Metabolizing enzyme localization and activities in the first trimester human placenta: the effect of maternal and gestational age, smoking and alcohol consumption

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BACKGROUND: The rationale for this study was to assess the expression, activity and localization of the enzymes uridine diphosphate glucuronosyltransferase (UGT), β-glucuronidase, cytochrome P450 1A (CYP1A) and cytochrome P450 2E1 (CYP2E1) in first trimester human placenta and to gauge the effects of maternal variables on placental metabolism. METHODS: CYP1A, CYP2E1, UGT and β-glucuronidase activities were assessed in 25 placentas using ethoxyresorufin, chlorzoxazone, 4-methylumbelliferone and 4-methylumbelliferone glucuronide respectively. Protein expression and localization were detected by immunoblot and immunohistochemistry. All statistics were non-parametric. RESULTS: UGT, β-glucuronidase and CYP1A activities were detected in all placentas sampled; CYP2E1 was undetectable. CYP1A, UGT1A UGT2B proteins were detected in all placentas (n = 6) tested and CYP2E1 in 4/6 placentas sampled and were localized to the syncytium. UGT and CYP1A activities were significantly elevated in the placentas of mothers who smoked (P < 0.05 and P < 0.001 respectively) and were greatest in women who both smoked and drank alcohol (P < 0.05 and P < 0.01 respectively). Enzyme activities were significantly negatively correlated with gestational age (P < 0.05, r = 0.54, UGT) and maternal age respectively (P < 0.001, r = 0.63, CYP1A). β-Glucuronidase activity did not differ with patient variables. CONCLUSIONS: Metabolism of compounds by the human placenta in the first trimester may be affected by maternal and environmental factors altering the activity of constitutive metabolizing enzymes.

Key words: alcohol consumption/CYP1A/placental metabolism/smoking/UDP-glucuronosyltransferase

Introduction

Many compounds ingested during pregnancy will enter the fetal circulation and therefore have the potential to affect the fetus (Morgan, 1997). These compounds must first pass through the placenta, the primary organ of communication between mother and fetus (Boyd and Kudo, 1994). This fetomaternal transport is established at ~5 weeks gestation (Briggs et al., 1994).

It is during the embryonic period (the first 2 months of gestation) that the organs and tissues of the embryo develop, and during the fetal period (2–9 months) that organ maturation and growth occurs (Sastry, 1991). The greatest risk of teratogenic effects occurs during the embryonic period, when the placenta is not fully mature or functional and the embryo is differentiating (Sastry, 1991). Unfortunately many women do not recognize that they are pregnant until after the fetal period, and consequently continue behaviours such as cigarette smoking and drinking alcohol that they would otherwise choose to avoid (Bar-Oz et al., 1999).

The mechanisms of teratogenicity are diverse and not fully elucidated. However, it has been proposed that some congenital defects could result from direct interference with placental growth, development and function at an early stage (Sastry, 1991). In addition, it has been suggested that some metabolizing enzymes may be involved in the differentiation and growth of the fetus. For example, constitutive absence of cytochrome P450 1B1 (CYP1B1) in embryonic tissues results in primary congenital glaucoma (Stoilov et al., 2001). Thus enzymes which have previously been designated ‘xenobiotic-metabolizing’ may play a role in morphogenesis and embryogenesis during pregnancy. Consequently, understanding the characteristics of placental metabolism and transfer of both xenobiotics (such as drugs and environmental compounds) and endobiotics (such as hormones), and defining the placenta’s role in both protective and harmful consequences for the fetus are essential.

The cytochromes P450 (CYP) are a structurally and functionally diverse superfamily of enzymes. They are involved...
extensively in oxidation processes throughout the body and are found in almost every cell type (Klaassen, 1996). Most of the oxidative metabolism of xenobiotics in the human body is catalysed by CYP isoforms (Lewis, 2000). Of the CYP isoforms, CYP1A and CYP2E1 are good candidates for study in the placenta due to their proven role in metabolism of cigarette smoke (Whyyatt et al., 1998) and alcohol (Song et al., 1990), both of which have been demonstrated to be deleterious to the fetus during pregnancy via maternal exposure (Jones and Smith, 1973; Chattinegius et al., 1993).

