Special Section on Pregnancy

Tissue Distribution and Relative Gene Expression of UDP-Glucuronosyltransferases (2B7, 2B15, 2B17) in the Human Fetus

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ABSTRACT

UDP-glucuronosyltransferases (UGTs) catalyze phase II conjugation reactions and play an important role in the inactivation and elimination of several drugs. It is well known that the UGT activity in fetal livers is low compared with the UGT activity in adult livers. In this study the mRNA expression levels of the three human subfamilies, 2B7, 2B15, and 2B17, were determined in 20 adult and 60 fetal liver tissue specimens. The expression profile in fetal kidneys ($N = 43$), adrenals ($N = 46$), and lungs ($N = 37$) was also determined. All fetal and adult samples were genotyped for the UGT2B17 deletion polymorphism. Adult liver contained 13–36 times higher levels of UGT2B mRNAs as compared with fetal livers. UGT2B7 was most abundant in fetal lungs and kidneys, whereas UGT2B15 and UGT2B17 were predominant in the liver. There was a significant correlation between UGT2B7 expression levels in lungs and kidneys, whereas for the other UGT2Bs no correlation between the different tissues was observed. Fetuses expressing two UGT2B17 alleles (ins/ins) displayed significantly higher levels of UGT2B17 mRNA compared to ins/del fetuses in lungs, whereas in the other tissues no gene dose-effect was observed.

Introduction

UDP-glucuronosyltransferases (UGTs) represent a family of enzymes that catalyze the glucuronidation of a variety of xenobiotics, including drugs and endogenous compounds (Tukey and Strassburg, 2000). There are 19 different functional human UGTs known today and they are divided into subfamilies 1A, 2A, and 2B based on evolutionary divergence and homology. In humans, seven functional UGT2B genes are expressed (UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, and UGT2B28) (Mackenzie et al., 2005).

Members of the UGT2B subfamily, in particular UGT2B7, 2B15, and 2B17 seem to be the most important enzymes in androgen inactivation (Belanger et al., 2003; Jakobsson et al., 2006). Of these, UGT2B7 is also an important catalyst of conjugation of several drugs, such as morphine, NSAIDs, and codeine (Coffman et al., 1997; Bowalgaha et al., 2005; Mano et al., 2007). Cumulating data indicate that UGT2B15 and 2B17 also are important in the elimination of various drugs including vorinostat, lorcaserin, etc. (Kang et al., 2010; Sadeque et al., 2012). Recently it was discovered that some individuals lack the UGT2B17 gene (Wilson et al., 2004) and that individuals homozygous for the deletion polymorphism excrete androgens and several drugs at a much lower rate compared with those expressing UGT2B17 (Jakobsson et al., 2006; Wong et al., 2011; Wang et al., 2012). The dual role in conjugation of androgens and therapeutic drugs opens the probability of drug-endobiotic interaction with the potential risk of endocrine disruption or therapeutic failure. This is of importance in therapy and in development of new drugs or new therapeutic principles not the least in infants and children.

The liver plays a central role in drug metabolism. Hepatic glucuronidation has been thoroughly studied in human liver samples, and a considerable difference in the glucuronidation activity during the human ontogenesis has been observed. A number of studies have shown that glucuronidation activity for several substrates is significantly lower in fetal than adult human liver (Leakey et al., 1987; Pacifici et al., 1989). In vitro studies using liver microsomes from human fetuses indicated that the glucuronidation activity against the UGT2B7- and UGT2B17-specific substrates morphine and testosterone is 10% and 3% of that seen in adults (Pacifici et al., 1982; Leakey et al., 1987).

Even though it is well known that the glucuronidation activity is low in fetal liver, the mRNA levels of the UGT enzyme genes in human fetal tissues are poorly studied. The aim of this study was to determine and compare the gene expression of three members of the UGT2B subfamily, 2B7, 2B15, and 2B17, in human fetus and adult livers. Moreover, the comparative mRNA expression profiles of the UGT2B enzymes were studied in fetal liver, lungs, adrenals, and kidneys.

