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Original article

Expression of *CYP1A1* and *CYP1A2* genes in human liver

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Immunoblot analysis of human livers using a monospecific antibody to rat CYP1A2§ demonstrated that the expression of CYP1A2 protein is highly variable in human liver. Quantitative PCR analysis was then employed to examine the interindividual variability of both CYP1A1 and CYP1A2 mRNAs in human liver. Hepatic content of CYP1A2 mRNA correlated significantly with levels of CYP1A2 protein as analysed by immunoblot analysis ($r = 0.58$; $p < 0.01$). CYP1A2 mRNA content varied >40-fold among individuals while CYP1A1 content varied >20-fold. CYP1A2 mRNA was higher than CYP1A1 mRNA (approximately two to 30-fold) in livers of different individuals. The individual with the highest CYP1A1 and CYP1A2 mRNA amounts was a current smoker, but mRNA expression in two other smokers was within the range observed among nonsmokers. The expression of the two CYP1A mRNAs correlated highly ($r = 0.72$; $p < 0.0005$) when smokers were included, but the correlation was less significant ($r = 0.62$; $p < 0.05$) in nonsmokers. We amplified a full-length *CYP1A2* cDNA clone by PCR from a liver which expressed extremely low amounts of CYP1A2 protein. Sequence analysis indicated that exon 4 was missing in this clone, but no other sequence changes were found. PCR analysis demonstrated that both the normally spliced mRNA and abnormally spliced mRNA could be detected in all human livers examined, but the normally spliced mRNA was more abundant than the splice variant. Therefore, sequence changes in the coding region of *CYP1A2* did not account for the poor expression of CYP1A2 in this individual.

Introduction

Multiple cytochrome P450 enzymes play a major role in the oxidative metabolism of a wide variety of chemically different endogenous and foreign substrates (Guengerich, 1991). To date, more than 150 cytochrome P450 genes and several pseudogenes have been identified in prokaryotic and eukaryotic organisms. Based on amino acid sequence similarities, these have been classified into 27 gene families. So far, the *CYP1* gene family comprises two members in all mammals studied, the *CYP1A1* and *CYP1A2* genes which encode the CYP1A1 and CYP1A2 proteins (Nebert *et al.*, 1991). CYP1A1 and CYP1A2 catalyse the activation of procarcinogens such as aromatic hydrocarbons and aromatic amines respectively (Butler *et al.*, 1989a,b; McManus *et al.*, 1990; Crespi *et al.*, 1991; Shimada & Guengerich, 1991). Therefore, these P450s have the potential to influence the development of chemically induced cancer in humans.

The expression of both *CYP1* genes is inducible by polycyclic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3-methylcholanthrene (3-MC) in both rodents and in human cells (Gonzalez, 1989). There is strong evidence that induction of *CYP1A1* is mediated through the aromatic hydrocarbon (*Ah*) receptor which binds as an inducer-receptor complex to *Ah*-responsive elements (*Ah*REs) in the 5′-flanking region of the gene (Nebert & Jones, 1989; Fisher *et al.*, 1990). The regulation of *CYP1A2* expression has not been explored as intensively as *CYP1A1*, but a 3-MC responsive regulatory element in the human *CYP1A2* gene has been reported (Quattrochi & Tukey, 1989). Despite an assumed common mechanism of induction, the *CYP1A1* and *CYP1A2* genes differ in their tissue-specific expression in

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§The abbreviations used are: PCR, polymerase chain reaction; CYP1A1, cytochrome P4501A1; CYP1A2, cytochrome P4501A2.

humans and in laboratory animals (Song *et al.*, 1985; McLemore *et al.*, 1990; Sesardic *et al.*, 1990; Pasanen *et al.*, 1990; McKinnon *et al.*, 1991). While CYP1A1 protein has generally been reported to be low or undetectable in human liver, it is induced in extrahepatic tissues of smokers. In contrast, expression of CYP1A2 appears to be restricted to the liver where it is expressed constitutively. The differential expression of these enzymes in hepatic versus extrahepatic tissues of humans resembles that reported earlier in rodents (Goldstein & Linko, 1984).

CYP1A2 has been reported to show considerable interindividual variation in human liver (Wrighton *et al.*, 1986; Sesardic *et al.*, 1988, 1990; Butler *et al.*, 1989b; Kalow & Tang, 1991). Since CYP1A1 and CYP1A2 are involved in the biological activation of procarcinogens, the mechanism responsible for the interindividual differences in the amounts of the two enzymes is of potential interest. In the present study, a quantitative assay for *CYP1A1* and *CYP1A2* expression was developed using the polymerase chain reaction (PCR) to quantitate the levels of the CYP1A1 and CYP1A2 mRNAs in human liver. Although CYP1A1 protein is at or below the limits of detection in human liver, CYP1A1 mRNA could be readily detected by PCR quantitation in the present study. CYP1A1 mRNA content of human liver was two- to 30-fold lower than CYP1A2 mRNA. We found a 40-fold variation in CYP1A2 mRNA in different human livers. The mRNA from the liver of an individual who expressed extremely low levels of CYP1A2 was amplified by PCR, and one clone was isolated which lacked exon 4. However, PCR analysis indicated that the normally spliced mRNA was more abundant than the splice variant, and no coding changes were found in CYP1A2 in this individual.

