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EXPRESSION OF XENOBIOTIC-METABOLIZING CYTOCHROME P450 FORMS IN HUMAN ADULT AND FETAL LIVER

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Abstract—Expression of human cytochrome P450 (CYP) genes in human adult and fetal liver were studied using the reverse transcriptase–polymerase chain reaction (RT–PCR) method. In adult liver mRNA of CYPs 1A1, 1A2, 2A6/2A7, 2B6/2B7, 2C8–19, 2D6, 2E1, 3A3/3A4 and 3A7 were detected while CYPs 2F1 and 4B1 were absent. In fetal liver mRNA of CYPs 2C8, 2D6, 3A3/3A4 and 3A7 were found but all other forms studied were undetectable. The results provide a comprehensive qualitative picture of the expression of CYP genes in families CYP1 through CYP4 in human adult and fetal liver.

Key words: cytochrome P450; xenobiotic; human liver; fetus; PCR

The CYP¶ superfamily consists of enzymes that carry out the oxidative metabolism of numerous endogenous and exogenous compounds [1]. Xenobiotics are mainly metabolized by families CYP1–4. It has been established that there are at least 19 CYP forms responsible for the metabolism of foreign substrates in humans [1]. Most of these are primarily expressed in the liver, but CYP1A1, CYP2F1 and CYP4B1 are mainly extrahepatic [1].

The human fetus is capable of metabolizing many xenobiotics relatively actively, usually at a lower rate than the adult [2, 3]. Human fetal liver contains multiple but fewer forms of cytochrome P450 as compared with adult liver [4]. There is evidence for the presence of mRNA or protein of members in the CYP1A [5–8] and CYP3A [9–12] subfamilies and CYP2D6 [13] and CYP17A [14] in human fetal liver.

The greatest difficulty in the study of fetal CYP system has been the low levels of individual forms present. RT–PCR allows detection of low quantities of mRNA with great sensitivity and specificity [15], making it very suitable for analysing human tissues. In this study the RT–PCR method was used to assess P450 gene expression in human adult and fetal liver.

MATERIALS AND METHODS

Liver samples. Six adult livers and 16 fetal livers were used in this study. The adult livers were obtained from the Department of Surgery, University of Oulu, Finland. Two of the samples were from organ donors who had died in accidents. Tissue from organ donors was removed within 30 min of death. The other four samples were biopsies taken during laparotomy in patients with liver disease.

Fetal liver tissues were obtained at abortions performed for socio-medical reasons. Nine of the fetal livers (U3–U43) were from Akademiska Hospital, Uppsala, Sweden. Seven fetal livers (K4–K13) were from Department of Obstetrics and Gynaecology, University of Kuopio, Finland. Clinical data of the fetal livers are summarized in Table 1. All the liver samples were frozen in liquid nitrogen and stored at −70° until mRNA extraction.

Table 1. Clinical data of human fetal livers used for RT–PCR analysis

Fetal liver	Gestational age (weeks)	Smoking status	Prostaglandin induced abortion
U3	24	?	+
U5	15	+	+
U9	21	+	+
U15	15	?	+
U18	13	?	+
U20	15	−	+
U29	17	+	+
U37	18	−	+
U43	19	+	+
K4	12	?	−
K5	12	−	−
K6	12	−	−
K7	12	+	−
K8	12	−	−
K10	11	−	−
K13	17	+	+

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¶ Abbreviations: CYP, cytochrome P450; RT–PCR, reverse transcriptase–polymerase chain reaction.