The CYP1A subfamily is comprised of two isoforms, CYP1A1 and CYP1A2, which catalyse metabolic activation and detoxification of aromatic hydrocarbons (CYP1A1) and aromatic amines (CYP1A2) (Hakkola et al., 1997; Whyyatt et al., 1998; Yueh et al., 2001). CYP1A is the most commonly and consistently expressed CYP isoform in the human placenta (Audus, 1999) and, although a variety of other CYP are present, they occur in varying amounts dependent upon gestational stage, health and exposure of the mother (Pelkonen, 1984; Hakkola et al., 1996a,b).

CYP2E1 is involved in the metabolism of low molecular weight pro-carcinogens (e.g. nitrosamines), organic solvents (including alcohol) and some drugs such as paracetamol and chlorozoxazone (Song et al., 1990; Hu et al., 1999). The expression of CYP2E1 in pregnancy has been investigated principally in relation to its induction/suppression profile in mothers consuming alcohol and the enzyme’s association with fetal alcohol syndrome—a collection of congenital abnormalities including low birth weight, small for gestational age babies, mandibular hyperplasia and developmental delay (Jones and Smith, 1973; Campbell and Fantel, 1983; Autti-Ramo and Granstrom, 1991).

The uridine 5’-diphosphate glucuronosyltransferases (UGT; EC 2.4.1.17) are, arguably, the most important enzymes involved in conjugative metabolism and elimination of compounds from the body. UGT catalyse the addition of glucuronic acid to a hydrophobic molecule, thus producing metabolites which are more polar and more readily eliminated (Mackenzie et al., 1997). Functional UGT are not observed in the fetal liver at measurable levels until some time after birth (Onishi et al., 1979; Coughtrie et al., 1988), and developmental deficiency of UGT is a major cause of jaundice among neonates (Tribelli and Ostrow, 1996). We hypothesize that the presence of UGT in the placenta may play a protective role during gestation through metabolism and clearance of potentially harmful compounds.

β-Glucuronidase (EC 3.2.1.31) is an enzyme which cleaves glucuronosyl bonds with a β-configuration (Sperker et al., 1997). It is present in many tissues including liver, spleen, kidney, intestine and lung as well as most blood cells and exhibits large inter-individual variability in activity and expression (Sperker et al., 1997). β-Glucuronidase-catalysed hydrolysis of glucuronides can cause, for example, enterohepatic recirculation (whereby glucuronide metabolites are degraded and the parent drug is reabsorbed) which may increase the exposure of patients to a drug (Sperker et al., 1997). Cleavage of glucuronides may also give rise to mutagenic and/or carcinogenic events, as seen with the glucuronide of 3-benzo(a)pyrene, which is cleaved by β-glucuronidase to a toxic intermediate (Kari et al., 1985). β-Glucuronidase expression and activity in the human placenta has been previously described (Desoye et al., 1992), although no correlations with maternal factors were reported.

In order to gain a better understanding of the placenta’s capacity for drug metabolism, and its response to xenobiotic exposure, we have studied the expression, localization and activity in the first trimester human placenta of CYP1A, CYP2E1, UGT and β-glucuronidase. In addition, the activity of these enzymes in relation to maternal variables including smoking, drinking, ethnicity (Caucasian versus other), maternal age and gestational age was investigated.

