

Polymorphic Expression of the UDP-Glucuronosyltransferase *UGT1A* Gene Locus in Human Gastric Epithelium

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Received May 13, 1998; Accepted July 2, 1998

This paper is available online at <http://www.molpharm.org>

ABSTRACT

The human UDP-glucuronosyltransferase (UGT) 1A (*UGT1A*) locus is regulated in a tissue specific fashion in liver and extrahepatic tissues. Three extrahepatic *UGT1A* proteins, *UGT1A7*, *UGT1A8*, and *UGT1A10*, have been discovered and are believed to contribute to the diversity of extrahepatic glucuronidation. UGTs eliminate by glucuronidation a broad variety of endobiotic and xenobiotic substrates, which include bilirubin, therapeutic drugs, and carcinogens. Human gastric mucosa represents a primary location of tissue contact with dietary constituents, pharmaceutical drugs, and environmental carcinogens. To study the role and regulation of *UGT1A* gene products in stomach *UGT1A* mRNA expression and UGT catalytic activities were investigated in a panel of 14 normal gastric mucosa/adenocarcinoma sample pairs. *UGT1A* mRNA levels were differentially regulated in stomach, a feature not found in

hepatic tissue. Normal gastric epithelium consistently expressed extrahepatic *UGT1A7* and *UGT1A10*. However, polymorphic expression of *UGT1A1* (29%), *UGT1A3* (21%), and *UGT1A6* (36%) was detected. Polymorphic *UGT1A* regulation was confirmed in adenocarcinoma samples with the additional observation of differential down-regulation of *UGT1A1*, *UGT1A3*, *UGT1A6*, and *UGT1A10* and up-regulation of *UGT1A7* mRNA. The polymorphic *UGT1A* regulation in stomach contrasts the homogeneous regulation of *UGT1A* gene products in human liver. Activity assays demonstrated 2- to 4-fold interindividual differences in UGT activity and qualitative differences between individuals. The polymorphic regulation of *UGT1A* gene products in gastric tissue may be the biological basis that determines interindividual differences in extrahepatic microsomal drug metabolism.

An important process of detoxification in human catabolic metabolism is performed by the UGTs localized in the endoplasmic reticulum. UGTs catalyze the formation of hydrophilic glucuronides, which facilitates the elimination of substrates from the body via urine or feces (Dutton *et al.*, 1980; Bock *et al.*, 1987; Mackenzie *et al.*, 1997). Two families of UGT proteins have been defined and termed *UGT1* and *UGT2* (Burchell *et al.*, 1991). The *UGT2* structural genes are located on chromosome 4 (Monaghan *et al.*, 1992). In contrast, the human *UGT1A* locus is located on chromosome 2, spans >160 kilobase pairs of DNA, with at least 12 individual first exon cassette sequences followed by exons 2–5 at the 3' end of the locus (Moghribi *et al.*, 1992; Ritter *et al.*, 1992; Mackenzie *et al.*, 1997). The 5' flanking region of each first exon cassette contains appropriate promoter elements. Transcription of each individual first exon leads to a strategy of exon sharing, combining the first exon sequences with common exons 2–5, a mechanism that can generate up to nine functional transferases (Mackenzie *et al.*, 1997). *UGT1A* pro-

teins eliminate by glucuronidation a broad array of endobiotic and exobiotic substrates, including reactive oxygen products of bioactivation, carcinogens, therapeutic drugs, complex phenolic compounds, and the heme synthetic by-product bilirubin (Ebner *et al.*, 1993; Ritter *et al.*, 1991; Green and Tephly, 1996; Mojarrabi *et al.*, 1996; Kim *et al.*, 1997; Strassburg *et al.*, 1998).

Investigation of *UGT1A* expression has focused primarily on the liver, which is considered to be the most important location of human glucuronidation. From liver RNA, five *UGT1A* cDNAs have been identified and cloned (Harding *et al.*, 1988; Ritter *et al.*, 1991; Wooster *et al.*, 1991; Mojarrabi *et al.*, 1996). It has been demonstrated that hepatocellular tissue expresses without interindividual variation *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6*, and *UGT1A9* gene transcripts (Strassburg *et al.*, 1997b). We have recently characterized the tissue specific regulation of the human *UGT1A* locus, which has led to the identification of three additional *UGT1A* proteins, selectively expressed in extrahepatic tissues (Strassburg *et al.*, 1997b; Strassburg *et al.*, 1998). The identification of *UGT1A7* expression in gastric epithelium, *UGT1A8* expression in colonic epithelium, and *UGT1A10* expression in gastric, biliary, and colonic epithelium but not

This work was supported by United States Public Health Service Grant GM49135 (R.H.T.) and Deutsche Forschungs Gemeinschaft Grant STR493/2-1 (C.P.S.). C.P.S. is a recipient of the Pete Lopiccola Award in Cancer Research, University of California, San Diego.

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; DRT-PCR, duplex reverse transcription polymerase chain reaction; bp, base pair(s);

in hepatocellular epithelium indicates a complex control of this gene locus and emphasizes a unique physiological role of glucuronidation in nonhepatocellular epithelial tissues.

Immediate and prolonged contact with ingested matter requires the stomach to exercise an effective defense against chemical and biological influences. The expression of individual UGT1A proteins in gastric mucosa may determine the stomachs epithelial glucuronidation capacity and may constitute an important factor for extrahepatic metabolism and first pass effects (Bock *et al.*, 1987; Bock and Lilienblum, 1994; Strassburg *et al.*, 1997a; Strassburg *et al.*, 1998). Therefore, variations in drug efficacy and of carcinogenic risk between individuals may be the result of interindividual differences of UGT1A regulation in extrahepatic surface tissue such as gastric epithelium. Because data on the regulation of the *UGT1A* locus in human gastric epithelium is scarce, we examined UGT1A mRNA regulation by quantitative DRT-PCR combined with UGT activity analysis.