Materials and methods

Tissue samples and preservation

Through a collaboration with Dr David Nagorney at the Mayo Clinic (Rochester, Minnesota), human liver samples from nontumour tissues were obtained from individuals undergoing resection for suspected liver cancer. Healthy liver tissue and tumour tissue were separated and rapidly frozen in liquid nitrogen immediately after excision and were stored at −70°C. Data concerning age, gender, smoking status and

Table 1. Summary of liver patients

Subject ID no.	*Age (years)*	*Gender*	*Smoking status*[a]	*PPD smoked*	*Pack years*	*Tumour type*[b]
401	61	M	F (<1.0)	2.0	90	HCC
402	38	F	Y	1.0	14	FNH benign
403	77	M	F (32)	1–2	50	HCC
404	73	M	F (5)	0.5	23	HCC
405	75	F	N	—	—	Stomach[3]
406	46	F	N	—	—	HCC
407	58	M	F (<1)	0.5	24	HCC
408	48	F	Y	0.3	8	HCC
409	55	M	Y	2.0	80	HCC/CA
410	29	F	F (7)	0.2	0.5	HCC/adenoma
411	76	M	N	—	—	CA
412	80	F	N	—	—	HCC
413	68	F	F (38)	0.1	0.8	CA
414	71	F	F (3)	1.0	38	CA
415	64	M	F (20)	1.0	26	HCC
416	53	F	N	—	—	HCC
417	70	F	N	—	—	HCC
418	63	F	F	0.15	2.3	AML (benign)
420	82	M	N	—	—	HCC
421	61	M	F (<1)	1.0	11	Pseudotumor
422	63	F	N	0	0	HCC

[a]Y, current smoker; N, nonsmoker; F, former smoker (years since patient stopped smoking); PPD = pack of cigarettes smoked per day.
[b]HCC, hepatocellular carcinoma; CA, cholangiocarcinoma; AML, angiomyolipoma (benign).
[c]Metastases from stomach.

Table 2. Oligonucleotide primers

	Sequences	*Size of PCR product*
A	5′-TAGACACTGATCTGGCTGCAG-3′(1641–1661)(16/20 mismatches with *CY1A2*)	146
B	5′-GGGAAGGCTCCATCAGCATC-3′(1767–1787)(7/20 mismatches with *CYP1A2*)	
C	5′-ATCGCCTCTGACCCAGCTTC-3′(445–464)(2/20 mismatches with *CYP1A1*)	201
D	5′-CTCATCGCTACTCTCAGGGA-3′(626–645)(14/20 mismatches with *CYP1A1*)	
E	5′-AAACTACCTTCAACTCCATC-3′(868–887)	163
F	5′-ATGATCTTGATCTTCATTGT-3′(1011–1030)	
G	5′-ACAGTTGGTACAGATGGCATTGTCCCAGTC-3′(−13 to +20)	
H	5′-CCCTGGCCTCAGAATGGTGGTGTCTTCTTC-3′(1547–1576)	1589 (full-length)
I	5′-GGACTTTGACAAGAACAGTGTC-3′(819–840)	285 (normally spliced)
J	5′-AAGTAGGGCAGCTGGGGTCT-3′(1084–1103)	195 (splice variant)

Numbers in parentheses denote position of the oligo with respect to initiation codon.
A, B: CYP1A1 cDNA (Omiecinski *et al.*, 1990).
C, D, G, H, I, J: CYP1A2 cDNA (Jaiswal *et al.*, 1986).
E, F: β-actin cDNA (Ponte *et al.*, 1984).

histological type of liver cancer are summarized in Table 1. One human liver donor sample (SR19) obtained from Stanford Research International (Menlo Park, CA) was used in some preliminary immunoblots because it expressed relatively high levels of CYP1A2.

Preparation of microsomes and immunoblotting

Microsomes from human livers were prepared by standard techniques. Rat CYP1A1, CYP1A2, and polyclonal antibodies against these cytochromes were prepared and purified as reported earlier (Goldstein *et al.*, 1982). Antibodies to CYP2C8 and CYP2C9 were a generous gift from Dr Judy Raucy (University of New Mexico) and were prepared in rabbits as previously described (Leo *et al.*, 1988). Microsomal protein content was determined by the method of Lowry *et al.* (1951), SDS-polyacrylamide gel electrophoresis of microsomes was performed according to Laemmli (1970). Immunoblots for CYP1A2 were carried out as previously described (Goldstein & Linko, 1984) and scanned with a laser densitometer (LKB Instruments). Immunoblots for CYP1A1 were performed similarly and also using the ECL (enhanced chemiluminescence) Western blotting kit from Amersham (UK). Recombinant human CYP1A1 and CYP1A2 proteins expressed in microsomes of the AHH-1/TK+/− cell line (~ 30 pmol mg^{-1}) were obtained from Gentest, Woburn, MA. Recombinant CYP1A2 overexpressed in *E. coli* microsomes (1.4 nmol mg^{-1}) was obtained from Dr Ronald Estabrook, University of Texas Southwestern Medical School.