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ABBREVIATIONS: PCR, polymerase chain reaction; UGT, UDP-glucuronosyltransferase.
Materials and Methods

Fetal and Adult Tissue Samples. Human adult liver specimens were collected as previously described (von Bahr et al., 1980). The study cohort used here included 20 adult livers of male (N = 9), female (N = 10), or unknown sex (N = 1), from patients between 30 and 75 years of age. The clinical background for surgery or death included cancer (N = 4), familial amyloidotic polyneuropathy (N = 6), hemorrhage (N = 8), asystole (N = 1), or unknown cause (N = 1).

Human fetal liver (N = 60), adrenal (N = 46), kidney (N = 43), and lung (N = 37) specimens from 60 fetuses were obtained at legal abortions performed for sociomedical reasons at the Karolinska University Hospital between 2000 and 2003. The fetal tissues were excised and immediately frozen in liquid nitrogen and stored at −70°C within 2 hours. The study was approved by the Ethics Review Board in Stockholm and by the National Board of Health and Welfare.

The gestational ages were determined by crown-rump length and ranged from 5 weeks to 12 weeks (median age 10.2 weeks). The sex of the fetuses was not determined at collection. None of the women reported any chronic or acute disease, regular drug use, or drug abuse. Smoking was reported in 22 women (37%), nonsmoking in 21 (35%), whereas for 17 there were no reports.

RNA and cDNA Preparations. Total RNA from 5–30 mg of fetal tissue samples and approximately 200 mg of adult liver tissue was prepared using AllPrep DNA/RNA and RNAeasy kit (Qiagen, Hilden, Germany), respectively.

RNA (0.5 μg) was reverse transcribed into cDNA with hexamer primer using SuperScript III (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol and diluted ten times.

Real-Time Polymerase Chain Reaction. The mRNA levels specific for UGT2B7, 2B15, and 2B17 in liver tissues were determined by real-time polymerase chain reaction (PCR) using the primer pairs described previously (Chouinard et al., 2006). 18S was chosen as endogenous housekeeping control gene (PN 4310893E, Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed using the 7500 Fast (Applied Biosystems). Reaction mixtures contained 2xSYBR green reaction mix (Applied Biosystems), 450 mmol of each primer and 1 μl cDNA template in a total volume of 15 μl. Thermal cycling conditions included activation at 95°C (10 minutes) followed by 40 cycles each of denaturation at 95°C (15-second) and annealing/elongation at 60°C (1 minute). Each reaction was performed in duplicates and no template controls were included in each experiment. Melting-curve analysis was performed to exclude the presence of nonspecific products and primer-dimers. For the comparison between adult and fetal liver samples, one adult liver sample was employed as a calibrator and the delta CT-formula was used as described (Livak and Schmittgen, 2001). When the UGT2B intraindividual expressions between different fetal tissues were studied, a kidney sample was employed as calibrator. The gene expression was quantified as the yield of the target gene relative to that of 18S.

Results

Expression of UGT2B Genes in Adult and Fetal Livers. Significantly higher levels of all three UGT mRNA transcripts were observed in the adult liver samples (N = 20) as compared with the fetal liver samples (N = 60). The mean UGT2B7 was 13 times higher in adult liver compared with fetal liver (mean 1.13 ± 0.33 versus 0.086 ± 0.016, P < 0.0001) (Fig. 1A). The mean UGT2B15 was 36 times higher in adult liver compared with fetal liver (mean 3.21 ± 1.68 versus 0.088 ± 0.029, P = 0.0006) (Fig. 1B). The mean UGT2B17 was 34 times higher in adult liver samples compared with fetal liver (mean 203.4 ± 68.5 versus 6.39 ± 2.83, P < 0.0001) (Fig. 1C).

The UGT2B7-, 2B15-, and 2B17-specific mRNAs were detectable in 97 (58), 80 (48), and 63% (38) of the fetal liver samples, respectively. In the adult livers UGT2B7 and UGT2B15 mRNA was abundant in all samples, whereas UGT2B17 was detected in 17 of the 20 individual samples.

Correlation Analysis. A significant correlation was observed between fetal age and the relative mRNA expression of UGT2B15 (R = 0.36, P = 0.02) (Fig. 2A). No correlations between gestational age and UGT2B7 or UGT2B17 mRNA expression were found.

Correlation analyses of the expression between the UGT2B genes were performed. There was no correlation between the expressions of the various UGT2B genes in the fetal liver samples.

There was no relation between fetal UGT2Bs mRNA expression and maternal smoking.

