The Effects of COX-Metabolites on Cyclooxygenase-2 Induction in LPS-treated Endothelial Cells

PRAVIT AKARASEREENONT, M.D., Ph.D.*,
SIRIKUL CHOTEWUTTAKORN, M.Sc.*,
KITIRAT TECHATRAISAK, M.D., Ph.D.,**
ATHIWAT THAWORN, Cert., M.S.T.*

Abstract

Cyclooxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs, also called COX-metabolites. COX exists as COX-1 and COX-2 isoforms. Each COX-metabolite has different characters and functions. The amounts of each COX-metabolite produced in cells are also different depending on cell type and mitogen stimulated cells. These were thought to be autoregulation among COX-metabolites. Here, we have investigated the effects of COX-metabolites, such as PGI_2, PGE_2, $PGF_{2\alpha}$ and U44069, on the induction of COX-2 in human umbilical vein endothelial cells (HUVEC) treated with LPS (1 μg/ml). COX activity was measured by the production of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$ and TXB_2 in the presence of exogenous arachidonic acids (10 μM for 10 min) using enzyme immunoassay (EIA). COX-1 and COX-2 protein was measured by immunoblotting using specific antibody. PGI_2, PGE_2, $PGF_{2\alpha}$ or U44069, did not affect on basal COX activity in untreated HUVEC (24 h incubation). Untreated HUVEC contained COX-1 protein but not COX-2 protein. When HUVEC were treated with LPS (1 μg/ml for 24 h), COX activity and COX-2 protein was increased in a dose dependent manner. The increased COX activity in LPS (1 μg/ml) treated HUVEC was inhibited with PGE_2 (0.03, 0.3 or 3 μM), but not PGI_2, $PGF_{2\alpha}$ or U44069, in a dose dependent manner. Similary, COX-2 protein expression in LPS treated HUVEC was also inhibited with PGE_2, but not PGI_2, $PGF_{2\alpha}$ or U44069, in a dose dependent manner. These results suggested that PGE_2, but not PGI_2, $PGF_{2\alpha}$ or TXA_2 is a key in feedback regulation of COX-metabolites produced in HUVEC.

Key word : COX-2, Prostaglandins, Endotoxin, Endothelium

AKARASEREENONT P, TECHATRAISAK K, CHOTEWUTTAKORN S, THAWORN A
J Med Assoc Thai 2001; 84 (Suppl 3): S696-S709

* Department of Pharmacology,
** Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Prostaglandins (PGs) have numerous cardiovascular and inflammatory effects[1]. Cyclooxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs[2,3]. COX exists in at least two isoforms. One is the constitutive enzyme, COX-1, producing regulatory prostanoids under physiological conditions[4], whereas the other, COX-2, is induced by mitogens[5,6], and proinflammatory cytokines[7,8] during pathological states such as inflammation. The main PGs or COX-metabolites produced in the body are prostacyclin (PGI_2), PGE_2, $PGF_{2\alpha}$, TXA_2 and PGD_2. Each PGs has different characters and functions. The amounts of each PGs produced in cells are also different depending on cell type and mitogen stimulated cells [9]. Among the PGs, PGE_2 is a potent lipid molecule with complex proinflammatory and immuno-regulatory properties[10]. PGE_2 is considered as a major contributor to the production and maintenance of immunosuppression after overwhelming injury[11]. PGE_2 is believed to modulate biochemical and immunological events leading to parturition[12]. PGE_2 also exerts a variety of biological activities for the maintenance of local homeostasis in the body [13]. Interestingly, the authors have shown in previous studies that the induction of COX-2 elicited by endotoxin (lipopolysaccharide, LPS) and interleukin-1β (IL-1β) in endothelial cells was inhibited by PGE_1[14] and PGE_2[15], respectively. However, the effects of other COX-metabolites, such as PGI_2, $PGF_{2\alpha}$ and TXA_2, on COX-2 expressed in endothelial cells are not known. Elucidation of the effects of COX-metabolites on COX isoform expressed in endothelial cells could lead to potential therapeutic interventions and understanding the feedback regulation of COX in endothelial cells. The authors investigated the effects of COX-metabolites using PGI_2, PGE_2, $PGF_{2\alpha}$ and U44069 (TXA_2 receptor agonist) on the induction of COX-2 in human umbilical vein endothelial cells (HUVEC) treated with LPS (1 μg/ml).

MATERIAL AND METHOD

Materials

PGE_2, PGI_2, $PGF_{2\alpha}$, U44069, DMSO, lipopolysaccharide (LPS), phosphate buffered saline (PBS; pH 7.4), Trizma base, EDTA, triton X-100, phenylmethylsulphonyl fluoride (PMSF), pepstatin A, leupeptin, glycerol, bromphenol blue, 2-mercaptoethanol, sodium dodecyl sulphate (SDS), anti-rabbit IgG antibody, goat IgG, premixed BCIP/NBT solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin G sodium and streptomycin were supplied by Sigma Chemical Company (U.S.A.). PGs (6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$ and TXB_2) and its respective acetyl-cholinesterase tracer and rabbit antiserum, pre-coated mouse anti-rabbit IgG microtitre plates (96-well) and Ellman's reagent were purchased from Cayman (Sapphire Bio-science, Australia). Human Endothelial-SFM Basal Growth Medium and foetal calf serum was obtained from GibThai (Thailand). Pure nitrocellulose membrane (0.45 micron) and filter paper were purchased from BIO-RAD (U.S.A.).

Cell culture

Human umbilical vein endothelial cells (HUVEC) were obtained from babies born to normal pregnant women (HUVEC) as previously described [16] and cultured in 96-well plates with Human Endothelial-SFM Basal Growth Medium (Gibco) containing 10 per cent foetal calf serum (Gibco), 100 units/ml penicillin G sodium and 100 mg/ml streptomycin. Cells were incubated at 37°C in a humidified incubator and grown to confluence before use.

