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Research Paper

Activity and Expression of Various Isoforms of Uridine Diphosphate Glucuronosyltransferase are Differentially Regulated During Hepatic Regeneration in Rats*

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Purpose. Glucuronidation pathway is very important in the detoxification of endogenous and exogenous compounds. The objective of this study was to evaluate the activity and expression of various hepatic uridine diphosphate glucuronosyltransferases (UGTs) in rats at various time points after initiation of hepatic regeneration by partial hepatectomy (PHx).

Methods. The mRNA expression of various UGTs was evaluated using real-time polymerase chain reaction (real-time PCR) with specific primers. The *in vitro* activity of UGTs was evaluated using different substrates such as estradiol (*UGT1A1*), acetaminophen (*UGT1A6/7*), morphine (*UGT2B1*), testosterone (*UGT2B1/3/6*), androsterone (*UGT2B2*), and (−)-borneol (*UGT2B12*).

Results. Whereas the activity and mRNA expression of *UGT1A1*, *UGT2B1*, *UGT2B1/3/6*, *UGT2B2*, and *UGT2B12* were lower, the activity and mRNA expression of *UGT1A6/7* were preserved during hepatic regeneration. The mRNA expression of *UGT2B8* was down-regulated, whereas the mRNA expression of *UGT1A5* and *UGT1A8* was not altered by PHx. The mRNA expression of *UGT1A2* and *UGT1A3* was increased during hepatic regeneration.

Conclusion. UGT-mediated drug-metabolizing ability of the liver was altered differentially in the regenerating rat liver. Individualized dosing regimen for different UGT substrates may be needed when using such substrates of these enzymes in patients with a regenerating liver, especially during the early postoperative period. However, the glucuronide conjugating capacity of the liver in the donor of a living donor liver transplantation is expected to completely return to normal with time after surgery.

KEY WORDS: hepatic regeneration; microsome; real-time PCR; regulation; UGT.

INTRODUCTION

Liver transplantation is an accepted treatment of choice for patients with end-stage liver diseases. The number of patients who require liver transplantation has increased 10-fold in the recent years, but the number of cadaveric organs that are available for transplantation has been stagnant. Living donor liver transplantation (LDLT) has emerged as an effective therapy for some patients and is a partial solution to the current severe shortage of cadaveric donor organs (1).

In LDLT, normally, the right hepatic lobe of the donor is transplanted into a recipient (2,3). After surgery, both the donor and the recipient have a small liver that grows in size because of hepatic regeneration to accommodate the requirements of the donor and the recipient. Hepatic regeneration after partial hepatectomy (PHx) involves proliferation of all the existing mature cells, including hepatocytes, biliary epithelial cells, fenestrated endothelial cells, Kupffer cells, and

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ABBREVIATIONS: *Beta-2-m*, beta-2-microglobulin; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; HPLC, high-performance liquid chromatography; IL-6, interleukin-6; LDLT, living donor liver transplantation; PCR, polymerase chain reaction; PHx, partial hepatectomy; TNF-α, tumor necrosis factor alpha; UGT, uridine diphosphate glucuronosyltransferase.

cells of Ito (4). Hepatic regeneration is believed to be triggered or activated by hepatocyte growth factor (HGF) (5), transforming growth factor-α (TGF-α) (6), tumor necrosis factor-α (TNF-α) (7), and interleukin-6 (IL-6) (8). Some cytokines are known to alter hepatic drug metabolism.

Living donor liver transplant recipients are normally treated with drugs such as tacrolimus, sirolimus, and cyclosporine, which are metabolized by phase 1 pathways, and mycophenolic acid, acetaminophen, and morphine, which are metabolized by phase 2 pathways (glucuronidation). Living donor recipients also receive drugs such as metaclopramide, pantoprazole, and ondansetron, which are metabolized by phase 1 pathways, and naloxane, hydromorphone, and oxycodone, which are metabolized by phase 2 pathways. In addition, several endogenous compounds such as bilirubin, estradiol, androsterone, and testosterone are metabolized by glucuronidation. Whereas the activity and expression of phase 1 enzymes have been reported to be decreased during hepatic regeneration (9–11), there is controversial and incomplete information on the activity and expression of uridine diphosphate glucuronosyltransferases (UGTs) during hepatic regeneration. Some publications indicated changes in the activity or expression of UGTs, and others indicated a lack of change (12–15). Moreover, published reports have used Northern blot method for the measurement of RNA expression. Real-time polymerase chain reaction (PCR) is a more sensitive, quantitative, accurate, and reliable assay for the measurement of mRNA. However, there are no published reports using real-time PCR to study multiple UGTs in rats.

Whereas hepatic regeneration has been of interest from a scientific point of view for several years, the implications of the changes in the activity of various xenobiotic metabolizing enzymes during hepatic regeneration from a clinical perspective have become a topic of great interest only recently with the increase in the use of living donors in the living donor liver transplantation. The clinically relevant questions are as follows:

1. Does UGT activity change during hepatic regeneration?
2. Does activity of all UGT isoforms change similarly?
3. Does UGT activity return to control values with time?