Extrahepatic mRNA Expression. The mRNA expression of the UGT2B genes was analyzed in fetal liver, kidneys, adrenals, and lungs. The UGT2B7 mRNA expression was significantly higher in the lungs (N = 37, mean = 3.84 ± 1.84) and the kidneys (N = 43, mean =

Fig. 1. Relative expression of (A) UGT2B7, (B) UGT2B15, and (C) UGT2B17 mRNA in liver tissues obtained from human fetuses (N = 60) and adults (N = 20). UGT2B7, 2B15, and 2B17 mRNA expression levels in adult livers were found to be significantly higher than in fetal livers. The boxes define the mean values and the 95% confidence intervals (CIs), and the lines, the minimum and maximum values using a logarithmic scale.
2.49 ± 1.47) as compared with the liver (N = 58, mean = 0.51 ± 0.76) and adrenal glands (N = 46, mean=0.43 ± 1.15), P < 0.001 (Fig. 3A).

The mRNA expression of the UGT2B15 gene in the liver (N = 48, mean = 184.6 ± 198.6) was significantly higher than in the kidneys (N = 21, mean = 20.4 ± 30.65, P < 0.001) and adrenals (N = 40, mean = 42.17 ± 36.63 P < 0.01). The expression in the lungs (N = 37, mean = 50.79 ± 27.57) was significantly higher than in the kidneys (P < 0.01) (Fig. 3B).

For UGT2B17 the expression was most abundant in the liver (N = 38, mean = 124.8 ± 333.7) and significantly higher than the expression in lung (N = 23 mean = 10.35 ± 5.99, P < 0.05). Expression of UGT2B17 in the adrenals and kidneys were only found in three and six of the subjects and therefore no statistical analysis was made (Fig. 3C).

Correlation analysis between the mRNA levels in different tissues was performed. There was no correlation between UGT2B15 and 2B17 in the different tissues. For UGT2B7 there was a significant correlation between the expression in lungs and kidneys (N = 35, R = 0.54, P = 0.0007) (Fig. 4).

Genetic Variation and mRNA Expression. As expected, individuals devoid of UGT2B17 allele (16 of 60 fetal and 3 of 20 adult specimens) expressed no detectable levels of UGT2B17-specific mRNA. There was no significant difference between the UGT2B17 mRNA levels in UGT2B17 ins/ins and ins/del fetal samples except for in the lung. The UGT2B17 expression in lung samples from ins/ins subjects was significantly higher (mean 12.79 ± 5.50) compared with the lung specimens obtained from ins/del fetuses (mean 6.55 ± 4.76, P = 0.01) (Fig. 5).

Discussion
Here we show for the first time in humans that the mRNA expression of UGT2B enzyme genes in the liver is significantly lower in fetuses than in adults with a 13- to 36-fold difference. Our data are in agreement with a recent study in which Finel et al. showed that adult livers express 17–28 times more UGT2Bs than a pool of 63 fetal liver samples commercially obtained (age 22–40 weeks) (Court et al., 2012).

Interestingly, we found that the extrahepatic gene expression profiles of the fetal enzymes differ. As expected, UGT2B15 and UGT2B17 were found at highest level in the fetal liver as is also the case in adults (Ohno and Nakajin, 2009; Court et al., 2012). In contrast, UGT2B7 appeared to be more abundant in the fetal lungs and kidneys than in the liver. High levels of UGT2B7 have also been found in the adult kidney (Nakamura et al., 2008; Ohno and Nakajin, 2009; Court et al., 2012), whereas in the adult lung UGT2B7 expression has been found to be low (Nakamura et al., 2008) or undetectable (Court et al., 2012). In addition to being highly abundant in the fetal lung, UGT2B7 was expressed in all fetal lung samples analyzed. These findings indicate that UGT2B7 may have an
important role in the organogenesis or in the intermediary metabolism of the lungs, at least in the first trimester. All fetal samples in this study were obtained in the first trimester, and it is possible that the transcriptional expression profiles of the UGT2B enzymes are altered later in pregnancy.

