphenol; Nitro-PhIP, 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine

**Figure 4: Immunofluorescence detection of UGT1A protein in the intestinal mucosa.** Indirect immunofluorescence using a rabbit anti human UGT1A antibody is shown with cryostat sections of ileum tissue. (A) UGT1A protein is localized to the epithelila cell layer and the crypts (magnification: 40x). UGT staining of the vili is homogeneous, the crypts exhibit a ring-like pattern. (B) high power magnification of a vilus section confirming staining of the epithelila cells but not of the submucosa (magnification: 400x). (C) High power magnification of the cross section of a mucosal crypt. UGT1A protein is concentrated in the apical portions of the crypt enterocytes towards the luminal surface (magnification: 400x). UGT protein is detected at the surfaces of direct contact between intestine and xenobiotic matter.
| tissue     | number | mean age (range) [years] | female/male | diagnosis                                                                 |
|------------|--------|--------------------------|-------------|---------------------------------------------------------------------------|
| duodenum   | 5      | 55.75 (51-62)            | 3/2         | pancreatic carcinoma                                                      |
| jejunum    | 5      | 54.4 (25-77)             | 2/3         | pancreatic carcinoma (n=2), gastric ulcer, gastric carcinoma, blind loop syndrome |
| ileum      | 8      | 62.14 (35-86)            | 4/4         | colon carcinoma (n=5), cholangiocellular carcinoma, inflammatory bowel disease, neuroendocrine tumor |
Table 2

Expression of UGT1A and UGT2B mRNA in Human Small Intestine and Liver

| UGT Isoform | Duodenum n=5 | Jejunum n=5 | Ileum n=8 | Liver n=16 |
|-------------|--------------|-------------|-----------|-----------|
| UGT1A1      | 3/5          | 1/5         | 3/8       | 16/16     |
| UGT1A3      | 3/5          | 5/5         | 5/8       | 16/16     |
| UGT1A4      | 4/5          | 4/5         | 7/8       | 16/16     |
| UGT1A5      | n.d.         | n.d.        | n.d.      | n.d.      |
| UGT1A6      | 4/5          | 1/5         | 6/8       | 16/16     |
| UGT1A7      | n.d.         | n.d.        | n.d.      | n.d.      |
| UGT1A8      | n.d.         | n.d.        | n.d.      | 16/16     |
| UGT1A9      | n.d.         | n.d.        | n.d.      | 16/16     |
| UGT1A10     | 5/5          | 5/5         | 8/8       | n.d.      |
| UGT2B4      | 1/5          | 2/5         | 5/8       | 16/16     |
| UGT2B7      | 2/5          | 3/5         | 2/8       | 16/16     |
| UGT2B10     | n.d.         | n.d.        | 1/8       | 16/16     |
| UGT2B15     | 3/5          | 5/5         | 8/8       | 16/16     |

Table 2 summarizes the expression of UGT transcripts detected by UGT isoform specific duplex RT-PCR. While a constitutive expression is demonstrated for UGT1A10 (bold type) and UGT2B15 (with the exception of two duodenal samples) in small intestine, the remaining transcripts are expressed in a polymorphic fashion. In contrast, hepatic UGT gene expression is not polymorphic. n.d. - not detected by DRT-PCR.
|            | duodenum | control | liver | control |
|------------|----------|---------|-------|---------|
|            | 1 2 3 4 5 | - +     | 1 2 3 4 5 | - +     |
| UGT1A1     |          |         |       |         |
| mRNA (DRT-PCR) | + + - - - + |         | + + + + + + |         |
| UGT2B7     |          |         |       |         |
| mRNA (DRT-PCR) | + + - - - - |         | + + + + + + |         |

55 kDa
Catalytic UGT-Activities in Small Intestine and Liver

[Graph showing enzymatic activities in different segments of the intestine and liver.]
Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine
Christian P. Strassburg, Susanne Kneip, Juliane Topp, Petra Obermayer-Straub, Ayse Barut, Robert H. Tukey and Michael P. Manns

*J. Biol. Chem.* published online July 31, 2000

Access the most updated version of this article at doi: [10.1074/jbc.M002180200](http://dx.doi.org/10.1074/jbc.M002180200)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/) to choose from all of JBC's e-mail alerts