Materials and Methods

Tissue samples. Gastric tissue samples were obtained from six female (mean age 58.67 ± 16.79 years) and eight male German patients (mean age 56.75 ± 11.68 years) with histologically confirmed gastric adenocarcinoma undergoing gastrectomy at the University of Hannover Medical Center, Hannover, Germany. The panel included 14 tissue sample pairs, each consisting of a sample of tumor tissue and a sample of healthy gastric mucosa from the same resection specimen. In each gastric tissue sample the mucosa was dissected from the underlying muscle layer and only mucosal tissue was used for the analyses. The normal gastric mucosa samples were microanatomically free of any detectable concurrent disease such as gastritis, *Helicobacter pylori* infection, or dysplasia and were all sampled from the area of the corpus/antrum junction. The patients did not receive chemotherapeutic compounds before resection and no drug therapy of any significance was administered. Patient records indicated absence of tobacco consumption for at least 6 months before sampling. Normal liver tissue samples were collected from 16 patients undergoing hemihepatectomy ($n = 14$) or liver transplantation ($n = 2$) for hepatocellular carcinoma ($n = 12$, 11 men, one woman, mean age 58.08 ± 10.44 years) or focal nodular hyperplasia ($n = 4$, four women, mean age 37.25 ± 14.48 years) (Strassburg *et al.*, 1997a). Tumor tissue was histologically confirmed and showed no signs of necrosis. In all instances, normal sampling was performed at the distal resection margin of the specimen and exhibited no signs of macroscopic deterioration such as necrosis or any histopathological abnormalities. All tissue was immediately frozen in liquid nitrogen and stored at -80° until use.

RNA Isolation, cDNA synthesis, and RNA purity analysis. Tissue was pulverized under liquid nitrogen and immediately lysed in acidic phenol/guanidinium-isothiocyanate solution (Tripure; Boehringer Mannheim, Mannheim, Germany) as described previously (Strassburg *et al.*, 1997b). RNA Concentrations were determined by spectrophotometry at 260 and 280 nm. Samples were stored in water at -80° until further analysis.

For cDNA synthesis, $3 \mu\text{g}$ of total RNA was denatured for 10 min at 70° in the presence of $0.5 \mu\text{g}$ of oligo(dT) primer and was then quick-chilled on ice. In a volume of $19 \mu\text{l}$ containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM MgCl_2 , 10 mM dithiothreitol, and 0.5 mM of each dNTP, the RNA was incubated at 42° for 5 min before the addition of 200 units of reverse transcriptase (SuperscriptII; Gibco BRL, Gaithersburg, MD). The final volume of $20 \mu\text{l}$ was incubated at 42° for 50 min, 70° for 15 min, and was then 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'-GGCGGCACCACCATGTACCCT-3' and the antisense primer 5'-

AGGGGCCGGACTCGTCATACT-3' (Strassburg *et al.*, 1997b) span the exon 4/intron 5/exon 5 junction of the β -actin gene. PCR with cDNA led to a 202-bp product, but contamination with genomic DNA template led to a 312-bp PCR product, which can be clearly distinguished from the 202-bp cDNA amplification product.

Intact RNA with an $A_{260 \text{ nm}}/A_{280 \text{ nm}}$ ratio between 1.5 and 1.9 was isolated from all 14 gastric carcinoma/normal tissue pairs. RNA was visualized in acridine orange-stained agarose gels to exclude tailing, as an additional quality control. In all samples, the RNA did not contain contaminating genomic DNA. Tailing was also not observed in the subsequent Northern blot analyses.

DRT-PCR for UGT1A transcripts. DRT-PCR of a 487-bp fragment of the conserved UGT1A exons 2–5 has been described previously in detail (Strassburg *et al.*, 1997b). Briefly, coamplification was carried out for six cycles of PCR synthesis with UGT1A primers at a concentration of 2 mM. After the addition of β -actin primers to a concentration of 0.4 mM, cycling was continued for a total of 32 cycles at 94° for 1 min, 59° for 1 min, and 72° for 1 min. PCR was preceded by a 3-min incubation of the reaction mixture at 94° and was followed by a 7-min elongation at 72° . DRT-PCR products were separated in a 2% agarose gel stained with ethidium bromide. Polaroid (Cambridge, MA) type 665 positive/negative film was used to quantify bands separated in 2% agarose using laser densitometry on a LKB 2222–020 UltroScan XL densitometer (LKB, Bromma, Sweden). Arbitrary units were calculated relative to β -actin products according to the following formula: (mean peak area for UGT/mean peak area for β -actin) $\times 100$ = relative arbitrary units. Independent and combined linear kinetics for both products during the amplification process were established as previously described in detail (Strassburg *et al.*, 1997b). Statistical analysis was performed using Student's *t* test from the GraphPad Prism software (GraphPad, San Diego, CA).

Exon-1-specific DRT-PCR. The *UGT1A* locus predicts the existence of UGT1A1 and UGT1A3–1A10. UGT1A2, -1A11, and -1A12 do not have an uninterrupted open reading frame and are therefore considered to be pseudogenes (Mackenzie *et al.*, 1997). DRT-PCR detection of all nine UGT1A transcripts predicted by the human *UGT1A* locus was performed using nine exon-1-specific sense primers and two antisense primers located within exons 2–5 or within a common portion of the 3' end of the first exons. As already described elsewhere (Strassburg *et al.*, 1997b), the primers 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. Briefly, coamplification of UGT1A first exon and β -actin sequences was performed using three cycling protocols: UGT1A1 and UGT1A6: 94° (1 min), 59° (1 min), 72° (1 min); UGT1A3, UGT1A4, UGT1A5: 94° (1 min), 56° (1 min), 72° (1 min); UGT1A7, UGT1A8, UGT1A9, UGT1A10: 94° (1 min), 64° (1 min), 72° (1 min). Each protocol was preceded by a 3-min incubation of the reaction mixture at 94° and was followed by a 7-min elongation at 72° . The specificity and kinetics of this assay have previously been reported (Strassburg *et al.*, 1997b). Experiments were performed in duplicate, and controls were performed without cDNA, primers, or thermophilic polymerase included. Quantification of products by laser densitometry was performed as described above.