Table 2. Primers used for PCR analysis

CYP	Sense primer location	Antisense primer location	PCR product (bp)	Reference
1A1	TCACAGACAGCCTGATTGAG 928–947	GATGGGTTGACCCATAGCTT 1341–1360	432	17
1A2	TGGCTTCTACATCCCCAAGAAAT 1199–1221	TTCATGGTCAGCCCGTAGAT 1488–1507	308	18
2A6/2A7	GTGTGGACATGATGCCGT 252–269	AGGACTTGAGGCGGAAGT 1385–1402	1151	19
2B6/2B7	CCATACACAGAGGCAGTCAT 1045–1064	GGTGTCAGATCGATGTCTTC 1402–1421	357	20
2C8–19	GCTAAAGTCCAGGAAGAGATTGA	TCCTGCTGAGAAAGGCATGAAGT	332	21–22
2D6	TGATGAGAACCTGCGCATAG 873–892	ACCGATGACAGGTTGGTGAT 1186–1205	332	23
2E1	AGCACAACTCTGAGATATGG 925–944	ATAGTCACTGTACTTGAACT 1271–1290	365	24
2F1	ATGAACTTGCCGCACCGCGT 1148–1167	AGCGAAAAGCTCTGCAGGAT 1412–1431	283	25
3A3/3A4	CCAAGCTATGCTCTTCACCG 1279–1298	TCAGGCTCCACTTACGGTGC 1583–1602	323	26,27
3A7	CTATGATACTGTGCTACAGT 1041–1060	TCAGGCTCCACTTACGGTCT 1496–1515	474	28
4B1	TGACCATGTGCATCAAAGGAG 1109–1128	AAAGCCATTCTTGGAGCGCA 1487–1506	397	29

The storing times for the Uppsala samples were between 2 and 4 years. The Kuopio samples were used within 1 year. The use of human tissues in this study was approved by the Ethics Committees of the Universities of Oulu, Kuopio and Uppsala.

mRNA and cDNA. mRNA was extracted from about 50 mg of tissue with QuickPrep Micro mRNA Purification Kit and cDNA was synthesized with First-Strand cDNA Synthesis Kit (both from Pharmacia P-L Biochemicals, Uppsala, Sweden). The cDNA was stored at −20° until the PCR reaction was performed.

PCR. The PCR reaction contained 1 μL of cDNA (out of 15 μL total), 2.5 U DynaZyme DNA polymerase (Finnzymes, Helsinki, Finland), 5 μL 10× DynaZyme reaction buffer, dNTP reaction mix (Pharmacia) at a final concentration of 400 μM, 20–50 pmol of each primer and water to a final volume of 50 μL. Twenty-five PCR cycles were performed: 1 min at 94°, 1 min at 55° and 2 min at 72°.

The primers were designed to hybridize only to the desired regions of cDNA. To exclude chances of cross-hybridization with other sequences, each primer was compared with the EMBL human gene bank using the Genetics Computer Group FASTA program [16]. The primers were also designed to amplify regions containing at least one intron in the gene to exclude contamination of cDNA with genomic DNA. The primers, their locations and the sizes of the PCR products are listed in Table 2. The following primers can detect more than one isoform: 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.

The PCRs with CYP2A6/2A7, CYP2B6/2B7, CYP2C8–19, CYP2E1 and CYP3A7 for adult liver and CYP3A7 for fetal liver were performed as co-amplifications in the same tube with β-actin primers to control the efficiency of reaction. The other CYP primers were found to interact with the β-actin primers and could therefore not be co-amplified. In every series there were two negative controls, one containing no cDNA and another in which the cDNA reaction was done without the template. In other respects these controls were identical to the sample reactions. After PCR reaction, 8 μL of the reaction solution was electrophoresed in an agarose gel and stained with ethidium bromide.

Identification of PCR products. The CYP2C8–19, CYP3A3/3A4 and CYP3A7 PCR products amplified from fetal livers were purified with gel purification (Gene Clean II, Bio 101, La Jolla, CA, U.S.A.) and sequenced with direct sequencing (Cycle Sequencing Kit, Pharmacia, Uppsala, Sweden).

RESULTS

Expression of CYP genes in families CYP1–4 was studied with RT–PCR method in human adult and fetal liver tissues. A representative amplification result by each pair of primers with adult liver is shown in Fig. 1.

CYP gene expression was studied in six adult liver samples (Table 3). The mRNA of CYP2F1 and CYP4B1 was not detected in any of the samples. Low contents of CYP1A1 mRNA were observed in three out of six adult livers. All other CYPs were readily detected—2A6/2A7, 2B6/2B7, 2C8–19 and 3A3/3A4 in all samples, 1A2 and 2D6 in four out of six samples, and 3A7 in five out of six samples.