Comparative mRNA quantitation by PCR

Total RNA and poly(A+) RNA from human livers were isolated as described (Aviv & Leder, 1972; Chomczynski & Sacchi, 1987). Messenger RNA (5 μg) was reverse transcribed into cDNA in a 20 μl reaction mixture containing 50 mM Tris-Cl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.25 mM each of dATP, dCTP, dGTP, dTTP, 0.5 μg oligo $d(T)_{18-20}$, 40 U RNAsin (Promega, Madison, WI), and 400 U MMLV reverse transcriptase (Gibco BRL, Grand Island, NY). The samples were incubated at 41°C for 60 min, heated to 94°C for 2 min, and then quick-chilled on ice. First strand cDNAs were diluted in water to various concentrations and amplified by PCR. Reactions were carried out in a total volume of 100 μl. CYP1A1, CYP1A2, and β-actin cDNAs were amplified in 1X PCR buffer (67 mM Tris-Cl (pH 8.8), 17 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 7 μM EDTA, 0.2 mg bovine serum albumin ml^{-1}), 50 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol each of 5′ and 3″ primers, 2.5 U AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT), and 10^6 cpm of ^{32}P-labelled 5′-primer. The $MgCl_2$ concentration was adjusted to 1 mM for the amplification of the β-actin cDNA and to 2 mM for the two CYP1A cDNAs, respectively. After heating at 94°C for 5 min, amplification was performed in sequential cycles with denaturing at 94°C for 1 min, primer annealing for 1.5 min at 54°C (1A1), 2 min at 55°C (1A2), or 1 min at 51°C (β-actin), and primer extension at 72°C for 2 min.

Primer sequences used for the amplification of CYP1A1 (A,B), CYP1A2 (C,D), and β-actin (E,F), cDNAs and the predicted sizes of PCR products are given in Table 2. After preliminary experiments with two-fold dilutions of mRNA and varying cycles from 15 to 30, the amplification reaction was found to be exponential through at least 23 cycles. Three concentrations of input mRNA were amplified for 23 cycles

in subsequent experiments as specified in Results. PCR products were separated on a 1% agarose gel and transferred to Nytran membranes (Micron Separation Inc., Westboro, MA) by standard methods (Maniatis *et al.*, 1982). The membranes were autoradiographed for different periods of time at −70°C to ensure linearity of the signal with exposure time. Autoradiograms were scanned with a laser densitometer (LKB Instruments) and the calculated area under each peak was plotted against the amount of input mRNA as described (Murphy *et al.*, 1990). To estimate the ratio of CYP1A1/CYP1A2 mRNA in each liver, the same cDNA aliquot was amplified using identical amounts of radiolabelled primers, and products were electrophoresed, blotted, and scanned simultaneously. CYP1A1 and CYP1A2 values were compared by Pearson correlation coefficients (Snedecor & Cochran, 1980).

PCR amplification, subcloning and sequencing of a full-length CYP1A2 cDNA from a liver expressing low amounts of CYP1A2

PCR was used to generate a full-length CYP1A2 cDNA from the liver of an individual (sample 406, Table 1) who appeared to express phenotypically low amounts of CYP1A2 protein based on Western blot analysis (see Fig. 1A). The reverse transcription reaction was carried out as described above. The resulting single stranded cDNAs were amplified by PCR in a two-step reaction: annealing and extension of 25 pmol of 30-mer 5′ and 3′ primers (Table 2, G,H) at 72°C for 3 min and DNA denaturation at 94°C for 1 min. The 100 µl reaction contained 1 µg reverse transcribed mRNA equivalent, 1X Taq-buffer (Perkin Elmer Cetus), 50 µM of dATP, dCTP, dGTP, and dTTP, and 2.5 U AmpliTaq DNA Polymerase (Perkin Elmer Cetus). After fifty cycles of amplification PCR products were extended for 10 min at 72°C.

Aliquots of full-length CYP1A2 PCR products were ligated into the plasmid pCR1000 (Invitrogen, San Diego, CA). Potential recombinant plasmids were purified with a plasmid purification kit (Qiagen Inc., Chatsworth, CA). After repeated screening, a single recombinant plasmid containing a CYP1A2 cDNA was recovered. The double-stranded cDNA was completely sequenced with Sequenase (U.S. Biochemical Corp., Cleveland, OH).

Amplification and direct sequencing through exon 4 of CYP1A2 cDNA

Reverse transcribed mRNA was amplified by PCR under identical conditions as described above. Oligo-nucleotide primers I and J (Table 2) were annealed at 59°C for 1 min and extended for 3 min at 72°C for 40 cycles. Amplified products were purified by agarose gel electrophoresis and resuspended in 50 µl water. Sequence analysis was carried out using 1–10 µl resuspended template DNA and one ^{32}P-end-labelled primer (1 pmol) in an asymmetric PCR.

Results

Immunoblot analysis of CYP1A1 and CYP1A2 in human liver

Microsomal fractions from twenty human nontumour liver samples were examined for CYP1A2 protein expression by immunoblotting using monospecific polyclonal antibody raised against the purified homologous rat protein. The antibody to rat CYP1A2 recognized a single immunoreactive protein in each of the human liver samples tested with a mobility on SDS polyacrylamide gels similar to that of rat CYP1A2 and human recombinant CYP1A2 (Fig. 1A). This antibody did not recognize recombinant human CYP1A1 (Fig. 1B), indicating that the protein in human microsomes is CYP1A2. Expression of CYP1A2 was extremely variable among the twenty individuals as indicated by large differences in the intensities of the immunostained protein bands. In contrast, Western blot analysis of CYP2C8 and CYP2C9 indicated that these two cytochromes were much less variable in the same samples than CYP1A2 (Fig. 1C). Three individuals (402, 405, 408) expressed high levels of CYP1A2, fifteen individuals expressed intermediate levels, and CYP1A2 was barely detectable in two individuals (406 and 407). Two of the high individuals (samples 402 and 408) and one low-intermediate individual (409) were smokers.