In both adult and fetal samples we found a large interindividual variation in hepatic mRNA expression. This variation has also been seen in other population studies of UGT2B mRNA (Izukawa et al., 2009; Court et al., 2012). UGTs are inducible by various compounds. Therefore, it is possible that intake of drugs, alcohol, food, and/or various levels of endogenous substrates may affect the gene expression of UGT2B enzymes. Previous studies have shown that smoking may induce the activity of several UGTs (Fleischmann et al., 1986), but we did not find any correlation between maternal smoking and UGT expression in our samples.

The interindividual variation in mRNA expression could also be due to genetic variation in UGT2B genes, and therefore we genotyped the samples for the functional UGT2B17-deletion polymorphism. Individuals homozygous for UGT2B17-insertion allele (ins/ins) have been shown in previous study not to differ in liver mRNA expression as compared with ins/del in adult human liver samples (Gallagher et al., 2010). In agreement with this, we did not observe any difference in UGT2B17 mRNA levels between ins/del and ins/ins in the fetal or in the adult liver samples. However, we found a significant difference in UGT2B17 mRNA levels between ins/del and ins/ins in fetal lung samples. There is one study indicating that individuals with one UGT2B17 allele (ins/del) express lower levels of UGT2B17 mRNA than do individuals homozygous for the insertion allele (ins/ins) in adult prostate tissue (Karypidis et al., 2008). Therefore one may speculate that the UGT2B17 deletion polymorphism has a higher impact on the UGT2B17 gene expression in extrahepatic tissues. In addition to genetic variation, gender has been shown to affect the mRNA expression levels of UGT2B7, 2B15, and 2B17 (Ramirez et al., 2008; Gallagher et al., 2010; Ekström et al., 2012). We did not observe any differences in UGT2B gene expression between the sexes among the adult liver samples. Unfortunately, the sex of the fetuses was not consistently determined at collection of tissue specimens used in our study. For obvious reasons a limitation with our study is the small size of tissue specimens available from the fetuses in the age range studied. Most of the material was consumed for RNA/DNA preparations. Because of this no protein- and UGT-activity measurements could be investigated. It is known that the UGT enzyme activity may be affected by posttranscriptional and posttranslational regulations. However, we believe that the inclusion by our study group of gene expression in different tissues obtained from 60 fetuses is unique and for the first time provides information about the interindividual and intrarandom UGT variation in gene expression in the first trimester.

It has been known for a long time that the glucuronidation activity in fetuses is markedly lower than in adults, but the reason for this has not been thoroughly elucidated. Here we provide evidence that the abundance of UGT2B7 transcripts is low in the fetus, which explains the low glucuronidation activity observed. The fetal levels of UGT2Bs as compared with the adult levels observed here and by Finel et al. (Court et al., 2012) correlate well with previous catalytic pathways studied in vitro. Thus, the activity in fetal microsomes for formation of testosterone and androsterone glucuronides was found to be 3 and 8% of the adult activity (Leakey et al., 1987), whereas the rate for morphine glucuronidation was 10-20% (Pacifici et al., 1982). The concentration of the cosubstrate UDPGlcUA may also be a rate-limiting factor in the fetus, which has been noted to be five-fold lower in the fetal liver compared with adult liver (Cappiello et al., 2000).

For some substrates the deficient glucuronidation capacity seems to be compensated for by development of sulfate conjugation pathways (Rollins et al., 1979; Pacifici et al., 1989). Drugs that in addition to UGT also are sulphotransferases substrates, e.g., acetaminophen, may be inactivated (at least partly) by these enzymes in the human fetus (N = 31), whereas drugs that are exclusively conjugated by UGTs may be less rapidly inactivated in the fetus, making them potentially more toxic. The prenatal developmental pattern of UGT enzymes may help us understand the neonatal disposition of drugs that are conjugated by UGT enzymes. Such drugs used in newborn infants include morphine.

In conclusion, we have shown that the hepatic mRNA expression of UGT2B7, 2B15, and 2B17 is significantly higher (13- to 36-fold) in adults compared with fetuses. We have also shown that the tissue-specific expression of the different UGT2Bs in the fetus is different, i.e., UGT2B17 and 2B15 are found at highest levels in the liver, whereas UGT2B7 is mainly expressed in lungs and kidneys. It is of great interest to further characterize and study-drug metabolism in the fetus, since fetal metabolism may contribute to the overall fetal-maternal clearance and have an impact on feto-toxic effects mediated by bioactivation.

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