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Differences in the expression and inducibility of cytochrome P450 2B isoenzymes in cultured rat brain neuronal and glial cells

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Abstract Studies initiated to investigate the distribution of cytochrome P450 2B (CYP2B) isoenzymes in rat brain cells revealed significant activity of CYP2B-dependent 7-pentoxyresorufin-O-dealkylase (PROD) in microsomes prepared from both, cultured rat brain neuronal and glial cells. Neuronal cells exhibited 2-fold higher activity of PROD than the glial cells. RT-PCR and immunocytochemical studies demonstrated significant constitutive mRNA and protein expression of CYP2B in cultured neuronal and glial cells. Induction studies with phenobarbital (PB), a known CYP2B inducer, revealed significant concentration dependent increase in the activity of PROD in cultured brain cells with glial cells exhibiting greater magnitude of induction than the neuronal cells. This difference in the increase in enzyme activity was also observed with RT-PCR and immunocytochemical studies indicating differences in the induction of CYP2B1 and 2B2 mRNA as well as protein expression in the cultured brain cells. Furthermore, a greater magnitude of induction was observed in CYP2B2 than CYP2B1 in the brain cells. Our data indicating differences in the expression and sensitivity of the CYP2B isoenzymes in cultured rat brain cells will help in identifying and distinguishing xenobiotic metabolizing capability of these cells and understanding the vulnerability of the specific cell types toward neurotoxins.

Keywords Brain cells · Primary culture · CYP2B · Phenobarbital · Immunocytochemistry · RT-PCR · Enzyme

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Introduction

Cytochrome P450 2B (CYP2B) is one of the important CYP primarly involved in the metabolism of drugs and environmental chemicals. CYP2B isoenzymes also catalyze the oxidation of endogenous substrates like testosterone, arachidonic acid, lauric acid, and various carcinogens, including aflatoxin B1 and benzo(a)pyrene [1]. CYP2B1 is non-constitutive and highly inducible by phenobarbital (PB), an anti-convulsant, used extensively for seizure control. CYP2B2 is constitutive but is also moderately induced by PB [2, 3]. The expression of CYP2B1, which is almost negligible in the control rat liver is induced almost 80–100 fold by PB while CYP2B2 is induced 10–20 fold by PB [4]. PB also induces the expression of CYP2B1 and 2B2 in extrahepatic tissues, although it induces the expression of CYP2B1 and 2B2 in liver to a much greater extent than in extrahepatic tissues [2, 3]. CYP2B subfamily also have a potential role in the etiology of glioma [5–7].

CYP2B isoenzymes have been shown to be expressed in mammalian brain. Constitutive expression of CYP2B1 has been reported in the brain [8–10]. Studies from our laboratory have shown that CYP2B1/2B2 specifically catalyze the O-dealkylation of PR in rat brain microsomes [11]. Several studies including immunocytochemistry and/or western blotting have reported high content of CYP2B

proteins in mammalian brain [8, 9, 12, 13], though its mRNA expression is reported to be extremely rare [14, 15]. Hedlund et al. [16] have earlier suggested that due to the low levels of CYPs in brain, the widespread staining reported with antibodies raised against several of the CYPs in brain is unlikely to be the reflection of the distribution of those CYP isoenzymes in the brain.

Recent studies have shown that significant regional differences exist in the expression of constitutive and inducible CYP2B1/2B2 isoenzymes in rat brain, which could be attributed to the differences in the cell type expressing these CYPs [15, 17–19]. Our earlier studies have also shown differences in the distribution of CYP dependent enzymes in rat brain cells [20]. Due to the cellular heterogeneity of the brain, cell specific functions can be understood only when the cells that contain the specific forms of CYPs have been identified. The understanding of their sites of expression is of importance as specific functions within the brain are assigned to certain regions as well as particular cell types. The cerebral localization of CYP2B along with the evidence of catalytic activity and the presence of respective mRNA species, is therefore, essential in understanding its role in xenobiotic as well as endogenous metabolism.

Studies were therefore attempted to investigate the expression and catalytic activity of CYP2B in rat brain primary cultures of neuronal and glial cells. In order to understand the cell specific sensitivity and vulnerability of brain cells to xenobiotics, studies were also carried out to investigate differences in the induction profile of CYP2B in these cultured brain cells following phenobarbital (PB) exposure.

