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SHORT COMMUNICATION

Detection of Cytochrome P450 Gene Expression in Human Placenta in First Trimester of Pregnancy

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ABSTRACT. Human first-trimester placentas were screened for the expression of xenobiotic-metabolizing cytochrome P450 (CYP) genes. mRNAs of CYP1A1, CYP1A2, CYP2C, CYP2D6, CYP2E1, CYP2F1, CYP3A4, CYP3A5, CYP3A7, and CYP4B1 were identified by reverse transcriptase-polymerase chain reaction (RT-PCR) in at least some of the six placental samples studied. CYP2A and CYP2B messages were absent in all samples. The level of all of these CYP mRNAs was lower compared to the corresponding levels in liver or lung. The catalytic activity marker (7-ethoxyresorufin O-deethylase) was inducible in the placentas by maternal cigarette smoking. Thus, the regulatory system of placental *CYP1A1*, mediated by the Ah-receptor, appears to be developed as early as the first trimester of pregnancy. Three immunoreactive bands from placental microsomes were detected by an antihuman CYP3A4 antibody, but no functional activity of CYP3A enzymes could be detected. These results show that placental tissue during the first trimester of pregnancy has the potential of expressing several CYP genes, and forms a basis for subsequent analysis of these forms at the protein and functional level. BIOCHEM PHARMACOL 52;2:379–383, 1996.

KEY WORDS. PCR; placenta; cytochrome P-450; early pregnancy; human; smoking

The human placenta is able to metabolize many foreign chemical compounds, although both the substrate and the metabolizing enzyme profiles are more restricted than those in liver [1]. Most of the studies of the xenobiotic-metabolizing capacity in the human placenta have been performed, obviously because of easy availability, with full-term placentas. There are, however, indications that gestation time has a significant effect on placental CYP[‖] gene expression. Placental AHH activity is induced by maternal cigarette smoking and parallels the induction of CYP1A1 [2, 3]. This induction is dependent on the stage of pregnancy and the activity is highest at term, and AHH activity is still very low at the end of the first trimester [4, 5]. CYP3A7 has been shown to be expressed in the first and second trimesters of pregnancy [6], but could not be detected at term [7]. Therefore, it seems that gestation time and maturity of placental tissue could affect expression of xenobiotic-metabolizing CYP forms in the placenta.

We have recently established a reverse transcriptase-polymerase chain reaction (RT-PCR) method to detect mRNAs of distinct xenobiotic-metabolizing forms in CYP1–4 families in human tissues. Using this approach, we were able to detect mRNA of several CYP forms in human full-term placentas [7]. In this study, we screened for the presence or absence of individual xenobiotic-metabolizing CYP mRNAs in first-trimester placentas. Our main interest was the CYP1A1 and CYP3A subfamily, in which protein products were also assessed using catalytic activity and immunoblot analysis.

MATERIALS AND METHODS

Human Placenta Tissue

Six placentas (gestation age 10–12 weeks) were obtained at abortions performed for sociomedical reasons at the Department of Obstetrics and Gynaecology, University of Kuopio. Placenta 6 was from a nonsmoker, and the smoking status of donor number 13 is unknown; the other placentas were from smokers. Placenta samples were frozen immediately in liquid nitrogen and stored at −70°C until used. Clinical data for the full-term placentas used for immunoblotting has been described earlier [7]. Use of human tissue in this study was approved by the Ethics Committees of the Universities of Oulu and Kuopio.

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‖ *Abbreviations:* CYP, cytochrome P450; RT-PCR, reverse transcriptase-polymerase chain reaction; AHH, aryl hydrocarbon hydroxylase; ECOD, 7-ethoxycoumarin O-deethylase; EROD, 7-ethoxyresorufin O-deethylase.