Therefore, the primary objective of this work is to address these questions from the perspective of the donor. Systematic study of the activity and expression of UGTs is necessary to thoroughly understand the regulation of UGT isoforms during hepatic regeneration. This information is important to optimize drug therapy and understand the metabolism of various exogenous and endogenous compounds in LDLT patients. In this study, partially hepatectomized rats, real-time PCR, and chromatographic methods were used to systematically study the effect of hepatic regeneration on the activity and expression of different UGTs using specific primers and specific markers.

MATERIALS AND METHODS

Chemicals

Estradiol, estradiol-3-glucuronide, acetaminophen, acetaminophen glucuronide, morphine sulfate, morphine-3-glucuronide, androsterone, testosterone glucuronide, (–)-borneol, and UDPGA were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Testosterone was obtained from Steraloids Inc. (Newport, RI, USA). UDP-[U-^{14}C] glucuronic acid was from MP Biomedicals (Irvine, CA, USA). Reagents for reverse transcription were purchased from Promega (Madison, WI, USA). Forward and reverse primers for mRNA measurements were synthesized by Applied Biosystems (Forest City, CA, USA). Rat IL-6 and TNF-α enzyme-linked immunosorbent assay (ELISA) kits were purchased from Pierce Biotechnology, Inc. (Rockford, IL, USA). All solvents used were of high-performance liquid chromatography (HPLC) grade.

Animals

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Partial hepatectomy was performed according to the method of Higgins and Anderson (16). Male Sprague–Dawley rats weighing 225–250 g were anesthetized with methoxyflurane inhalation, and the ventral surface was shaved along the midline and swabbed with Betadine. A midline incision of 3–4 cm was made. Blood vessels supplying the medial and left lateral lobes of the liver were sutured, and the lobes were excised. This resulted in the removal of 65–75% of the total liver, leaving the right lateral lobe and the small caudate lobe. For the sham operation (served as paired controls), the liver was manipulated similar to the partial hepatectomy procedure, but was not excised. After surgery, the rats had free access to food and water and were maintained on a 12-h light and 12-h night cycle. Rats were sacrificed by CO_2 inhalation at 24 h, day 6 or day 14 after PHx. On the day of the sacrifice, the livers from rats were perfused with ice-cold 0.15 M KCl, frozen in liquid nitrogen immediately, and stored at −80°C for extraction of total RNA and preparation of microsomes. To minimize the variance, all rats for each time point were ordered on the same day. Twelve rats were ordered every time (six rats for PHx; six rats for sham), and the surgery was conducted between 9:00 AM and 11:00 AM. For the rats that were sacrificed at 24 h, 3 ml blood was collected at the time of sacrifice. The whole blood was centrifuged immediately after collection at 3000 rpm for 10 min, and the serum was stored at −20°C for cytokine measurements.

IL-6 and TNF-α Measurement

The serum concentration of IL-6 and TNF-α was measured using rat IL-6 and TNF-α ELISA kits (Pierce Biotechnology) according to the instructions of the manufacturer.

Preparation of Microsomes

Liver microsomes were prepared by differential centrifugation procedure as per Tian *et al.* (17). The protein content was determined by Lowry's method using bovine serum albumin as the standard (18). Microsomes were stored at −80°C until the incubation studies were performed.

Incubation of UGT Substrates in Microsomes

The microsomes were incubated with various substrates to measure the *in vitro* activity (estradiol as a *UGT1A1* marker, acetaminophen as a *UGT1A6/7* marker, morphine as

a *UGT2B1* marker, testosterone as a *UGT2B1/3/6* marker, androsterone as a *UGT2B2* marker, and (−)-borneol as a *UGT2B12* marker) (19–24). Incubation conditions were the same as those reported in literature: estradiol (150 μM) (25), acetaminophen (10 mM) (26), morphine (10 mM), and testosterone (150 μM) (27).

For androsterone (200 μM; reported K_m, 7.2 μM) (28) and (−)-borneol (500 μM; reported K_m, 36 μM) (29), the incubation procedure was established as follows. A solution (250 μl) containing 1 mg/ml microsomal protein (activity was linear from 0.25 to 1 mg/ml of protein), 2 mM UDPGA (including 0.2 μCi UDP-[U-^{14}C]glucuronic acid/reaction for androsterone or 0.1 μCi UDP-[U-^{14}C]glucuronic acid/reaction for (−)-borneol), 10 mM $MgCl_2$, Brij 58 (0.1 mg/mg protein), and 200 μM androsterone or 500 μM (−)-borneol was incubated for 60 min (linear from 15 to 60 min) at 37°C in a shaking water bath. Then 25 μl of 6% perchloric acid was added to the incubation solution. After centrifugation at 13,000 rpm for 5 min, 100 μl of supernatant was analyzed by HPLC.

