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## PGE2 調控 cytochrome P450 2B1 表現之機制探討(3/3)

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# Prostaglandin $E_2$ down-regulation of cytochrome P-450 2B1 expression induced by phenobarbital is through $EP_2$ receptor in rat hepatocytes

**Abstract**

Cytochrome P-450 is an important bioactivation-detoxification system in vivo. Its expression is regulated by foreign chemicals and dietary factors, and lipids have been found to regulate its gene expression. We showed previously that prostaglandin $E_2$ ($PGE_2$), a fatty acid metabolite, down-regulates cytochrome P-450 2B1 (CYP 2B1) expression induced by phenobarbital. The objective of the present study was to determine whether $PGE_2$ type 2 receptor ($EP_2$)—which is coupled to Gs-protein when bound by $PGE_2$, leading to cAMP production—is involved in this down-regulation. We also determined the possible roles of $EP_2$ downstream pathways in this down-regulation. We used a primary rat hepatocyte culture model in which $EP_2$ was shown to be present to study this question. The intracellular cAMP concentration in primary rat hepatocytes was significantly higher after treatment with 1 μM $PGE_2$ than after treatment with 0, 0.01, or 0.1 μM $PGE_2$. Butaprost, an $EP_2$ agonist, down-regulated CYP 2B1 expression in a dose-dependent manner. SQ22536, an adenylate cyclase inhibitor, reversed the down-regulation by $PGE_2$ as did H-89, a protein kinase A inhibitor. These results suggest that $EP_2$ and the downstream pathways of cAMP and protein kinase A are involved in the down-regulation of CYP 2B1 expression by $PGE_2$ in the presence of phenobarbital.

Keywords: Cytochrome P-450 2B1; $EP_2$; cyclic AMP; protein kinase A; hepatocytes

The liver is the major organ involved in detoxification, and the detoxification system is composed mostly of phase I and phase II enzymes. Cytochrome P-450s (CYPs)[3] [1] and epoxide hydrolases [2] are phase I enzymes, and glutathione-S-transferases [3] and UDP-glucuronosyltransferases [4] are phase II enzymes. CYPs are composed of about 40 to 50 isoenzymes [5]. These enzymes are found mainly in the liver and the gut. They are involved in the biosynthesis and catabolism of endogenous compounds, such as fatty acids and steroid hormones, and play an important role in the metabolic activation of various foreign compounds [5,6].

In rat liver, cytochrome P-450 2B1 (CYP 2B1) has been shown to be highly inducible by many chemical agents [7], especially phenobarbital, which results in significant induction within a few hours [8]. In rodents, hepatic CYP 2B1 activity was found to be affected by both chemicals and dietary factors, such as dietary lipids and soy protein [9,10]. It has also been shown that increased cAMP suppresses the induction of CYP 2B1 by phenobarbital in primary rat hepatocyte cultures [11].

Prostaglandin $E_2$ ($PGE_2$) is a cyclooxygenase (COX) metabolite of arachidonic acid that has been suggested to show diverse physiologic and pathologic effects on different cell types [12,13]. In our previous study, we found that $PGE_2$ plays a down-regulatory role in the α-tocopheryl succinate modulation of CYP 2B1 expression induced by phenobarbital [14]. $PGE_2$ has been shown to modulate the expression of several genes, such as the down-regulation of neuronal nitric oxide synthase messenger RNA [15] and intercellular adhesion molecule 1 gene expression [16] and the up-regulation of *HER-2* oncogene expression [17].

The physiologic effect of $PGE_2$ is mediated through prostaglandin E receptors (EPs), which regulate the intracellular secondary messenger system and trigger different signal transduction

pathways [18]. There are 4 subtypes of EPs: $EP_1$, $EP_2$, $EP_3$, and $EP_4$, all of which belong to the family of G-protein coupled receptors [19,20]. $EP_2$ is known to couple to $G\alpha_s$ and activate adenylate cyclase, resulting in increased intracellular cAMP [21]. In the present study, we used a primary rat hepatocyte culture to study the mechanism involved in the $PGE_2$ down-regulation of CYP 2B1 expression induced by phenobarbital and whether $EP_2$ is involved in this down-regulation.