Using a very sensitive chemiluminescence method for immunoblots, antibody to rat CYP1A1 recognized recombinant human CYP1A1 and recognized a very faint band with a similar mobility in a few human samples (SRI9, 402, and 408) as shown in Fig. 1B for SRI9. However, this antibody was cross-reactive (~10%) to recombinant human CYP1A2 with a modified N-terminus expressed at high levels in *E. coli* (Fig. 1B, middle panel). Comparison of immunoblots of recombinant human CYP1A1 and CYP1A2 expressed in AHH/TK-cells indicated that the unmodified human proteins have very similar mobilities on SDS polyacrylamide gels (7.5–10%) (Fig. 1B, right panel). Therefore, we were unable to conclude whether or not CYP1A1 protein is expressed in human liver.

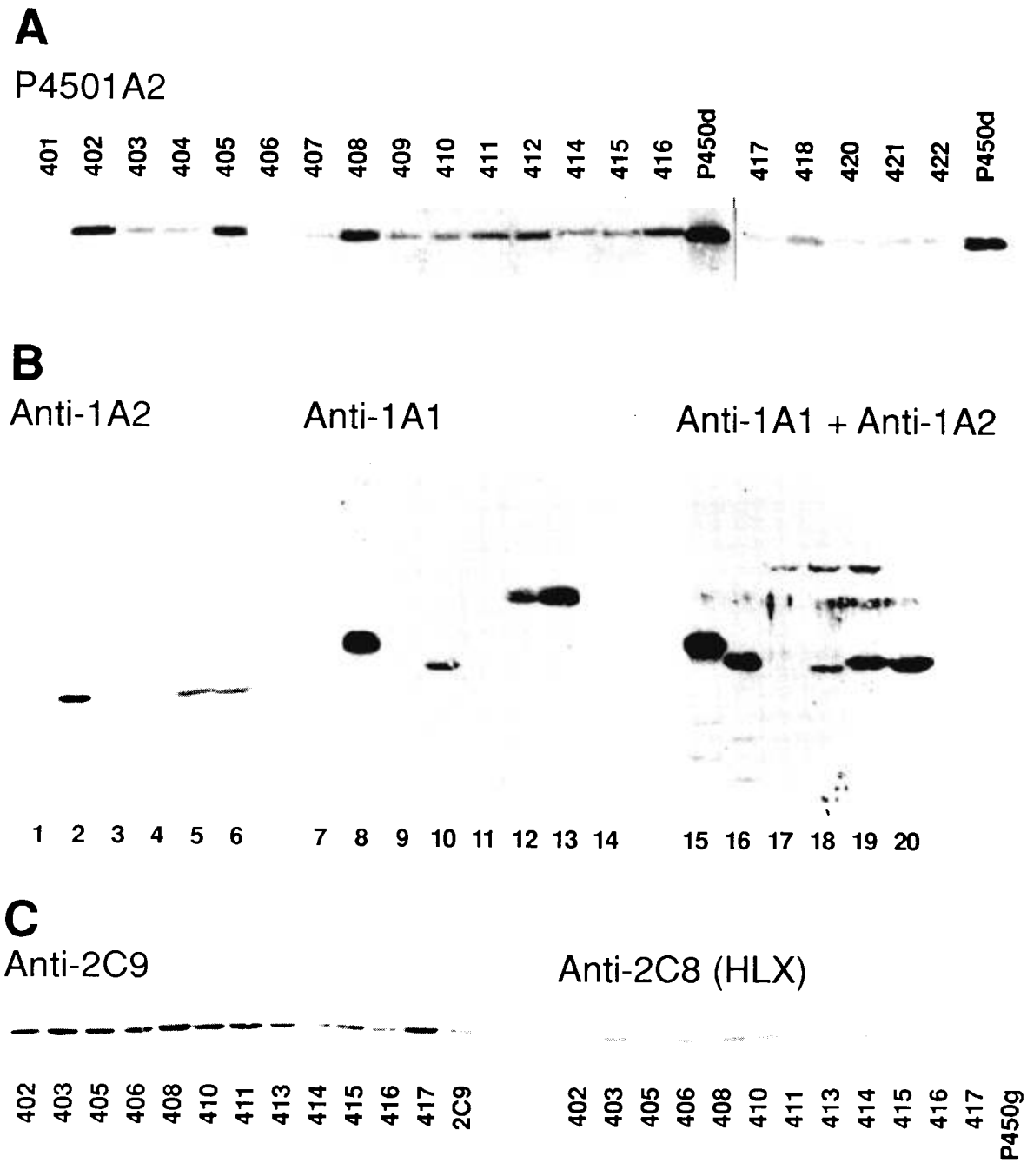

Fig. 1. Immunoblot analysis of human liver microsomal fractions.