Materials and methods

The following reagents were used: 4-methylumbelliferone (4-MU), chlorozoxazone (both ICN Biomedicals, Aurora, OH, USA); 4-methylumbelliferone glucuronide (Lancaster Chemical Company, Lancaster, Lancs, UK); acrylamide (Biorad, Hercules, CA, USA); enhanced chemiluminescence reagent and NEN TSA 700A Immunohistochemistry Kit (NEN Lifesciences, Boston, MA, USA); Hybrid-P PVDF (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England); uridine diphosphate glucuronic acid (UDPGA; Roche Diagnostics Ltd, Auckland, New Zealand); secondary biotinylated antibody and normal donkey serum (Jackson Laboratories, Westgrove, PA, USA); 96-well Spectraplate® microtitre plates (Whatman, London, UK); bromophenol blue, diaminobenzidine (Sigma Chemical Co., St Louis, MO, USA), bovine serum albumin (BSA; free fatty acid, fraction V; Life Technologies, Auckland, New Zealand); antibodies to the UGT1A subfamily, UGT2B7 and human lymphoblasts expressing UGT2B7 (Gentest Corp., Woburn, MA, USA). Ethoxyresorufin was synthesized by Dr Brian Palmer, Cancer Research Laboratories, The University of Auckland, New Zealand. Antibody to UGT2B4 was a gift from Drs Jacques Magdalou, Sylvie Fourmél-Gigleux and Anna Radominska Pandya and was generated by Mohamed Ouzine (University of Nancy, France and University of Arkansas, USA). UGT2B antisera was a gift from Dr Alain Bélanger (Université Laval, Quebec, Canada). All other reagents were obtained from a variety of commercial sources and were analytical grade or better.

Tissue collection procedure and protein preparation

Twenty-five villous placental tissue samples were collected by blunt dissection within 30 min of delivery from women (age range 15–41 years) undergoing elective termination of pregnancy during the first trimester (gestational age 8–12 weeks) at Epsom Day Unit, Auckland, New Zealand. All samples were collected under ethical approval from the Auckland Human Ethics Committee and all participants gave informed consent. Placental tissue was dissected, placed in ice-cold 67 mM phosphate buffer with 1.15% KCl, pH 7.4, homogenized immediately and centrifuged to prepare microsomes as previously described (Collier et al., 2002). Protein content was measured with the bichinchoninic acid method using BSA as the protein standard (Smith et al., 1985).

Western blotting

Microsomal proteins extracted from placenta (30 µg), HEK293 cells (negative control, 15 µg) and female human liver (positive control, 10 µg) were resolved on 7.5 and 10% sodium dodecyl sulphate–polyacrylamide electrophoretic gels under reducing conditions, transferred to Hybond-P PVDF membrane and blocked in 5% non-fat milk powder overnight. Membranes were incubated with primary antibody (1:2000 UGT2B and CYP2E1, 1:1000 UGT2B7, UGT1A
and CYP1A and 1:250 UGT2B4) for 2 h at room temperature, washed and incubated with a secondary, biotinylated antibody (1:10 000) for 1 h. Antibody detection was subsequently performed using streptavidin–horseradish peroxidase complex (1:3000), visualized by enhanced chemiluminescence and exposed to X-ray film. Individual blots were performed for each enzyme or isofrom.

**Immunohistochemistry**

Immunohistochemistry was performed with tyramide amplification using an NEN 700A Signal Amplification Immunohistochemistry Kit as per the manufacturer’s instructions as previously described (Collier et al., 2002).

**Measurement of UGT activity with 4-MU**

UGT activity was measured with a fluorescent microplate assay as previously described (Collier et al., 2000). Female human liver microsomes were used as a positive control.

**Measurement of β-glucuronidase activity with 4-methylumbelliferone glucuronide**

We used a modification of a previously published method (Trubetskoy and Shaw, 1999). Briefly, 60 µg microsomal protein was placed in a 96-well microtitre plate with pre-warmed (37°C) 0.1 mol/l Tris–HCl pH 7.3 and 20 µl 4-methylumbelliferone glucuronide (0–1000 µmol/l final concentration) such that the final volume was 200 µl. Fluorescence was observed over time at 355 nm excitation and 460 nm emission (15 nm bandwidth) in a Victor Multiplate reader (Wallac) with appearance of fluorescent substrate indicating the cleavage of the glucuronide by β-glucuronidase. Results were transformed to pmol/min/mg protein by using a standard curve generated with 4-MU (0–1000 µmol/l).

**Measurement of CYP1A activity with ethoxyresorufin**

The method used was a modification of a published procedure (Dutton and Parkinson, 1989). Human placental microsomes (0.1 mg) were added to a 96-well microtitre plate with 170 µl of ethoxyresorufin (0–1000 µmol/l final concentration) in 0.05 mol/l Tris buffer, pH 7.4 at 37°C and MgCl₂ (25 mmol/l). The plate was placed in a fluorimeter and NADPH (5 mmol/l final concentration) added before reading. The final volume was 200 µl. Fluorescence (excitation 530 nm; emission 585 nm) was monitored for appearance of the fluorescent resorufin product. Results were transformed to pmol/min/mg protein using a standard curve generated with resorufin (0–1000 nmol/l).

