Cytochrome P450 (CYP) Expression in Human Myeloblastic and Lymphoid Cell Lines

Fusako NAGAI, Yasuko HIYOSHI, Kumiko SUGIMACHI, and Hiro-omi TAMURA*

Kyoritsu College of Pharmacy, Minato-ku, Tokyo 105–8512, Japan.

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To explore the physiological roles of cytochrome P450 (CYP) in peripheral blood cells, we examined which isoforms of CYP families were expressed in human myeloid leukemia cell lines (U937, HL-60 and K562) and lymphoid cell lines (BALL-1, MOLT-4 and Jurkat) by RT-PCR. We observed relatively high expression of CYP1A1, CYP1B1, CYP2A6, CYP2A7, CYP2D6, and CYP2E1 in all cell types, but CYP2A13 and CYP2C9 expression was not detected. Expressions of aryl hydrocarbon (Ah) receptor and Ah receptor nuclear translocator (ARNT), which mediate induction of the CYP1 family, were also detected in all cell types. Cell-type specific expression of CYP3A4 and CYP3A5 was observed in MOLT-4 and K562 cells. Weak, but significant, expression of CYP3A7 was detected in K562 cells. The profile of CYP expression in the culture cells reported here provides information that furthers our understanding of the physiological roles of CYP enzymes in human blood cells.

Key words cytochrome P450; U937; MOLT-4; Jurkat; HL-60; K562

Metabolism of foreign compounds in the body to polar, hydrophilic metabolites is an important prerequisite for detoxification and elimination of xenobiotics from the body. A major family of enzymes involved in the metabolism of xenobiotics is cytochromes P450 (CYPs). CYPs also catalyze the bioactivation and inactivation of a wide variety of endogenous compounds, including steroid hormones and eicosanoids. The CYP is a superfamily of similar proteins with common characteristics.^{1,2} Approximately 70% of human liver CYPs are accounted for by CYP1A2, CYP2A6, CYP2B6, CYP2C, CYP2D6, CYP2E1, and CYP3A enzymes.³ Most of these enzymes are located in the liver, however, some of the enzymes are expressed in extrahepatic tissues, such as the small intestine, lungs and kidneys. Among these extrahepatic tissues, little is known about CYP expression in peripheral blood cells. CYP1A1 enzymes in human peripheral blood lymphocytes might be responsible for aryl hydrocarbon hydroxylation, which is reported to correlate positively with susceptibility to lung cancer from exposure to tobacco smoke.⁴ It has been reported that CYP3A enzymes are expressed in human polymorphic neutrophils.⁵ Recently, Starkel *et al.* reported CYP3A induction in human lymphocytes by rifampicin.⁶ The importance of the CYP enzymes is most clearly established with respect to the metabolism of xenobiotics, particularly medications. However, possible CYP expression in human blood cells has yet to be fully investigated. Since it is clinically important to clarify whether CYP is expressed or not in human blood cells, we selected several established human myeloblastic and lymphoid cell lines and determined the level of CYP expression using reverse transcription (RT)-PCR.

MATERIALS AND METHODS

Materials Reagents for cDNA synthesis were purchased from Stratagene (California, U.S.A.). Taq DNA polymerase was obtained from Perkin-Elmer (California, U.S.A.). Media and supplements for cell culture were purchased from GIBCO BRL (New York, U.S.A.). Other reagents were obtained from Wako Chemicals (Tokyo, Japan). Human cell lines were purchased from RIKEN cell bank (Wako, Saitama,

Japan).

Cells and Cell Culture HL-60 is a human promyelocytic leukemia cell line; K562 is a human erythroleukemia cell line; U937 is a human monocyte tumor cell line; BALL-1 is a human B-cell lymphoblastoid cell line; and MOLT-4 and Jurkat are human T-cell lymphoma cell lines. The human myeloid leukemia cell lines HL-60, U937, and K562 and human lymphoid cell lines BALL-1, MOLT-4, and Jurkat were cultured in suspension in RPMI1640 medium (Life Technologies, Inc., Gaithersburg, MD, U.S.A.) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO₂ in air. Human hepatoma HepG2 cells were maintained in DMEM supplemented with 10% fetal bovine serum.