(A) Human liver microsomal proteins from 20 individuals were separated on a 10% denaturing polyacrylamide gel, blotted and stained with monospecific polyclonal antibody raised against purified rat CYP1A2 (A, 20 μg protein per sample). Lane 'P450d' contains purified rat CYP1A2.

(B) Shows the specificity of the antibody to rat CYP1A2 (left panel) and antibody to rat CYP1A1 (middle panel) for recombinant human CYP1A2 and CYP1A1 on immunoblots. The right panel is an immunoblot developed with both antibodies. Lanes 1, 8, and 15 contained 2 pmol of purified rat CYP1A1, lanes 2, 7, and 16 contained 2 pmol purified rat CYP1A2, lanes 3, 9, and 17 contained control AHH TK+/− cell microsomes (100 μg), lanes 4, 10, and 18 contained 100 μg AHH TK+/− cell microsomes containing recombinant human CYP1A1 (~3 pmol), lanes 5, 11, and 19 contained 100 μg AHH TK+/− cell microsomes containing recombinant human CYP1A2 (~3 pmol), and lanes 6, 14, and 20 human liver microsomes (100 μg) from sample SRI9, Standford Research Institute. Lanes 12 and 13 contained recombinant *E. coli* membranes containing 30 and 150 pmol of CYP1A2 with a modified N-terminus.

(C) Some of the same human liver microsomes shown in (A) were blotted with antibody to human CYP2C8 and antibody to human CYP2C9. Lanes '2C9' and 'P450g' in (C) contain purified human 2C9 and purified rat CYP2C13.

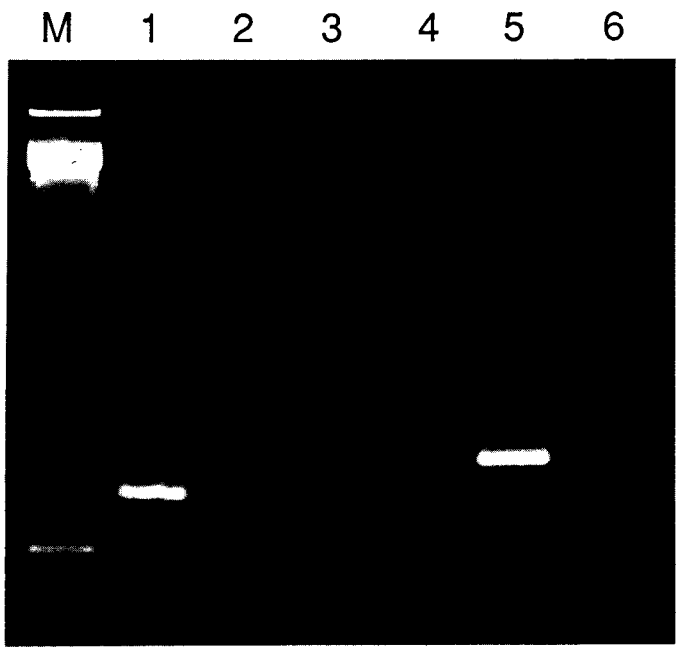

Fig. 2. Amplification products of reverse transcribed mRNAs. Ethidium bromide-stained agarose gel containing PCR products for β-actin with (lane 1), CYP1A1 (lane 3), and CYP1A2 (lane 5). As controls, lanes 2 (β-actin), 4 (CYP1A1), and 6 (CYP1A2) contained all reagents including mRNA which had not been reverse transcribed. Forty nanograms of cDNAs were amplified for 40 cycles and 16 µl of each reaction were analysed on an agarose gel. M, 123 bp-ladder. The sizes were identical to those predicted in Table 1.

PCR analysis of CYP1A1 and CYP1A2 mRNAs in human liver

Because of the difficulties in detecting human CYP1A1 protein in human liver, we utilized a sensitive PCR technique to examine the expression of CYP1A1 and CYP1A2 using specific primers to amplify short regions of both mRNAs. The PCR products with the predicted sizes for the amplified cDNAs for CYP1A1 and 1A2 mRNA (Table 2) are shown on an ethidium bromide-stained agarose gel (Fig. 2, lanes 3 and 5). In addition, restriction of the CYP1A1 PCR fragment with *Bam* HI yielded the expected fragments with sizes of 93 and 53 base pairs (bp) (data not shown), while CYP1A2 has no *Bam* HI restriction sites. Restriction of the CYP1A2 PCR fragment with *Mbo* I resulted in the expected fragments with sizes of 130 and 71 bp, but *Mbo* I did not cut CYP1A1. These results confirm the specificity of the PCR primers used for amplification of both CYP1A1 and CYP1A2 and verify that CYP1A1 mRNA is expressed in human liver. The absence of PCR products when the PCR reaction was performed on mRNA which was not reverse transcribed indicates that these bands represent CYP1A1 and CYP1A2 mRNAs and are not due to genomic DNA or laboratory contamination (Fig. 2). However, the level of CYP1A1 mRNA appeared much lower than the level of CYP1A2 mRNA as indicated by the different intensities of the stained PCR products. β-actin mRNA, amplified from the same cDNA synthesis in a separate PCR reaction (lane 1), was used to normalize CYP1A1 and CYP1A2 mRNA levels in subsequent comparative quantification studies. Actin was amplified in a separate reaction because simultaneous amplification of actin and either CYP1A1 or CYP1A2 mRNAs resulted