## Materials and methods

**Chemicals.** Cell culture medium (RPMI-1640) was obtained from GIBCO-BRL (Gaithersburg, MD); $ITS^+$ and Matrigel (insulin, transferrin, selenium, bovine serum albumin, and linoleic acid) were from Collaborative Biomedical Products (Bedford, MA); collagenase type I was from Worthington Biochemical Corporation (Lakewood, NJ); phenobarbital and *N*-(2-[*p*-bromocinnamylamin]ethyl)-5-isoquinolinesulfonamide hydrochloride (H-89) were from Sigma Chemical Company (St Louis, MO); [9-(tetrahydro-2'-furyl)adenine] (SQ22536) was from Calbiochem (San Diego, CA); and $PGE_2$ was from Cayman Chemical Company (Ann Arbor, MI).

**Hepatocyte isolation and culture.** Male Sprague-Dawley rats (weighing 250 to 300 g) were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). Hepatocytes were isolated by a modification of the two-step collagenase perfusion method described previously [22]. After isolation, hepatocytes ($3 \times 10^6$ cells per dish) were plated on collagen-coated 60-mm plastic tissue dishes in RPMI-1640 medium (pH 7.38) supplemented with 10 mM HEPES, 1% $ITS^+$, 1 μM dexamethasone, 100 IU penicillin/mL, and 100 μg streptomycin/mL. Cells were incubated at 37°C in a humidified incubator containing 5% $CO_2$. After 4 h, the cells were washed with PBS to remove any unattached or dead cells, and the same medium supplemented with Matrigel (233 mg/L) and 0.1 μM dexamethasone was added. Thereafter, the medium was changed daily. The protocol for each experiment is described in the corresponding figure legend.

**cDNA preparation and RT-PCR analysis.** Forty hours after attachment, hepatocytes were treated with or without 1 μg/mL lipopolysaccharides (LPS) for 18 h, then total RNA was isolated and was subjected to RT-PCR with specific $EP_2$ or COX-1/2 primers. Amounts of 0.1 to 0.25 μg total RNA was reversely transcribed with superscript II reverse transcriptase (Stratagene, Heideberg, Germany) in a 20-μL final volume of the reaction buffer, which consisted of 5 mM $MgCl_2$, 1 mM of each deoxyribonucleotide triphosphate, 2.5 units RNase inhibitor, and 2.5 mM oligo(dT). For the synthesis of complementary DNA, the reaction mixtures were incubated at 45°C for 15 min; the reaction was stopped by denaturing the reverse transcriptase by heating the mixture to 99°C for 5 min. To the cDNA sample, a PCR mixture containing 4 mM $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate, a dilution of the cDNA preparation, 5 μL of 10x buffer, 0.2 units Taq polymerase, and 0.6 pmol $EP_2$ primer was added to a total volume of 50 μL. The sequences of the RT-PCR $EP_2$ primers were as follows: 5'-CGCTACCTCGCCATCGGA CACC-3'(forward) and 5'-GGGAGCATACAGCGAAGGTGAT-3' (reverse). The sequences of the RT-PCR COX-1 primers were as follows: 5'-CTTCCGTGTGCCAGATTACCC-3'(forward) and 5'-GGCTGGCCTAGAACTCACTGC-3' (reverse) which amplify a 474 bp fragment (bp 1795－ 2268) of the COX-1 (GenBank accession nos. **U03388**), and COX-2 primers were as follows: 5'-TCTCCCTGAAACCTTACACAT-3'(forward) and 5'-GTTGAACGCCTTTTGA TTAGT-3'(reverse) which amplify a 439 bp fragment (bp 1367－1805) of the COX-2 (GenBank accession nos. **L20085**). For $EP_2$ PCR amplification, the samples were heated to 94°C for 3 min and then immediately cycled 30 times through a 0.5-min denaturing step at 94°C, a 0.5-min annealing step at 60°C, and a 0.5-min elongation step at 72°C. After the final cycle, a 5-min elongation step at 72°C was carried out. For COX-1/2 PCR amplification, the samples were heated to 94°C for 5 min and then immediately cycled 35 times through a 1-min denaturing step

at 94°C, a 1-min annealing step at 60°C, and a 1-min elongation step at 72°C. After the final cycle, a 10-min elongation step at 72°C was carried out. The amplified PCR products of $EP_2$ and COX-1/2 mRNA were separated on 1.5%-agarose gels alongside markers and were further confirmed by sequencing.

