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RESEARCH ARTICLE

Evidence for cytochrome P450 2B1/2B2 isoenzymes in freshly prepared peripheral blood lymphocytes

Kumar Saurabh and Devendra Parmar

Developmental Toxicology Division, CSIR-Indian Institute of Toxicology Research, Lucknow, UP, India

Abstract

Cytochrome P450 2B1 and 2B2, the major hepatic drug metabolizing enzymes belonging to CYP2 family and associated constitutive androstane receptor (CAR) were found to be expressed in peripheral blood lymphocytes (PBL) isolated from rats. As observed in liver, pretreatment of phenobarbital (PB) or phenytoin were found to increase the expression of CYP2B1, CYP2B2 and associated enzyme activity in PBL. Like in liver, blood lymphocyte CYP2B1/2B2 catalyzed the activity of 7-pentoxyresorufin O-dealkylase (PROD). The present data, demonstrating similarities in the regulation of blood lymphocyte CYP2B-isoenzymes with the liver enzymes, suggests that blood lymphocyte CYP2B-isoenzymes could be used as a biomarker to monitor tissue levels.

Keywords: Lymphocyte, liver, CYP, PB, induction, expression

Introduction

Cytochrome P450s (CYPs), a superfamily of proteins, is involved in the oxidative metabolism of a wide variety of xenobiotics, including drugs (Omiecinski et al. 1999; Anzenbacher & Anzenbacherová 2001; Shimada 2006). It has been shown that the levels of CYPs that are primarily involved in xenobiotic metabolism are modulated by exposure to different drugs and chemicals (Gerhold et al. 2001; Hamadeh et al. 2002). Genetic polymorphism is also known for the xenobiotic metabolizing CYPs which may alter the functional activity of CYPs (Miller et al. 2001; Ingelman-Sundberg 2002; Vineis 2002). The expression of CYP1-family, primarily involved in the metabolic activation of procarcinogens and promutagens to reactive species, is known to increase several fold following exposure to polycyclic aromatic hydrocarbons, PAHs (Omiecinski et al. 1999; Shimada 2006). Likewise, phenobarbital (PB) and other PB-like inducers, e.g. phenytoin are known to increase the expression of CYP2B-isoenzymes (Nelson et al. 1996; Czekaj 2000), while pretreatment of ethanol and other low molecular weight solvents increase the activity of CYP2E1 (Lipscomb et al. 2004; Wu & Cederbaum 2005; Cederbaum 2009).

The CYPs have also been established as biomarkers of susceptibility with individuals carrying variant genotypes of CYPs being more susceptible to the adverse effects of drugs or environmental chemicals (Miller et al. 2001; Vineis 2002; Thier et al. 2003; Boccia et al. 2007). However, as opposed to measuring genetic polymorphism and correlating them with other determinants of risk, another approach has been to measure interindividual variation in the expression of CYPs, involved in xenobiotic metabolism, and develop CYPs as biomarkers of exposure or effect. Peripheral blood lymphocytes (PBL) have been shown to express several of the CYPs involved in xenobiotic metabolism (Hukkanen et al. 1997; Dey et al. 2001; Furukawa et al. 2004; Haas et al. 2005; Saurabh et al. 2010). Thus, measuring expression profiles of CYPs in PBL for identifying the alterations in the activity of CYPs in the tissues, including liver, has several advantages and could be potentially used as a biomarker in population studies (Lucier & Thompson 1987; Harris 1989; Vanden Heuvel et al. 1993; Rumsby et al. 1996; Raucy et al. 1997, 1999; Nguyen et al. 2000; Siest et al. 2008). Using qRT-PCR, Krovat et al. (2000) observed that though the human blood cell lines exhibit

Address for Correspondence: Dr Devendra Parmar, Developmental Toxicology Division, CSIR-Indian Institute of Toxicology Research (IITR), P.O. Box 80, M. G. Marg, Lucknow-226 001, UP, India. Tel: +91 522 2627586 Ext. 261. Fax: +91 522 2628227, 2621547.
E-mail: parmar_devendra@hotmail.com

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poor response to CYP inducers, *in vivo* patterns of CYP and microsomal epoxide hydrolase (mEH) gene expression were well maintained in cultured blood cells. Further the constitutive patterns of CYP and mEH expression in PBL exhibited relatively low levels of variation among individuals. However, a poor correlation was also reported between CYP mRNA expression and systemic enzyme activity following induction (Finnström et al. 2001; Haas et al. 2005).