Measurement of COX activity

Confluent HUVEC were gently washed twice with PBS and replaced with fresh medium (200 μl/well) before use. Cells were treated with no addition, LPS (1 μg/ml), LPS (1 μg/ml) plus COX-metabolites (PGE_2, PGI_2, $PGF_{2\alpha}$ or U44069) or COX-metabolites alone for 24h after which time the medium was removed and washed twice with PBS. COX activity was measured by the production of four COX metabolites e.g. 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2), PGE_2, $PGF_{2\alpha}$ and TXB_2 (a stable metabolite of TXA_2) in the replaced fresh medium containing exogenous arachidonic acid (10 μM for 10 min) using enzyme immunoassay (EIA). Briefly, 50 μl of standard PGs or samples were added to pre-coated mouse anti-rabbit IgG microtitre plates (96-well). Then, PGs acetylcholinesterase tracer (Clayman; 50 μl) and rabbit antiserum of PGs were added. The plate was covered with plastic film and incubated for 18 h at 4°C, after which time the wells were emptied and rinsed five times with wash buffer (PBS containing 0.05% Tween). Ellman's reagent

(Clayman; 200 µl) was added to each well and the plates were shaken on a microtitre plate shaker. The reaction was taken after about 90 min. A yellow colour developed which was read using a microplate reader (BIORAD; OD 415 nM).

Immunoblot (Western blot) analysis

HUVEC which were untreated, treated with LPS (1 µg/ml), LPS (1 µg/ml) plus COX-metabolites (PGE_2, PGI_2, $PGF_{2\alpha}$ or U44069) or COX-metabolites alone were cultured in 6-well culture plates (37°C; for 24 h). After 24h incubation, cells were extracted and analysed by immunoblotting using specific antibodies for COX-1 and COX-2 protein (Clayman, USA) as previously described[17].

Measurement of cell viability

Cell respiration, an indicator of cell viability, was assessed by the mitochondrial dependent reduction of 3-(4,5-dimethylthi-azol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[18]. At the end of each experiment, cells in 96-well plates were incubated (37°C; 1 h) with MTT (0.2 mg/ml) dissolved in culture medium. After 1 h incubation, the medium was removed by aspiration and cells were solubilized in DMSO (200 ml each well). The extent of reduction of MTT to formazan within cells was quantitated by the measurement of optical density at 650 nm (OD650) using a microplate reader (BIORAD, USA).

Statistical analysis

The results are shown as mean ± SEM of triplicate determinations (wells) from at least four separate experimental days (n=12). Student's paired or unpaired *t*-tests, as appropriate, were used for the determination of significance of differences between means and a p-value of less than 0.05 was taken as statistically significant.

RESULTS

The effects of COX-metabolites on COX activity as measured by the production of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$ and TXB_2 in HUVEC treated with LPS (1 µg/ml)

Untreated HUVEC in the presence of arachidonic acid (10 µM for 10 min) released lower amounts of 6-keto-$PGF_{1\alpha}$ (3.4 ± 0.1 ng/ml), PGE_2 (0.4 ± 0.04 ng/ml), $PGF_{2\alpha}$ (0.8 ± 0.01 ng/ml) and TXB_2 (0.04 ± 0.01 ng/ml). In LPS (0.001, 0.01, 0.1 and 1 µg/ml) treated HUVEC, the production of 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ were increased but not TXB_2 (Fig. 1). The production of 6-keto-$PGF_{1\alpha}$ in HUVEC treated with LPS (0.001, 0.01, 0.1 and 1 µg/ml) was increased significant in a dose dependent manner (Fig. 1). This increase was significant at 0.001 ng/ml of LPS for the production of 6-keto-$PGF_{1\alpha}$. The others, PGE_2 and $PGF_{2\alpha}$, was only increased significantly in HUVEC treated with LPS 1 µg/ml (Fig. 1).

In HUVEC treated with either PGE_2 (3 µM), PGI_2 (3 µM), $PGF_{2\alpha}$ (2 µM), or U44069 (3 µM) alone, COX-metabolite production did not change significantly when compared to untreated HUVEC (Fig. 2 to 5). Interestingly, the increased 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ in LPS (1 µg/ml) treated HUVEC was significantly inhibited by PGE_2 (0.003, 0.03, 0.3 or 3 µM) in a dose dependent manner (Fig. 2). This inhibition was significant at 0.3, 0.003 and 0.003 µM of PGE_2 for the production of 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$, respectively. However, PGI_2, $PGF_{2\alpha}$ and U44069 did not affect COX-metabolites produced by LPS activated endothelial cells (Fig. 3 to 5) besides $PGF_{2\alpha}$ at 2 µM which was shown to inhibit PGE_2 produced by LPS (1 µg/ml) activated endothelial cells (Fig. 4B).

LPS (1 µg/ml) alone, PGE_2 (3 µM) alone, PGI_2 (3 µM) alone, $PGF_{2\alpha}$ (2 µM) alone, U44069 (3 µM) alone, and LPS (1 µg/ml) plus either PGE_2 (3 µM), PGI_2 (3 µM), $PGF_{2\alpha}$ (2 µM) or U44069 (3 µM) did not affect cell viability when compared to the untreated control cells over a 24 h incubation period (all above 90% of untreated cells).

The stability of PGE_2 (3 µM) in cultured medium up to 24 h was not changed significantly after 3 (2.9 ± 0.2), 6 (2.9 ± 0.1), 12 (2.9 ± 0.2) and 24 (2.9 ± 0.2) hours incubation of PGE_2.

The stability of PGI_2 (3 µM) and U44069 (3 µM) in cultured medium up to 24 h was also tested and a low level of 6-keto-$PGF_{1\alpha}$ (less than 0.004 ng/ml) and TXB_2 (less than 0.001 ng/ml), respectively was detected.