Assays

The concentration of the glucuronide metabolite in the supernatant was measured based on published HPLC methods, with minor modifications (21,25–28). The correlation coefficients (r^2) for the standard curves were ≥0.98, and the coefficient of variations were less than 3% ($n = 3$) for all the assays. The peaks for both androsterone glucuronide and borneol glucuronide were identified by comparing the incubation samples with the controls in which substrate, UDPGA (both nonlabeled and radiolabeled), or radiolabeled UDPGA was omitted. No standard curves were established for the measurement of the radioactivity of glucuronide of androsterone and borneol; however, the radioactivity was measured within the linear range of the detector.

Extraction of RNA and Reverse Transcription

Total RNA was extracted from 50 to 100 mg of liver using Trizol (Invitrogen, Carlsbad, CA, USA) according to the instructions of the manufacturer. Extracted RNA was quantitated spectrophotometrically at 260/280 nm, and the integrity was evaluated using agarose gel. After removal of DNA using RNase-Free DNase, 2 μg of RNA was reversely transcribed using 0.5 μg of random hexamer (Promega) heated to 70°C for 5 min, and then cooled to 4°C. A reaction mixture containing 200 U Moloney murine leukemia virus reverse transcriptase, 1 mM dNTPs, and 25 U RNasin (Promega) was added to the previous mixture and incubated at 37°C for 60 min. The resulting cDNA was diluted 10-fold and stored at −20°C. The control samples were also prepared using the same procedure but by replacing the reverse transcriptase with water.

Real-Time PCR

Polymerase chain reaction was performed on Applied Biosystems 7700 cycler using 5 μl of cDNA, 7.25 pmol of forward and reverse primers, and 12.5 μl of SYBR Green PCR Master Mix (Applied Biosystems) for a total volume of 25 μl. Forward and reverse primers for *UGT1A* and *UGT2B* family were designed using the combination of nucleotide–nucleotide Blast, Align two sequences (http://www.ncbi.nlm.nih.gov/BLAST/), Amplify 1.2 (freeware from http://engels.genetics.wisc.edu/amplify/), and Primer Express 2.0 (Applied Biosystems) (Table I). The specificity of each pair of primers was first evaluated using nucleotide–nucleotide Blast to confirm that there was no match between the primers in Table I and the rat genome except for UGTs. The primer specificity was further evaluated (using Amplify 1.2) for any potential amplification of other UGT isoforms except the target isoform. Because of the high homology between *UGT2B3* and *UGT2B6*, the primers were designed to amplify both isoforms simultaneously. Cycling conditions were the same as per Komoroski *et al.* (30). The relative cDNA content was determined in duplicate using standard curves with *beta-2-m* from cDNA and normalized to *beta-2-m* for each sample. For each pair of primers, the control without reverse transcriptase was also used for PCR reactions in duplicate to confirm that there was no genomic DNA contamination in the cDNA samples.

Data Analysis

All data are reported as mean ± SD. Comparisons between groups were made by Student's t test ($p < 0.05$).

RESULTS

Serum Concentration of IL-6 and TNF-α

The serum concentration of IL-6 was significantly increased 24 h after hepatic regeneration (PHx-24 h *vs.* sham, 124 ± 28 *vs.* 47 ± 18 pg/ml). However, the serum concentration of TNF-α was not altered 24 h after initiation of hepatic regeneration (PHx-24 h *vs.* sham, 89 ± 20 *vs.* 95 ± 18 pg/ml).

Selection of Control Genes

The mRNA expression of *beta-actin* and *beta-2-m* at different time points during hepatic regeneration is shown in Table II. The mRNA expression of *beta-actin* almost doubled at 24 h (sham *vs.* PHx, 0.191 ± 0.0258 *vs.* 0.318 ± 0.0610). The mRNA level of *beta-actin* in the PHx group was similar to the paired sham group by day 6. The mRNA expression of *beta-2-m*, on the other hand, was relatively stable during hepatic regeneration and was not significantly different between paired sham groups and PHx groups. Therefore, *beta-2-m* was chosen as the normalization gene for the study of other target genes.

Activity and Expression of UGT1A1 during Hepatic Regeneration

The activity of *UGT1A1* was decreased 24 h and 6 days after PHx and recovered by day 14 (Fig. 1, panel A). The mRNA expression of *UGT1A1* was also decreased 24 h and 6 days after initiation of regeneration and returned to normal level by day(14 (Fig. 1, panel B).