Isolation of microsomal protein from tumor and normal gastric tissue. Approximately 200 mg of tissue was pulverized under liquid nitrogen, resuspended in 1 ml of buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgCl_2) and homogenized with a Potter-Elvehjem tissue grinder. The tissue homogenate was centrifuged at $10,000 \times g$ for 5 min at 4° in a microcentrifuge and the supernatant was collected. The pellet was resuspended in 0.5 ml of buffer, centrifuged, and the supernatant was collected. The combined supernatants were centrifuged at $150,000 \times g$ for 60 min at 4° in a TL100 ultracentrifuge (Beckman, Palo Alto, CA) and the pellet was resuspended in 0.2 ml of buffer. Protein concentration was determined by the method of Bradford *et al.* (1976). Microsomal protein was stored at -80° .

UGT enzymatic activity assay. 1-naphthol, 4-nitrophenol, 4-methylumbelliferone, 4-isopropylphenol, octylgallate, estriol, estrone, naringenin, hydoxycholeic acid (all from Sigma, St. Louis, MO), and 8-hydroxy-benzo[a]pyrene were suspended in methanol, 7-hydroxy-benzo[a]pyrene was suspended in acetone (-pyrenes from National Cancer Institute Chemical Carcinogen Repository, Midwest Research Institute, Kansas City, MO). Microsomal protein (25 μ g) in reaction buffer (50 mM Tris-Cl, pH 7.6, 10 mM $MgCl_2$) were incubated in the presence of 0.1 mM UDP-glucuronic acid, 0.08 mg/ml phosphatidylcholine, 0.04 μ Ci of ^{14}C -labeled glucuronic acid, and 0.1 mM of test substrate for 60 min at 37°. Protein was precipitated by the addition of ethanol and subsequent centrifugation. Lyophilized supernatants were resuspended in 50 μ l of methanol and separated by thin layer liquid chromatography with *n*-butanol/acetone/acetic acid/water (35:35:10:20%) as the running solvent. The production of ^{14}C -labeled glucuronides was detected by autoradiography. To determine specific catalytic activities the ^{14}C -labeled glucuronides were quantified by liquid scintillation counting and expressed as picomoles of glucuronide formed per minute per milligram of microsomal protein.

Western blot analysis. Hepatic and gastric microsomal protein (25 μ g) were boiled for 90 sec in loading buffer (2% sodium dodecyl sulfate, 62.5 mmol/liter Tris-HCl, pH 6.8, 10% glycerol, and 0.001% bromophenol blue) with 2% β -mercaptoethanol and separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis before electrotransfer onto nitrocellulose membrane. As a control, 15 μ g of baculovirus-expressed UGT1A1 protein (Strassburg *et al.*, 1996) was included. Immunodetection was performed according to previously published protocols (Strassburg *et al.*, 1996). UGT1A protein was detected using a rabbit anti-UGT1A antiserum raised against the peptide SSLHKDRPVEPLDLA located between amino acids 441 and 455 of exon 5 of the constant UGT carboxyl-terminal portion (Strassburg *et al.*, 1998). Visualization was achieved with an alkaline phosphatase conjugated goat anti-human IgG diluted at 1:1000.

Results

Quantitative variability of gastric UGT1A mRNA regulation. Total RNA from gastric carcinoma and corresponding normal tissue was analyzed for the expression of UGT1A transcripts by quantitative DRT-PCR (Fig. 1). A 487-bp fragment from the *UGT1A* constant exons 2–5 was amplified by RT-PCR and quantified relative to a coamplified 202-bp fragment of β -actin, as shown in Fig. 1. Overall UGT1A expression was lower in gastric tissue compared with hepatic tissue. Between the 14 samples of gastric adenocar-

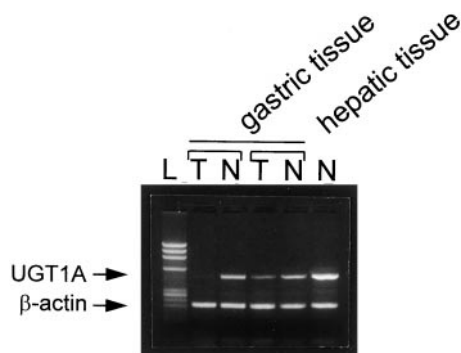


Fig. 1. UGT1A mRNA expression in human gastric epithelium and adenocarcinoma. UGT1A mRNA was detected in normal gastric epithelium and corresponding adenocarcinoma by DRT-PCR. The photograph of an ethidium bromide-stained gel demonstrates an example of UGT1A mRNA expression in two gastric mucosa/adenocarcinoma sample pairs and normal human liver. Quantification was performed by laser densitometry to assess UGT1A mRNA levels in all studied samples. *standard*, *HaeIII* digest of ϕ X174 DNA.

cinoma and normal gastric epithelium, UGT1A expression was found to be significantly ($p = 0.013$) down-regulated, indicating that a quantitative differential regulation of UGT1A mRNA exists between hepatic and gastric tissue, but also between gastric adenocarcinoma tissue and healthy gastric epithelium (Fig. 2).