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RT-PCR

mRNA was extracted and cDNA was synthesized as described previously [8]. The PCR reaction was performed according to an earlier study [7]. The CYP primers and their locations have been described previously [7], except CYP1A1, sense primer: CCTTTGAGAAGGGCCACATC, antisense primer: GATGGGTTGACCCATAGCTT. The primers were designed to hybridize only to the desired regions of the cDNA. The specificity of the primers was characterized in an earlier study [7]. The following primers can detect more than one form: CYP2A6/2A7, CYP2B6/2B7, and CYP3A3/3A4. The CYP2C(8–19) primers were designed to detect all known human CYP2C cDNAs (i.e. 2C8, 2C9, 2C10, 2C18, and 2C19. For CYP3A3 and CYP3A4, only the designation CYP3A4 is used in this report.

In every series of PCR reactions, there were 2 negative controls, 1 containing cDNA synthesis reaction performed without template (negative control I) and another containing mRNA extraction reagents, but no cDNA template (negative control II). To control the efficiency of PCR reactions, a positive control (human liver or lung sample) was used in all PCR series. After amplification, 8 μL of the reaction mixture was electrophoresed in an agarose gel and stained with ethidium bromide. To control for reproducibility, all amplifications were repeated at least once. To ensure the identity of the PCR products, the DNA was transferred to Qiabrane nylon filter (Qiagen, Chatsworth, CA, U.S.A.) and hybridized with specific, ^{32}P-labeled cDNA probes.

cDNA Probes

CYP2F1, CYP2E1, CYP3A4, and CYP3A5 full-length cDNAs were generously provided by Dr. F. J. Gonzalez (Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD, U.S.A.). CYP1A1, CYP1A2, CYP2C8, CYP2D6, CYP3A7, and CYP4B1 cDNAs were prepared by using RT-PCR to generate 600–1000 bp amplification products from human liver or placental RNA. PCR products were subsequently cloned into pCR™II (TA-cloning Kit, Invitrogen, San Diego, CA, U.S.A.) and sequenced to verify the correct identity of the cDNAs.

Microsomes and Enzyme Assays

Microsomes were prepared by the standard ultracentrifugation technique, as described earlier [9]. Enzyme assays were performed in duplicate according to earlier reports: aromatase [10], testosterone 6β-hydroxylase and androstendione formation from testosterone [11], 7-ethoxycoumarin O-deethylase (ECOD) [12], and 7-ethoxyresorufin O-deethylase (EROD) [13].

Immunoblot Experiments

Immunoblotting was performed as described earlier [7]. CYP3A4 (formerly named P-450 NiF or P450-5) was purified from adult human liver and polyclonal antibody raised in rabbits [14]. This antibody reacts with all members of the CYP3A subfamily. The horseradish peroxidase-labeled second antibody (ECL Western blotting kit, Amersham, Buckinghamshire, U.K.) was used. Chemiluminescence-based detection of proteins was performed according to the manufacturer's protocol, and autoradiograph film (Hyperfilm-MP, Amersham, Buckinghamshire, U.K.) was exposed to the sheet for a suitable length of time.

RESULTS AND DISCUSSION

PCR

Expression of cytochrome P450 genes in *CYP1–CYP4* families was investigated in 6 individual placenta samples by RT-PCR. mRNAs in each subfamily, excluding CYP2A and CYP2B, were found to be present in at least some of the samples (Fig. 1).

All three CYP3A forms (CYP3A4, CYP3A5, and CYP3A7) gave a much stronger PCR amplification product in sample number 13 compared to the others. Although this qualitative PCR assay was not designed to produce quantitative data, this strong difference in amplification suggests that CYP3A mRNAs were more abundantly present in sample 13 than in the other placentas. Because a similar difference was not seen in the other CYP forms, this difference is not likely to be due to the higher quality of mRNA or cDNA. There was no maternal drug therapy that could have functioned as a metabolic inducer.

Altogether, 10 of the 12 CYP forms studied were detected in at least some of the samples. The present result gives a comprehensive initial picture of CYP enzymes potentially present in the early-term placenta, and provides evidence that subfamily CYP2A and CYP2B forms are absent in placental tissue.