Materials and methods

Chemicals

7-pentoxyresorufin-O-dealkylase (PROD) phenylmethylsulphonylfluoride (PMSF), NADPH, bovine serum albumin (BSA), dithiotheritol (DTT), antibiotic/antimycotic, potassium chloride, poly-L-lysine (PLL), MTT (tetrazolium bromide salt) and fetal bovine serum were procured from Sigma- Aldrich, St.. Louis, MO, U.S.A. Phenobarbital sodium salt (PB) was a gift from Biodeal Laboratories (India). DMEM media, Trizol LS, fetal bovine serum (FBS), glucose, trypsin-EDTA were obtained from Life technologies, USA. Monoclonal antibodies for GFAP (Chemicon International, U.S.A) and β-III-tubulin (Concave Co., California) and Secondary Fluorescence antibodies FITC and Rhodamine (TRITC), (Jackson Immunoresearch Laboratories, Inc., U.S.A) were kindly gifted by Dr. Eugene Major, National Institute of Neurological Disorders and Stroke (NINDS) Bethesda, Maryland. The polyclonal antibody for CYP2B1/2B2 was procured from Chemicon, International, Inc., U.S.A. The antibody reacts only with human and rat cytochrome P450 enzyme CYP2B1/2B2 in hepatic microsomal fractions. No cross reactivity is reported with other cytochrome P450 enzymes in these species. Oligo$(dT)_{20}$, RNAse out, dNTP mix, Thermoscript RT, Taq DNA polymerase and all other reagents for PCR were procured from Invitrogen, USA. All the other chemicals used were of highest purity commercially available and procured either from E. Merck, India or SISCO Research Laboratories Pvt. Ltd., India.

Neuronal and Glial cell culture

Pregnant albino Wistar rats weighing 175–200 gm (~8 week old) were obtained from Industrial Toxicology Research Centre breeding colony and raised on a commercial pellet diet and water *ad libitum.* Animals were cared for in accordance to the policy laid down by Animal Care Committee of Industrial Toxicology Research Centre and the Ethical Committee of the Centre approved animal experimentation. For neuronal cell culture, 14 days old embryos and for glial cells, 0–1 day old pups were used as described in our earlier study by [21]. In brief, brain tissue was minced, trypsinised and incubated with DMEM media containing 12% FBS. Cells were then dissociated and the resulting cell suspension were filtered through a 50 µm diameter nylon mesh and pelleted by centrifugation. Approximately, 30 million cells in 12–15 ml medium were plated in each PLL coated flask. More than 90% of the neurons were attached to the PLL coated surface within about 5 min. Unattached cells were then transferred to second and third set of PLL coated flask after which the unattached glial cells were transferred to a fresh PLL coated flask. Only the first and the last batches of flasks were used as a source of neuronal (N) and glial (G) cells respectively. The purity of neuronal and glial cells preparation, identified by counting multiple fields after staining with cell specific markers, was 90–95%.

Assessment of purity of neuronal and glial cells by immunocytochemistry

The purity of neuronal and glial cultures was assessed as described by Kapoor et al. [21]. In brief, the cultured cells were fixed, permeabilized and then incubated with primary antibody (anti- β-III tubulin or anti-GFAP). The cells were then washed and incubated with anti-rabbit FITC labeled secondary antibody which recognize anti-GFAP antibody, for 1 h. The cells were then washed again and incubated with secondary anti-mouse TRITC labeled antibody, which recognize anti-β-III tubulin antibody. The cells were then

mounted with the mounting media and visualized and analyzed under fluorescence microscope (Leica,Germany) using Leica Q Fluoro Software.

Treatment of brain cells with PB and cytotoxicity assessment

The cultured cells were treated with different concentrations of PB (0.5 mM, 1.0 mM, 2.0 mM, 4.0 mM) for different time intervals (12, 24, 48, 72, 96 h). After treatment, the pure cultured cells were processed for cytotoxicity assessment and for the isolation of microsomes and mRNA.

Cytotoxicity was assessed by a colorimetric assay based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye as described by Kapoor et al. [21]. In brief, after the exposure of PB, MTT was added to each well and left for 4–6 h. The medium containing the MTT was aspirated carefully and 150 µl of DMSO was added to each well and incubated for 30 min. The solution in each well was mixed well and the absorbance read on a microplate reader at 550 nm.