Analysis of UGT1A mRNA in the individual tissue samples also indicated that gastric *UGT1A* gene expression was characterized by significant interindividual variation compared with the more uniform UGT1A mRNA levels found in hepatic tissue. This feature of a greater range of UGT1A mRNA levels is consistently found in both normal gastric epithelium and gastric adenocarcinoma tissue (Fig. 2). Interestingly, UGT1A expression in two tumor samples was undetectable. This is probably caused by differential regulation of the *UGT1A* locus in these patients and not to genomic instability of the locus or other mutational events that could affect expression. These conclusions are made from previous analysis of the *UGT1A* locus in hepatic adenoma, hepatocellular carcinoma, and focal nodular hyperplasia in which there was no evidence of mutational events in the regulation of these genes (Strassburg *et al.*, 1997a). As a result of this variability, experiments were conducted to define the contribution of the individual *UGT1A* gene transcripts in each of the human gastric samples.

Polymorphic regulation of UGT1A isoform expression in human gastric tissue. First-exon sequences encoded by the *UGT1A* locus share extensive sequence similarity, with 94% similarity among UGT1A7, UGT1A8, UGT1A9, and UGT1A10. Therefore, a transcript specific DRT-PCR assay was employed, capable of detecting all nine predicted UGT1A transcripts (Fig. 3 and Table 1). Gastric mucosa and carcinoma samples were characterized by a unique UGT1A expression pattern. Analysis of the healthy gastric tissue samples revealed UGT1A7 expression in 12 of the 14 samples, whereas UGT1A10 was expressed in all 14 normal tissue samples (Table 1). In contrast, the expression of UGT1A1 was identified in four samples, UGT1A3 in only

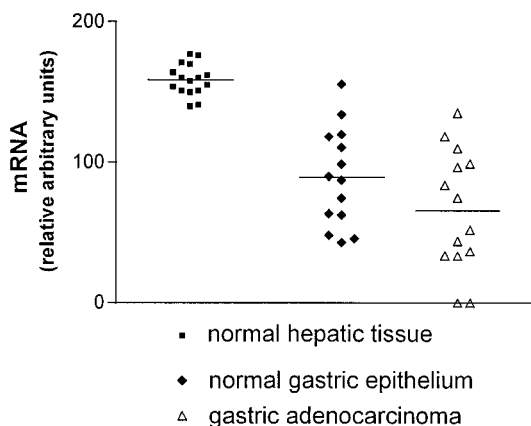


Fig. 2. Graphic representation of the quantitative interindividual UGT1A regulation in hepatic, normal gastric, and gastric adenocarcinoma tissue. UGT1A transcript levels were determined by quantitative DRT-PCR. In 16 samples of hepatic tissue, UGT1A mRNA expression levels were homogeneous among subjects. In normal gastric epithelium and gastric adenocarcinoma tissue, differential quantitative regulation of UGT1A mRNA was evident among individual samples and differential down-regulation was observed in gastric adenocarcinoma. Interindividual differences of UGT1A mRNA regulation in human gastric tissue are preserved despite neoplastic transformation to adenocarcinoma.

three samples, and UGT1A6 in five samples. Sequences of the PCR products were confirmed by dideoxy sequencing. The expression of UGT1A4, UGT1A5, UGT1A8, and UGT1A9 was not detected in any of the tissues. The patterns identified in the healthy gastric tissue specimens were confirmed in the corresponding adenocarcinoma tissue samples. In these samples, UGT1A7 and UGT1A10 were expressed in 13 of 14 samples, UGT1A1 in three of 14 samples, UGT1A3 in one of three samples and UGT1A6 in four of 14 samples, whereas UGT1A4, UGT1A5, UGT1A8, and UGT1A9 were also not detected. These findings indicate a polymorphic expression of UGT1A isoforms in human gastric tissue that is present in both normal epithelium and adenocarcinoma tissue from the same subject.

Analysis of the UGT1A mRNA expression data identified several individual expression patterns in human gastric mucosa samples, examples of which are demonstrated in Fig. 3. Pattern A (Fig. 3A), which was characterized by the exclusive expression of UGT1A7 and UGT1A10, was seen in five of 14

samples. Expression of UGT1A1, UGT1A7, and UGT1A10 was detected in two of the 14 tissues, as shown in Fig. 3B. Transcripts encoding UGT1A1, UGT1A6, UGT1A7, and UGT1A10 were detected in two of 14 samples (Fig. 3C), whereas a pattern expressing UGT1A3, UGT1A6, UGT1A7, and UGT1A10 was detected in two of 14 samples (Fig. 3D). In addition, one sample expressed UGT1A3, UGT1A7, and UGT1A10 and one sample expressed UGT1A6, UGT1A7, and UGT1A10 (not shown). An additional sample exhibited only UGT1A3, UGT1A6, and UGT1A7 transcripts but no UGT1A10 mRNA (not shown). These data demonstrate the interindividual heterogeneity of *UGT1A* gene expression in human gastric mucosa and contrast the regulation of the *UGT1A* locus in human liver, in which UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 transcripts were identified in each of the 16 samples (Strassburg et al., 1997b) (Table 1).

Normal gastric epithelium and adenocarcinoma samples were consistent with respect to the regulation of individual UGT1A isoform mRNAs. However, UGT1A1 ($p = 0.15$), UGT1A3 ($p = 0.049$), UGT1A6 ($p = 0.38$), and UGT1A10 ($p = 0.02$) mRNA levels were differentially down-regulated in the adenocarcinoma tissue compared with surrounding normal mucosa (Figs. 3 and 4). Although the comparisons for UGT1A3 and UGT1A10 were statistically significant, UGT1A1 and UGT1A6 displayed a trend toward down-regulation. In contrast, UGT1A7 mRNA ($p = 0.19$) showed a trend toward up-regulation, which may indicate a reciprocal mode of regulation for this isoform. These findings confirm an independent regulation of individual *UGT1A* genes. Although differential regulation within the sample pairs was demonstrated, interindividual variation of transcript levels prevented statistical significance of UGT1A1, UGT1A6, and UGT1A7 comparisons.