RT–PCR of 16 fetal liver samples was used to determine which of the CYP genes expressed in

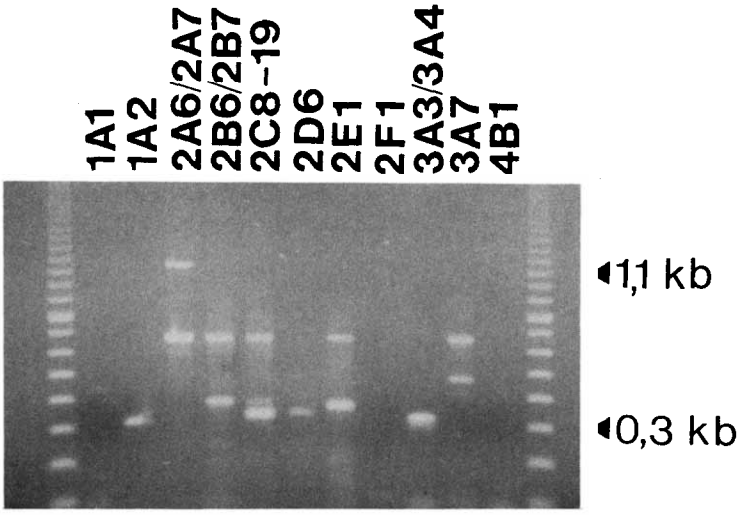

Fig. 1. A representative PCR amplification result by each pair of primers with an adult liver. The PCRs with CYP2A6/2A7, CYP2B6/2B7, CYP2C8–19, CYP2E1 and CYP3A7 were performed as co-amplifications in the same tube with β-actin primers. The 661 bp band in these lanes represent β-actin. The sample lanes are flanked by DNA molecular weight markers (100-base pair ladder).

Table 3. Summary of RT–PCR amplification results

	CYP										
	1A1	1A2	2A6/2A7	2B6/2B7	2C8–19	2D6	2E1	2F1	3A3/3A4	3A7	4B1
Adult liver											
5	−	+	+	+	+	+	+	−	+	−	−
6	+	+	+	+	+	+	+	−	+	+	−
8	−	+	+	+	+	+	+	−	+	+	−
10	−	+	+	+	+	+	+	−	+	+	−
15	+	−	+	+	+	−	+	−	+	+	−
16	+	−	+	+	+	−	+	−	+	+	−
Fetal liver											
U3	−	−	−	−	−	−	−	−	−	+	−
U5	−	−	−	−	−	−	−	−	−	−	−
U9	−	−	−	−	−	−	−	−	−	−	−
U15	−	−	−	−	−	−	−	−	+	+	−
U18	−	−	−	−	−	−	−	−	+	−	−
U20	−	−	−	−	−	−	−	−	−	−	−
U29	−	−	−	−	−	−	−	−	−	−	−
U37	−	−	−	−	−	−	−	−	+	−	−
U43	−	−	−	−	−	−	−	−	−	−	−
K4	−	−	nd	−	−	nd	−	nd	−	+	−
K5	−	−	nd	−	+	nd	−	nd	+	+	−
K6	−	−	nd	−	+	nd	−	nd	+	+	−
K7	−	−	−	−	+	+	nd	nd	+	+	nd
K8	−	−	−	−	+	+	nd	nd	+	+	nd
K10	−	−	−	−	+	+	nd	nd	+	+	nd
K13	−	−	−	−	+	+	nd	nd	+	+	nd

A right size band seen on gel is marked with +. A negative result is marked with −. Not determined (nd).

adult were also expressed in fetal liver (Table 3). Examples of positive amplifications using primers detecting members in CYP2C, CYP2D and CYP3A subfamilies are shown in Figs 2 and 3. CYP3A7 and CYP3A3/3A4 yielded the highest amounts of amplification products. They were both observed in nine out of 16 fetal livers. In the Uppsala material (U3–U43, N = 9), only members of 3A subfamily