**Measurement of intracellular cAMP concentrations.** Intracellular cAMP concentrations were measured by using the cAMP EIA kit (Cayman Chemical Company). The cells were incubated with various amounts of $PGE_2$ for 20 h. Cell extracts were prepared as described by Beck and Omiecinski [23].

**Northern blotting for CYP 2B1.** RNA was extracted from rat primary hepatocytes with 0.5 mL TRIzol reagent (Invitrogen Corporation, Carlsbad, CA). The extract was allowed to react at room temperature for 5 min, 0.1 mL chloroform was added, and the sample was incubated for an additional 3 min. The samples were centrifuged at 12,000 × *g* for 15 min at 4°C. The aqueous phase was transferred to a fresh tube, and the RNA was precipitated by the addition of 0.5 mL isopropyl alcohol. The RNA samples were allowed to sit at room temperature for 10 min and were then centrifuged at 12,000 × *g* for 20 min at 4°C. The resulting RNA pellets were washed twice with 75% ice-cold ethanol. For Northern blot analysis, 20 µg of each RNA sample was electrophoresed on a 1%-agarose gel containing 6% formaldehyde and were then transferred to a Hybond-N nylon membrane (Amersham, Little Chalfont, United Kingdom) as previously described [24]. For hybridization with cDNA, the membrane was prehybridized at 42°C for 2 h in a solution containing 10x Denhardt's reagent (0.2% Ficoll, 0.2% polyvinypyrolidone, and 0.2% bovine serum albumin), 5x saline-sodium phosphate-EDTA buffer (SSPE, 750 mM NaCl,

50 mM $NaH_2PO_4$, and 5 mM EDTA), 2% sodium dodecyl sulfate (SDS), 50% formamide, and 100 mg/L of single-stranded sheared salmon sperm DNA. The membrane was then hybridized in the same solution with $^{32}$P-labeled 2B1 cDNA probe at 42°C overnight. The hybridized membrane was washed once or twice in 2x saline-sodium citrate buffer (SSC/0.05% SDS) at room temperature and then at 55°C for 10 min in 0.1x SSC/0.1% SDS. Autoradiography was performed by exposing the membrane to Kodak SuperRx X-ray film (Pierce, Rockford, IL) at 80°C with an intensifying screen.

**Western blotting for CYP 2B1.** SDS polyacrylamide gels made with 7.5% acrylamide were prepared as described by Laemmli [25]. For CYP 2B1, 7.5 µg of microsomal protein was applied to each gel. After electrophoresis, the separated proteins were transferred to polyvinylidene difluoride membranes. The nonspecific binding sites on the membranes were blocked with 5% nonfat dry milk in 15 mM tris–150 mM NaCl buffer (pH 7.4) at 4°C overnight. Polyclonal antibody against CYP 2B1 was obtained from Chemicon International (Temecula, CA). A goat peroxidase-conjugated anti-rabbit IgG was used to detect the immunoreactive bands. Incubation with primary and secondary antibodies was performed at 37°C for 30 min. For color development, hydrogen peroxide and 3,3'-diaminobenzidine tetrachloride were used as the substrates for peroxidase.

**Statistical analysis.** Data were analyzed by using analysis of variance (SAS Institute Inc. Cary, NC). The significance of the difference between mean values was determined by one-way analysis of variance and Tukey test; $P$ values $< 0.05$ were taken to be statistically significant.