In contrast, studies from our laboratory have demonstrated that freshly prepared PBL isolated from control rats or rats treated with specific inducers expressed mRNA and protein corresponding to CYP1A1, 1A2 or 2E1 or 3A isoenzymes. These CYPs expressed in PBL were catalytically active and exhibited similar pattern of regulation as observed in the liver (Dey et al. 2001, 2002, 2005, 2006; Saurabh et al. 2010; Khan et al. 2011). The levels of CYP1A1 and 2E1 were found to be increased in blood lymphocytes isolated from patients suffering from tobacco-induced lung cancer and alcoholic liver cirrhosis, respectively (Shah et al. 2009; Khan et al. 2011). Individuals with variant genotypes of CYP1A1 (Msp1-T/C at 3' non-coding region and Ile/Val-A/G in exon 7) exhibited relatively higher magnitude of induction in CYP1A1 levels as compared to those with wild type genotype (Shah et al. 2009).

However, to develop the expression profiles of CYPs as possible biomarkers of exposure or effect, studies are needed to validate and characterize the expression of other CYPs, involved in xenobiotic metabolism, in blood lymphocytes. As compared to PAH- and ethanol-metabolizing CYPs, very limited information is available on the expression of CYP2B1/2B2, the major hepatic drug-metabolizing enzymes, in PBL (Hukkanen et al. 1997; Baron et al. 1998; Furukawa et al. 2004; Hannon-Fletcher & Barnett 2008). Therefore, attempts were made to understand the status and regulation of CYP2B1 and CYP2B2 isoenzymes in freshly prepared PBL, by characterizing the mRNA and protein expression of CYP2B1 and CYP2B2 isoenzymes along with its catalytic activity in freshly prepared PBL isolated from control and PB pretreated rats.

Materials and methods

Chemicals

The 7-pentoxyresorufin, phenytoin sodium salt, resorufin, histopaque 1077, phenylmethyl sulfonyl fluoride (PMSF), NADPH, dithiothreitol (DTT), protease inhibitor cocktail, 3-methylcholanthrene (MC), metyrapone, SKF-525A, acrylamide, goat anti-rabbit IgG conjugated to horseradish peroxidase, etc. were procured from Sigma-Aldrich, St. Louis, MO, USA. Immobilon-P nitrocellulose membrane was procured from Millipore Corp. (MA, USA). For western blotting, anti-rat hepatic CYP2B1/2B2 developed in rabbit was obtained from Chemicon, USA. Phenobarbitone sodium salt (PB) was a gift from Biodeal Laboratories, India. All the other chemicals used were of highest purity and were procured either from E. Merck, India or SISCO Research Laboratories Pvt. Ltd., India.

Animals and treatment

Adult male albino Wistar rats (6–8 week old) were procured from Indian Institute of Toxicology Research breeding colony and raised on animal pellet diet and water *ad libitum*. Animal care and experimentation were in accordance with the policy approved by the Animal Care Committee of the Center. For studying PB responsive CYPs in blood lymphocytes, the rats were divided into seven groups, containing 10 animals each. The animals in the groups, I to III were treated i.p. with 40, 60, and 80 mg/kg body weight of PB, dissolved in normal saline, respectively, for five consecutive days. Rats in fourth group were treated i.p. with 100 mg/kg body weight of phenytoin, dissolved in normal saline, for five days. The animals in fifth group were treated with 30 mg/kg body weight of MC, dissolved in corn oil, daily, i.p. for five consecutive days. Rats in the sixth and seventh groups served as controls and received an equivalent amount of corn oil or normal saline. The animals were sacrificed 24 h after the last dose and blood was drawn from the heart and processed for the isolation of lymphocytes.