**Measurement of CYP2E1 activity with chlorzoxazone**

Microsomal incubations were carried out in a 1 ml reaction mixture containing 0.4 mg microsomal protein, 600 µl potassium phosphate buffer (pH 7.4) and 400 µmol/l chlorzoxazone. After pre-incubation at 37°C for 3 min, NADPH co-factor (1 mmol/l final concentration) was added to initiate the reaction and the mixture incubated for 40 min in a shaking water bath. The reaction was terminated by adding 50 µl of 43% phosphoric acid, the internal standard (15 µg phenacetin) added and the incubation mixture extracted with 2 ml chloroform/2-propanol (85:15 v/v). After centrifugation at 3000 g for 10 min, the organic phases were removed, dried down in a Speedvac SC200 (Savant) and the products analysed by high-performance liquid chromatography (Lucas et al., 1996).

**Data collection and statistical analyses**

Maternal data were collected and recorded by social workers at the clinic at the time of tissue collection and analysed retrospectively. Patients were asked a series of questions by social workers to assess whether they smoked, drank alcohol and took medication during their pregnancy. Enzyme kinetic modelling of data from individual placenta was performed by fitting results to Michaelis–Menten curves. Individual curves for each placenta were generated in triplicate and means were derived from the parameters for each curve. Non-parametric Spearman’s rank-sum correlation for co-variance or Mann–Whitney U-tests for differences between groups were performed for the UGT, β-glucuronidase and CYP1A enzymes. For the comparison of enzyme data with maternal details, non-parametric Kruskal–Wallis tests with Dunn’s post analysis were performed. All curve-fitting and statistical analyses were performed using GraphPad Prism 3.0 (San Diego, CA, USA).

**Results**

**Protein expression**

Both the UGT1A subfamily (Figure 1A, 55 kDa) and the UGT2B (Figure 1B, 55 kDa) subfamily were detected with Western blotting in all six placentas sampled. The UGT2B subfamily isoforms UGT2B4 and UGT2B7 were also detected in all placentas (Figure 1C, 52 kDa and Figure 1D, 56 kDa respectively). Protein products for CYP1A (55 kDa) were observed in all placentas sampled and CYP2E1 protein (54 kDa) was observed in 4/6 placentas sampled (Figure 1E).
Maternal variables affect placental metabolic activity

Figure 2. (A–H) Immunohistochemical analysis of first trimester placenta sections using specific primary antibodies (1:100 in Tris-buffered saline-Tween-20) visualized by diaminobenzidine staining. Uridine diphosphate glucuronosyltransferase (UGT)1A, 2B, 2B4 and 2B7 were present in the syncytial layer of the placental trophoblast covering the placental villi (A–D respectively). Cytochrome P450 1A (CYP1A) and cytochrome P450 2E1 (CYP2E1) were also present in the syncytium (E and F respectively). Specific staining is denoted by the arrow. Positive (anti-hCG; G) and negative (normal rabbit serum; H) controls are included for comparison and show some non-specific mesenchymal staining although nuclei are visible due to counter staining, they are not specific. Scale bar = 0–0.1 mm.

and F respectively). All proteins were observed in the human liver, including CYP2E1.

**UGT, CYP1A and CYP2E1 protein localization**

UGT1A, 2B, 2B4 and 2B7 were detectable with immunohistochemistry in each placenta tested and were localized to the syncytium of the placental villi (Figure 2A–D respectively). CYP1A and CYP2E1 (Figure 2C and D respectively) were also observed in the syncytium. Positive (anti-hCG; Figure 2G) and negative (Figure 2H) controls are included for comparison. No significant staining was noted in the fetal endothelium (the lining of the fetal capillaries) or the villous mesenchyme.