Table I. Primers for Real-time PCR Analysis of mRNA Expression

Gene	GenBank No.	Primers	Base positions	Amplicon size
Beta-actin	NM_031144	Forward 5′-ctggcctcactgtccacctt-3′	1117–1136	65
		Reverse 5′-gggccggactcatcgtact-3′	1163–1181	
Beta-2-m	NM_012512	Forward 5′-cgtgcttgccattcagaaaa-3′	58–77	76
		Reverse 5′-gaagttgggcttcccattctc-3′	113–133	
UGT1A1	NM_012683	Forward 5′-gccatgcagcctggattt-3′	549–567	64
		Reverse 5′-ctcttgggcacgtaggacaac-3′	592–612	
UGT1A2	D38066	Forward 5′-cgcaaattcttgtgcagctcta-3′	368–390	76
		Reverse 5′-accacatcgaaggaactggaa-3′	423–443	
UGT1A3	D38067	Forward 5′-ggccatgtacctgcgtgttc-3′	473–493	71
		Reverse 5′-tgcttcaaattccagttcacaga-3′	521–543	
UGT1A5	AF461734	Forward 5′-tcgacagttctcttaaggtcttgtatg-3′	395–422	78
		Reverse 5′-aaggagctggaattcagatgct-3′	451–472	
UGT1A6	NM_057105	Forward 5′-ccgctatcgctcctttgg-3′	356–374	73
		Reverse 5′-ctgtactctcttagaggagccatcag-3′	403–428	
UGT1A7	NM_130407	Forward 5′-cagaccccggtgactatgaca-3′	750–771	73
		Reverse 5′-caacgtgaagtctgtgcgtaaca-3′	800–822	
UGT1A8	NM_175846	Forward 5′-gagggcatgaggtggtggta-3′	154–174	72
		Reverse 5′-cacggtaaaattcagcgactttc-3′	203–225	
UGT2B1	M13506	Forward 5′-ctgaagcagagccctgagaga-3′	1626–1647	76
		Reverse 5′-gggaaggcactggcatga-3′	1684–1701	
UGT2B2	J02589	Forward 5′-ggcagggcagcagtcatc-3′	2182–2200	86
		Reverse 5′-cctacttcttgctcactctctgctt-3′	2243–2267	
UGT2B3/6	M31109 (2B3)	Forward 5′-atgccaagaaatgggatcca-3′	717–736	72
		Reverse 5′-tgcccattgtctcagctaagg-3′	768–788	
	M33746 (2B6)	Same primers as the pair for 2B3	728–747[a]	72
			779–799	
UGT2B8	U27518	Forward 5′-tgaacaaaatgttcgggcaat-3′	363–384	75
		Reverse 5′-aagttccttgtttgaaacaacttctct-3′	411–437	
UGT2B12	U06273	Forward 5′-tgctgcaaataagtttctgctttaa-3′	33–58	74
		Reverse 5′-tgactatattccatcggccatacc-3′	83–106	
CAR	NM_022941	Forward 5′-cggagtataaacagcgcatactca-3′	1190–1213	72
		Reverse 5′-aagcagcggcatcatagca-3′	1243–1261	
PXR	NM_052980	Forward 5′-cggctacctgcggtgttt-3′	725–742	63
		Reverse 5′-caacagtgaggcctgcagaa-3′	768–787	
HNF1	X54423	Forward 5′-ctcctcggtactgcaagaaacc-3′	3061–3082	73
		Reverse 5′-ttgtcaccccagcttaagactct-3′	3111–3133	
C/EBP α	NM_012524	Forward 5′-tatagacatcagcgcctacatcga-3′	183–206	76
		Reverse 5′-ccggctgtgctggaagag-3′	241–258	

[a] One mismatch at position 738.

Activity and Expression of UGT1A6/7 During Hepatic Regeneration

The activity of *UGT1A6/7* was preserved at all time points studied (Table III). The mRNA expression of both *UGT1A6* and *UGT1A7* during hepatic regeneration also stayed at the normal level as measured in paired sham group (Table IV).

Activity and Expression of UGT2B1 During Hepatic Regeneration

The activity of *UGT2B1* was decreased 24 h after PHx (Fig. 2, panel A). The mRNA expression of *UGT2B1* was also decreased 24 h after regeneration and returned back to normal level as measured in paired sham group by day 6 (Fig. 2, panel B).

Table II. The mRNA Expression of Control Genes at Different Time Points after Initiation of Regeneration ($n = 4$)

	24 h		6 days		14 days	
	Sham	PHx	Sham	PHx	Sham	PHx
Beta-actin	0.191 ± 0.0258	0.318 ± 0.0610**	0.192 ± 0.0596	0.204 ± 0.0114	0.198 ± 0.0321	0.213 ± 0.0380
Beta-2-m	0.260 ± 0.0370	0.233 ± 0.0280	0.318 ± 0.0260	0.240 ± 0.0680	0.297 ± 0.0270	0.303 ± 0.0380

The arbitrary mRNA values were determined by real-time PCR as described in Materials and Methods. All data are expressed as mean ± SD. Sham: pooled lobes from sham groups; PHx: pooled regenerated lobes after PHx; UGT: uridine diphosphate glucuronosyltransferases; PCR: polymerase chain reaction.
**$p < 0.01$ *vs.* sham (Student's *t* test).