The effects of COX-metabolites on COX isoform expressed in HUVEC treated with LPS

Untreated HUVEC contained unchanged COX-1 protein (Fig. 7 and 9, lane 1) and no COX-2 protein (Fig. 6 and 8, lane 1). Either PGE_2 (3 µM), PGI_2 (3 µM), $PGF_{2\alpha}$ (2 µM) or U44069 (3 µM) treated HUVEC also contained unchanged COX-1

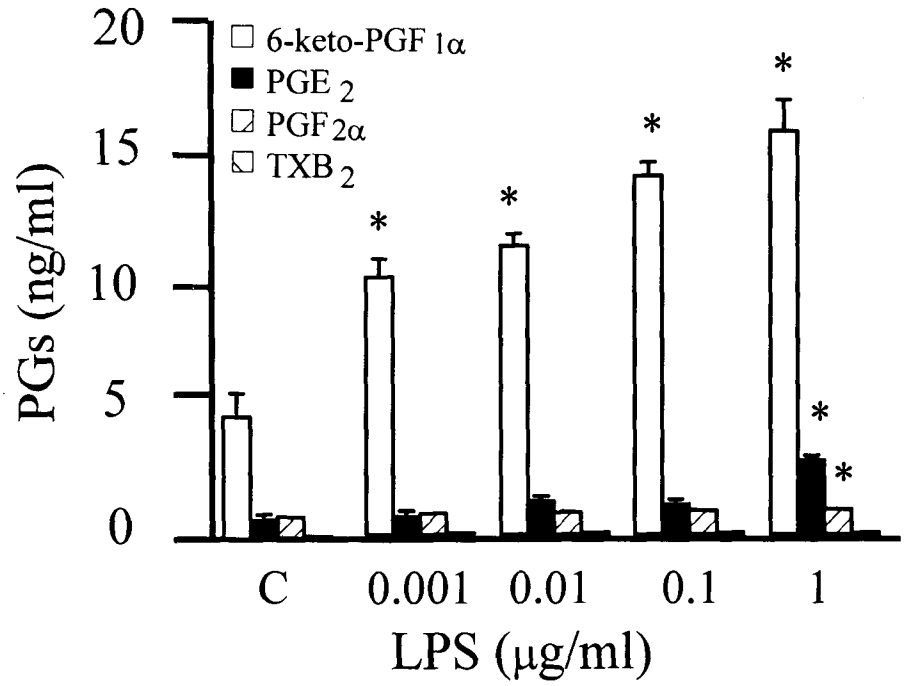

Fig. 1. The effects of LPS (1 μg/ml) on COX activity in HUVEC. COX activity was measured by the formation of 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$ and TXB_2 in the presence of exogenous arachidonic acid (10 μM; 10 min). Data are expressed as mean ± SEM of twelve determinations from at least four separate experimental days. *p < 0.05 when compared to untreated HUVEC at 24 h (C).

protein (Fig. 7 and 9, lane 2) and no COX-2 protein (Fig. 6 and 8, lane 2). COX-2 protein was expressed in HUVEC treated with LPS (1 μg/ml; Fig. 6 and 8, lane 3) for 24 h, whereas, COX-1 protein was unchanged (Fig. 7 and 9, lane 3). Interestingly, this induction of COX-2 in HUVEC treated by LPS (1 μg/ml) was inhibited by PGE_2 (0.03, 0.3 or 3 μM) in a dose dependent manner (Fig. 6, lane 4 to 6). The amounts of COX-1 protein expressed in HUVEC treated with LPS (1 μg/ml) plus PGE_2 (3 μM) did not change when compared to untreated HUVEC (Fig. 7, lane 4 to 6).

Similar to COX activity, PGI_2 (0.03, 0.3 or 3 μM; Fig. 8 and 9, panel A), $PGF_{2\alpha}$ (0.02, 0.2 or 2 μM; Fig. 8 and 9, panel B) and U44069 (0.03, 0.3 or 3 μM; Fig. 8 and 9, panel C) did not affect either COX-2 or COX-1 protein expressed in LPS (1 μg/ml) treated HUVEC (Fig. 8 and 9, lane 4 to 6, respectively).

DISCUSSION

The authors have shown that the induction of COX-2 elicited by LPS in HUVEC can be inhibited by PGE_2, but not by PGI_2, $PGF_{2\alpha}$ or U44069, in a dose dependent manner. Moreover, PGE_2, including PGI_2, $PGF_{2\alpha}$ and U44069, did not affect on COX-1 either protein or activity. The results suggested that i) PGE_2, but not PGI_2, $PGF_{2\alpha}$ or U44069, can be negative feedback regulation in the induction of COX-2 elicited by LPS in endothelial cells and ii) the therapeutic uses of PGE_2 or their analogues in the condition which COX-2 has been involved may play a role.

PGs induce a wide range of biological actions which are mediated through specific membrane-bound receptors. Among the PGs, PGE_2 is considered to exert a variety of biological activities such as the maintenance of local homeostasis in the body[13], a major contributor to the production