RNA extraction

Total RNA was extracted from whole blood isolated from control and pretreated rats by TRIzol LS and from liver by TRIzol reagent (Life Technologies, USA) according to protocol provided by manufacturer.

qRT-PCR analysis

For qRT-PCR, cDNA was synthesized by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) as described by Shah et al. (2009). The sequences of primers used for CYP2B1, CYP2B2, constitutive androstane receptor (CAR), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) have been described in the literature (Wyde et al. 2005; Baldwin et al. 2006; Qin & Meng 2006). The PCR reaction mixture for CYP2B1, CYP2B2, CAR, and GAPDH in 20 μL contained 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 10 pM of each gene primer, 4 pM of each gene probe, 2 μL cDNA, and rest Nuclease-free H_2O. TaqMan assays for each gene target were performed in triplicates on cDNA samples in 96-well optical plates on an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems). The PCR conditions were as follow: 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. For absolute quantification of CYP2B1, 2B2 and CAR, the number of copies of mRNA was calculated by interpolation from the standard curve generated using a known amount of full length cDNA clones.

Isolation of lymphocytes

Lymphocytes were isolated by an established method described earlier (Dey et al. 2001). In brief, 4.0 mL of whole blood was diluted with 4.0 mL of phosphate buffered saline (PBS), pH 7.4, and carefully layered over 2.0 mL of histopaque 1077. After centrifugation at 400 × *g* for 30 min at room temperature, the opaque interface containing mononuclear cells was transferred into a clean centrifuge

tube. After repeated washing with PBS and recentrifugation at 250 × *g*, the lymphocyte pellet was resuspended in 0.5 mL of PBS. The number of lymphocytes was counted using a hemocytometer and the viability of the cells was assayed by the trypan blue exclusion test.

Preparation of microsomes and enzymatic analysis

Microsomal preparations from liver and blood lymphocytes were prepared by methods reported earlier (Parmar et al. 1998; Hannon-Fletcher & Barnett 2008). The microsomal pellets were resuspended and then stored at –70°C until further analysis. The activity of 7-pentoxyresorufin-O-deethylase (PROD), marker enzyme of CYP2B1 and CYP2B2 isoenzymes were determined in rat liver and blood lymphocytes by the modified method of Parmar et al. (1998). *In vitro* inhibition studies with polyclonal antibody specific for CYP2B1/2B2 and inhibitors for CYPs (SKF-525A and metyrapone) were carried out as described earlier (Parmar et al. 1998). Protein content of the samples was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the reference standard.

Immunoblot analysis

CYP2B1/2B2 isoenzymes were identified by western blot analysis in liver and lymphocyte solubilized preparations isolated from control and treated animals as described earlier (Parmar et al. 1998). In brief, the membranes after transfer were incubated with primary antibody (1:500 dilutions) overnight at 32°C. The membranes after washing were incubated with secondary antibody (horseradish peroxidase conjugated goat anti-rabbit) at 1:2000 dilutions for 60 min at room temperature. After incubation, the membranes were washed and then processed for chemiluminescence with luminol and p-coumaric acid. Images were developed with Versa Doc imaging system and densitometric analyses of the bands were carried out using Quantity One Quantitation Software version 4.3.1 (Biorad, USA).

Statistical analysis

Students "*t*"-test was employed to calculate the statistical significance between control and treated groups; $p < 0.05$ was considered to be significant when compared with the controls.

Results

Approximately, 2–3 × 10^6 cells were present in 0.5 mL of rat blood lymphocyte suspension. The viability of these cells was above 95%.

mRNA expression studies

The mRNA expression of GAPDH, a housekeeping gene was found to be uniform in all the samples (control and treated) analyzed. Absolute quantification studies revealed that mRNA for CYP2B1 and 2B2 were expressed at very low levels in PBL isolated from control rats. The expression of CYP2B1 and 2B2 was found to be 10- and 25-fold lower in PBL when compared to liver. Similarly, the expression of CAR, the transcription factor involved in CYP2B induction was found to be six-fold lower in PBL when compared to liver (Table 1). The mean copy number values (basal expression) revealed that the expression of CYP2B2 in PBL was higher than CYP2B1 and CAR. A similar pattern of expression of CYP2B isoenzymes and CAR receptor was observed in liver (Table 1).

Pretreatment with different doses of PB was found to significantly increase the mRNA expression of CYP2B1 and CYP2B2 isoenzymes in a dose-dependent manner in PBL. The magnitude of induction was several-fold higher for CYP2B1, when compared to CYP2B2 in PBL. A similar dose-dependent induction of CYP2B-isoenzymes was observed in liver after pretreatment of different doses of PB, though the magnitude of induction was several-fold higher in liver when compared to PBL (Table 2). The PB pretreatment was also found to increase the mRNA

Table 1. Absolute quantification of RNA isolated from lymphocytes and liver of control and PB pretreated rats by qRT-PCR.