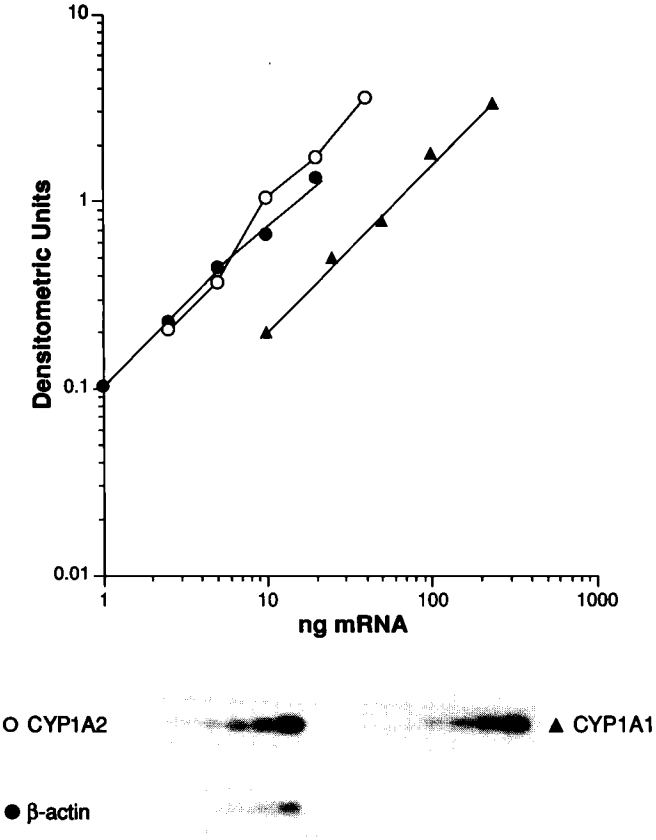

Fig. 3. Quantitative analysis of β-actin, CYP1A1, and CYP1A2 mRNAs in human liver. Five µg of mRNA from liver 402 was reverse transcribed. Serial dilutions of the cDNA were amplified for 23 cycles by PCR using specific primers for CYP1A1, CYP1A2, and β-actin. ^{32}P-labelled 5′-primers were used to quantitate the PCR products. For CYP1A1, the amounts of cDNA amplified were 10, 25, 50, 100, and 240 ng, and for CYP1A2, the amounts amplified were 1, 2.5, 5, 10, 20, and 40 ng, and for β-actin, the amounts amplified were 0.5, 1, 2.5, 5, 10, and 20 ng. Sixteen microlitres of each reaction were analysed on agarose gels and then blotted as described in Materials and methods. After autoradiography, detectable bands were scanned on a densitometer and the area under each peak was calculated and plotted. Photographs of the autoradiograms are shown beneath the corresponding graphs.

Table 3. PCR quantitation of CYP1A1 and CYP1A2 mRNAs in human liver. Three cDNA dilutions for each mRNA were amplified. 1A1/actin ratios and 1A2/actin ratios were calculated after autoradiography and scanning densitometry. Only values in the linear range were used. The very low actin values for samples 415 and 417 precluded their inclusion

Sample	*1A1/actin*	*1A2/actin*
401	0.0165	0.450
402	0.126	1.267
403	0.037	0.914
404	0.066	0.530
405	0.028	0.353
406	0.012	0.029
407	0.037	0.084
408	0.066	0.213
409	0.022	0.123
410	0.041	0.384
411	0.023	0.238
412	0.017	0.397
413	0.013	0.075
414	0.0058	0.052
416	0.036	0.175
418	0.074	0.484
420	0.060	0.392
421	0.057	0.313
422	0.083	0.597

in lower levels of PCR products than independent amplification.

Quantification of CYP1A1 and CYP1A2 mRNA levels

To determine the relative expression of the two *CYP1A* genes, we used PCR with a ^{32}P-end-labelled 5′ primer for mRNA quantification. The results presented in Fig. 3 illustrate the quantitation of CYP1A1 and CYP1A2 mRNA levels in one representative liver sample (409). Using 23 cycles, amplification of human β-actin, CYP1A1 and CYP1A2 mRNAs was linear with serial dilutions of mRNA. The amounts of PCR products doubled with successive two-fold increases of input mRNA. Thus, PCR amplification proceeded exponentially with a constant efficiency, and the exponential phase of amplification could be utilized to compare the relative amounts of CYP1A1 and CYP1A2 mRNAs normalized for β-actin mRNA.

As presented in Table 3, the two *CYP1A* genes were variably expressed among individuals. The expression of CYP1A2 mRNA varied at least 40-fold among individuals, while the variation of CYP1A1 mRNA was 20-fold. This data correlated reasonably well with the amounts of CYP1A2 protein levels estimated by scanning of immunoblots (data not shown) ($r = 0.58$; $p < 0.01$). The amount of CYP1A2 mRNA was approximately two- to 30-fold higher than that of CYP1A1 mRNA in these human livers. However, these ratios should be considered as approximations, since many variables can influence the efficiency of PCR amplification including the efficiency of particular primer sets (Wang *et al.*, 1989). There was a statistically significant correlation between the amount of CYP1A1 and CYP1A2 mRNAs in nineteen human livers ($r = 0.72$; $p < 0.0005$, Fig. 4). When active smokers were excluded, however, the correlation was somewhat weaker ($r = 0.62$; $p < 0.05$).