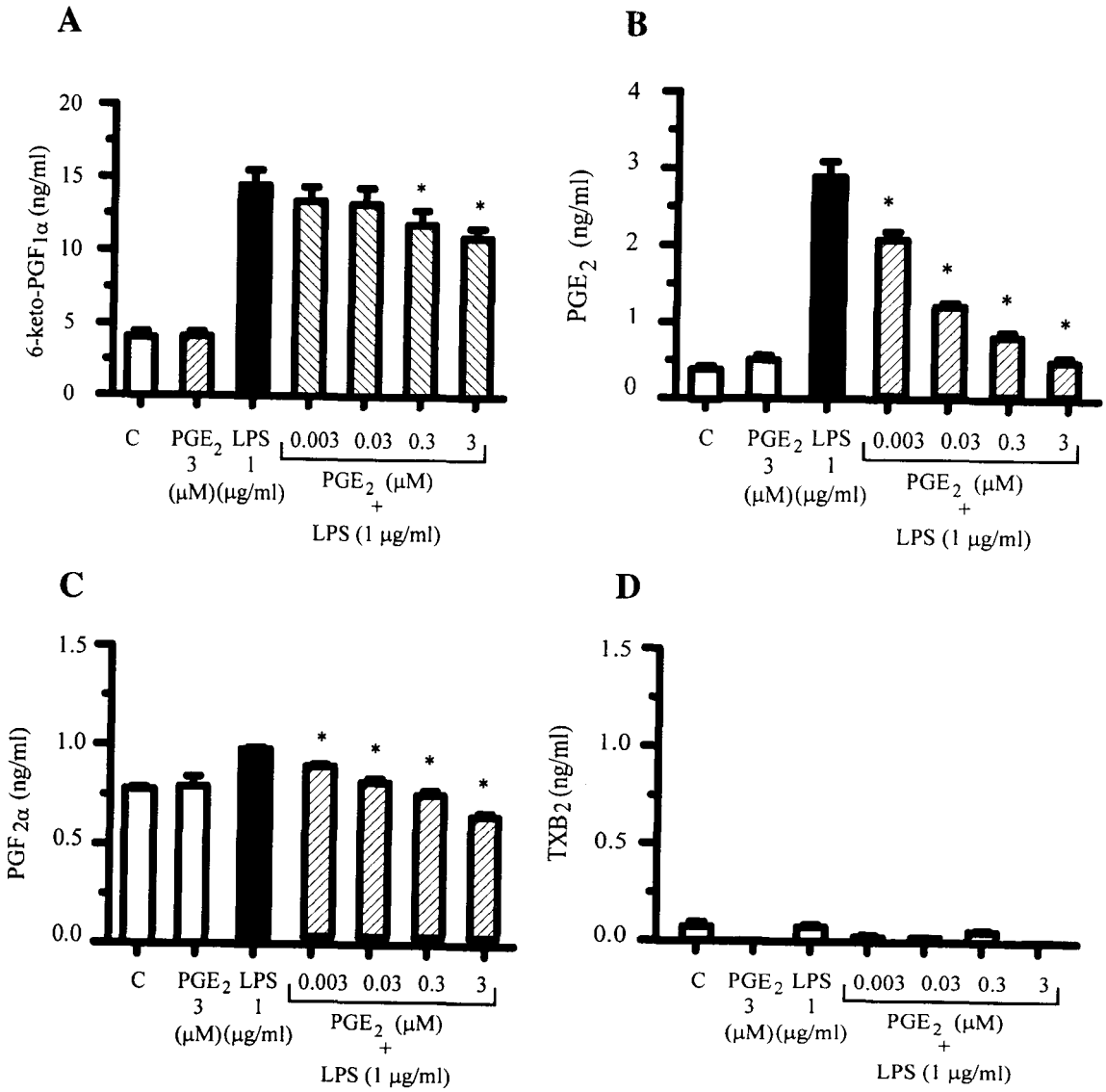

Fig. 2. The effects of PGE_2 (0.003, 0.03, 0.3 or 3 μM) on COX activity in LPS (1 μg/ml) treated HUVEC. COX activity was measured by the formation of 6-keto-$PGF_{1\alpha}$ (panel A), PGE_2 (panel B), $PGF_{2\alpha}$ (panel C) and TXB_2 (panel D) in the presence of exogenous arachidonic acid (10 μM; 10 min). Data are expressed as mean ± SEM of twelve determinations from at least four separate experimental days. *p < 0.05 when compared to LPS treated HUVEC at 24 h.

and maintenance of immunosuppression after overwhelming injury[11] and an important factor for implantation and decidualization[19]. Therefore, PGE_2 is a lipid molecule with complex inflammatory modulation and immunoregulatory properties. The present results have support that PGE_2 can act as antiinflammation and immunosuppression in the induction of COX-2 in endothelial cells by LPS.

The exact mechanisms by which PGE_2 inhibited COX-2 induction in endothelial cells acti-

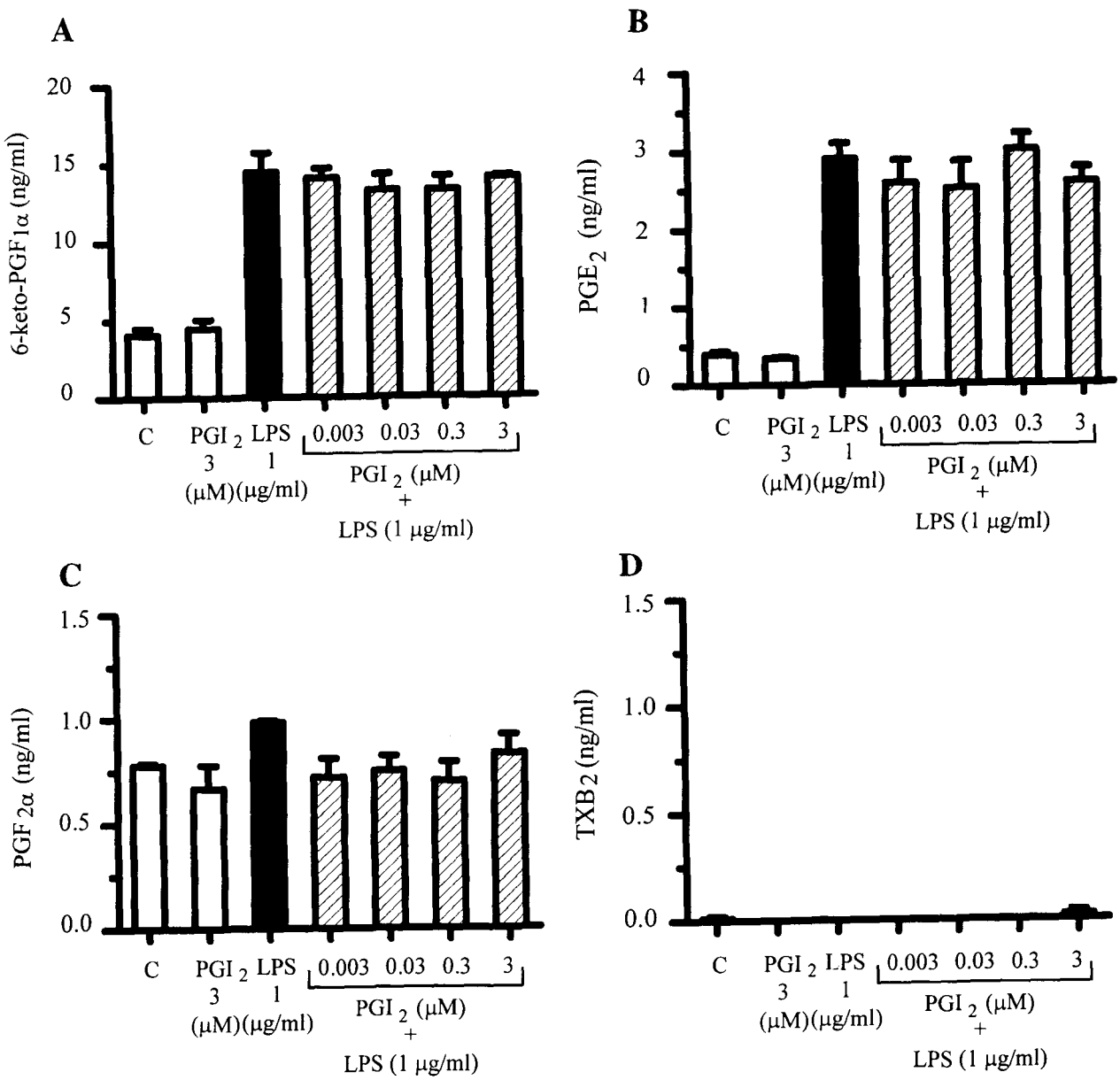

Fig. 3. The effects of PGI_2 (0.003, 0.03, 0.3 or 3 μM) on COX activity in LPS (1 μg/ml) treated HUVEC. COX activity was measured by the formation of 6-keto-$PGF_{1\alpha}$ (panel A), PGE_2 (panel B), $PGF_{2\alpha}$ (panel C) and TXB_2 (panel D) in the presence of exogenous arachidonic acid (10 μM; 10 min). Data are expressed as mean ± SEM of twelve determinations from at least four separate experimental days. *p < 0.05 when compared to LPS treated HUVEC at 24 h.