Enzyme activity

The neuronal and glial cells, after treatment with PB were processed for isolation of microsomes as described in our earlier study [21]. The activity of 7-pentoxyresorufin-O-deethylase (PROD) were determined in cultured rat brain neuronal and glial cell microsomes by the method of Parmar et al. [11] using a Perkin Elmer LS 55 Luminescence spectrometer. As isoforms other than CYP2B1 and 2B2 may constitute CYPs in control brain [10, 22], antibody inhibition studies with anti-CYP2B1/2B2 were also carried out to further identify the role of CYP2B1/2B2 in catalyzing the activity of PROD in brain cells as described earlier [11].

RT-PCR analysis

Total RNA was extracted from cultured brain cells treated with and without PB by Trizol LS (Life Technologies, U.S.A.) according to the protocols described earlier [21]. cDNA was synthesized as described in our earlier study [21] and used for subsequent PCR. Prior to the amplification of CYP2B isoenzymes, normalization was carried out with glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the housekeeping gene. PCR reactions for CYP2B1, 2B2 and GAPDH were carried out as described by Johri et al. [23]. The details of the primers used for CYP2B1, 2B2 and GAPDH have been described earlier [24, 25]. PCR products were analysed in VERSA DOC Imaging system, Model 1000 (Bio-Rad, USA). Densitometric analysis of the bands was carried out using Quantity One Quantitation Software version 4.3.1 (BioRad, U.S.A).

Immunocytochemistry

For immunocytochemistry dual staining, the cultured neuronal and glial cells were fixed with 4% formaldehyde for 20 min. Fixed cells were then permeabilized with PBS containing 0.1%BSA, 0.02%Triton X 100 for 15 min. The neuronal cells were incubated with primary antibody (1:100 dilution) specific for neuronal (β-III tubulin) and anti-CYP2B1/2B2, and the glial cells were incubated with monoclonal anti-GFAP (specific for glial cells, 1:100 dilution) and anti-CYP2B1/2B2 (1:100 dilution) for 1 h at 4°C. The cells were then washed with PBS and incubated with (1:100 dilution), anti-rabbit FITC labeled secondary antibody, which recognize CYP2B1 and 2B2 for 1 h. The cells were then washed with PBS, and then incubated for a further one hour with secondary anti-mouse TRITC labeled antibody, which recognized β-III tubulin and GFAP raised in mouse. The cells were then mounted with the mounting media and visualized under fluorescence microscope (Leica Qfluro Standard, Leica Microsystems Imaging Solutions Ltd., Germany). Experiments were performed at least three times, and, on average, 20 fields were evaluated for double blind scoring on each slide.

Statistical analysis

Student's t-test was employed to calculate the statistical significance between control and treated groups. $P < 0.05$ was considered to be significant.

Results

Assessment of purity of neuronal and glial cells by immunocytochemistry

Simultaneous staining of the neuronal cells with β-III tubulin (neuronal marker) and GFAP (glial marker) gave positive immunofluorescence for β-III tubulin (90–95%), whereas GFAP staining was only 5%. Similarly when glial cells were simultaneously stained with β-III tubulin and GFAP, positive immunofluorescence was seen for GFAP (90–95%), whereas, β-III tubulin stained only 5% of the cells (data not shown).

Cytotoxicity assessment of PB and PROD activity in brain cells

When the neuronal and glial cells were exposed to 0.5, 1.0, 2.0, or 4.0 mM PB for 12, 24, 48, 72 or 96 h and assessed by the MTT assay for cytotoxicity, none of the concentrations used were found to be toxic (data not shown).

Microsomes from untreated cultured neuronal and glial cells were found to catalyze the CYP2B dependent N-dealkylation of 7-pentoxyresorufin. The specific activity of PROD in control neuronal cells was approximately 2 fold greater as compared to the glial cells (Table 1). An increase in the activity of PROD was also observed in the cultured brain cells with an increase in the concentration of PB and with the increase in the duration of exposure (data not shown). However, based on the linear response and to compare the sensitivity of the cultured neuronal or glial cells to PB under identical conditions, a concentration of 1.0 mM and time interval of 48 h were chosen for the further induction studies. Addition of PB (1.0 mM) for 48 h was found to produce a significant increase in the PROD activity in cultured neuronal and glial cells. The percentage of induction in the glial cells (140%) was more than that observed in neuronal cells (47%).