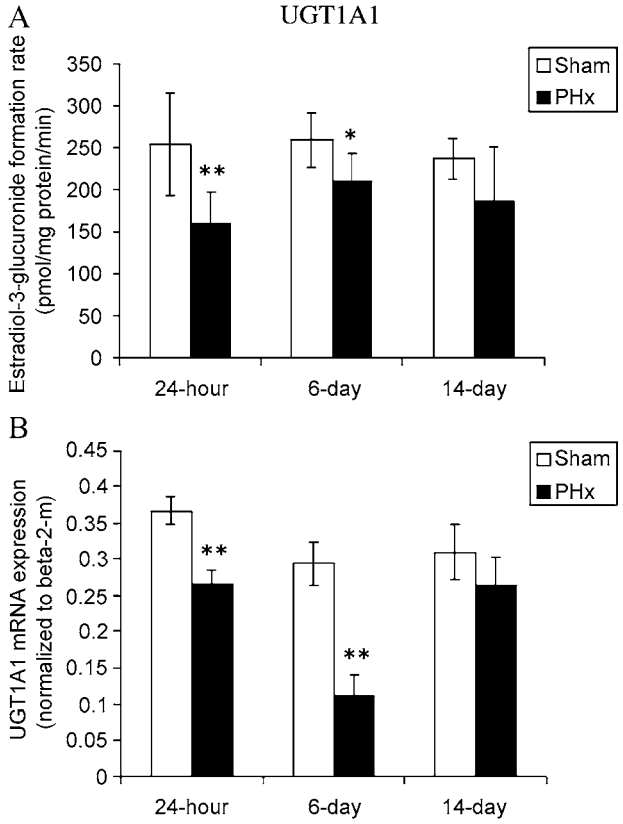

Fig. 1. The activity and mRNA expression of *UGT1A1* at different time points after PHx. Sham: liver lobes from sham groups; PHx: the regenerated liver lobes after PHx. The activity was measured using liver microsomes prepared as described in Materials and Methods. The relative mRNA level was determined by real-time polymerase chain reaction (PCR) as described in Materials and Methods using pooled cDNAs generated from total RNAs from six normal livers as the standard. The arbitrary mRNA values were normalized with their respective *beta-2-m* values. All data are expressed as mean ± SD. $^{**}p < 0.01$ *vs.* sham; $^{*}p < 0.05$ *vs.* sham (Student's *t* test). $n = 4–6$.

Activity and Expression of UGT2B1/3/6 During Hepatic Regeneration

The activity of *UGT1/3/6* measured using testosterone was decreased 24 h and 6 days after PHx and recovered by day 14 (Table III). The mRNA expression of *UGT2B3/6* was also lower at 24 h and 6 days (Table IV).

Activity and Expression of UGT2B2 During Hepatic Regeneration

Both the activity and mRNA expression of *UGT2B2* were decreased after initiation of hepatic regeneration and recovered completely by day 6 (Fig. 3).

Activity and Expression of UGT2B12 During Hepatic Regeneration

Both the activity and mRNA expression of *UG2T2B12* were decreased after initiation of hepatic regeneration and recovered completely by day 6 (Fig. 4).

Expression of Other UGTs (UGT1A2, UGT1A3, UGT1A5, UGT1A8, and UGT2B8) During Hepatic Regeneration

UGT1A2 and *UGT1A3* mRNA was up-regulated compared to sham groups during hepatic regeneration (Table IV). The mRNA expression of *UGT1A5* and *UGT1A8* was not altered at any time during the regeneration process (Table IV). *UGT2B8* was down-regulated compared to sham groups (Table IV).

The mRNA Expression of CAR, PXR, HNF1, and C/EBP α During Hepatic Regeneration

CAR, *PXR*, and *HNF1* genes were expressed stably 24 h after PHx. The *C/EBP α* gene was down-regulated 24 h after initiation of hepatic regeneration (Fig. 5) and recovered back to normal level by day 6.

DISCUSSION

Limited and conflicting data have been published on the effect of hepatic regeneration on the expression and the activity of UGTs. The activity of *UGT1A1* (bilirubin conjugation) has been reported to be preserved (12), whereas the *UGT1A1* mRNA expression has been shown to be decreased after PHx in rats (14). In rats, *UGT1A6* activity (*p*-nitrophenol conjugation) was reported to be not altered after PHx in two studies (12,15); however, Iversen *et al.* (13) reported decreased and induced *UGT1A6* activity for the glucuronidation of naphthol at different time points after PHx. *UGT2B1* activity (glucuronidation of morphine) was reported to be decreased, whereas increased *UGT2B1* mRNA expression has been reported after PHx in rats (13).

Table III. The Activity of *UGT1A6/7* and *UGT2B1/3/6* at Different Time Points after PHx ($n = 4–6$)

	24 h		6 days		14 days	
Sham	Sham	PHx	Sham	PHx	Sham	PHx
UGT1A6/7 (acetaminophen glucuronide formation rate, nmol/mg protein/min)	1.15 ± 0.303	0.819 ± 0.281	1.12 ± 0.243	1.25 ± 0.301	1.07 ± 0.216	0.885 ± 0.323
UGT2B1/3/6 (testosterone glucuronide formation rate, nmol/mg protein/min)	2.73 ± 0.559	1.47 ± 0.452**	2.53 ± 0.249	1.45 ± 0.398**	2.20 ± 0.425	1.73 ± 0.627

All data are expressed as mean ± SD.
Sham: liver lobes from sham groups; PHx, the regenerating liver lobes after PHx.
$^{**}p < 0.01$ *vs.* sham (Student's *t* test).