vated with LPS is not known. These may involve binding to specific cell surface receptors and influencing second messenger systems through G-proteins. Indeed, these should be complex because the effects of PGE_2 are exerted by a variety of PGE receptors which are different in their signal transduction properties[20]. There are at least four subtypes of PGE receptors. The EP1 and EP3 receptors are coupled to Ca^{2+} mobilization and the inhibition of adenylate cyclase, respectively, and the EP2 and EP4 receptors

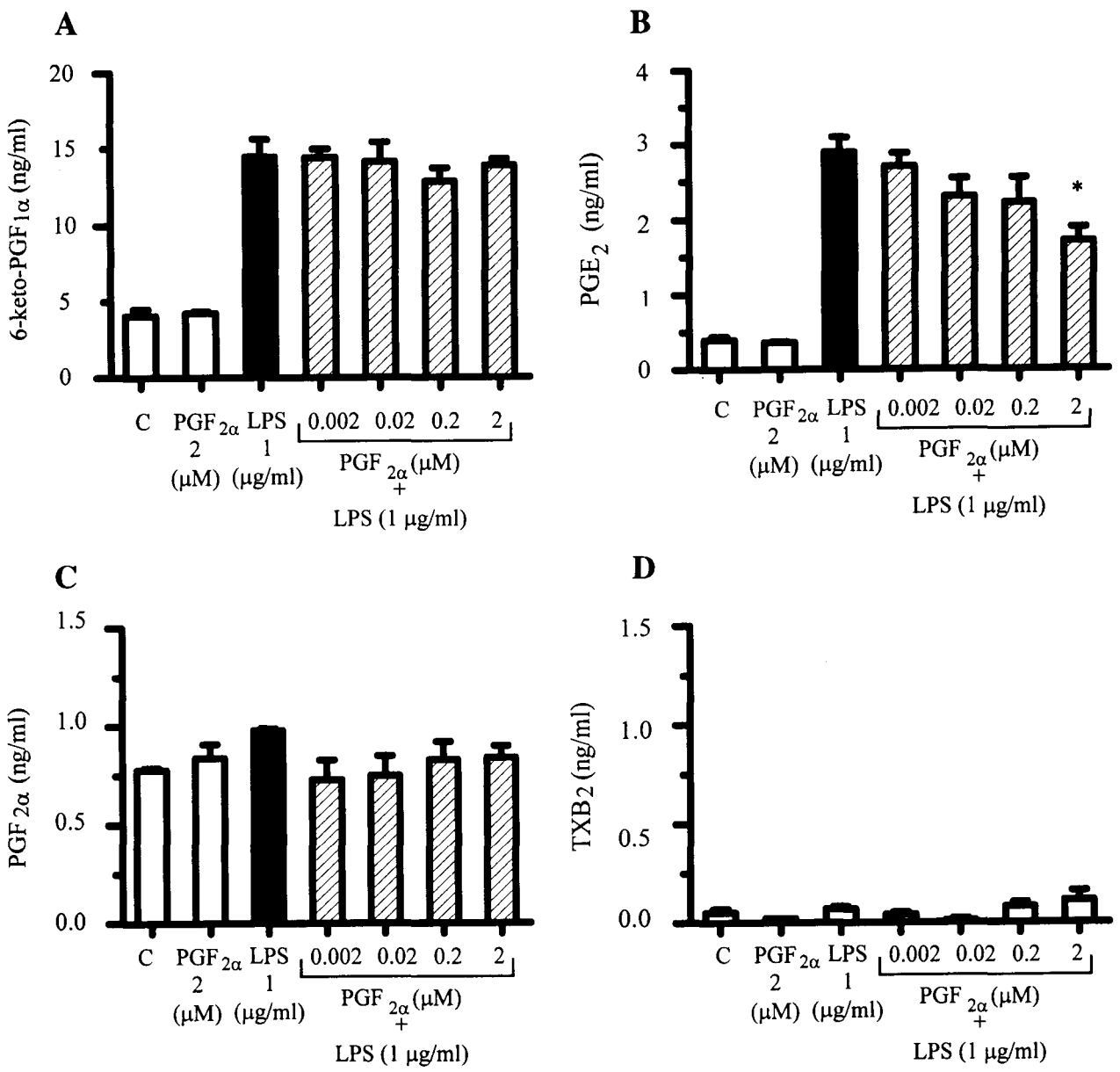

Fig. 4. The effects of $PGF_{2\alpha}$ (0.002, 0.02, 0.2 or 2 μM) on COX activity in LPS (1 μg/ml) treated HUVEC. COX activity was measured by the formation of 6-keto-$PGF_{1\alpha}$ (panel A), PGE_2 (panel B), $PGF_{2\alpha}$ (panel C) and TXB_2 (panel D) in the presence of exogenous arachidonic acid (10 μM; 10 min). Data are expressed as mean ± SEM of twelve determinations from at least four separate experimental days. *p < 0.05 when compared to LPS treated HUVEC at 24 h.

are coupled to the same signal transduction pathway, stimulation of adenylate cyclase[21]. Therefore, which EP receptors mediated the inhibiton of PGE_2 on COX-2 induced in LPS treated HUVEC should be elucidated.