	Number of mRNA molecules/μg of total RNA	
	Lymphocytes	Liver
CYP2B1	$1.8 \times 10^4 \pm 1.13 \times 10^3$	$1.7 \times 10^5 \pm 1.41 \times 10^4$
CYP2B2	$2.6 \times 10^4 \pm 1.97 \times 10^3$	$6.3 \times 10^5 \pm 3.41 \times 10^4$
CAR	$3.4 \times 10^3 \pm 4.71 \times 10^2$	$1.9 \times 10^4 \pm 1.44 \times 10^3$

Note: Values represent mean ± S.E. of three experiments (each reaction was performed in triplicate).
Number of copies of mRNA was calculated by interpolation from the standard curve generated using known amount of full length cDNA clone of CYPs and CAR with slope ranges from –3.1 to –3.3 approximately and R^2 ranges ~0.98–0.99.

Table 2. Relative quantification of RNA isolated from lymphocytes and liver of control and pretreated rats by qRT-PCR.

	Fold change					
	Lymphocytes			Liver		
	CYP2B1	CYP2B2	CAR	CYP2B1	CYP2B2	*CAR*
PB (40 mg/kg)	5.18 ± 0.62*	1.92 ± 0.31	1.21 ± 0.18	46.62 ± 3.9*	8.83 ± 0.72*	2.18 ± 0.13*
PB (60 mg/kg)	11.25 ± 1.13*	6.32 ± 0.26*	1.82 ± 0.19*	133.2 ± 11.7*	17.18 ± 2.11*	4.07 ± 0.37*
PB (80 mg/kg)	24.08 ± 3.39*	13.83 ± 0.98*	3.3 ± 0.31*	240.3 ± 30.5*	30.5 ± 4.33*	7.83 ± 0.82*
PHT (100 mg/kg)	15.36 ± 1.32*	7.46 ± 0.63*	2.29 ± 0.25*	226.0 ± 24.2*	77.83 ± 6.53*	6.89 ± 0.51*

Each reaction was performed in triplicate on cDNA samples in 96-well optical plates. The threshold cycle value (C_t values) of each sample was normalized with C_t values of endogenous control (GAPDH) [ΔC_t]; fold change is calculated from $\Delta\Delta C_t^{\#}$ value of each sample.
$^{\#}\Delta\Delta C_t$, ΔC_t of Treated – ΔC_t of Control.
*$p < 0.05$ when compared with the controls.

expression of CAR in PBL and liver in a dose-dependent manner (Table 2). Similar to that observed with PB, pretreatment of phenytoin increased the mRNA expression of CYP2B1, CYP2B2, and CAR receptor in PBL and liver (Table 2).

Immunoblot analysis

Western blot analysis of microsomes isolated from liver of control rats revealed significant cross-reactivity co-migrating with rat liver CYP2B1 and 2B2. In contrast, very faint immunoreactivity, co-migrating with the liver isoenzymes, was observed in PBL isolated from control rats (Figure 1). The PB pretreatment was found to produce a significant increase in the expression of CYP2B1 and 2B2 in the liver microsomes as indicated by a several-fold increase in immunoreactivity co-migrating with these CYP isoenzymes in the liver. A similar increase in the immunoreactivity was observed in freshly prepared PBL isolated from rats pretreated with PB (Figure 1). Due to extremely low levels of expression of CYP2B1 and CYP2B2 in PBL and high analogy of nucleotide and amino acid sequences in CYP2B1 and CYP2B2 (Waxman & Azaroff 1992), it was not possible to differentiates between CYP2B1 and CYP2B2 in PBL. Densitometric analysis has shown that the increase in the expression of CYP2B1/2B2 isoenzymes after PB treatment was several-fold lower in blood lymphocytes when compared to the liver (data not shown).