Analysis of CYP1A2 mRNA sequence in sample 406

To determine whether sequence changes in the mRNA were responsible for the poor expression of CYP1A2 in liver 406, a cDNA for the full coding region of CYP1A2 was amplified by PCR from this sample and subcloned. Due to the low amounts of CYP1A2 mRNA in this sample, this was the only CYP1A2 cDNA recovered from this liver after repeated screening. Sequencing indicated that exon 4 was completely absent in this clone. Three possible point mutations in this clone were found to be PCR errors by direct sequencing of the genomic DNA from this liver (data not shown). Reverse transcribed mRNA from all of the human liver samples was amplified by PCR using primers from exon 3 and exon 5 (I and J from Table 2). These primers generated a major 285 bp PCR and a second minor 195 bp product in all of the samples including sample 406. Direct sequencing of the two PCR fragments showed that the 285 bp fragment included exon 4 while exon 4 was missing from the 195 bp fragment. The ratio of the minor 195 bp fragment to the 285 bp fragment appeared to be similar in all samples. The fact that the splice variant was not unique to sample 406 indicates that abnormal splicing is not responsible for the low expression of CYP1A2 in this tissue.

Discussion

CYP1A1 and CYP1A2 proteins in normal human liver

The expression of CYP1A2 was investigated in human liver by immunoblot analysis using monospecific antibody to rat CYP1A2. This antibody recognized recombinant human CYP1A2 but did not recognize human CYP1A1. The CYP1A2 antibody detected a single immunoreactive band in all liver samples with a mobility on SDS polyacrylamide gels comparable to that of purified rat CYP1A2 protein and human recombinant CYP1A2. There were striking variations in levels of CYP1A2 in these human liver samples in both smokers and nonsmokers. Three of the livers in

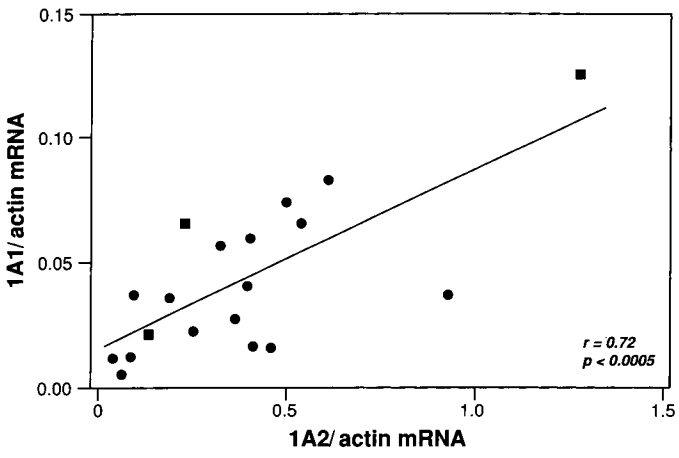

Fig. 4. Relation between CYP1A1 and CYP1A2 mRNA expression. ● represent values for nonsmokers, and ■ represent values for smokers. The abscissa represents the values for CYP1A2 mRNA amounts normalized to β-actin content, the ordinate represents the values for the CYP1A1 mRNA amounts normalized for β-actin content, and the line is the calculated regression line ($r = 0.72$, $p < 0.0005$).

our study were from individuals with a reported current smoking history. Two of these samples expressed high levels of CYP1A2, but one expressed low levels of this protein. Although smoking has been reported to induce CYP1A2 protein expression and correlated enzyme activity in human liver, other laboratories have reported considerable variation of both parameters among smokers and nonsmokers (Wrighton *et al.*, 1986; Sesardic *et al.*, 1988, 1990; Butler *et al.*, 1989b; Kalow & Tang, 1991; Shimada *et al.*, 1989; Ratanasavanh *et al.*, 1991). Ten-fold inter-individual variations in CYP1A2 content of human liver were reported by Wrighton *et al.* (1986) while phenacetin O-deethylase, caffeine 3-demethylation, and aminobiphenyl *N*-oxidation (primarily CYP1A2 dependent enzymes) were reported to vary from 57- to 130-fold in humans (Butler *et al.*, 1989b). Although the majority of the samples in our study were from patients with primary liver cancer, surrounding nontumour tissue was used for these analyses. Moreover, Guengerich & Turvy (1991) reported that metastatic liver cancer had no effect on the hepatic level of CYP1A2 in humans. The levels of CYP1A2 were much more variable in the present study than the levels of two constitutive human P450 enzymes, CYP2C8 and CYP2C9, in the same samples. The striking interindividual variability of CYP1A2 is consistent with the possibility of a genetic polymorphism as suggested by other groups (Minchin *et al.*, 1985; McManus *et al.*, 1990). On the other hand, Vistisen *et al.* (1992) recently reported that CYP1A2 mediated demethylation of caffeine has a log normal distribution, with no evidence of outliers indicative of a polymorphism. Other environmental influences such as diet and exercise have also been shown to affect CYP1A2 mediated metabolism (Vistisen *et al.*, 1992).