RNA Isolation and Reverse Transcription-PCR (RT-PCR) Total RNA was isolated from the cultured cells by the guanidium thiocyanate phenol-chloroform extraction method.⁷ First strand cDNA was synthesized from 10 µg of total RNA by 1 unit M-MLV reverse transcriptase with oligo(dT) primers according to the manufacturer's protocol. PCR was carried out using the cDNA as a template by AmpliTaq Gold polymerase (Perkin-Elmer). Primers used for PCR to amplify human CYP cDNAs are listed in Table 1. PCR cycles (30–40 cycles) were as follows: 1 min at 94 °C, 1.5 min at 54–58 °C and 2 min at 72 °C. To amplify trace amount of transcripts, the PCR mixtures were further amplified by second round PCR. Primers for β -actin, CYPs, and others were designed from the published sequences (Table 1).

RESULTS AND DISCUSSION

Expression of CYP1 Family The human CYP1 family consists of 3 subfamilies and 3 genes and has no known endogenous substrates. However, these enzymes are inducible by certain polycyclic hydrocarbons, some of which are found in cigarette smoke and charred food, and they can activate compounds to carcinogens.^{8–10} We observed high levels of CYP1A1 expression in all cell types, as much as that observed in HepG2 cells, a hepatic control (Fig. 1A, Table 2). Expression of CYP1A2 was detected in U937 cells, whereas other cells showed weak or no CYP1A2 expression. High

* To whom correspondence should be addressed. e-mail: tamura-hr@kyoritsu-ph.ac.jp

Table 1. Oligonucleotide Primers for PCR Amplifications

Target mRNA	Sense (5' to 3')	Antisense (5' to 3')	Fragment size (bp)
CYP1A1	TAG ACA CTG ATC ATG GCT GCA G	GGG AAG GCT CCA TCA GCA TC	146
CYP1A2	CTT TGA CAA GAA CAG TGT CCG	AGT GTC CAG CTC CTT CTG GAT	226
CYP1B1	AAC GTC ATG AGT GCC GTG TGT	GGC CGG TAC GTT CTC CAA ATC	360
CYP2A6	TCA AAG GCT ATG GCG TGG TA	GCT GAC GGT CTC GGT GCC CC	589
CYP2A7	GAA CAC AGA GCA CAT ATG TG	GCT GAC GGT TCT CCG TGC CTG	774
CYP2A13	ACA AAG AGT TCC TGT CAC TG	CCG CAA AGA AGA GGT TCA GG	320
CYP2C9	ACA TTG ACC TTC TCC CCA CCA GCC	CAA ATC CAT TGA CAA CTG GAG TGG	357
CYP2D6	TCA ACA CAG CAG GTT CA	CCA TCC ATG TTT GCT TC	419
CYP2E1	ACC TGC CCC ATG AAG CAA ACC	GAA ACA ACT CAT GCG AGC C	246
CYP3A4	CCT TAC ACA TAC ACA CCC TTT GGA AGT	AGC TCA ATG CAT GTA CAG AAT CCC CGG TTA	353
CYP3A5	CCC AGT TGC TAT TAG ACT TGA	GGG GCA CAG CTT TCT TGA AGA CCA	432
CYP3A7	AGT ATA GAA AAG TCT GGG GTA TTT ATG ACT	TAT TGA GAG AAC GAA TGG ATC TAA TGG	447
AhR	GTG ACT TGT ACA GCA TAA TG	ATC TTC TGA CAC AGC TGT TG	316
arnt	GAA TTG GAC ATG AGTA CCA GC	AAG CTG ATG GCT GGA CAA TG	326
β -actin	GCC GTC TTC CCC TCC ATC GT	TGT CAC GCA CGA TTT CCC TC	550

Nucleotide sequences were adopted from the Genbank database. Genbank accession IDs are as follows; CYP1A1 (XM.007727), CYP1A2 (NM.000761), CYP1B1 (U56438), CYP2A6 (NM.000762), CYP2A7 (U22029), CYP2A13 (U22028), CYP2C9 (NM.000771), CYP2D6 (NM.000106), CYP2E1 (AF182276), CYP3A4 (NM.017460), CYP3A5 (NM.000777), CYP3A7 (NM.000765), AhR (NM.0016219), arnt (XM.002027), β -actin (X00351).