**Enzyme activity**

Collated results for enzyme activity are presented in Table I. UGT, β-glucuronidase and CYP1A activities were present in all placentas tested and a positive skew in UGT activity was
Table 1. Table of derived enzyme kinetic parameters in the first trimester human placenta

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$V_{\text{max}}$ (nmol/min/mg protein)</th>
<th>$K_M$ (µmol/l)</th>
<th>CLint (min/mg×l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT</td>
<td>65.2 ± 47 (10.7–172)</td>
<td>1168 ± 620 (187–2230)</td>
<td>$6\times10^{-5} ± 1\times10^{-5}$ (2.5–8×10^{-5})</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>0.011 ± 0.004 (0.004–0.023)</td>
<td>9.20 ± 13.4 (0.54–49.0)</td>
<td>$5\times10^{-6} ± 5\times10^{-7}$ (4×10^{-7}–1.5×10^{-5})</td>
</tr>
<tr>
<td>CYP1A</td>
<td>0.057 ± 0.037 (15.7–151)</td>
<td>268 ± 209 (19.5–859)</td>
<td>$3.27\times10^{-7} ± 2.1\times10^{-7}$ (9.9×10^{-9}–5.4×10^{-7})</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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Uridine diphosphate glucuronosyltransferase (UGT), β-glucuronidase and cytochrome P450 1A (CYP1A) were assessed using the fluorometric probe substrates 4-methylumbelliferone (0–1000 µmol/l), 4-methylumbelliferone glucuronide (0–1000 µmol/l) and ethoxyresorufin (0–1000 µmol/l) respectively. CYP2E1 was assessed with high-performance liquid chromatography using chlorzoxazone at a single substrate concentration (400 µmol/l). Results are a mean ± SD of 25 individual placentas, each assessed in triplicate with the range for all 25 placentas included. CLint = intrinsic clearance ($V_{\text{max}}/K_M$ ratio); ND = not detected.

Figure 3. First trimester placenta shows significant correlations with maternal variables. Gestational age correlates negatively with uridine diphosphate glucuronosyltransferase (UGT) activity (A, $P < 0.05$, Spearman’s rank-sum correlation) while maternal age correlates negatively with cytochrome P450 1A (CYP1A) activity (B, $P < 0.001$). Levels of UGT (C) and CYP1A (D) activity were significantly increased in smokers compared with non-smokers, when no other maternal variables were taken into account (Mann–Whitney U-test). When the effects of smoking and drinking were analysed separately, an apparent synergistic effect on UGT (E) and CYP1A (F) activities was observed (Kruskal–Wallis with Dunn’s post test). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

2568
observed, similar to that previously reported by us in the term placenta (Collier et al., 2002). Compared with human liver, the first trimester placenta had lower β-glucuronidase and CYP1A activity but higher UGT activity (on a per milligram or protein basis).

CYP2E1 activity was not detectable with chlorzoxazone in first trimester human placentas but was detectable in human liver microsomes (used as a positive control). The limit of sensitivity for the assay was 0.51 ± 0.27 nmol of product \((n = 5)\) and the limit of quantification 10 nmol of product \((n = 5, \text{coefficient of variation } 13.4\%)\).

**Correlation of enzyme activities to patient data**

Variation in enzyme activities for UGT, β-glucuronidase and CYP1A were analysed with respect to maternal smoking, drinking, age and ethnicity (Caucasian, 16 versus other, 9) as well as gestational age. Although data on patient’s medications were available there were insufficient of any one group to make meaningful comparisons.

UGT activity from first trimester placentas did not vary significantly with maternal ethnicity \((P = 0.07, \text{Caucasian versus non-Caucasian, Mann–Whitney U-test})\) nor did it correlate with either maternal age \((P = 0.70, \text{Spearman’s correlation})\) or alcohol consumption \((P = 0.10, \text{Mann–Whitney U-test})\). However, there was a significant negative correlation \((P = 0.04, r = 0.54, \text{Spearman’s correlation, Figure 3B})\) between UGT activity and gestational age. Significantly greater UGT activity was also observed in smoking mothers \((P = 0.015, \text{Mann–Whitney U-test, Figure 3C})\). A significant relationship \((P = 0.0189; \text{Kruskal–Wallis with Dunn’s multiple comparison test, Figure 3E})\) was observed between placental UGT activity from mothers who were (i) non-smokers or drinkers, \(n = 7\); (ii) drinkers only, \(n = 3\); (iii) smokers only, \(n = 9\); or (iv) both drinkers and smokers, \(n = 6\). This analysis showed that placentas from mothers who both smoked and drank had significantly greater UGT activity \((P < 0.05)\) than non-smokers and drinkers.