Effect of anti-CYP2B1/2B2 on cultured rat brain cell PROD activity

Addition of polyclonal antibody raised against rat liver CYP2B1/2B2 isoenzymes to the microsomes isolated from control neuronal and glial cells did not produce any significant inhibition in the PROD activity at lower concentrations. Significant inhibition in the enzyme activity was observed only at the highest concentration (Table 1). Almost similar magnitude of inhibition in the enzyme activity was observed in both cultured neuronal or glial cells (Table 1). Addition of preimmune IgG had no significant effect when added in vitro to the microsomes isolated from control neuronal or glial cells (Table 1). In vitro addition of anti-CYP2B1/2B2 to the microsomes isolated from PB pretreated neuronal and glial cells produced a significant concentration dependent inhibition of the enzyme activity with much higher inhibition of the enzyme activity being observed in the cultured brain cells treated with PB. Approximately 80–90% inhibition occurred in the PROD activity with anti-CYP2B1/2B2 at the higher concentration (Table 1).

RT-PCR analysis

RT-PCR analysis with primers specific for rat liver GAPDH resulted in the formation of PCR products of expected band size (194bp) in the RNA isolated from the rat brain neuronal and glial cells cultured in DMEM alone or in DMEM plus PB (Fig. 1). Densitometric analysis of the PCR products revealed that PCR amplification resulted in the formation of products of almost equal intensity in untreated and PB treated cell cultures (Fig. 1). Using specific primers for CYP2B1 and CYP2B2, PCR analysis of the RT product obtained from the RNA isolated from rat brain neuronal and glial cells cultured in DMEM alone or in DMEM plus PB after normalization with GAPDH, produced a distinct band visible by agarose gel electrophoresis and of the correct size of 109bp (CYP2B1) and 163bp (CYP2B2) respectively (Figs. 2 and 3). The product of similar size was obtained from the RNA isolated from control rat liver. The basal expression of CYP2B1 and CYP2B2 mRNA in the neuronal and glial cells was determined by densitometry, and as seen in the Figs. 2 and 3, the constitutive level of CYP2B1 and CYP2B2 in the cultured neuronal cells was greater as compared to the cultured glial cells.

Treatment of the cultured brain cells with PB produced a significant increase in CYP2B1 and CYP2B2 mRNA expression in both cultured neuronal and glial cells, relative to the corresponding untreated cells. Densitometric analysis further revealed that the percentage of induction was more in glial cells as compared to the neuronal cells (Figs. 2 and 3). Furthermore, as evident from Figs. 2 and 3,

Table 1 PROD[a] activity in cultured brain cells and in vitro effect of anti-CYP2B1/2B2 on enzyme activity

	Neuronal cells		Glial cells	
	Control	Treated	Control	Treated
None	27.2 ± 1.3	40.1 ± 2.7*	10.0 ± 1.2	24.1 ± 3.5*
Preimmune IgG				
0.15 mg	26.5 ± 1.4	38.3 ± 2.8	9.31 ± 1.1	23.2 ± 3.4
0.30 mg	25.1 ± 1.6	36.5 ± 3.2	8.52 ± 1.2	22.1 ± 3.3
0.80 mg	22.5 ± 1.5	34.3 ± 3.5	7.52 ± 1.1	20.4 ± 3.2
Anti CYP2B1/2B2				
0.15 mg	23.2 ± 1.5	19.8 ± 2.1*	7.50 ± 1.0	11.6 ± 1.5*
0.30 mg	20.1 ± 1.5	8.72 ± 0.9*	5.21 ± 0.9	4.45 ± 0.9*
0.80 mg	13.5 ± 1.4*	3.43 ± 0.8*	3.41 ± 0.8*	1.92 ± 0.4*