Detection of Functional Proteins

After initial screening, the next step was to assess whether or not the mRNAs found are translated to biologically meaningful amounts of active protein. Elevated expression of CYP1A1 in full-term placentas from smoking mothers has been well characterized in a number of studies. In addition, there have been suggestions that at least one constitutive form, responsible for ECOD activity, would be expressed in placenta [1]. Recently, we showed by RT-PCR that at least six CYP genes (*CYP1A1*, *CYP2E1*, *CYP2F1*, *CYP3A4*, *CYP3A5*, and *CYP4B1*) were expressed in human full-term placenta. However, only CYP1A1 could be detected by catalytic activity or immunoblot methods [7].

Enzyme assays confirmed the presence of functional CYP enzymes in human placenta during early pregnancy (Table 1). Aromatase activity and androstendione formation from testosterone were detected and, thus, demonstrated the presence of functionally active proteins in all samples.

CYP1A1

ECOD and EROD activities were readily detected from placentas (Table 1). EROD activity most likely reflects the

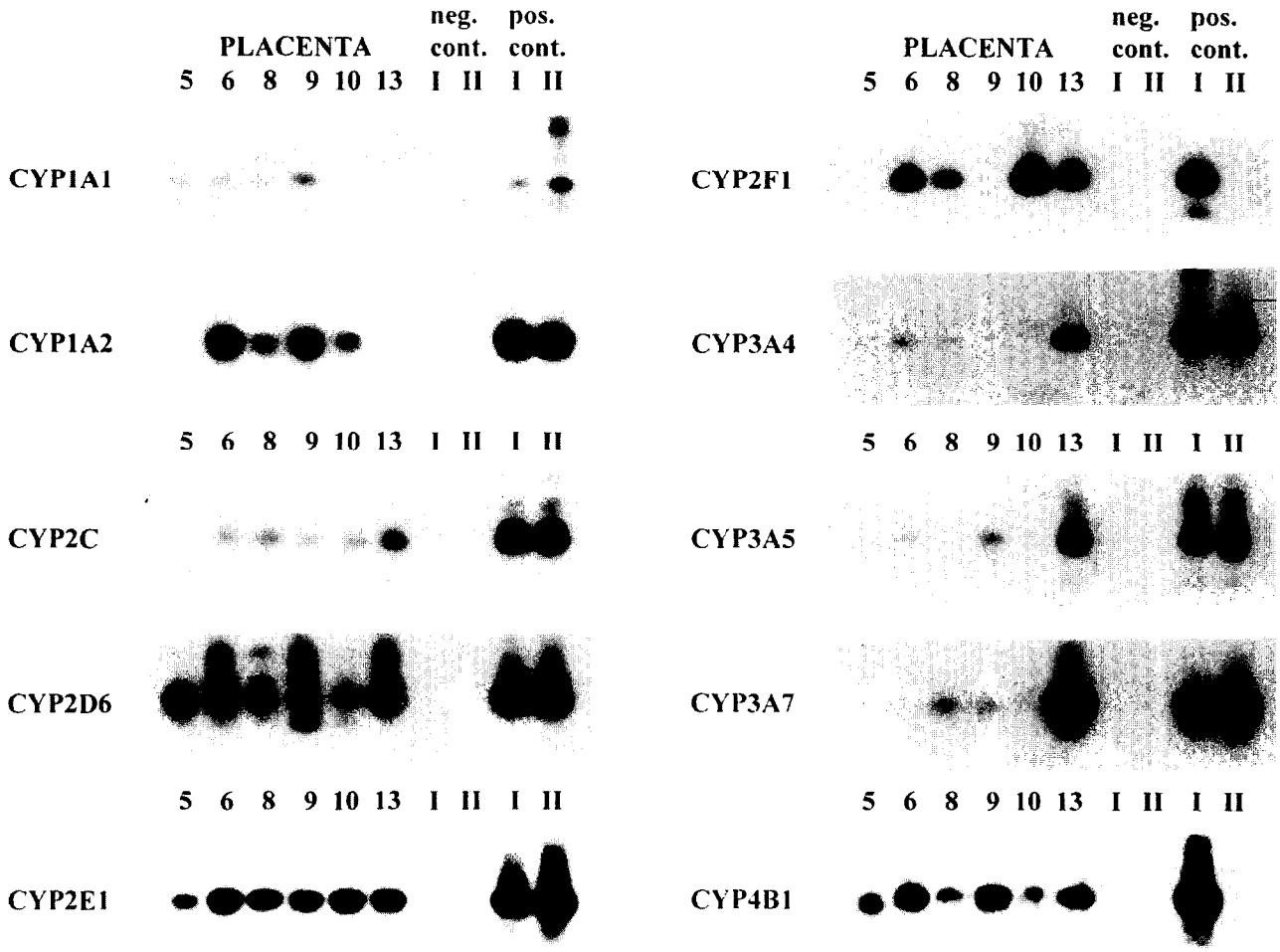

FIG. 1. Autoradiographs of the hybridized PCR products of CYP forms. Negative controls have been described in Materials and Methods. Positive controls are liver samples, except in CYP2F1 and CYP4B1: I, lung sample; II, empty line. Intensities of the bands in the different blots should not be compared because the exposure times vary.