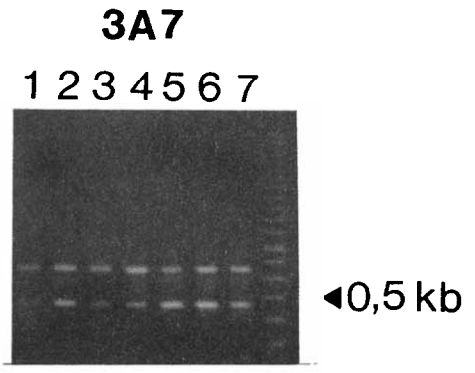

Fig. 2. Detection of CYP3A7 in seven fetal livers. PCRs were performed as co-amplifications with β-actin primers. The molecular weights of the CYP3A7 and β-actin products were 432 and 661 base pairs, respectively. Lane 1, fetal liver K4; lane 2, K5; lane 3, K6; lane 4, K7; lane 5, K8; lane 6, K10; lane 7, K13.

were detected. In the Kuopio material (K4–K13, N = 7), also 2D6 (all four livers studied) and members of the 2C subfamily (six out of seven) were detected. mRNA of CYP1A, CYP2A and CYP2B subfamilies, and CYP2E1, CYP2F1 and CYP4B1, were constantly undetectable. The decreased detection in the Uppsala material was ascribed to partial degradation of mRNA, due obviously to prostaglandin treatment and longer storage times at −70° in comparison with the Kuopio material. Thus, the CYP3A members could be detected in only some of the Uppsala samples and the CYP2C and CYP2D6 forms, which also produced weaker bands in Kuopio samples, were below detection level in the Uppsala material.

Direct sequencing of fetal PCR products yielded unequivocally the expected sequences, i.e. CYP3A3/3A4 (identical sequences in the amplified area) and CYP3A7 from respective amplification products. The sequence obtained from the CYP2C product proved to be CYP2C8.

DISCUSSION

Members of the CYP3A subfamily are considered to be major constituents in human adult and fetal liver [11]. Kitada *et al.* [9] purified P450HFLa (CYP3A7) from fetal liver. They showed, using an antiserum against P450HFLa, that in fetal liver this protein accounted for over 36% of total CYP content, but only 5% of that in adult liver. Wrighton *et al.* [30] detected CYP3A5 in one out of ten human fetal livers. Komori *et al.* [31] showed by northern blot analysis that CYP3A7 mRNA could be detected in fetal livers but not in adult livers. In contrast, CYP3A3 and CYP3A4 were detected only in adult liver [31]. With the RT–PCR method used here both CYP3A7 and CYP3A3/3A4 mRNA in both adult and fetal liver were able to be detected. It has been shown that CYP3A4 accounts for the majority of CYP3A species in human adult liver [32], making it likely that the CYP3A3/3A4 PCR product represents CYP3A4 mRNA. The PCR results suggest that in addition to CYP3A7 other members of CYP3A subfamily may also be translated to protein in fetal liver.

The presence of CYP1A1 in human adult liver has, until recently, been unclear. McKinnon *et al.* [33] demonstrated CYP1A1 mRNA in northern blot analysis in 11 out of 23 human liver samples. This PCR result, in which CYP1A1 mRNA was seen in three out of six liver samples, supported this observation. Appearance of CYP1A1 mRNA did not correlate with whether the patients smoked or not. Murray *et al.* [34] showed with anti-human CYP1A1 antibody that CYP1A1 protein was undetectable in human adult liver. These results suggest that CYP1A1 protein may not be constitutively present in human liver and that the expression may mainly be post-transcriptionally regulated.