Functional polymorphism of UGT catalytic activity in gastric microsomal protein. To assess the functional impact of the observed polymorphic expression of UGT1A gene products in gastric epithelium, four microsomal protein preparations of normal gastric tissue samples expressing different UGT1A transcript patterns were analyzed (Table 2). In Table 2, sample 1 expressed Fig. 3, pattern C, sample 2 expressed pattern B, sample 3 expressed pattern D, and sample 4 expressed pattern A. Microsomal glucuronidation activities were examined with a panel of nine substrates

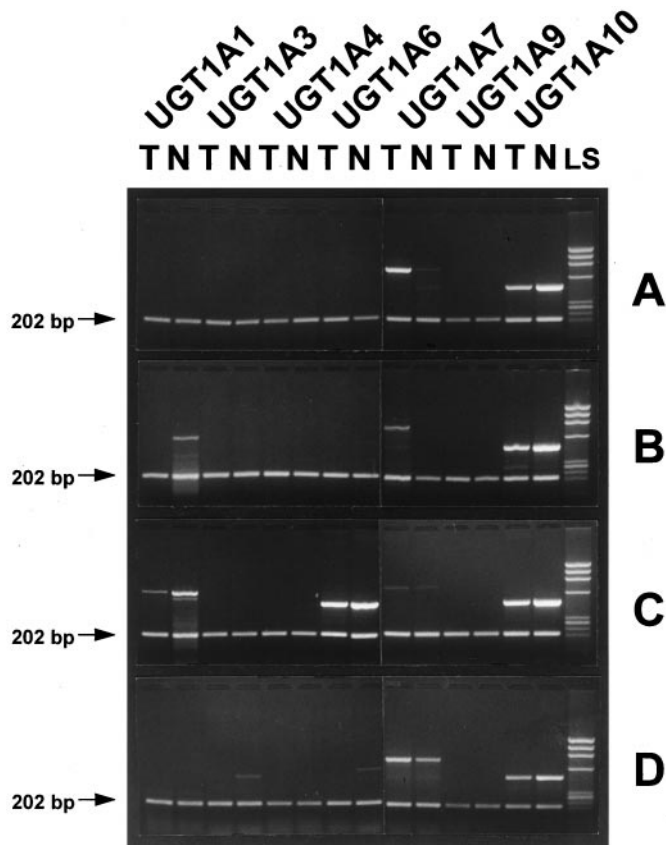


Fig. 3. Polymorphic regulation of UGT1A mRNA in human gastric tissue. DRT-PCR specifically detected the expression of UGT1A transcripts in gastric carcinoma (T, tumor sample) and corresponding normal mucosa (N, normal mucosa) and yielded the PCR products of expected sizes (as described in Materials and Methods). Assays for UGT1A5 and UGT1A8 were negative in all samples and are not shown. A-D, Each panel demonstrates the DRT-PCR results obtained from a single patient tumor/normal tissue sample pair and is an example of different UGT1A isoform expression patterns found between the studied individuals. A, major UGT1A expression pattern found in five of 14 sample pairs. B-D, major UGT1A expression patterns found in two of 14 sample pairs. Up-regulation of UGT1A7 and down-regulation of all other UGT1A transcripts is demonstrated. The same subjects demonstrated in this figure were analyzed for specific gastric microsomal UGT activity and are reported in Table 2. Products were separated in a 2% agarose gel stained with ethidium bromide. LS, molecular length standard.

TABLE 1
Expression pattern of the *UGT1A* locus in gastric carcinoma and normal gastric mucosa

Studied samples were collected as pairs of tumor tissue and surrounding healthy mucosa from the same organ. Total denotes presence of specific UGT1A transcripts in either tumor or normal sample of the individual patient. Polymorphic regulation of UGT1A1, UGT1A3, and UGT1A6 are noted in human gastric epithelium. In contrast, the analysis of a panel of 16 hepatic RNAs [as reported in Strassburg et al. (1997b)] displays no polymorphic UGT1A expression in human liver.

UGT	Gastric tissue (n = 14)		Total	Hepatic tissue (n = 16) normal
	Tumor	Normal		
UGT1A1	3	4	4	16
UGT1A3	1	3	3	16
UGT1A4	0	0	0	16
UGT1A5	0	0	0	0
UGT1A6	4	5	5	16
UGT1A7	13	12	13	0
UGT1A8	0	0	0	0
UGT1A9	0	0	0	16
UGT1A10	13	14	14	0

ranging from planar and complex phenols to steroid hormones, flavones, bile acid metabolites, and hydroxylated benzo[*a*]pyrene. Interindividual glucuronidation activity levels varied up to 4-fold among the four tissue protein samples (1-naphthol, 4-nitrophenol, 4-methylumbelliferone), which is in agreement with the quantitative differences found at the transcript level (Fig. 2). Sample 3 displayed the highest level of 1-naphthol, 4-nitrophenol, 4-methylumbelliferone, and 4-isopropylphenol glucuronidation. On the mRNA level, this sample is characterized by an abundant expression of *UGT1A7* (compare DRT-PCR analysis of this sample in Fig. 4D). This result is in agreement with the recently described specific activities of *UGT1A7* that favor phenolic substrates (Strassburg *et al.*, 1998). Interestingly, hyodeoxycholic acid,

which has been described as a substrate of *UGT2B4* (Jackson *et al.*, 1987; Fournel-Gigleux *et al.*, 1991) and has not been found to be glucuronidated by *UGT1* proteins was glucuronidated by one of the four samples (Table 2, sample 1). Taken together, these data provide evidence for the polymorphic regulation of the human *UGT1A* locus at the RNA transcript and functional levels, in addition to demonstrating that *UGT2B* proteins are potentially regulated in a polymorphic fashion in human gastric tissue.