PGE_2 is one of the PGs or COX metabolites, such as PGI_2, PGE_2, $PGF_{2\alpha}$ and TXA_2, synthesized by COX-1 and COX-2 which are involved in physiology and pathology[4-8], respectively. Each COX isoform can produce different COX metabo-

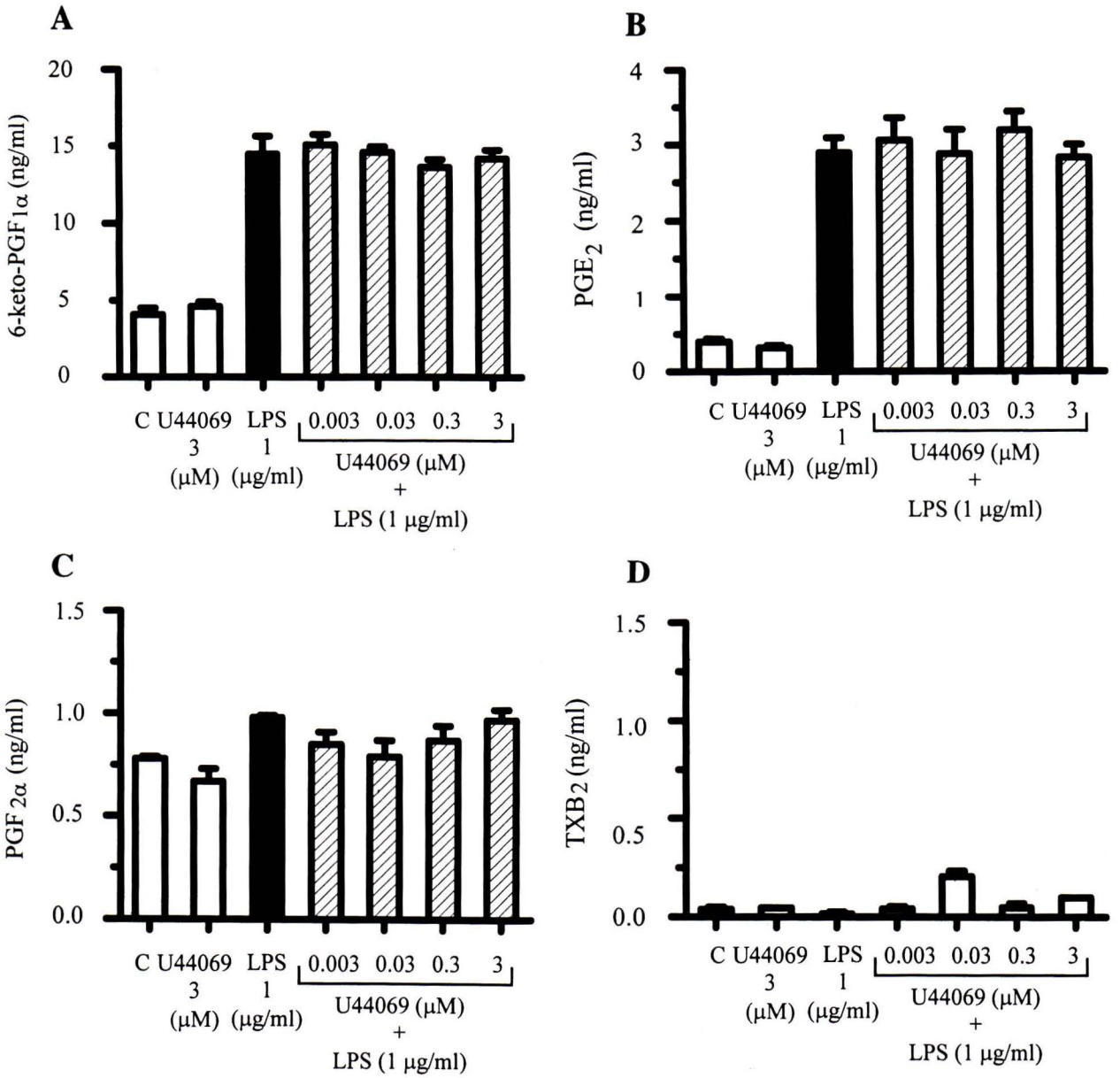

Fig. 5. The effects of U44069 (0.003, 0.03, 0.3 or 3 μM) on COX activity in LPS (1 μg/ml) treated HUVEC. COX activity was measured by the formation of 6-keto-$PGF_{1\alpha}$ (panel A), PGE_2 (panel B), $PGF_{2\alpha}$ (panel C) and TXB_2 (panel D) in the presence of exogenous arachidonic acid (10 μM; 10 min). Data are expressed as mean ± SEM of twelve determinations from at least four separate experimental days. *p < 0.05 when compared to LPS treated HUVEC at 24 h.

lites in different cell types such as PGI_2 which is a major COX-1 and COX-2 metabolite in endothelial cells while PGE_2 is a major COX-2 metabolite in macrophages[22]. The difference in COX metabolite production in different cell types may result from the feedback regulation of each released COX metabolites. The present results showed that PGE_2 (0.3 μM), but not PGI_2, $PGF_{2\alpha}$ and U44069, inhibited PGE_2 production (60% inhibition; Fig. 2B) more than PGI_2 production (20% inhibition; Fig.