Table IV. The mRNA Expression of Various UGTs at Different Time Points after PHx ($n = 4–6$)

	24 h		6 days		14 days	
	Sham	PHx	Sham	PHx	Sham	PHx
UGT1A2	0.000260 ± 0.0000500	0.00271 ± 0.000900**	0.000230 ± 0.0000500	0.000410 ± 0.000130*	0.000280 ± 0.0000800	0.000410 ± 0.000130*
UGT1A3	0.000800 ± 0.000100	0.00171 ± 0.000710*	0.000640 ± 0.000170	0.000580 ± 0.000150		
UGT1A5	0.0440 ± 0.0122	0.0428 ± 0.0146	0.0337 ± 0.00260	0.0384 ± 0.00550		
UGT1A6	0.00744 ± 0.00364	0.00656 ± 0.00264	0.0149 ± 0.00203	0.0120 ± 0.00240		
UGT1A7	0.00138 ± 0.000350	0.000990 ± 0.000220	0.00161 ± 0.000340	0.00201 ± 0.000770		
UGT1A8	0.000570 ± 0.000160	0.000630 ± 0.000120	0.000320 ± 0.000130	0.000570 ± 0.000180		
UGT2B3/6	0.590 ± 0.0661	0.294 ± 0.0292**	0.624 ± 0.0508	0.454 ± 0.0977*	0.594 ± 0.0840	0.650 ± 0.00950
UGT2B8	0.000224 ± 0.0000600	0.000118 ± 0.0000230*	0.000679 ± 0.000164	0.000330 ± 0.000203*	0.000509 ± 0.000109	0.000622 ± 0.000233

The relative mRNA level was determined by real-time PCR as described in Materials and Methods using pooled cDNAs generated from total RNAs from six normal livers as the standard. The arbitrary mRNA values were normalized with their respective *beta-2-m* values. All data are expressed as mean ± SD.
*$p < 0.05$ *vs.* sham (Student's *t* test).
**$p < 0.01$ *vs.* sham (Student's *t* test).

Fig. 2. The activity and mRNA expression of *UGT2B1* at different time points after PHx. Sham: liver lobes from sham groups; PHx: the regenerating liver lobes after PHx. The activity was measured using liver microsomes prepared as described in Materials and Methods. The relative mRNA level was determined by real-time PCR as described in Materials and Methods using pooled cDNAs generated from total RNAs from six normal livers as the standard. The arbitrary mRNA values were normalized with their respective *beta-2-m* values. All data are expressed as mean ± SD. **$p < 0.01$ *vs.* sham; *$p < 0.05$ *vs.* sham (Student's *t* test). $n = 4–6$.

The activity of rat *UGT2B3* (testosterone glucuronidation) has been reported to be altered after initiation of hepatic regeneration in rats (13), whereas Pellizzer *et al.* (14) reported no change in the *UGT2B3* mRNA expression after PHx in rats. In these studies, the activity of different UGT isoforms during hepatic regeneration was completely inconsistent with the mRNA expression of these isoforms. Conclusive information about the regulation of UGTs cannot be obtained from published studies because of lack of systematic studies.

In this study, several currently available specific UGT markers were used to evaluate the activity of different UGT isoforms at different time points after initiation of hepatic regeneration. Because real-time PCR provided more accurate, sensitive, and reliable measurements of the mRNA expression compared to Northern blot method used in previous studies, this study used real-time PCR to assess the effect of hepatic regeneration on the mRNA expression of these UGT isoforms at the same time points. In addition, the mRNA expression of additional UGTs using real-time PCR, where specific substrates or antibodies are not available, was also measured.

A major concern in mRNA measurements is the specificity of primers designed because of the high homology of different UGTs. In this study, specific primers for several