## Results

**$EP_2$ mRNA expression.** We showed previously in rat primary hepatocytes that $PGE_2$ had down-regulatory effect on CYP 2B1 expression induced by phenobarbital [14]. To clarify whether $EP_2$ is involved in this down-regulation, we used RT-PCR to detect $EP_2$ in rat primary hepatocytes, rat spleen, and kidney. $EP_2$ was shown to be present in hepatocytes (Fig. 1, lane 1) and rat spleen (lane 3), but not in kidney (lane 2). The RT-PCR was performed using a forward primer [5'-GCTACCTCGCCATCGGACACC-3'] and reverse primer [5'-GGGAGCATACAG CGAAGGTGAT-3'] which amplify a 430 bp fragment cording nucleotide 445 to 874 of the $EP_2$ of male Sprague-Dawley rats (GenBank accession nos. **U94708**).

**COX mRNA expression in hepatocytes.** To determine whether the hepatocyte preparation was contaminated with Kupffer cells, the hepatocyte preparation was treated with 1 μg/mL LPS, which induces COX-2 gene expression in Kupffer cells. As shown in Fig 2, the constitutive COX-1 mRNA was detected in hepatocytes treated with (lane 1) or without (lane 3) LPS, but no inducible COX-2 mRNA was detected with or without LPS treatment (lane 2 and lane 4).

**Effect of $PGE_2$ on intracellular cAMP concentrations.** To study whether the down-regulatory effect of $PGE_2$ on CYP 2B1 expression is mediated through cAMP, we assessed the effect of $PGE_2$ on the intracellular cAMP concentration. As shown in Table 1, the intracellular cAMP concentration was increased by $PGE_2$, and the significantly increase was seen after treatment with 1 μM $PGE_2$.

**Effect of $PGE_2$ on CYP 2B1 gene expression.** In our previous study [14], phenobarbital induction of CYP 2B1 gene expression in rat primary hepatocytes was down-regulated by $PGE_2$. In the present study, same result was found. As shown in Fig. 3A, 1 μM $PGE_2$ significantly down-regulated CYP 2B1 protein expression. The CYP 2B1 mRNA expression pattern was similar to that of the protein (Fig. 3B).

**$EP_2$ agonist effect on $PGE_2$** down-regulation **of CYP 2B1 expression.** To determine the role of $EP_2$ in the down-regulation of CYP 2B1 expression by $PGE_2$, butaprost, an $EP_2$ agonist, was used. CYP 2B1 protein expression was down-regulated by butaprost in a dose-dependent manner (Fig. 4A). In addition, CYP 2B1 mRNA expression was down-regulated by butaprost at concentration of 0.1 and 1 μM (Fig. 4B).

**Adenylate cyclase effect on $PGE_2$** down-regulation **of CYP 2B1 expression.** SQ22536, an inhibitor of adenylate cyclase, reversed the $PGE_2$ down-regulation of CYP 2B1 protein expression in a dose-dependent manner; the maximum effect was observed with 0.8 mM (Fig. 5A). A similar pattern was found for mRNA expression (Fig. 5B).

**Protein kinase A inhibitor effect on $PGE_2$** down-regulation **of CYP 2B1 expression.** Previous studies showed that activation of the cAMP-dependent protein kinase A (PKA) pathway is involved in the regulation of CYP 2B1 gene expression [11]. To further determine whether PKA plays a key role in the down-regulation of CYP 2B1 gene expression by $PGE_2$, the PKA inhibitor H-89 (7.5 μM) was used. As shown in Fig. 6, H-89 reversed the down-regulation of CYP 2B1 gene expression by $PGE_2$ in the presence of phenobarbital.

## Discussion

Previous studies showed that the cAMP-PKA pathway can regulate many cellular processes, such as cell metabolism [26], proliferation [27], and gene transcription [28]. It has also been shown that the phenobarbital induction of CYP 2B1/2 and 3A1 expression in rat primary hepatocytes is suppressed by activators of cAMP and PKA [11]. In our previous study, we found that $PGE_2$ down-regulated CYP 2B1 expression induced by phenobarbital in rat primary hepatocytes [14]; however, the mechanism for this down-regulation was not clear.