There is some evidence for the expression of CYP1A1 in human fetal liver. Kitada *et al.* [7] showed that an anti-rat CYP1A1 antibody cross-reacted with P450HFLb purified from human fetal livers. They also showed that this form was distinct from CYP1A2. Omiecinski *et al.* [6] detected mRNA

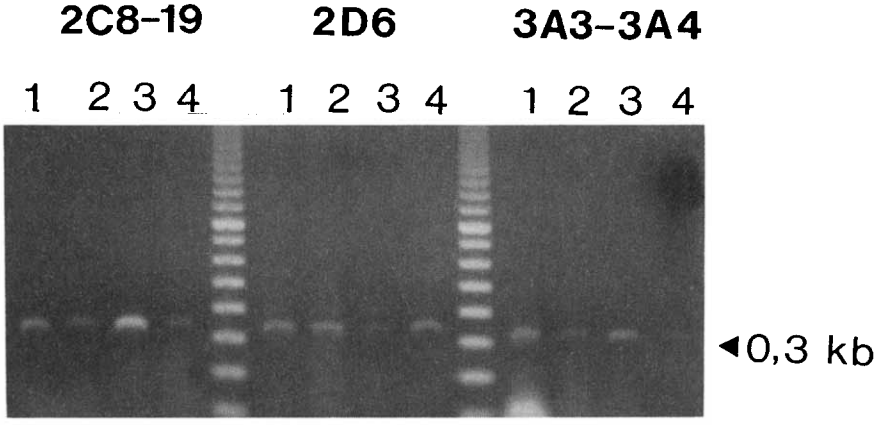

Fig. 3. Detection of CYP2C8–19, CYP2D6 and CYP3A3/3A4 in four fetal livers. Lanes 1, fetal liver K7; lanes 2, K8; lanes 3, K10; lanes 4, K13.

of CYP1A1 in fetal liver using PCR and observed an increasing content with increasing fetal age. In this study detection of CYP1A1 in fetal liver samples studied was not possible. The assay used by Omiecinski *et al.* [6] was more sensitive since more cycles and Southern blotting of amplification products were used. This facilitated the detection of extremely low contents of mRNA.

Ladona *et al.* [35] did not detect any protein in immunoblots of fetal liver with anti-human P450 2D6 antibody. Also, the metabolic data in the same study supported the absence of protein. Treluyer *et al.* [13] detected CYP2D6 mRNA in fetal liver. They also reported low CYP2D6 protein contents in some of the fetal livers. The protein was observed later than RNA, usually only after the 20th gestational week. Presence of this enzyme seemed to be associated more often than not with spontaneous abortion [13]. Here it is shown that CYP2D6 mRNA was present in at least a subset of fetal livers as early as 11 weeks of gestation.

There is very little evidence for the presence of members in the CYP2C subfamily in fetal liver. Ratanasavanh *et al.* [36] observed very low quantities of CYP2C with immunohistochemical, immunoblotting and mRNA blotting techniques in some fetal liver samples. Cresteil *et al.* [37] and Pons *et al.* [38] reported that anti-human CYP2C9 or anti-rat CYP2C11 antibodies did not cross-react with any proteins in fetal liver. Also, CYP2C9 related mRNA was not detected in any fetal liver [39]. Mephenytoin hydroxylase activity, which is associated with the CYP2C subfamily, was also very low [40]. Mäenpää *et al.* [41] showed that anti-mouse CYP2C antibody cross-reacted with one to three proteins in the fetal livers. The present study confirmed the existence of mRNA of CYP2C subfamily members in fetal liver. Sequencing of the CYP2C PCR product from fetal liver yielded the CYP2C8 sequence. Obviously, this form is the major, if not the only, isoenzyme among the CYP2C subfamily in fetal liver. Whether any other forms in CYP2C subfamily are expressed in fetal liver requires analysis with PCR primers, detecting individual members in this subfamily.

Jones *et al.* [42] reported that CYP2E1 RNA was not detectable with a full-length cDNA probe in fetal liver samples. The present results are in agreement with this.

CYP2F1 and CYP4B1 have been identified through cDNA cloning in human lung [25, 29], but neither one has been detected in human liver. Consistent with this, it was not possible in this study to amplify either CYP2F1 or CYP4B1 transcripts in adult or fetal livers.

It should be emphasized that in this study identical PCR conditions were used for the detection of CYP forms in both adult and fetal liver. This permitted direct comparison of adult and fetal CYP gene expression to be made. In conclusion, this is the first report to describe a comprehensive panel of xenobiotic-metabolizing CYP expression in fetal liver at early gestation (<24 weeks).

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