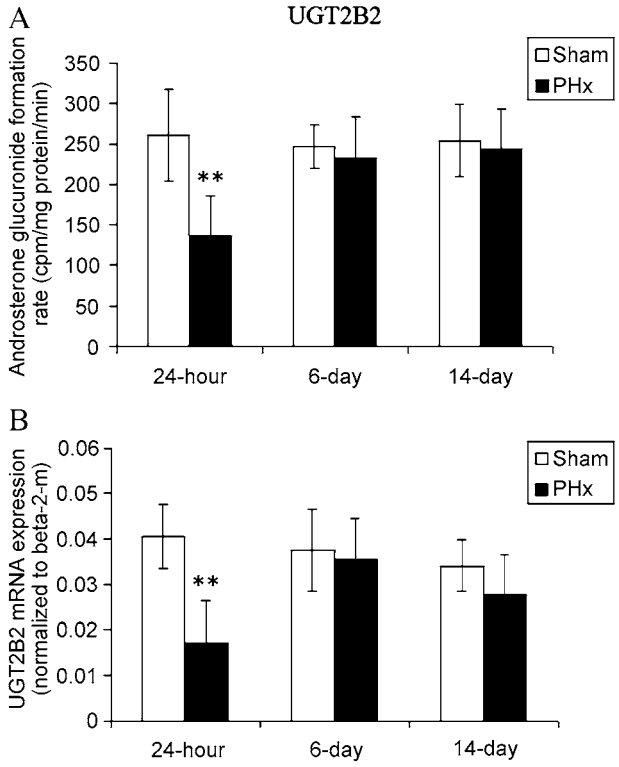

Fig. 3. The activity and mRNA expression of *UGT2B2* at different time points after PHx. Sham: liver lobes from sham groups; PHx: the regenerating liver lobes after PHx. The activity was measured using liver microsomes prepared as described in Materials and Methods. The relative mRNA level was determined by real-time PCR as described in Materials and Methods using pooled cDNAs generated from total RNAs from six normal livers as the standard. The arbitrary mRNA values were normalized with their respective *beta-2-m* values. All data are expressed as mean ± SD. $^{**}p < 0.01$ *vs.* sham (Student's *t* test). $n = 4$–6.

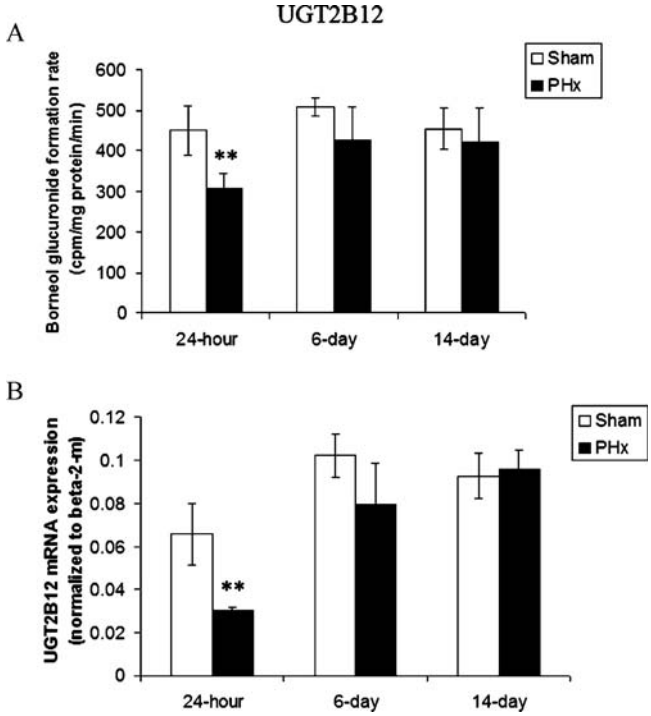

Fig. 4. The activity and mRNA expression of *UGT2B12* at different time points after PHx. Sham: liver lobes from sham groups; PHx: the regenerating liver lobes after PHx. The activity was measured using liver microsomes prepared as described in Materials and Methods. The relative mRNA level was determined by real-time PCR as described in Materials and Methods using pooled cDNAs generated from total RNAs from six normal livers as the standard. The arbitrary mRNA values were normalized with their respective *beta-2-m* values. All data are expressed as mean ± SD. $^{**}p < 0.01$ *vs.* sham; $^{*}p < 0.05$ *vs.* sham (Student's *t* test). $n = 4$–6.

rat UGTs were designed, and the mRNA expression of these UGTs was measured during hepatic regeneration. To the best of our knowledge, this is also the first study to measure various UGT isoforms in rats using real-time PCR with specific primers. Because the levels of *beta-actin* changed during hepatic regeneration, *beta-2-m* was used as the normalization gene in this study.

The formation of estradiol-3-glucuronide has been used as a marker of the activity of human *UGT1A1* (19). There was no direct documentation of the specificity of estradiol as a substrate for the *UGT1A1* activity in rats; however, rat and human *UGT1A1* share more than 70% identity in their deduced primary amino acid sequences. Accordingly, rat and human *UGT1A1* exhibited similar enzymatic efficiencies toward estrogens (including estradiol), flavonoids, phenols, and several other classes of chemicals (20). It has been concluded that rat and human *UGT1A1* are functionally similar and can be considered orthologous enzymes (20). Consequently, it is highly possible that estradiol is also a probe substrate for rat *UGT1A1*. Based on this information, we used the formation rate of estradiol-3-glucuronidation as the marker of the *UGT1A1* activity in rats.