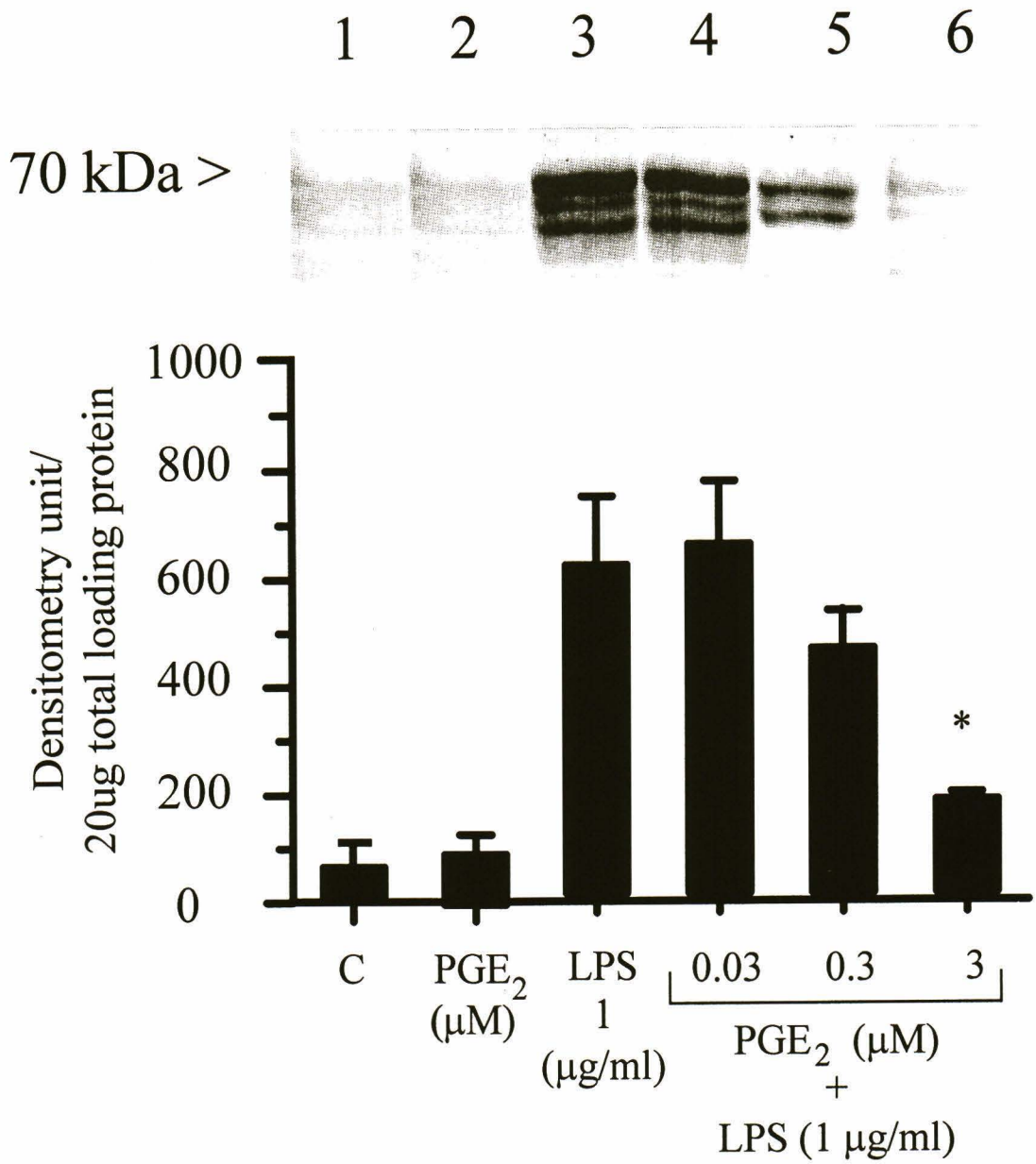

Fig. 6. The effects of PGE_2 on COX-2 protein expressed in LPS (1 μg/ml) treated HUVEC. COX-2 protein was detected by Western blots using specific antibodies to COX-2. Equal amounts of protein (20 μg/lane) were loaded in each lane. The significant differences between each band were compared by scanner densitometry using image 1D program (densitometry unit).

2A) and $PGF_{2\alpha}$ production (25% inhibition; Fig. 2C) in LPS treated endothelial cells. This may explain the COX metabolites produced in LPS treated endothelial cells that PGI_2 released in highest amounts and the lesser extent of PGE_2, $PGF_{2\alpha}$ and TXA_2, respectively. Thus, elucidation of the feedback regu-