function of the CYP1A1 enzyme in human placenta [3], although, in other tissues, other enzymes mediate EROD activity [15]. Thus, the inducibility of CYP1A1, mediated by the Ah-receptor complex, seems to be already developed in first trimester placentas. Earlier studies have suggested that AHH activity is poorly elevated by inducers during the first trimester [4, 5]. A more recent study, however, shows that placental AHH is inducible by maternal cigarette smoking as early as gestation age 8 to 11 weeks [16], a result that is in agreement with the present findings.

TABLE 1. Enzyme activities of placental microsomes

Placenta	AROM	ANDR	ECOD	EROD
5	44	27	15	nd
6	32	20	7.5	3.2
8	48	34	20	111
9	46	32	17	44
10	44	27	15	6.4
13	19	23	6.7	4.3

Aromatase (AROM), pmol/mg protein/min; androstendione formation from testosterone (ANDR), pmol/mg protein/min; ECOD, pmol/mg protein/min; EROD, pmol/mg protein/min; nd, not determined.

CYP3A

CYP3A4 is the most abundant P450 form in human liver, and metabolizes many clinically important drugs and chemical carcinogens [17]. CYP3A7 is the predominant form in fetal liver, and probably accounts for the major part of the fetal capacity for metabolising xenobiotics [18]. CYP3A7 has been detected by immunoblotting from human placentas during the first two trimesters of pregnancy [6]. CYP3A4 and CYP3A5, but not CYP3A7 mRNA, could be detected from full-term placentas by RT-PCR [7]. In the same study, antipeptide CYP3A4 antibody (which probably also recognizes CYP3A7) failed to detect any proteins in full-term placentas [7].

A polyclonal antihuman CYP3A antibody was used for detection of proteins in microsomal fractions of both full-term and early placentas (Fig. 2). Three immunoreactive bands (52 kD, 51 kD, and 49.5 kD) near the size expected