Detection of *UGT1A* protein in gastric microsomal fractions. Overall *UGT1A* protein was detected with an antibody directed against a 15-mer epitope within exon 5 of the common *UGT1A* carboxyl-terminal portion. Normal liver microsomal protein was compared with a pair of gastric ad-

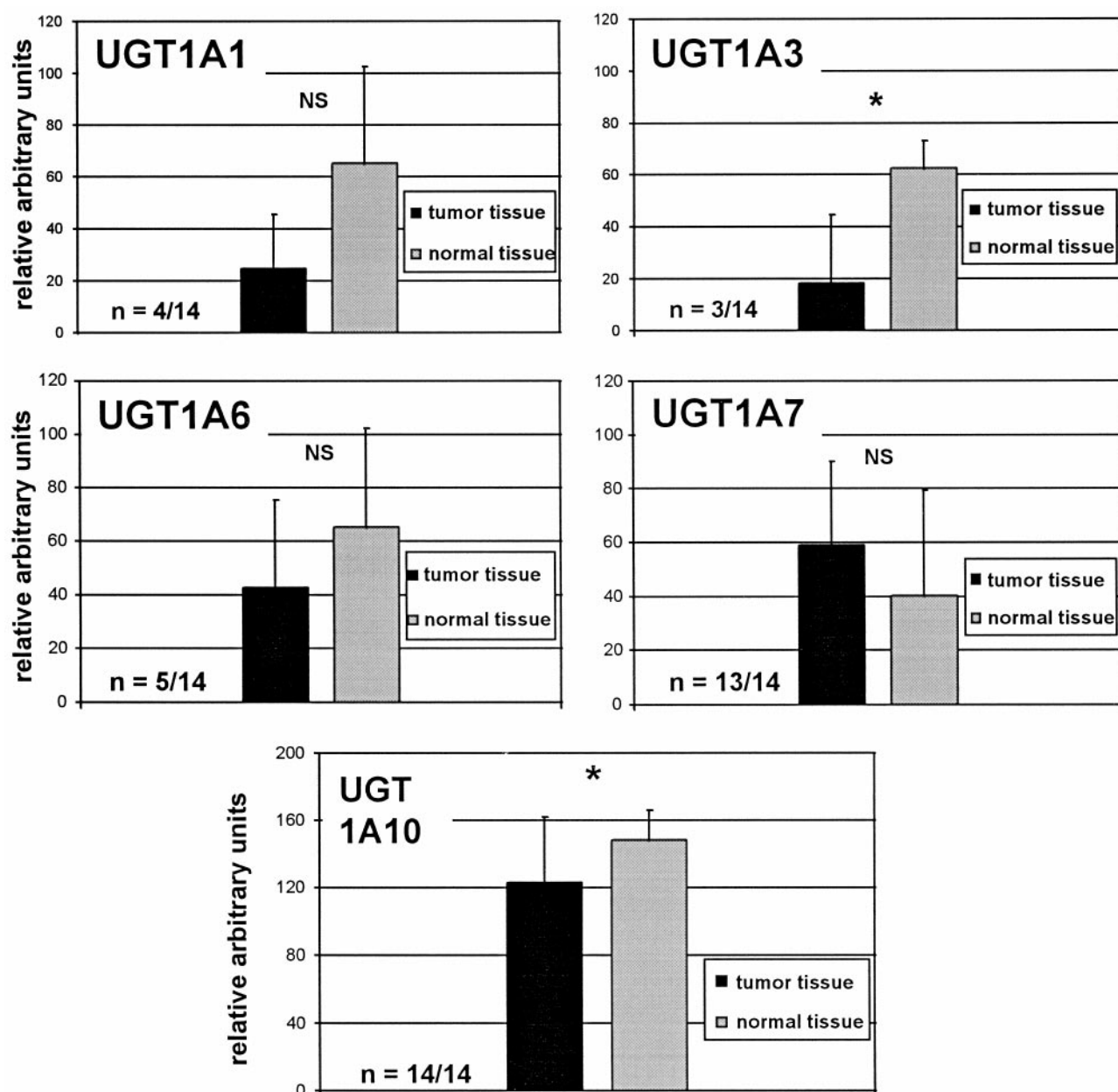


Fig. 4. Differential down-regulation of the *UGT1A* transcripts expressed in gastric carcinoma. *UGT1A* transcripts were quantified by DRT-PCR as outlined in Fig. 2. All comparisons except *UGT1A7* were characterized by down-regulation of *UGT1A* transcripts in gastric carcinoma (■) versus normal surrounding gastric mucosa (□). *, Down-regulation of *UGT1A3* ($p = 0.049$) and *UGT1A10* ($p = 0.02$) was statistically significant (Student's *t* test). *n*, number of samples out of the 14 studied that expressed each individual *UGT1A* isoform (also summarized in Table 1).

enocarcinoma/normal gastric mucosa microsomes. In agreement with the observed differences of UGT1A isoform mRNA expression detected by DRT-PCR between the two tissues, band mobility was unique to each tissue type (Fig. 5, lanes 3–5). In addition, the analysis of pairs of adenocarcinoma and normal gastric mucosa tissue demonstrated lower levels of overall UGT1A protein in the adenocarcinoma samples. This finding is in agreement with the results obtained by DRT-PCR on the mRNA level (Fig. 1 and Fig. 2) indicating that down-regulation of UGT1A mRNA leads to down-regulation of UGT1A protein in these samples.