Unlike previous conflicting reports, our results showed consistent changes in both the activity and the mRNA expression for all the isoforms studied, suggesting the observed changes to be at the transcriptional level. In addition, we also observed that *UGT1A2* and *UGT1A3* mRNA was up-regulated during hepatic regeneration. The significance of this finding is unknown at this time because of

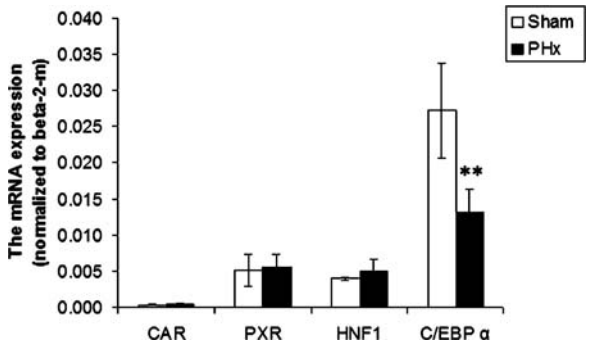

Fig. 5. The mRNA expression of *CAR*, *PXR*, *HNF1*, and *C/EBP α* 24 h after PHx. The relative mRNA level was determined by real-time PCR as described in Materials and Methods using pooled cDNAs generated from total RNAs from six normal livers as the standard. The arbitrary mRNA values were normalized with their respective *beta-2-m* values. Sham: liver lobes from sham groups; PHx: the regenerating liver lobes after PHx. All data are expressed as mean ± SD. $^{**}p < 0.01$ *vs.* sham (Student's *t* test). $n = 5$.

the lack of probe substrates for rat *UGT1A2* and *UGT1A3* and unknown clinically relevant drugs metabolized by these isoforms. Similarly, because of the lack of known clinically relevant drugs metabolized by *UGT2B8*, the significance of the down-regulation of *UGT2B8* is unknown at this time.

Differential regulation of different UGT isoforms has also been reported in other systems. Acute-phase response induced by turpentine injection led to no reduction in glucuronidation of *p*-nitrophenol (*UGT1A6*) (12), whereas it impaired glucuronidation of testosterone (*UGT2B1/3/6*) (31). Strasser *et al.* (31) suggested that the promoter regions of some UGT isoforms may contain specific regulatory elements capable of responding to certain cytokines. Cytokines such as TNF-α and IL-6 were reported to inhibit the activity of UGT (31,32). IL-6 can suppress the mRNA expression of the two most abundant UGT isoforms, *UGT1A1* and *UGT2B3*, in rat hepatocytes (31). Several cytokines including TNF-α and IL-6 are involved in the initiation of hepatic regeneration (4). Our data showed that the serum concentration of IL-6 was significantly increased 24 h after initiation of hepatic regeneration, which is also consistent with the published data (8,33,34). Therefore, IL-6 is likely to be a contributor to the decreased activity and expression of several UGTs after initiation of hepatic regeneration.

Two nuclear receptors *CAR* and *PXR* have been implicated in the acute phase response-mediated decrease in CYP activity (35). Because both *CAR* and *PXR* are also involved in the regulation of UGTs (36), we expected altered levels of *CAR* and *PXR* in rat livers during hepatic regeneration. However, there was no change in *CAR* or *PXR* in the regenerating livers.

There is also direct evidence showing that *C/EBP α* knockout is the cause for the loss of expression of *UGT1A1* (bilirubin UGT) in mouse liver. In addition, *HNF1 α* binding site has been found in human *UGT2B7* (37,38). This would imply that *C/EBP α* and *HNF1 α* could also regulate the expression of UGTs (36). In this study, the expression of *C/EBP α* and *HNF1* in rat livers was evaluated during hepatic regeneration. Only *C/EBP α* was down-regulated significantly after PHx. This finding is consistent with published data (39) and suggests that *C/EBP α* is possibly an important factor responsible for the lower expression of UGTs during hepatic regeneration, even if not the only reason.

Finally, although the activity and expression of CYPs and UGTs are altered during hepatic regeneration, in general, the recovery of UGT activity seems to be more rapid than the recovery of CYP3A activity as measured by the metabolism and clearance of tacrolimus and mycophenolic acid (17).

CONCLUSION

Our study points to several important observations and conclusions. (a) The activity and mRNA expression of several UGTs were decreased during hepatic regeneration, whereas mRNA expression of some UGTs remained unaltered, and mRNA expression of *UGT1A2* and *UGT1A3* was increased. (b) The mRNA expression of *UGT1A1*, *UGT2B1*, *UGT2B2*, and *UGT2B12* changed in the same direction as the activity of the corresponding isoforms. (3) The mRNA expression and activity returned to control values by day 6 in most of the cases and by day 14 in all the cases. Whereas it is possible that changes in mRNA expression in partial hepatectomized rats may not be the same as those in LDLT donors, these observations suggest that the dose of drugs metabolized by UGTs must be adjusted during the early phase of LDLT. Different magnitude of adjustment in the dose of drugs metabolized by different UGT isoform is also necessary. However, the glucuronide-conjugating capacity of the liver in a LDLT will completely return to normal with time after surgery.

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