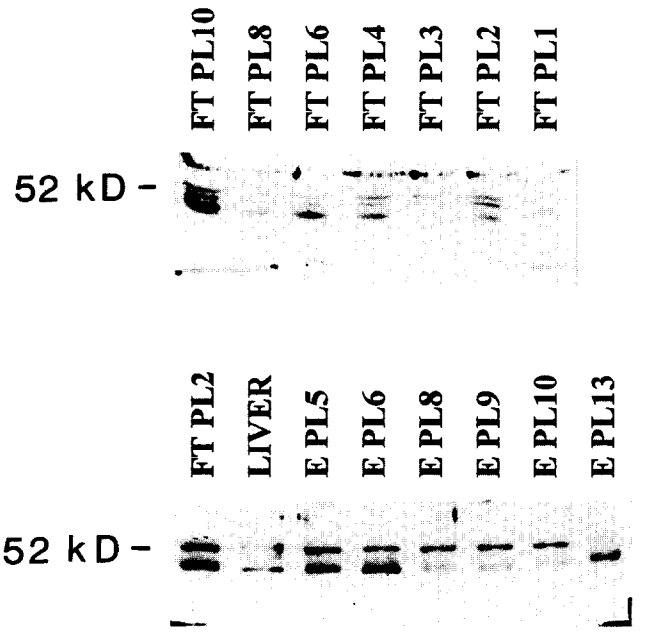

FIG. 2. Immunoblot analysis of microsomes from 7 human full-term placentas (FT PL), 6 placentas from early pregnancy (E PL) and one human liver. Immunoblots were developed with an antihuman CYP3A antibody. 20 μg of microsomal protein was used for placental samples and 0.2 μg for the liver sample. Three immunoreactive bands, 52 kD, 51 kD and 49.5 kD, were detected. For some unknown reason, the band in the middle was weaker in the lower blot and is poorly visible in the figure.

for the CYP3A proteins [19], were detected in both materials. Equal sized bands were detected also in human liver. The levels of these proteins in placentas were about 1/100 of the amount in liver. Some of these bands may represent CYP3A apoproteins. However, testosterone 6β-hydroxylase, an activity marker for CYP3A forms, was below the level of detection in all placental samples (data not shown) (detection level about 0.5 pmol/mg protein/min). The first trimester placenta number 13 did not differ in protein level from that in the other samples.

Clearly, a discrepancy exists in some of the CYP3A results. Our PCR results, and the immunoblot assay with polyclonal anti-CYP3A antibody in this study, together with earlier PCR results [7] and immunoblot data by Schuetz *et al.* [6], support the possibility that the CYP3A family is expressed in human placenta. However, the lack of CYP3A detection in full-term placentas using a specific antipeptide antibody [7] and, especially, undetected enzyme activity suggest that only very little functional protein exist. This inconsistency may be a consequence of different sensitivities of the methods. All together, these findings, however, suggest that at least the constitutive expression of CYP3A forms in human placenta is extremely low and functional activity is still unknown.

In conclusion, this study shows that several CYP mRNAs can be detected in placenta by RT-PCR as soon as the first trimester of pregnancy. This underscores the fact that RT-PCR is an extremely sensitive method that can detect mRNA levels far below the corresponding levels in the liver [20]. The number of different mRNAs detected decreases towards the end of pregnancy. During the first trimester of pregnancy the mitotic frequency is high, the placenta grows at an exponential rate, and rapid functional and structural changes take place. Therefore, the large number of P450 forms expressed in an early stage of pregnancy could be due to the development process, and only a few forms are expressed in the fully matured and functionally stable placenta at term. A tissue specimen used for extraction of mRNA always contains, in addition to trophoblast cells, other cell types. Blood vessels, especially are abundantly present in placental tissue. Human umbilical vein endothelial cells have been shown to possess CYP forms [21] and, also, several other studies have detected different forms of P450 in human blood vessels [20]. Thus, some of the CYP forms detected in this study may be derived from endothelial or other nontrophoblastic cells, and may partially explain the low-level expression of several CYP forms. It remains to be determined as to which forms expressed at the mRNA level actually produce enzyme activity, and whether or not forms other than CYP1A1 can be induced by drugs or environmental compounds. It is, hypothetically, possible that even very low activities could have local toxic or physiological consequences, justifying a more detailed analysis of the putative protein products of the CYP mRNAs present in the placenta.

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