Although CYP1A1 has been reported in extrahepatic tissues of smokers, previous studies have indicated that this protein is apparently low or absent in human liver (Sesardic *et al.*, 1988, 1990; McLemore *et al.*, 1990; Guengerich & Turvy, 1991). For example, McManus *et al.* (1990) reported that a polyclonal antibody to rabbit CYP1A1 detected recombinant human 1A1 protein on immunoblots, but did not detect CYP1A1 protein in any of eighteen human liver microsomes. One study (Wrighton *et al.*, 1986) reported that an antibody to rat CYP1A1 recognized both CYP1A2 and a second protein on immunoblots, in one of 14 human liver samples. However, the mobility of this protein on SDS polyacrylamide gels was less than that of CYP1A2. The fact that both our studies and those of McManus *et al.* (1990) found that recombinant human CYP1A1 has a mobility almost identical to or barely greater than that of CYP1A2 on SDS polyacrylamide gels suggests that the second protein was not CYP1A1. Our results indicate the possible presence of extremely low amounts of CYP1A1 in some human liver microsomes on immunoblots using an enhanced chemiluminescence technique. However, this protein is at the limits of detection, and the presence of some cross-reactivity of the antibody to human CYP1A2 precludes the absolute identification of the protein as CYP1A1.

Comparative PCR analysis of CYP1A1 and CYP1A2 expression in human liver

Because of its specificity and sensitivity, PCR is the method of choice to investigate low abundance gene transcripts (Murphy *et al.*, 1990; Wang *et al.*, 1989), especially when the amount of available tissue is limited, e.g. human clinical samples. Although the presence of CYP1A1 protein in human liver has been debated, CYP1A1 mRNA was readily detected in human liver in the present study by the use of PCR. Restriction analysis verified the identity of the PCR products. CYP1A1 mRNA was approximately 10-fold lower than CYP1A2 mRNA (ranging from two- to 30-fold) in human liver. In addition to the improved sensitivity of PCR, the use of specific primers in the PCR and the relative ease of proving the identity of the PCR products circumvents problems of reliable detection of the desired gene products especially when they

are as closely related as the two *CYP1A* genes. In contrast to PCR analysis, Northern blot analysis detected CYP1A1 mRNA expression in only one of 12 human liver samples in earlier studies (Ikeya *et al.*, 1989). McKinnon *et al.* (1991) were able to detect CYP1A1 in only half of 23 samples using riboprobes. They were even unable to detect CYP1A1 mRNA in the liver of one subject who was an extensive smoker.

In the present study, the expression of the two *CYP1A* genes varied greatly among individuals. The liver of one smoking subject (402) contained by far the highest amounts of both CYP1A1 and CYP1A2 mRNA; however, CYP1A1 and CYP1A2 mRNA levels in two other smokers (408 and 409) were in the range of those observed in nonsmokers. Therefore, smoking history did not completely account for the observed variability. The weak positive correlation between CYP1A1 and CYP1A2 mRNA amounts (Fig. 4) indicates that some of the variability in CYP1A1 and CYP1A2 may reflect coordinate induction of both genes in human liver by smoking. However, the weak correlation in nonsmokers is consistent with previous suggestions (Minchin *et al.*, 1985; McManus *et al.*, 1990) that genetic differences could play a role in the variability of CYP1A2 expression.

Detection of an abnormally spliced CYP1A2 mRNA in human liver

We isolated a cDNA derived from an abnormally spliced RNA lacking exon 4 from an individual who expressed low amounts of CYP1A2 protein. However, both the normal mRNA and the splice variant could be detected in all of the livers examined, indicating that the low expression of CYP1A2 in this liver was not due to abnormal expression of an alternatively spliced product. No sequence changes could be verified in the mRNA in this individual. These results indicate that the sequence of the coding region of *CYP1A2* is normal in the individual expressing the lowest amounts of this gene product. Additional studies will be required to determine whether sequence changes in the noncoding region of the gene account for the variability in expression of this protein. Although abnormal splice variants of CYP1A2 have not been reported previously, splice variants which are not known to be associated with abnormal splice sites have been reported for human CYP2C8, CYP2C9, and CYP2B6 (Okino *et al.*, 1987; Miles *et al.*, 1988; Yasumori *et al.*, 1992).

In conclusion, our results show a striking variability in both CYP1A2 protein and CYP1A2 mRNA in human liver. The levels of CYP1A1 protein in human liver are at or below the detection limits of the present methodology. In contrast, CYP1A1 mRNA was readily demonstrated in human liver using PCR analysis. There was a 40-fold variability in CYP1A2 mRNA and a 20-fold variability in CYP1A1 mRNA in human liver. Hepatic content of CYP1A2 mRNA correlated significantly with levels of CYP1A2 protein as analysed by immunoblot analysis ($p < 0.01$). The high variability of CYP1A2 expression could reflect induction by environmental factors and/or suggest genetic differences between individuals. An alternately spliced CYP1A2 mRNA was identified, but this mRNA did not contribute to the differences in expression of CYP1A2 protein. Moreover, the sequence of the coding region of *CYP1A2* was unaltered in an individual expressing extremely low amounts of CYP1A2 in the present study.

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