Placental CYP1A activity in the first trimester did not correlate significantly with gestational age or ethnicity \((P = 0.88 \text{ and } P = 0.94, \text{Spearman’s correlation and Mann–Whitney U-test respectively})\). However, a significant negative correlation of activity with maternal age in years \((P = 0.0009, r = 0.63, \text{age range } 15–41, \text{Figure 3A})\) was observed, with younger mothers showing greater placental CYP1A activity than older mothers. There was no significant difference in the ages of women who smoked compared with non-smokers in our study \((P = 0.12, \text{unpaired } t\text{-test})\) which rules out the potential confounding effect of younger women being more likely to smoke and thus skewing the data in relation to maternal age and smoking. CYP1A activity from the placentas of smoking mothers was also significantly greater than CYP1A activity from non-smokers \((P < 0.0001, \text{Mann–Whitney U-test, Figure 3D})\).

Placental CYP1A activity was compared in mothers who (i) didn’t drink or smoke, \(n = 7\); (ii) drank only, \(n = 3\); (iii) smoked only, \(n = 9\); and (iv) both drank and smoked, \(n = 6\); during their pregnancy (Kruskal–Wallis test with Dunn’s multiple comparison test). Highly significant differences were observed \((P < 0.001, \text{Figure 3F})\). Placentas from drinkers did not differ from controls, whereas placentas from smokers showed significantly greater CYP1A activity than those from controls or women who drank during pregnancy \((P < 0.05)\). Placentas from women who both smoked and drank during their pregnancy showed significantly greater CYP1A activity than women who did not drink or smoke and women who only drank \((P < 0.01 \text{ and } P < 0.05 \text{ respectively})\) and also significantly greater CYP1A activity than women who only smoked during their pregnancy \((P < 0.05)\). These data suggest that drinking and smoking may have synergistic effects on CYP1A activity in human first trimester placentas.

β-Glucuronidase activity did not vary significantly with any maternal variables.

**Discussion**

**Expression, localization and activity of xenobiotic-metabolizing enzymes in the first trimester placenta**

We have previously demonstrated that the human placenta at term expresses RNA and protein from the UGT2B subfamily but not the UGT1A family and that the UGT2B isoforms were active and present in the syncytiotrophoblast at term (Collier et al., 2002). In the current study we have shown for the first time that both the UGT1A and 2B subfamily proteins (including the isoforms UGT2B4 and UGT2B7) are present in first trimester human placenta, as are the CYP isoforms CYP2E1 and CYP1A. All of these enzymes are localized in the syncytiotrophoblast of the villous placenta at the maternal–fetal interface and are not observed in the fetal endothelium. As UGT2B4 and UGT2B7 have ~85% primary sequence homology (Tukey and Strassburg, 2000), it is impossible to rule out cross-reactivity of the antibodies in the tissues. Our contention that protein products for both isoforms are present therefore requires confirmation with alternative technologies.

We have also demonstrated greater UGT enzyme activity in the first trimester than in term placenta as previously reported by us (Collier et al., 2002). A similar positive skew in the data is evident.

The greater level of activity toward the probe substrate 4-MU in first trimester placenta compared with term may be ascribed to two major causes. Firstly, although the substrate 4-MU is considered to be ‘non-specific’ for UGT activity, it is metabolized primarily by the UGT1A family (Burchell et al., 1995; Cheng et al., 1998; Strassburg et al., 1998). Some members of the UGT2B family including UGT2B4, UGT2B11 (Jin et al., 1993), UGT2B15 (Green et al., 1994) and UGT2B7 (Ritter et al., 1990; Jin et al., 1992; Chen et al., 1993) have also been reported to metabolize 4-MU in vitro although at rates ~10-fold lower than the UGT1A isoforms. Thus, the greater activity towards 4-MU in first trimester placental tissue compared with that reported at term may be due to the presence of the UGT1A subfamily.