Catalytic activity of CYP2B1/2B2 isoenzymes in blood lymphocytes

The PBL was found to catalyze the O-dealkylation of pentoxyresorufin, the substrate specific for CYP2B1/2B2 isoenzymes in liver. As compared to the liver, low activity of 7-pentoxyresorufin-O-dealkylase (PROD) was observed in rat blood lymphocytes (Table 3). The rate of resorufin formed was linear up to 30 min at an optimum pH of 7.5 and protein quantity of 100 μg in the presence of NADPH. The enzyme activity was found to be NADPH dependent. As with the liver enzyme, addition of NADH (0.50 mM) to the reaction mixture containing NADPH did not significantly affect the enzyme activity and NADH alone could not catalyze the O-dealkylation of PR in rat blood lymphocytes. Similar to that observed in liver microsomes, addition of SKF-525A to the complete system inhibited the enzyme activity. Likewise, when the reaction mixture was saturated with carbon monoxide (CO), significant inhibition in the activity of PROD was observed in the blood lymphocytes. Similar inhibition in the enzyme activity was observed in liver microsomes when saturated with CO (Table 3).

Pretreatment with different doses of PB significantly increased the activity of PROD in liver microsomes as well as in blood lymphocytes in a dose-dependent manner. Though the magnitude of induction was higher in rat liver microsomes when compared to blood lymphocytes, statistically significant increase was observed in the activity of PROD in freshly prepared blood lymphocytes isolated from rats pretreated with different doses of PB (Table 4). Likewise, as observed in liver, pretreatment with phenytoin significantly increased the activity of PROD in PBL, though the magnitude of induction was much higher in liver (Table 4). In contrast, pretreatment with MC, an inducer of CYP1A isoenzymes did not produce any effect on the activity of PROD in blood lymphocytes or liver microsomes (Table 4).

To further investigate similarities in the activity of CYP2B-dependent PROD activity in blood lymphocytes with the tissue enzyme, enzyme kinetics was also studied in rat blood lymphocytes. The O-dealkylation of 7-pentoxyresorufin (PR), catalyzed by blood lymphocytes isolated from control rats, exhibited a monophasic pattern as revealed by Lineweaver–Burk plot (Table 5).

Table 3. Cofactor requirement of O-dealkylation of 7-pentoxyresorufin by CYP2B1/2B2 isoenzyme in rat blood lymphocytes.

	Lymphocytes	Liver
	pmoles resorufin/min/mg protein	
Complete system (CS)	2.28 ± 0.11	19.7 ± 1.9
Complete system (boiled enzyme)	ND	< 0.5
Complete system (-) NADPH + NADH	ND	< 0.5
Complete system + NADH	2.31 ± 0.09	20.3 ± 2.0
Complete system + SKF525A	1.44 ± 0.07*	12.2 ± 1.3*
Complete system + CO	0.07 ± 0.05*	2.1 ± 0.1*

Whole liver microsomal (0.025 mg) and lymphocytes (0.025 mg) protein were incubated at 37°C with 1.0 mM NADPH, 2.0 mg BSA, and 1.5 μM PR and 0.1M sodium phosphate buffer in a final reaction mixture of 1.25 mL. This represents the complete system; 1.0 mM NADH was substituted or added to the reaction mixture; SKF-525A was added at the final concentration of 0.001 M. All the values are mean ± SE of three experiments. CS, complete system; ND, Not detected.

*$p < 0.05$ when compared with the controls.

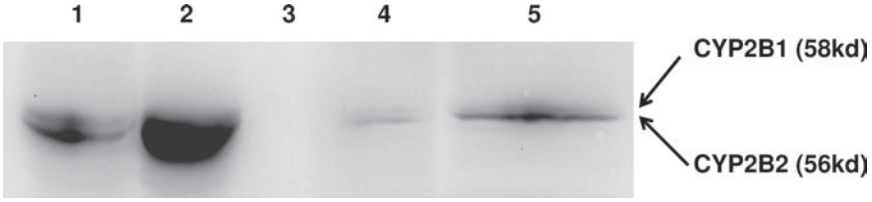

Figure 1. Western Blot analysis of CYP2B1/CYP2B2 in blood lymphocytes and liver of rats pretreated with PB (80 mg/kg). Lanes 1 and 2 contains 25 μg of microsomal protein isolated from liver of control and PB pretreated rats, respectively. Lane 3 is blank. Lanes 4 and 5 contains 500 μg of microsomal protein isolated from lymphocytes of control and PB pretreated rats, respectively.