The physiologic effects of $PGE_2$ are mediated through interaction with G-protein-coupled EP receptors [29]. Four rat prostanoid EP receptor subtypes have been cloned and characterized [19, 20], and they have been shown to have tissue specificity [30]. In the present study, the Gs-linked $EP_2$ receptor was detected in primary rat hepatocyte cultures by RT-PCR (Fig. 1). Using density gradient centrifugation, Fennekohl et al. [31] reported that hepatocyte preparations may be contaminated with 0.1% to 1% Kupffer cells and that $EP_2$ mRNA is expressed in Kupffer cells but not in hepatocytes. To confirm that our hepatocyte preparation was not contaminated with Kupffer cells, we treated the preparation with lipopolysaccharide, which induces cyclooxygenase-2 gene expression in Kupffer cells [32, 33]. The presence of Kupffer cells in our hepatocyte preparation was excluded, because no lipopolysaccharide-induced cyclooxygenase-2 gene expression was found (Fig. 2). We also confirmed the presence of $EP_2$ in hepatocytes; thus, our results differ from those of Fennekohl et al. [31]. The expression of EP receptors may vary under different culture conditions [34, 35].

$PGE_2$-mediated cAMP production is involved in the regulation of various cellular physiologic responses [36] and signal transduction pathways [37]. Sidhu and Omiecinski [11] showed that an increased intracellular cAMP concentration inhibits phenobarbital-induced CYP

2B1 gene expression in rat primary hepatocytes and suggested that cAMP plays a negative regulatory role in phenobarbital gene induction [38, 39]. Therefore, we examined the ability of $PGE_2$ to stimulate intracellular cAMP. As shown in Table 1, 1 μM $PGE_2$ raised the intracellular cAMP concentration significantly more than did the other treatments, and 1 μM $PGE_2$ showed the greatest down-regulation of CYP 2B1 gene expression in the presence of phenobarbital (Fig. 3). These results suggest that the effect of $PGE_2$ down-regulation of phenobarbital-induced CYP 2B1 gene expression may be through an elevation in the intracellular cAMP concentration.

Butaprost is an $EP_2$-specific agonist that is used pharmacologically to define the role of $EP_2$ in various tissues and cells [40, 41]. In the present study, the down-regulatory effect of butaprost on CYP 2B1 expression paralleled the effect of $PGE_2$ (Figs. 3 and 4). These results suggest that $EP_2$ plays an important role in the down-regulation of CYP 2B1 gene expression by $PGE_2$.

PKA is activated as a result of interaction with cAMP, a well-studied secondary messenger signal that is generated through G-protein-coupled receptor stimulation of adenylate cyclase [42]. Activation of a cAMP-dependent PKA pathway results in suppression of phenobarbital-inducible CYP gene expression [11, 43]. In the present study, the PKA inhibitor H-89 and the adenylate cyclase inhibitor SQ22536 reversed the down-regulatory effect of $PGE_2$ on CYP 2B1 in the presence of phenobarbital (Figs. 5 and 6). These results suggest that the cAMP-dependent PKA pathway is involved in the down-regulation of CYP 2B1 by $PGE_2$.

Several studies propose the liver-enriched orphan nuclear receptor (e.g., constitutive androstane receptor, CAR), plays a central role in PB-induction of CYPs [44,45,46], such as human CYP 2B6, mouse CYP 2B10 and CYP 2B1/2B2. There are PB-responsive element (PBRE) in their promotors which contain the conserved nuclear receptor binding sites for CAR [47, 48]. Moreover, the activity of CAR has been suggested to depend upon phosphorylation

steps [48]. Several serine/threonine protein kinases or phosphatases have been characterized to implicate in PB-induced gene expression [49], however, whether CAR is one of these targets is unclear. Future work is warranted to determine whether CAR is involved in cAMP-dependent PKA inhibition of CYP 2B1 expression.

In summary, the results of the present study indicate that $PGE_2$ decreases CYP 2B1 expression in the presence of phenobarbital in rat primary hepatocytes through $EP_2$ and that cAMP and the downstream cAMP-dependent PKA pathway are involved in this down-regulation.