Fig. 1. Analyses of Expression of CYP1 and Related Genes

(A) Total RNA was isolated from cultured cells and subjected to specific RT-PCR analysis (1A1, CYP1A1; 1A2, CYP1A2; 1B1, CYP1B1). (B) Expression of AhR, arnt and β -actin. Lane 1, HepG2; lane 2, U937; lane 3, BALL-1; lane 4, MOLT4; lane 5, Jurkat; lane 6, HL-60; lane 7, K562. The sequences of primers and the sizes of products are listed in Table 1.

levels of CYP1A2 expression have been linked to an increased risk of colon cancer.⁸⁾ We also observed CYP1B1 expression in all cells as CYP1A1 (Fig. 1A). CYP1B1 is found exclusively in extrahepatic tissues and is activated by dioxins such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).⁸⁾ An experiment using CYP1B1-null mice established that CYP1B1 mediates the carcinogenicity of 7,12-dimethylbenz[*a*]anthracene (DMBA).¹¹⁾ The high level expression of the CYP1 family, especially of CYP1A1 and CYP1B1, in the myeloblastic and lymphoid cell lines indicates that CYP1 might correlate to risk of carcinogenesis of blood cells. In fact, Fung *et al.* reported that CYP1A1 was responsible for bioactivation of benzo[*a*]pyrene in rat peripheral blood lymphocytes.⁴⁾ The members of the CYP1 family are induced by aromatic hydrocarbons.^{8,11)} The activation involves a specialized receptor called the Ah receptor (AhR) and its nuclear translocator (ARNT).¹²⁾ As shown in Fig. 1B, all cells expressed high levels of AhR and ARNT genes. These results suggest induction of the CYP1 family by aromatic hydrocarbons in human blood cells, affecting cyto- and/or geno-toxicity of chemicals toward the cells.

Expression of CYP2 Family Although members of the CYP2A subfamily are known to metabolize several promutagens, procarcinogens, and pharmaceuticals, little is known about the tissue-specific expression of CYP2A genes in humans.¹³⁾ As shown in Fig. 2, we observed CYP2A6 and CYP2A7 expression in all cell types, as much as that observed in HepG2 cells, whereas no CYP2A13 expression was

Table 2. Expression Profile of CYPs in Culture Cells Derived from Human Blood

CYP	U937	BALL-1	MOLT4	Jurkat	HL-60	K562	HepG2
1A1	+	+	+	+	+	+	+
1A2	+	—	—	+	—	—	+
1B1	+	+	+	+	+	+	+
2A6	+	+	+	+	+	+	+
2A7	+	+	+	+	+	+	+
2A13	—	—	—	—	—	—	+
2C9	—	—	—	—	—	—	+
2D6	+	+	+	+	+	+	+
2E1	+	+	+	+	+	+	+
3A4	— ^{a)}	—	+	— ^{a)}	—	+	+
3A5	— ^{a)}	— ^{a)}	+	— ^{a)}	— ^{a)}	+	+
3A7	—	—	—	—	—	+	+

^{a)} Visible after second round PCR.

detected (data not shown). CYP2A6 is known to be expressed in livers at relatively high levels and may have some involvement in metabolism of nicotine and procarcinogen activation, as well as in the metabolism of drugs such as coumarin, halothane, disulfiram.^{14,15)} From our data we speculate that CYP2A6 is expressed in human blood cells and is involved in activation of procarcinogens and detoxification of drugs.