Table 4. Effect of CYP inducers on rat blood lymphocytes and liver 7-pentoxyresorufin-O-deethylase (PROD) activity.

	Lymphocytes	Fold induction	Liver	Fold induction
Control	2.28 ± 0.11	–	19.72 ± 1.9	–
PB (40 mg/kg)	2.64 ± 0.31	–	155.59 ± 15.3*	7.89
PB (60 mg/kg)	3.35 ± 0.33*	1.47	263.06 ± 21.4*	13.34
PB (80 mg/kg)	6.53 ± 0.27*	2.86	340.21 ± 18.3*	17.25
PHT (100 mg/kg)	7.02 ± 0.64*	3.08	388.68 ± 27.2*	19.71
MC	1.89 ± 0.08	–	17.88 ± 1.6	–

All the values are mean ± S.E. of three experiments. The enzyme activity is expressed as pmoles resorufin/min/mg protein.
*$p < 0.05$ when compared to the controls.

Table 5. Apparent kinetic constants for rat blood lymphocyte PROD.

	K_m	V_{max}
Control	6.25 ± 0.24	1.62 ± 0.08
PB (80 mg/kg)	11.11 ± 1.05*	5.88 ± 0.18*

Note: K_m is expressed in μM and V_{max} in pmoles resorufin formed/min/mg protein. Values represent data ± SE of three experiments.
*$p < 0.05$ when compared with the controls.

Pretreatment of PB resulted in a significant increase in the affinity for the substrate for the enzyme (apparent K_m) in blood lymphocytes. Kinetic studies also revealed that PB pretreatment resulted in significantly increased apparent V_{max} for the O-dealkylation of PR in rat blood lymphocytes (Table 5).

To further identify that CYP2B1 and CYP2B2 isoenzymes expressed in blood lymphocytes catalyze the O-dealkylation of PR, *in vitro* studies were carried out using inhibitors of CYP2B1 and CYP2B2 and antibody raised against rat liver CYP2B1/2B2. As evident from Table 6, *in vitro* addition of acetone or ethanol to the blood lymphocytes isolated from control or PB pretreated rats, did not produce any effect on the activity of PROD. *In vitro* addition of SKF-525A, an inhibitor of CYP catalyzed reactions, to the reaction mixture containing lymphocytes from control rats produced a significant concentration-dependent decrease in the activity of PROD. In contrast, *in vitro* addition of metyrapone, an inhibitor of PB-inducible CYP catalyzed reactions to the blood lymphocytes isolated from control rats produced a small decrease in the activity of PROD (Table 6). However, as seen with control blood lymphocytes, *in vitro* addition of metyrapone to lymphocytes isolated from PB pretreated rats, produced a significant concentration-dependent inhibition in the activity of PROD (Table 6). Interestingly, the inhibition observed in the PROD activity on addition of SKF-525A to the lymphocytes isolated from PB pretreated rats was less when compared to the inhibition observed with control lymphocytes further demonstrating that SKF-525A is not a specific inhibitor of CYP2B1 and CYP2B2 catalyzed reactions. Similarly, *in vitro* addition of polyclonal antibody raised against CYP2B1/2B2 isoenzymes to the reaction mixture containing lymphocytes isolated from control rats produced inhibition of the enzyme activity, which was significant at higher concentration (0.8 mg). In contrast, addition of preimmune IgG to the reaction mixture containing blood lymphocytes isolated either from control or PB pretreated rats did not produce any appreciable effect on the activity of PROD (Table 6). Addition of polyclonal antibody of CYP2B1/2B2 to the reaction mixture containing blood lymphocytes isolated from PB pretreated rats produced significant concentration-dependent inhibition of PROD activity at both the concentrations studied demonstrating that CYP2B1 and CYP2B2 isoenzymes expressed in rat blood lymphocytes catalyze the activity of PROD (Table 6).

Table 6. Effect of inhibitors on PROD activity in blood lymphocytes isolated from control or PB (80 mg/kg) pretreated rats.