Discussion

Human *UGT1A* genes are regulated in a tissue-specific fashion in hepatic and extrahepatic tissue (Strassburg et al., 1997a; Strassburg et al., 1998). This distribution of individual UGT1A isoforms is believed to determine tissue specific metabolic and detoxification requirements. Although most UGT1A cDNAs have been cloned and characterized using hepatic tissue (Harding et al., 1988; Ritter et al., 1991; Wooster et al., 1991; Mojarrabi et al., 1996), the regulation and function of extrahepatic UGTs is less well established. As an organ of the digestive tract characterized by prolonged and immediate contact to dietary constituents, including xenobiotics, carcinogens, and therapeutic drugs, human gastric epithelium is likely to play a significant role in extrahepatic detoxification (Peters et al., 1989; McDonnell et al., 1996). Previous studies have identified UGT1A1 and UGT1A4 expression in the extrahepatic gastrointestinal tract (McDonnell et al., 1996) in addition to significant bilirubin UGT activity in small intestine (Peters et al., 1989).

In the present study, we provide evidence for a polymorphic regulation of the *UGT1A* locus in gastric mucosa and carcinoma. In a panel of 14 normal gastric epithelium/adenocarcinoma tissue sample pairs that were examined, UGT1A7 was expressed in 13 and UGT1A10 was expressed in all samples, confirming UGT1A7 and UGT1A10 expression as the predominant extrahepatic UGT1A gene products in human gastric tissue (Strassburg et al., 1997b). However,

in contrast to findings obtained with a panel of hepatic tissue samples, gastric tissue was not characterized by a uniform presence of UGT1A gene products consistent among individuals. Analysis of the overall UGT1A mRNA in gastric epithelium displayed significant interindividual differences. In addition, the expression of UGT1A1 mRNA was observed in four of 14 samples, UGT1A3 mRNA in three of 14 samples, and UGT1A6 mRNA in five of 14 samples. This finding documents a polymorphic expression of the *UGT1A* locus within a single tissue type. Because the history of the sampled subjects indicates no relevant drug or nicotine use, and all samples were obtained from the area of the corpus/antrum junction, these findings are not likely to be the result of sampling or external factors. In addition to examining normal gastric epithelium, corresponding specimens of adenocarcinoma tissue from the same organ were analyzed for UGT1A mRNA expression. The polymorphic expression of UGT1A mRNAs was confirmed. Moreover, differential down-regulation of UGT1A1, UGT1A3, UGT1A6, and UGT1A10 was observed in the tumor specimens. Interestingly, UGT1A7 mRNA was up-regulated in gastric adenocarcinoma.

The results also demonstrate that in normal gastric epithelium and paired adenocarcinoma samples, there occurs significant differential regulation of UGT1A gene products. This regulation includes the consistent expression of predominant and tissue-specific UGT1A gene products such as UGT1A7 and UGT1A10, the variable coexpression of individual *UGT1A* genes such as UGT1A1, UGT1A3, and UGT1A6 in selected individuals, as well as down-regulation and up-regulation of *UGT1A* gene expression in the course of neoplastic transformation to gastric adenocarcinoma. Combined, the data provides compelling evidence for individual regulation of the human *UGT1A* gene products in gastric tissue.

TABLE 2

Glucuronidation activity in gastric microsomes

Gastric microsomal protein (25 µg) prepared from four individual gastric tissue specimens (1–4) were analyzed for glucuronidation activity as described in Materials and Methods. Sample 1 corresponds to Figure 3C, sample 2 to Figure 3B, sample 3 to Figure 3D, and sample 4 to Figure 3A. ¹⁴C-labeled glucuronides were separated by thin layer liquid chromatography and visualized after exposure to X-ray film. The glucuronides were scraped and quantified by liquid scintillation counting to determine specific catalytic activities. Only sample 1 exhibits catalytic activity toward hydroxycholeic acid. The other specific activities vary 2- to 4-fold among the four samples, demonstrating a polymorphism of catalytic UGT activity in human gastric epithelium.

Substrate (100 µM)	1	2	3	4
	pmol/mg/min			
1-Naphthol	182.9	74.3	299.3	71.8
4-Nitrophenol	86.3	28.18	120.2	64.8
4-Methylumbelliferone	82.5	30.2	129.1	62.4
4-Isopropylphenol	31.8	8.2	29.5	25.2
Octyl-gallate	35.3	16.2	26	25.5
Estrilol	13.8	8.1	11.9	14.3
Estrone	13.9	5	12.9	16.9
Naringenin	23.8	8.7	23.7	32.2
7-OH-benzo[a]pyrene	33.5	9.3	27	36.5
Hydroxycholeic acid	147.1	N.D.	N.D.	N.D.

N.D., not detected.

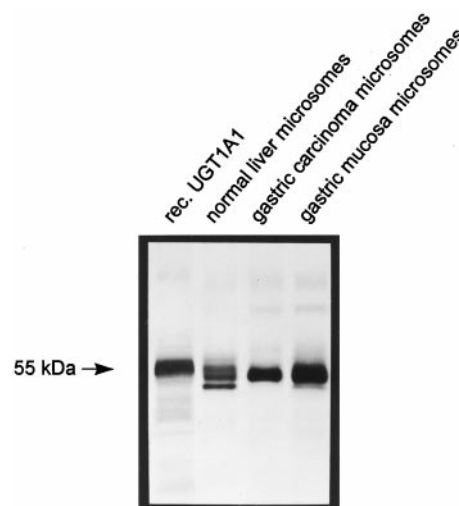


Fig. 5. Western blot analysis of UGT1A protein in hepatic and gastric microsomal protein. Normal microsomal protein from liver and gastric microsomal protein show a tissue-specific banding pattern for each tissue, which is consistent with the differential expression of UGT1A isoform mRNA detected by DRT-PCR. The pattern is consistent among gastric adenocarcinoma and normal mucosa samples. However, differential quantitative expression of UGT1A mRNA detected by DRT-PCR (Fig. 1 and 2) results in down-regulation of UGT1A protein in gastric adenocarcinoma compared with the surrounding normal tissue (two right lanes). Recombinant UGT1A1 (Strassburg et al., 1996) was included as positive control (rec. UGT1A1).