Secondly, at term the placenta is functionally an end-stage organ and the trophoblast as a percentage of placental mass decreases (Fox, 1997). As immunohistochemical analysis
revealed that the UGT enzymes were localized to the syncytiotrophoblast layer bordering the placental villi, it would be reasonable to expect greater activity in the first trimester placenta on a per milligram of protein basis due to the extracted microsomes containing a greater percentage of trophoblast-derived protein.

On a per milligram of protein basis, the first trimester human placenta has greater UGT activity than the human liver. This is consistent with placental UGT activity previously reported in rats (Litterst et al., 1975). However, due to the lower yield of protein from placental tissue compared with liver tissue (1 versus 10 mg/g), the tiny size of the placenta in the first trimester (~6 g at 12 weeks) and the reported inaccuracies of scaling UGT data from in vitro to in vivo (Miners et al., 2000), the intrinsic clearance contribution by the first trimester placenta to total maternal clearance would not be significant.

β-Glucuronidase enzyme activity was also observed in the first trimester human placenta, but at a rate ~10 000-fold less than UGT. No skew was observed in β-glucuronidase activity data. Likewise, CYP1A activity and protein expression was detectable in all first trimester placentas sampled with no appreciable skew in the data.

It is interesting that, although we were able to detect weak expression of CYP2E1 with immunoblot and immunohistochemistry, we were unable to detect activity of the enzyme even in mothers who both smoked and drank alcohol. These findings are consistent with other researchers who have variously reported positive RT–PCR (Farin et al., 1994), Southern blots (Hakkola et al., 1996b) and immunoblots (Rasheed et al., 1997) but undetectable CYP2E1 enzyme activity (Rasheed et al., 1997; McRobie et al., 1998). To our knowledge, the only other researcher to present immunoblots of placental CYP2E1 did so with samples of term placenta from mothers with heavy alcohol consumption (Rasheed et al., 1997). This suggests that although CYP2E1 has been shown to be readily inducible in human liver with ethanol consumption (Koop and Tierney, 1990), the mechanisms of induction in the human placenta differ and may be more influenced by genetic rather than environmental factors.

**Correlation of enzyme activities and maternal variables**

The significant negative correlation between UGT activity and gestational age in the first trimester placentas reinforces the immunochemical and biochemical data which demonstrated a decline in UGT1A protein and a fall in UGT activity across gestation.

The highly significant correlation between CYP1A activity and maternal age in the first trimester placenta is interesting. It is well documented that fertility and pregnancy outcomes deteriorate with age and some congenital syndromes such as Down’s syndrome occur in greater frequency as the mother ages (Sigler et al., 1965; Bardham, 1966; Kane, 1967). The greater activity of an enzyme such as CYP1A in placentas from younger mothers may indicate that placental function also declines with maternal age and it is possible that this is a factor in the increase in negative pregnancy outcomes in older women. Although information regarding maternal age-related changes in placental function is scarce, one study has reported lower hCG secretion in the first trimester placenta of older women compared with younger women, although the decrease was not statistically significant and the pregnancies were complicated by Down’s syndrome (Weinans et al., 2001). Data on parity for each woman was not obtained in our study, thus it is possible that the number of previous pregnancies experienced by each woman may contribute to the trends observed.