		Control	PB
		pmoles resorufin/min/mg protein	
None		2.27 ± 0.09	6.57 ± 0.27
Acetone		2.25 ± 0.10 (100%)	6.44 ± 0.25 (100%)
SKF-525A	1×10^{-5}M	1.80 ± 0.07* (80%)	4.83 ± 0.22 (75%)
	1×10^{-4}M	1.57 ± 0.07* (70%)	4.19 ± 0.20* (65%)
	1×10^{-3}M	1.02 ± 0.05* (55%)	3.22 ± 0.19* (50%)
Ethanol		2.25 ± 0.09 (100%)	6.46 ± 0.29 (100%)
Metyrapone	1×10^{-5}M	1.98 ± 0.08* (88%)	3.10 ± 0.14 (48%)
	1×10^{-4}M	1.58 ± 0.08* (70%)	2.39 ± 0.11* (37%)
	1×10^{-3}M	1.18 ± 0.07* (52%)	1.55 ± 0.07* (24%)
Preimmune IgG	0.3 mg	2.24 ± 0.10 (100%)	6.40 ± 0.23 (100%)
	0.8 mg	2.20 ± 0.10 (100%)	6.35 ± 0.23 (100%)
Anti-CYP2B1/2B2	0.3 mg	1.34 ± 0.06* (60%)	3.84 ± 0.21* (40%)
	0.8 mg	0.66 ± 0.06* (31%)	0.67 ± 0.07* (11%)

Values represent data ± SE of three experiments.
*$p < 0.05$ when compared with the controls. Figures in parenthesis indicate percentage activity remaining as compared to their respective controls.

Discussion

The presence of CYP2B-mRNA and protein and associated enzyme activity in freshly prepared PBL isolated from control rats have provided evidence for the expression of CYP2B isoenzymes in PBL. The specific requirements of NADPH, significant inhibition of the enzyme activity by SKF-525A and CO have demonstrated that like in liver, the activity of PROD in PBL is catalyzed by CYPs. Low but detectable protein expression and associated PROD activity in PBL isolated from control rats have provided support to the earlier studies demonstrating that as observed with the other CYPs (Hannon-Fletcher & Barnett 2008), low levels of CYP2B-mRNA leads to catalytically active protein in blood lymphocytes. Though CYP2B-isoenzymes have not been characterized in PBL, reports are available demonstrating CYP2B6 mRNA in PBL isolated from human samples (Hukkanen et al. 1997; Baron et al. 1998;

Furukawa et al. 2004). Comparing blood CYP expression levels obtained using DNA arrays to the values for CYP expression in liver reported using immunochemical methods (Hanna et al. 2000; Lamba et al. 2003; Hesse et al. 2004), Nyugen et al. (2000) observed exact concordance/correspondence between CYP2A6, 2B6 and 2E1 among the two methods. Even though the interindividual variations were reported to be high among different CYPs in PBL isolated from healthy individuals, close associations were observed among expression levels of CYP1A1, 1A2, 2B6, and 2E1 in blood samples isolated from hepatocellular carcinoma (HCC) patients suggesting that basal levels of CYP gene expressions mainly indicate the constitutive expression (Furukawa et al. 2004).

The expression of CAR in blood lymphocytes and dose-dependent increase in the mRNA expression of CAR by PB have further provided support to the earlier studies demonstrating that transcription factors involved in CYP regulation are expressed in blood lymphocytes (Siest et al. 2008; Saurabh et al. 2010). Further, as observed in liver, increase in the expression of CAR in PBL by pretreatment of phenytoin have demonstrated similarities in the responsiveness of CAR in PBL and liver. Phenytoin has shown to be a selective activator of CAR mediating induction of CYP2B6 gene expression in primary human hepatocytes (Wang et al. 2004). The DNA array studies in PBL isolated from healthy individuals have also shown that transcription factors, including CAR, pregnane X receptor (PXR), aromatic hydrocarbon receptor (AhR), AhR nuclear translocator (Arnt), Vitamin D receptor (VDR) and glucocorticoid receptor (GR) involved in CYP regulation, are expressed in the majority of subjects (Nyugen et al. 2000; Siest et al. 2008). These receptors are not only known to modulate expression of individual CYPs but also mutually influence their expression (Siest et al. 2008; Saurabh et al. 2010).