[a] Specific activity is expressed in pmoles resorufin/min/ mg protein

All values are mean ± S.E. of 3 experiments

*$P < 0.05$ when compared to the controls

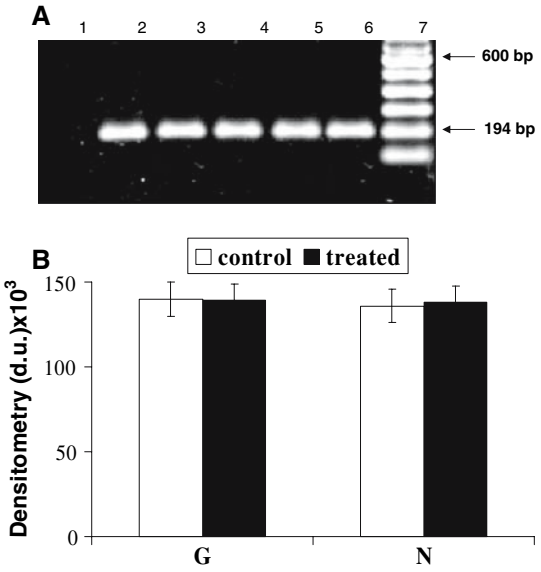

Fig. 1 (**a**) Ethidium bromide stained agarose gel showing rat GAPDH mRNA in cultured neuronal and glial cells. Lane 1 contains 5 µl of the RT-PCR product without RNA. Lanes 2 & 3 contains 5 µl of the RT-PCR product isolated from control and PB treated glial cells. Lanes 4 & 5 contain 5 µl of the RT-PCR product of RNA isolated from control and PB treated neuronal cells. Lanes 6 and 7 contains 5 µl of the RT-PCR product isolated from control liver and 1.0 Kb DNA ladder respectively. (**b**) Densitometric analysis of RT-PCR products (mean ± S.E., $n = 3$ gels). G and N corresponds to the intensity of RT-PCR product of RNA isolated from cultured glial and neuronal cells respectively

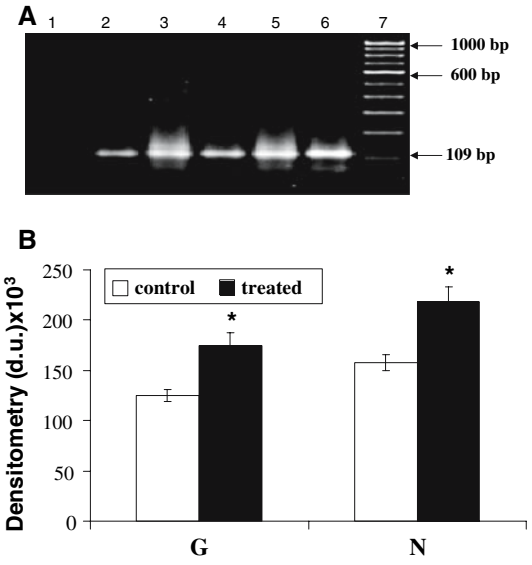

Fig. 2 (**a**) Ethidium bromide stained agarose gel showing rat CYP 2B1 mRNA in cultured neuronal and glial cells. Lane 1 contains 5 µl of the RT-PCR product without RNA. Lanes 2 & 3 contains 5 µl of the RT-PCR product isolated from control and PB treated cultured glial cells. Lanes 4 & 5 contains 5 µl of the RT-PCR product from control and PB treated cultured neuronal cells. Lanes 6 and 7 contains 5 µl of the RT-PCR product from control liver and 1.0 Kb DNA ladder respectively. (**b**) Densitometric analysis of RT-PCR products (mean ± S.E., $n = 3$ gels). G and N corresponds to the intensity of RT-PCR product of RNA isolated from cultured glial and neuronal cells respectively

the magnitude of increase in CYP2B2 mRNA expression was found to be more when compared to that observed with CYP2B1.

Western blot analysis

Immunoblot analysis of the solubilised microsomal proteins isolated from the untreated or treated neuronal and glial cells with hepatic anti-CYP2B1/2B2 did not exhibit significant immunoreactivity co-migrating with the liver isoenzyme in either control rat brain cells or cells cultured in the presence of PB (data not shown).

Assessment of CYP2B expression in cultured neuronal and glial cells by immunocytochemistry