The expression and activity of CYP1A in the human placenta and CYP2E1 in the human liver have previously been shown to be modified by environmental exposure to cigarette smoke and alcohol respectively (Conney, 1986; Terelius et al., 1991; Rasheed et al., 1997). Both smoking and alcohol consumption have been implicated in adverse outcomes for the neonate. With smoking, placental vascular abnormality, low birthweight and pre-term birth have been identified (Piran and MacGillivray, 1978; Pasanen et al., 1986; Cnattingius et al., 1993; Moore and Zaccaro, 2000) and with alcohol consumption, the fetal alcohol syndrome (Campbell and Fantel, 1983; Autti-Ramo and Granstrom, 1991; Rasheed et al., 1997). It has also been shown that UGT can be induced in vitro by polycyclic aromatic hydrocarbons, which are components of cigarette smoke (Grove et al., 2000), but to our knowledge, no data have been reported to substantiate in-vivo effects of smoking on UGT expression and activity. Likewise, it has been demonstrated that transcriptional induction of UGT of both the 1A and 2B family can be caused by ethanol in rats (Li et al., 1999; Kardon et al., 2000), yet no data have been reported in humans. We have shown that both UGT and CYP1A enzymes had greater levels of activity in first trimester placentas from smoking mothers. The induction of CYP1A enzymes in the placentas of smoking mothers is entirely consistent with the literature (Conney, 1986; Sesardic et al., 1990; Hakkola et al., 1997; Whyatt et al., 1998). However, the induction of UGT by smoking in vivo is a novel observation. Mechanistically, the transcriptional activation of both CYP1A and some of the UGT isoforms (UGT 1A6 and 1A9) may be facilitated in part by the Ah-receptor (Bock et al., 1999). Components of cigarette smoke have previously been shown to interact with this receptor (Hakkola et al., 1997, 1998; Whyatt et al., 1998).

The apparent synergistic effect of smoking and drinking which correlates with greater activity of both CYP1A and UGT is also novel, if not entirely unexpected. Evidence from chronic ethanol consumption studies in CYP2E1 knockout mice has demonstrated that many CYP including CYP1A, 2A12, 3A and 2B, are able to be up-regulated in the liver by chronic ethanol consumption (Kono et al., 1999). Acute ethanol consumption studies in rats have also found that CYP1A was increased in the liver in response to ethanol (Lechevrel and Wild, 1997). Thus the observed synergism may well be due to components of cigarette smoke and alcohol having differential but complementary actions in increasing CYP1A and UGT activities.

A caveat to our study design is that mothers were not monitored throughout pregnancy nor were biomarkers of smoking and drinking [such as cotinine levels for smoking mothers (Pasanen et al., 1986)] available. As smoking and
drinking were ascertained through a questionnaire, absolute exposure to cigarette smoke and alcohol could not be verified, leading to an element of self-reporting error. Furthermore there may be some under-reporting bias in our data due to the current, negative social climate towards drinking and smoking during pregnancy. The magnitude of this bias is likely to be small for first trimester patients because these women did not carry their babies to term and the potential damage to the fetus would not be expected to be a significant factor.

Smoking and drinking have also shown to be additive behaviours, especially among women, and it is known that smokers who drink are more likely to be heavier smokers than those who abstain from alcohol (Ma et al., 2000; Hoffman et al., 2001). It is possible that an increased exposure to cigarette smoke in women who both smoked and consumed alcohol during their pregnancies caused the apparent increases in CYP1A and UGT activity rather than a synergistic mechanism.

The role of CYP activity in the placenta and its relative contribution to toxic versus non-toxic outcomes is controversial. It is well recognized that CYP1A can bioactivate many compounds, especially polycyclic aromatic hydrocarbons. However, the consistent observation of CYP1A in developing tissues has led to the proposal that the enzyme is an essential part of cell cycle control mechanism during differentiation (Beresford, 1993; Delescluse et al., 2000). Induction of CYP1A in the placenta in response to environmental stimuli may be regarded in this light, as a protective response in maintaining growth and developmental processes. Furthermore, our observation of a significant negative correlation of CYP1A activity with maternal age suggests that maternal genetic factors are implicated in the regulation of enzyme activity in the human placenta.

Evidence of a detoxification role with regard to xenobiotics in the first trimester placenta can be extrapolated from the low activity of β-glucuronidase compared with UGT. Comparison of the enzymes’ activities, which is appropriate as essentially the same probe substrate (4-MU and its glucuronide metabolite) was used, indicates that glucuronide metabolism in the placenta during early gestation is shifted towards conjugation and elimination. Differential expression of UGT enzymes across gestation (with the UGT1A family present in the first trimester but not at term) suggests developmental regulation of the enzymes and supports our hypothesis that UGT in the placenta are primarily fetoprotective during the critical embryogenic and organogenic stages prior to 12 weeks gestation.

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