CYP2D6 is well known to exhibit polymorphism in drug metabolism.¹⁶⁾ CYP2D6 is of particular clinical importance because a number of commonly prescribed drugs are sub-

Fig. 2. Analysis of CYP2 Expression

Total RNA was isolated from cultured cells and subjected to specific RT-PCR analysis (2A6, CYP2A6; 2A7, CYP2A7; 2D6, CYP2D6; 2E1, CYP2E1). Lane 1, HepG2; lane 2, U937; lane 3, BALL-1; lane 4, MOLT4; lane 5, Jurkat; lane 6, HL-60; lane 7, K562. The sequences of primers and the sizes of products are listed in Table 1.

Fig. 3. Analysis of CYP3 Expression

Total RNA was isolated from cultured cells and subjected to the specific RT-PCR analysis (3A4, CYP3A4; 3A5, CYP3A5; 3A7, CYP3A7). Lane 1, HepG2; lane 2, U937; lane 3, BALL-1; lane 4, MOLT4; lane 5, Jurkat; lane 6, HL-60; lane 7, K562. The sequences of primers and the sizes of products are listed in Table 1.

strates of this enzyme. Significant CYP2D6 expression was observed in blood-derived cells (Fig. 2), suggesting that this class of CYP plays a defensive role against the side effects of commonly used drugs in human blood cells.

We detected CYP2E1 expression in all cells examined (Fig. 2). Human lymphocyte is reported to express CYP2E1 and its level is increased in alcohol abusers.¹⁷⁾ Taken together with our data, we assume that high level CYP2E1 expression is a common feature in human blood cells.

Expression of CYP3 Family The CYP3A subfamily consists of 3A4 and 3A5 isozymes in adult liver, and of 3A7 isozyme in fetal liver. It is one of the most important drug metabolizing families in humans.¹⁹⁾ CYP3A4 is the most abundantly expressed P450 in human liver,¹⁸⁾ and has been estimated to be involved in the oxidative metabolism of more than half of the drugs commonly used in humans.¹⁹⁾ As shown in Fig. 3A, we detected CYP3A4 expression in MOLT-4 and K562 cells, as much as that detected in HepG2 cells. Second round PCR revealed weak expression of CYP3A4 in U937 and Jurkat cells (data not shown). These findings suggest the possibility of CYP3A4 expression in T lymphocytes and macrophages, with CYP3A4 playing a defensive role against many kinds of drugs and chemicals. In fact, it has been reported that CYP3A enzymes are expressed in human polymorphic neutrophils.⁶⁾ We also detected CYP3A5 expression in MOLT-4 and K562 cells as CYP3A4 (Fig. 3). Second round PCR showed weak expression of CYP3A5 in all other cells (data not shown). The spectrum of substrates and the catalytic activity of CYP3A5 are known to be similar to those of CYP3A4. These two CYP3A subfam-

lies might work together to act as a defensive barrier against toxic drugs and chemicals in human blood cells. CYP3A7 expression was scarce in the most cells (Fig. 3). CYP3A7 expression was detected in K562 cells after second round PCR (data not shown), indicating weak CYP3A7 expression.

Here we described several features of CYP expression in various human culture cells originating from blood-stem cells. We found expression of CYP1A1, CYP1B1, CYP2A6, CYP2D6 and CYP2E1 in all cell types, and cell-type specific expression of CYP3A subfamilies. These characteristics might correlate with the susceptibility of the cells to drugs or other toxic chemicals. It should be noted that the expression level of mRNA does not always correlate to that of its product.²⁰⁾ Further analysis to show expression of CYP proteins in the cells must be carried out. The data obtained here helps further our understanding of the physiological roles of CYP enzymes in human blood cells. Clarification of these findings must be sought by further analysis of CYP expression using separated human blood cells. In addition, the culture cells expressing different sets of CYPs might be useful for analysis and prediction of CYP-mediated metabolism of drugs and chemicals.

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