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**Figure legends**

Fig. 1. RT-PCR analysis of the expression of $EP_2$ receptor mRNAs in rat primary hepatocytes. Total RNA was isolated distinctively from hepatocytes (lane1), kidney (lane 2) and spleen (lane 3) tissues and were subjected to RT-PCR with specific $EP_2$ primer, as described in materials and methods. RNA from rat kidney was used as negative control and RNA from rat spleen was used as positive control. $EP_2$ RT-PCR products were separated by 1.5% agarose gel and stained with ethidium-bromide. A 100-bp ladder used as a molecular marker is indicated on the right (lane 4), and the $EP_2$ PCR product is indicated by the arrow on the left.

Fig. 2. RT-PCR analysis of the expression of COX mRNAs in rat primary hepatocytes. Forty hours after attachment, hepatocytes were treated with or without 1 μg/mL LPS for 18 h then total RNA was isolated from hepatocytes and was subjected to RT-PCR with specific COX-1 (lane 1 and lane 3) or COX-2 (lane 2 and lane 4) primers, as described in materials and methods.

Fig. 3. Effect of various concentrations of $PGE_2$ on CYP 2B1 expression in the presence of phenobarbital (PB). Forty hours after attachment, hepatocytes were incubated with $PGE_2$ for 15 min before the addition of phenobarbital; the cells were then incubated with phenobarbital for another 20 h. (A) Western blot analysis. (B) Northern blot analysis. Uniform RNA loading was demonstrated by the ethidium bromide staining of the 18S rRNA band which was used as the standard. One representative experiment out of three independent experiments was shown.

Fig. 4. Effect of various concentrations of butaprost on CYP 2B1 expression in the presence of phenobarbital (PB). Forty hours after attachment, hepatocytes were incubated with butaprost for 15 min before the addition of phenobarbital; the cells were then incubated with phenobarbital for another 20 h. (A) Western blot analysis. (B) Northern blot analysis. Uniform RNA loading was

demonstrated by the ethidium bromide staining of the 18S rRNA band which was used as the standard. One representative experiment out of three independent experiments was shown.

Fig. 5. Effect of various concentrations of SQ22536 on CYP 2B1 expression inhibited by $PGE_2$ in the presence of phenobarbital (PB). Forty hours after attachment, hepatocytes were pretreated with SQ22536 for 1 h before the addition of $PGE_2$. After $PGE_2$ addition for 15 min, phenobarbital was added and the cells were incubated for another 20 h. (A) Western blot analysis. (B) Northern blot analysis. Uniform RNA loading was demonstrated by the ethidium bromide staining of the 18S rRNA band which was used as the standard. One representative experiment out of three independent experiments was shown.

Fig. 6. Effect of H-89 on CYP 2B1 expression inhibited by $PGE_2$ in the presence of phenobarbital (PB). Forty hours after attachment, hepatocytes were pretreated with 7.5 μM H-89 for 1 h before the addition of $PGE_2$. After $PGE_2$ addition for 15 min, phenobarbital was added and the cells were incubated for another 20 h. (A) Western blot analysis. (B) Northern blot analysis. Uniform RNA loading was demonstrated by the ethidium bromide staining of the 18S rRNA band which was used as the standard. One representative experiment out of three independent experiments was shown.

**TABLE 1**

Effect of $PGE_2$ on the intracellular cAMP concentration [1]

| Treatment | cAMP (%) |
|---|---|
| Control | 100 [b] ± 0 |
| $PGE_2$ (0.01 μmol/L) | 102.3 [b] ± 5.9 |
| $PGE_2$ (0.1 μmol/L) | 105.2 [b] ± 15.1 |
| $PGE_2$ (1 μmol/L) | 132.4 [a] ± 16.2 |

[1] Values are means ± SD, $n = 3$. Forty hours after attachment, hepatocytes were incubated with various concentrations of $PGE_2$ for 20 h. No $PGE_2$ was added to the control. The cells were then washed twice with cold PBS and lysed and scraped into 800 mL of ice-cold 70% ethanol. Cell debris was pelleted at 2,000 × *g*, and the resulting supernatant fluid was lyophilized and stored at -20°C until analyzed. cAMP concentrations in the control are expressed as 100% , and the concentrations in the other groups were calculated in comparison with the control. Values not sharing a same letter are significantly different ($P < 0.05$).