Both neuronal and glial cells cultured with DMEM medium alone or treated with PB, grown on PLL coated glass slides, when fixed, and stained with anti-CYP2B antibody and with anti-β-III tubulin or anti-GFAP, and the secondary antibodies labeled with FITC or TRITC showed positive staining for CYP2B. As shown in Fig. 4, all the neuronal cells exhibiting staining with anti-β-III tubulin also expressed CYP2B1 as judged by similar pattern of staining with anti-CYP2B1. However, some anti-β-III tubulin negative cells were immunopositive for CYP2B1/2B2. Likewise, all glial cells exhibiting staining with anti-GFAP also expressed CYP2B as judged by staining with anti-CYP2B (Fig. 5). Some of the glial cells, which did not exhibit any staining with anti-GFAP, were found to be immunoreactive for CYP2B. As the primary culture used in the present study was a mixed culture consisting of oligodendrocytes, microglia and astrocytes and the monoclonal anti- GFAP used recognizes only the astrocytes and Bergman glia cells, cells other than above such as microglia and oligodendroctyes could be positive for CYP2B, but negative for GFAP [26]. Similarly some anti- β-III tubulin negative cells, which were immunopositive for CYP2B, could be the endothelial or fibroblast cells contaminating the neuronal cultures. Densitometric analysis revealed that treatment of PB resulted in an increase in the intensity of FITC fluorescence (Figs. 4 and 5). The induction appeared to be more in glial cells, as compared to the neuronal cells.

Discussion

The present study indicating constitutive as well as inducible mRNA and protein expression of CYP2B isoenzymes along with the evidence of its catalytic activity

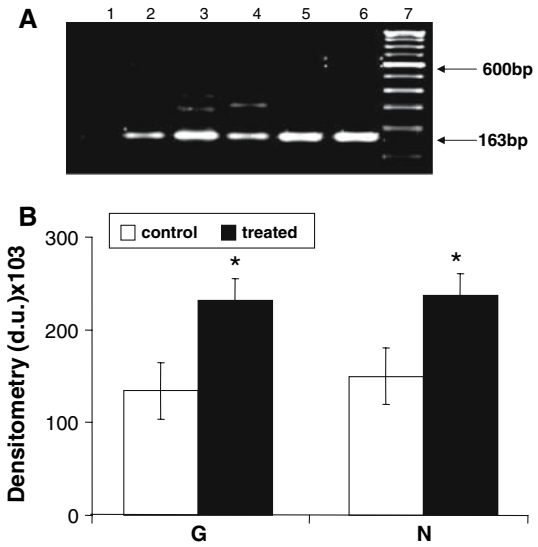

Fig. 3 (**a**) Ethidium bromide stained agarose gel showing rat CYP 2B2 mRNA in cultured neuronal and glial cells. Lane 1 contains 5 µl of the RT-PCR product without RNA. Lanes 2 & 3 contains 5 µl of the RT-PCR product isolated from control and PB treated cultured glial cells. Lanes 4 & 5 contains 5 µl of the RT-PCR product from control and PB treated cultured neuronal cells. Lanes 6 and 7 contains 5 µl of the RT-PCR product from control liver and 1.0 DNA ladder respectively. (**b**) Densitometric analysis of RT-PCR products (mean ± S.E., $n = 3$ gels). G and corresponds to the intensity of RT-PCR product of RNA isolated from cultured glial and neuronal cells respectively

in cultured neuronal and glial cells has clearly demonstrated expression of CYP2B isoenzymes in rat brain [9, 10, 19, 27]. Earlier studies have also shown that the constitutive androstane receptor (CAR), involved in the induction of CYP2B isoenzymes, and the other nuclear receptors, pregnane X receptor (PXR) and retinoid X receptor (RXR) are expressed in murine and human brain [28–30]. Furthermore, significant increase in the CYP2B dependent PROD enzyme activity in cultured brain cells following treatment of phenobarbital (PB) have indicated that the responsiveness to PB is retained in the primary cultures. Interestingly, though the constitutive levels of CYP2B were higher in neurons, PB treatment resulted in a greater fold induction in glial cells as compared to the neuronal cells.

Immunocytochemical studies were also consistent with enzyme data indicating higher immunoreactivity with anti-CYP2B in the cultured neuronal cells as compared to the glial cells. Rosenbrock et al. [31] reported that CYP2B in brain is primarily localized in neurons. Studies with rat brain and human medulla also showed CYP2B1 immunoreactivity in the rat neuronal cell bodies [12, 32]. Kempermann et al. [26] however, demonstrated that CYP2B1/2B2 immunoreactivity in astrocytes both in vivo and in vitro. Immunocytochemical studies have further indicated that the increase observed in the activity of PROD after treatment with PB in cultured brain cells could be due to the increase in the expression of CYP2B isoenzymes. Miksys et al. [18] also reported that nicotine, a major constituent of tobacco, increases CYP2B1 immunoreactivity in brain, the pattern of which was found to be highly region and cell type specific. They concluded that the high degree of localization of CYP2B1 in specific cell types and brain region after nicotine induction may be sufficient to alter nicotine metabolism thereby contributing to the development of the central tolerance to nicotine observed both in animal models and in human smokers.