Metabolism by glucuronidation in human gastric tissue may therefore be determined by a regulatory polymorphism that may potentially represent the molecular biological basis of interindividual differences in gastric UGT activity. These differences could impact on interindividual rates of glucuronidation of drugs that could potentially be absorbed through the gastrointestinal lining. With few exceptions, all drugs can be classified as uncharged drugs, organic acids, or organic amines. In an acidic environment such as that of the stomach, amines would exist predominately as ionized and protonated molecules that are not readily accessible for transport into the gastric epithelium. It is interesting to note that UGT1A4, which has specificity toward the metabolism of tertiary amines to form quaternary ammonium glucuronides (Green and Tephly, 1996), is not expressed in gastric epithelium. However, other agents, such as weak acids, would be protonated, uncharged, and would have a high oil/water partition coefficient, allowing for efficient absorption, thus serving as excellent candidates for glucuronidation in the stomach. The interindividual variation observed with the expression of UGT1A1, UGT1A3, UGT1A6, UGT1A7, and UGT1A10 could potentially impact on drug disposition as a result of variations in first-pass metabolism through the gastric mucosa. The variations observed in stomach are in contrast to the consistent UGT1A mRNA regulation in human liver (Strassburg *et al.*, 1997a), which further highlights the differences between hepatic and extrahepatic regulation of the *UGT1A* locus.

The polymorphic regulation of a group of xenobiotic and drug metabolizing enzymes is an interesting finding in view of the stomach's physiological role as a first site of prolonged immediate contact with exobiotic compounds. These differences could potentially modify the cellular defense potential of the stomach against diet-borne cytotoxic and mutagenic compounds, thereby affecting gastric cancer predisposition (Kim *et al.*, 1997; Strassburg *et al.*, 1997a). The analysis of the catalytic ability of gastric microsomal protein to glucuronidate different substrates was exploited to further elucidate a functional impact of the discovered polymorphic regulation. In normal gastric microsomal samples, a 2- to 4-fold difference in specific activities was noted. In particular, phenolic 4-nitrophenol, 4-methylumbelliferone, and 1-naphthol UGT activities catalyzed by UGT1A proteins (Ebner *et al.*, 1993; Ebner and Burchell *et al.*, 1993; Green and Tephly, 1996; Burchell and Brierley 1998; Strassburg *et al.*, 1998), were highly variable. The considerable overlap of specific substrate activities of the majority of UGT1A proteins prevents an exact assignment of specific activity to an individual UGT1A isoform when several UGT1A proteins are simultaneously expressed in a tissue. This is exemplified by the substrate estrone, which has been found to be glucuronidated by UGT1A3 (Mojarrabi *et al.*, 1996) and UGT1A10 (Strassburg *et al.*, 1998). Because expression of both isoforms was demonstrated in gastric tissue in this study, their individual contribution to microsomal estrone activity cannot be estimated by catalytic activity assays using microsomal protein preparations. However, the activity of UGT1A7, which has been found to be uniquely expressed in stomach, was found to be associated with higher specific activities for phenolics such as 1-naphthol, 4-methylumbelliferone, and 4-nitrophenol (Fig. 4D; Table 2, sample 3), all of which have recently been identified as preferred substrates of UGT1A7 (Strass-

burg *et al.*, 1998). The panel included the putative tobacco carcinogen metabolite 7-hydroxy-benzo[*a*]pyrene (Strassburg *et al.*, 1998), which was also glucuronidated with specific activities that varied 4-fold. This may contribute to interindividual differences in cytoprotection and genoprotection. Interestingly, a qualitative differential activity was demonstrated for the glucuronidation of hyodeoxycholic acid. Because hyodeoxycholic acid has not been identified as a substrate for the UGT1A gene products but has been found to be conjugated by UGT2B4 (Fournel-Gigleux *et al.*, 1991), the polymorphic patterns of catalytic activities detected here are very likely to include UGT2B proteins.

The findings presented in this study differ from the classical genetic polymorphisms of drug metabolizing enzymes, which have been documented for such cytochrome P450 enzymes as CYP2D6, as well as glutathione *S*-transferase, *N*-acetyltransferases, and methyltransferases (Daly, 1995; Meyer and Zanger, 1997). Most genetic polymorphisms have been discovered as a result of bifunctional distribution of drug metabolizing enzyme function, which becomes clinically evident in adverse drug reactions. The genetic basis of this feature is the presence of monogenic traits in the normal population, which is represented as two phenotypes. As a result, drug metabolizing enzyme function can be substantially altered by homozygous combination of "loss of function alleles" or enhanced by duplication or amplification events. Other than in the investigation of Crigler Najjar's and Gilbert's diseases (Mackenzie *et al.*, 1997) genetic polymorphisms have not been documented for the majority of UGT proteins. We report here a differing observation leading to polymorphic expression of *UGT1A* gene product levels and isoform expression, not following a bimodal distribution. This seems to be the result of tissue-specific polymorphic regulation of the human *UGT1A* gene locus in gastric tumor as well as in normal gastric epithelium. In contrast to the homogeneous expression of *UGT1A* gene products in human hepatic tissue, this also implies that interindividual regulatory mechanisms may affect the function of drug metabolizing enzymes in the stomach and thus determine interindividual variations in metabolism.

In summary, a quantitative and qualitative polymorphism of *UGT1A* gene regulation in human gastric epithelium has been demonstrated. Interindividual differences in UGT activity included a 4-fold quantitative variation and the qualitative variations of specific substrate activities. The regulation of UGT1A transcripts in gastric tissue follows a complex mode different from that in liver, which may indicate a unique control of human extrahepatic glucuronidation.

Acknowledgments

We wish to thank the late Professor R. Pichlmayr, as well as Dr. J. Jähne, Dr. R. Raab and Dr. J. Klempnauer, Department of Abdominal and Transplant Surgery, Medizinische Hochschule Hannover, for their support in tissue sample procurement.

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