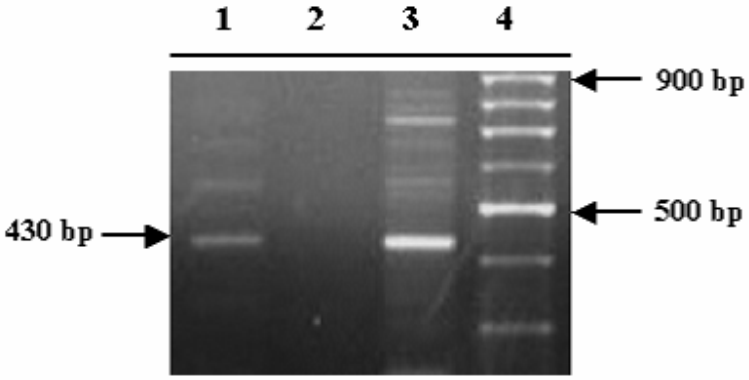


**Fig. 1**

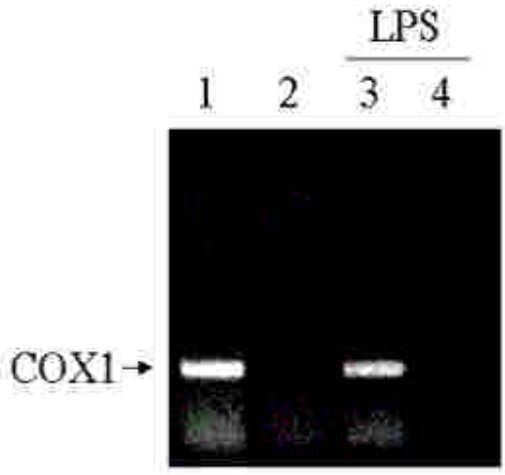


**Fig. 2**

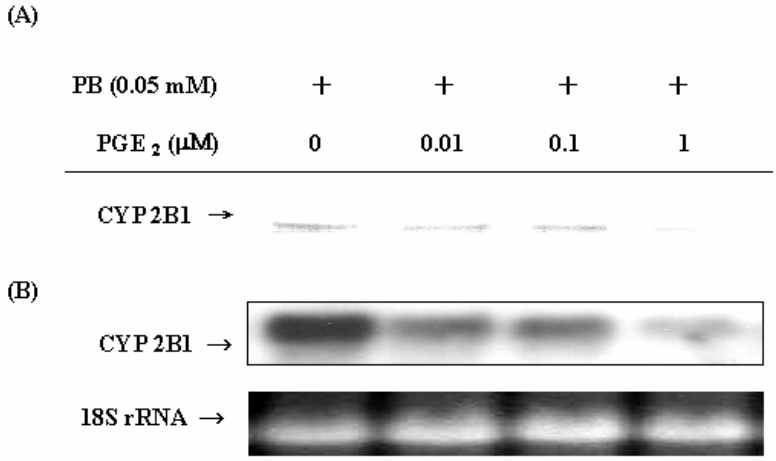
(A)
PB (0.05 mM) + + + +
PGE 2 (μM) 0 0.01 0.1 1
CYP2B1 →
(B)
CYP2B1 →
18S rRNA →