Similarity in the pattern of the dose-dependent increase in CYP2B1 and CYP2B2 mRNAs and associated PROD activity in PBL and liver isolated from PB pretreated rats have demonstrated that responsiveness of CYP2B-isoenzymes is retained in blood lymphocytes. The present study indicated a significant increase in the CYP2B1/2B2 protein and PROD activity in blood lymphocytes isolated from PB or phenytoin pretreated rats and have further shown that like in liver, increase in CYP2B-mRNA in blood lymphocytes is associated with several-fold increase in CYP2B1 and CYP2B2 protein and enzyme activity. Though very limited information is available on the induction of CYP2B-isoenzymes in PBL in rodents, pretreatment of rats with MC or ethanol or dexamethasone is known to increase the expression of CYP1A- or CYP2E1- or CYP3A-isoenzymes in freshly isolated PBL (Dey et al. 2001, 2005, 2006). Hannon-Fletcher and Barnett (2008) have reported a significant increase in expression of the lymphocyte CYP2B, 2E, 3A and 4A proteins on pretreatment of rats with specific CYP inducers. Significant increase in the mRNA expression of several CYPs including CYP2B6 has also been demonstrated in PBL or liver biopsy samples isolated from patients suffering from hepatocellular carcinoma (HCC) indicating that the major regulatory mechanisms of CYP gene induction by exogenous stressors are common for CYPs expressed in PBL (Furukawa et al. 2004). This was further supported by our data indicating a similar pattern of increase in the mRNA expression of CAR in PBL and liver isolated from PB or phenytoin pretreated rats. A very weak PXR and CAR-mRNA expression levels have also been reported in peripheral blood mononuclear cells isolated from healthy individuals when incubated with PB or rifampicin (Manceau et al. 2010).

Further evidence that CYP2B isoenzymes are expressed in PBL and are catalytically active was provided by our data indicating a dose-dependent increase in the activity of PROD in PBL isolated from PB pretreated rats. Earlier studies from our laboratory have demonstrated that CYP1A1/1A2 and 2E1 expressed in PBL catalyze the dealkylation of 7-ethoxresorufin, 7-methoxyresorufin and demethylation of N-nitroso-dimethylamine (Dey et al. 2001, 2002, 2005, 2006; Saurabh et al. 2010). Further, the increase in blood lymphocyte PROD activity after PB administration in PBL was found to be associated with a significant increase in the apparent V_{max} and the affinity of the substrate for the lymphocyte enzyme, suggesting that as reported in liver, PB pretreatment results in enrichment of CYP2B/2B2 isoenzymes, which may catalyze the O-dealkylation of 7-pentoxyresorufin (PR) in blood lymphocytes. The CYP2B-isoenzymes catalyze the activity of PROD in PBL was further supported by enzymatic studies using specific inhibitors. *In vitro* studies showing concentration dependent inhibition in the activity of blood lymphocyte PROD on addition of metyrapone or polyclonal antibody specific for CYP2B1/2B2 isoenzymes to the lymphocyte protein isolated from PB pretreated rats have demonstrated that like in liver, the activity of PROD in PBL is catalyzed by CYP2B1 and CYP2B2 isoenzymes.

In contrast, some of the earlier studies failed to provide a significant correlation between CYP mRNA content in blood lymphocytes and systemic enzyme activity (Finnström et al. 2001; Haas et al. 2005). This lack of correlation could be attributed to the use of general inducer of multiple drug metabolizing enzymes and to the probes that lack enzyme specificity. Furthermore, lack of information on whether the CYPs expressed in blood lymphocytes are actually translated into functional proteins has hampered the use of mRNA expression assays for xenobiotic metabolizing CYPs in PBL as surrogates for identifying changes in systemic enzyme activity. The present study is, therefore, significant as it attempts to simultaneously monitor the mRNA and protein expression of CYP2B-isoenzymes along with its transcription factor and enzyme activity in PBL.

In conclusion, the results of the present study have provided evidence for the mRNA expression of CYP2B1, 2B2, and its associated CAR receptor in blood lymphocytes and are in support of the earlier studies demonstrating that majority of CYPs, involved in toxication–detoxication mechanisms are expressed in PBL and like in tissues are responsive to exposure of other drugs

and chemicals. Further, the present study demonstrating protein expression of CYP2B isoenzymes and the role of CYP2B isoenzymes in catalyzing the activity of PROD in blood lymphocytes has convincingly shown that CYP2B isoenzymes expressed in blood lymphocytes could be used as a biomarker for predicting exposure and toxicity of environmental chemicals.

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Declaration of interest

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