The inability to detect CYP2B1/2B2 protein expression by immunoblotting in either control or PB treated cultured neuronal and glial cells could be because of the relatively lower protein expression of CYP2B1/2B2 in brain cells which maybe beyond the levels of detection of the antibody used in the present study. It has been further reported that CYP2B1/2B2 do not constitute majority of the PB-inducible CYPs in brain [22]. However, the ability to detect

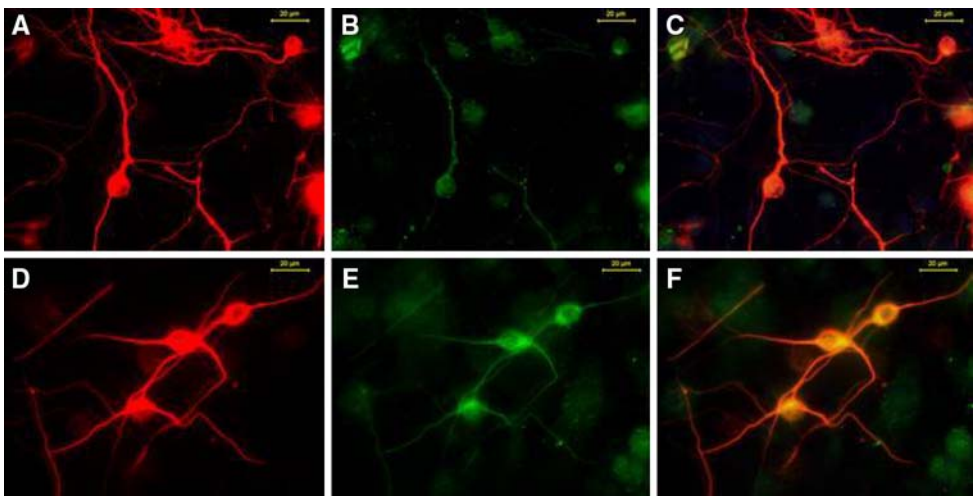

Fig. 4 Immunocytochemical detection of CYP2B in cultured rat brain neuronal cells. **A**, **B** & **C** represent primary cultures of neuronal cells in DMEM. D, E & F represent cultures of neuronal cells in DMEM+PB. A & D show cells in culture that are positive for β-III tubulin (red-TRITC), a neuronal marker. **B** & **E** show immunoreactivity in the same neuronal cells with anti-CYP2B1 (green-FITC). C & F represent an overlay of the two images control and PB treated respectively. Original magnification × 400, scale 20 µm

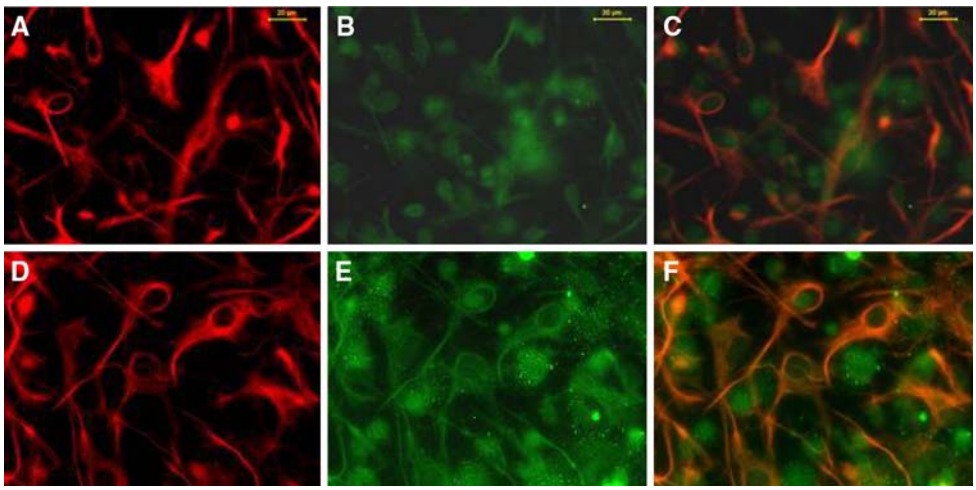

Fig. 5 Immunocytochemical detection of CYP2B in cultured rat brain glial cells. A, B & C represent primary cultures of glial cells in DMEM. D, E & F represent cultures of glial cells in DMEM+PB. **A** & **D** show cells in culture that are positive for GFAP (red-TRITC), a glial marker. **B** & **E** show immunoreactivity in the same glial cells with anti-CYP2B1 (green-FITC). C & F represent an overlay of the two images control and PB treated respectively. Original magnification × 400, scale 20 μm