**Fig. 3**

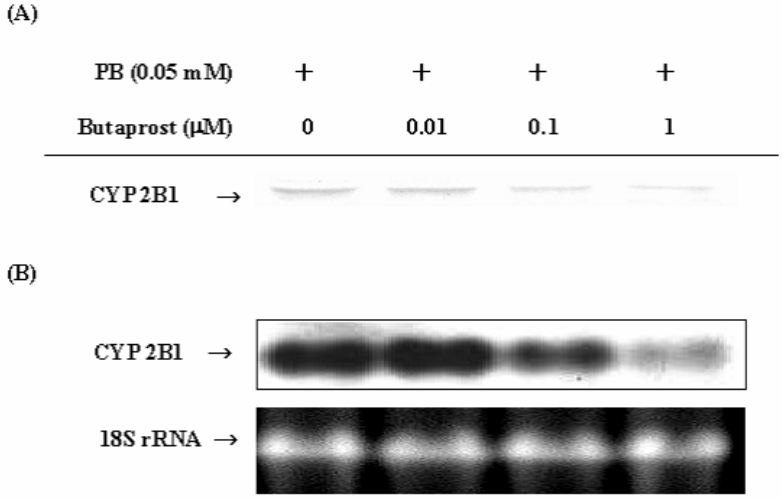
(A)
PB (0.05 mM) + + + +
Butaprost (μM) 0 0.01 0.1 1
CYP2B1 →
(B)
CYP2B1 →
18S rRNA →

**Fig. 4**

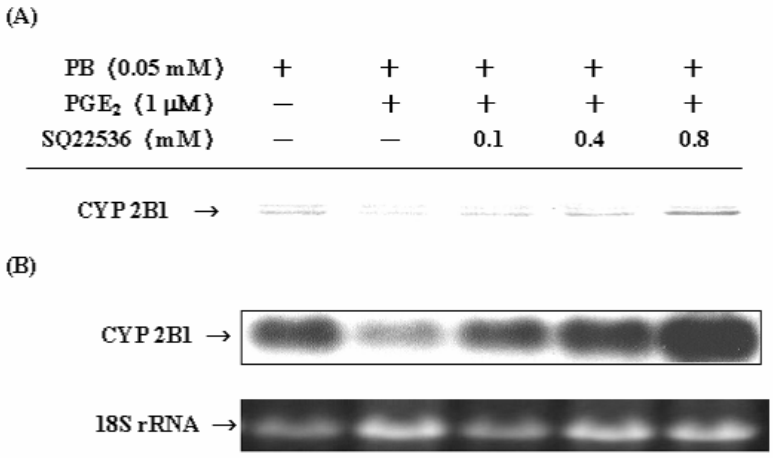
(A)
PB (0.05 mM) + + + + +
PGE2 (1 μM) − + + + +
SQ22536 (mM) − − 0.1 0.4 0.8
CYP 2B1 →
(B)
CYP 2B1 →
18S rRNA →

**Fig. 5**

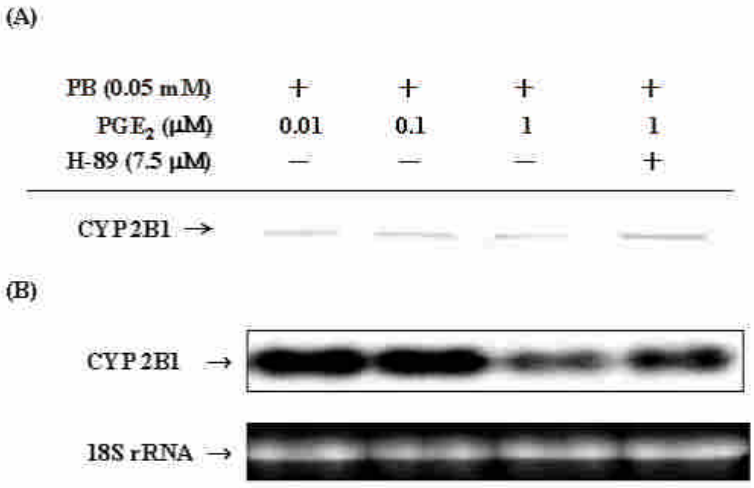
(A)
PB (0.05 mM) + + + +
PGE2 (μM) 0.01 0.1 1 1
H-89 (7.5 μM) − − − +
CYP 2B1 →
(B)
CYP 2B1 →
18S rRNA →

**Fig. 6**