responsiveness of cultured brain cells to PB by immunocytochemistry or PCR could be attributed to the enhanced sensitivity of those assays where even the increase in the expression of single cell could be detected as compared to identifying increase in protein expression from a pool of microsomal proteins by western blotting. Likewise, relatively higher catalytic activity measured in the cultured brain cells could be attributed to the endogenous factors that may regulate CYP2B catalytic activity. Similar inconsistency and lack of correlation in enzyme content as measured by Western blotting and enzyme activity has been shown for CYP1A2 in liver and has been attributed to the endogenous factors that may modulate enzyme activity rather than enzyme expression [33]. Recent studies using reporter gene protocol have shown that ligands of peripheral benzodiazepine receptor (PBR) or $GABA_A$ receptor induce CYP2B activity that is mediated through the phenobarbital response unit (PBRU) and the nuclear receptor binding sites [34].

RT-PCR studies have also shown that PB treatment induced the mRNA expression of CYP2B1 and CYP2B2 isoenzymes in both the neuronal and glial cells. CYP2B2 was however found to be induced to a greater extent as compared to CYP2B1, suggesting that different mechanism of induction might be existing for the two isoforms, in these cell types, as compared to the liver. Ibach et al. [35] demonstrated increase in CYP2B1 mRNA expression in primary rat astrocyte cultures after phenytoin treatment, though CYP2B2 was not detected at any age of astrocyte cultures. They suggested that various CYP inducers differentially regulate CYP2B2 gene expression in whole brain and astrocytes and tissue inherent cellular interactions could be essential to induce CYP2B2 expression in vivo. Schilter et al. [36] also observed tissue-specific and time dependent enhancement in the CYPs following PB treatment and attributed it to distributional or dispositional kinetic differences between brain and liver. Substantial and almost comparable activation of CYP2B1 and CYP2B2 was also reported in cerebellum and striatum after repeated administration of PB while no change or even slightly decreased levels of CYP2B1 and CYP2B2 was observed after 1 or 2 days of PB treatment. Furthermore, as compared to the cultured hepatocytes, where matrigel is required to maintain the expression and induction of CYP2B1/2B2, the activity of CYP2B isoenzymes was maintained and induced following addition of PB to cultured neuronal and glial cells without any matrigel support, suggesting that difference in the induction of CYP2B gene even exist under in vitro conditions.

The results of the present study indicating greater sensitivity of glial cells to PB have suggested that these cells play a protective and decisive role in biotransformation of xenobiotics that reach the CNS [37, 38]. Hagemeyer et al. [39] suggested CYP expression in astrocytic population within the olfactory bulb and the forebrain, in the smooth muscle cells covering micro vessels, in ependymal cells in the choroids plexus, which actively constitute the functional components of the blood-brain barrier. As the glial cells, along with the endothelial cells constitute a major part of the blood-brain barrier, the greater fold induction in CYP2B levels in the glial cells, following PB exposure, as compared to the neuronal cells, provides evidence that these cells play a significant role in the detoxification mechanism. Meyer et al. [38] who studied the role of astrocyte CYP in the metabolic degradation of phenytoin observed that CYPs in astrocytes fulfil a mediatory detoxification function by degrading phenytoin to keep the drug response of the neurons in balance.

In conclusion, results of the present study have provided evidence for the expression of CYP2B1 and CYP2B2 in cultured neuronal and glial cells. Relatively high levels of CYP2B dependent catalytic activity in pure cultures of

neuronal and glial cells suggests the role of endogenous factors in regulating CYP2B activity in brain. These studies have further shown differences in the responsiveness of the cultured brain cells to the inductive effects of PB with glial cells exhibiting greater sensitivity to PB mediated induction. The enhanced sensitivity of CYP2B in the glial cells is of significance as these cells primarily constitute the blood-brain barrier. Furthermore, greater magnitude of induction of CYP2B2 have suggested that different mechanism of induction maybe occurring in brain as compared to liver.

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