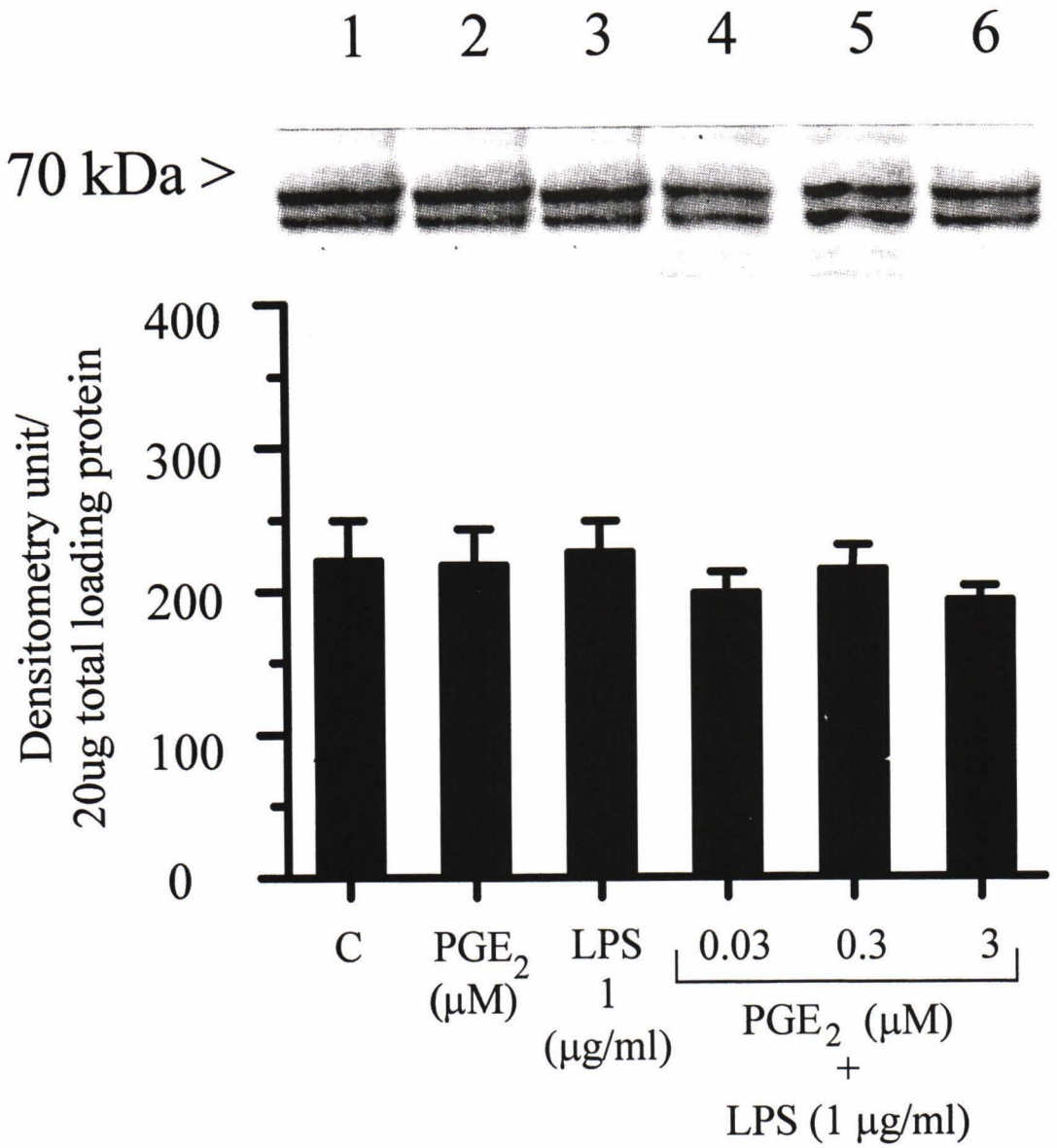

Fig. 7. The effects of PGE_2 on COX-1 protein expressed in LPS (1 μg/ml) treated HUVEC. COX-1 protein was detected by Western blots using specific antibodies to COX-1. Equal amounts of protein (20 μg/lane) were loaded in each lane. The significant differences between each band were compared by scanner densitometry using image 1D program (densitometry unit).

lation of each COX metabolites will help to understand the variety in COX metabolites produced in different cells and may lead to potential therapeutic interventions. For the present study, the authors have shown that PGE_2 is a negative feedback regulation of the induction of COX-2, but not COX-1, in

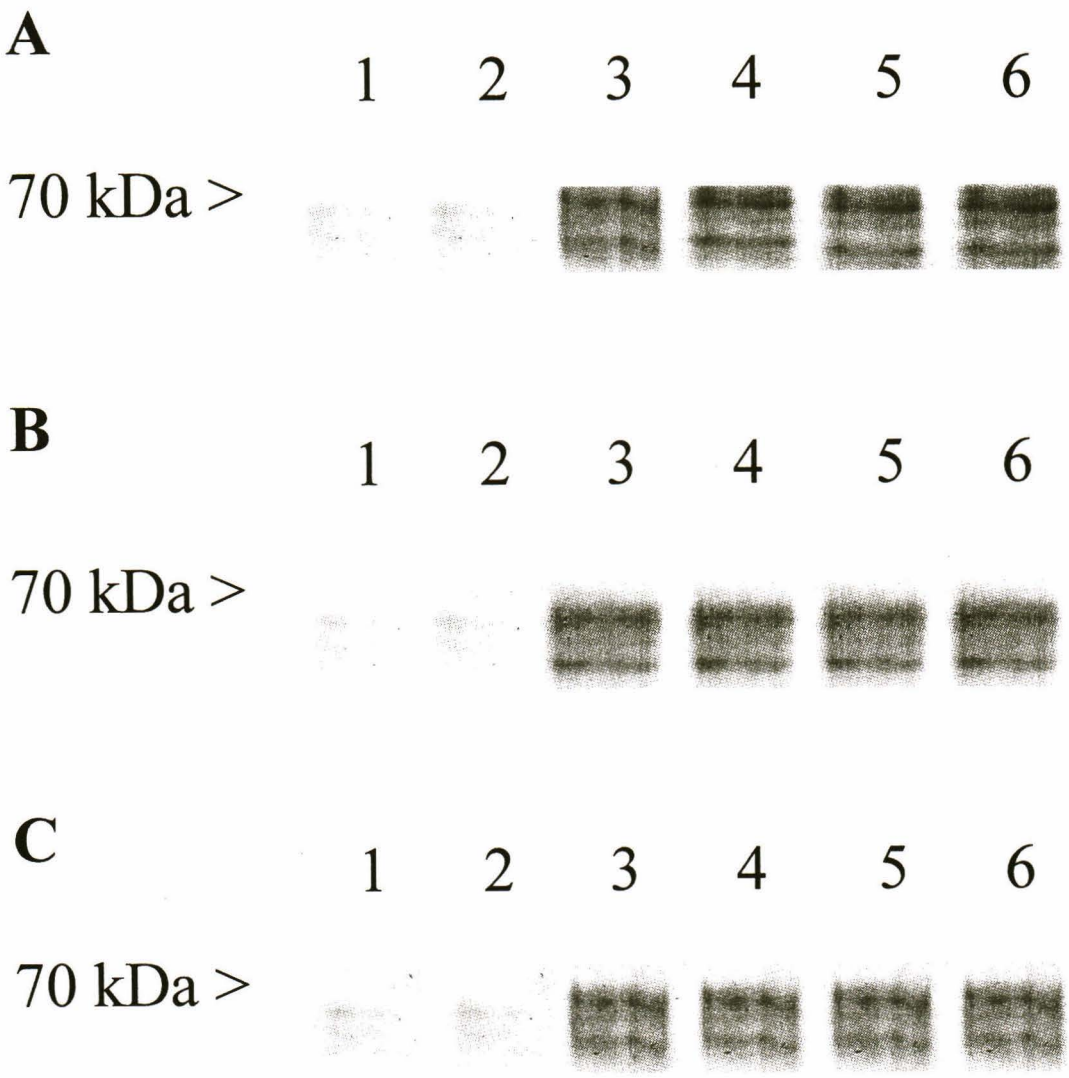

Fig. 8. The effects of PGI_2 (panel A), $PGF_{2\alpha}$ (panel B) and U44069 (panel C) on COX-2 protein expressed in LPS (1 μg/ml) treated HUVEC. COX-2 protein were detected by Western blots using specific antibodies to COX-2. Equal amounts of protein (20 μg/lane) were loaded in each lane. Similar results were obtained with cell extracts from 3 separate batches of cells.

endothelial cells activated with LPS. However, PGI_2, $PGF_{2\alpha}$ and U44069 did not affect either COX activity and protein in HUVEC activated with LPS. This suggests that the PGE series may have negative feedback regulation of COX-2 induction in endothelial cells as shown in the authors' previous study, that PGE_1 and PGE_0 can inhibit the induction of COX-2 in endothelial cells activated with LPS [14]. PGE series have been used in clinical disorders such as peripheral vascular occlusive diseases[23], NSAIDs-induced gastric ulcer[24], abortion[25] and impotence[26]. Thus, the authors propose that the therapeutic uses of PGE_2 in conditions which COX-2 has been involved may play a role and the effects

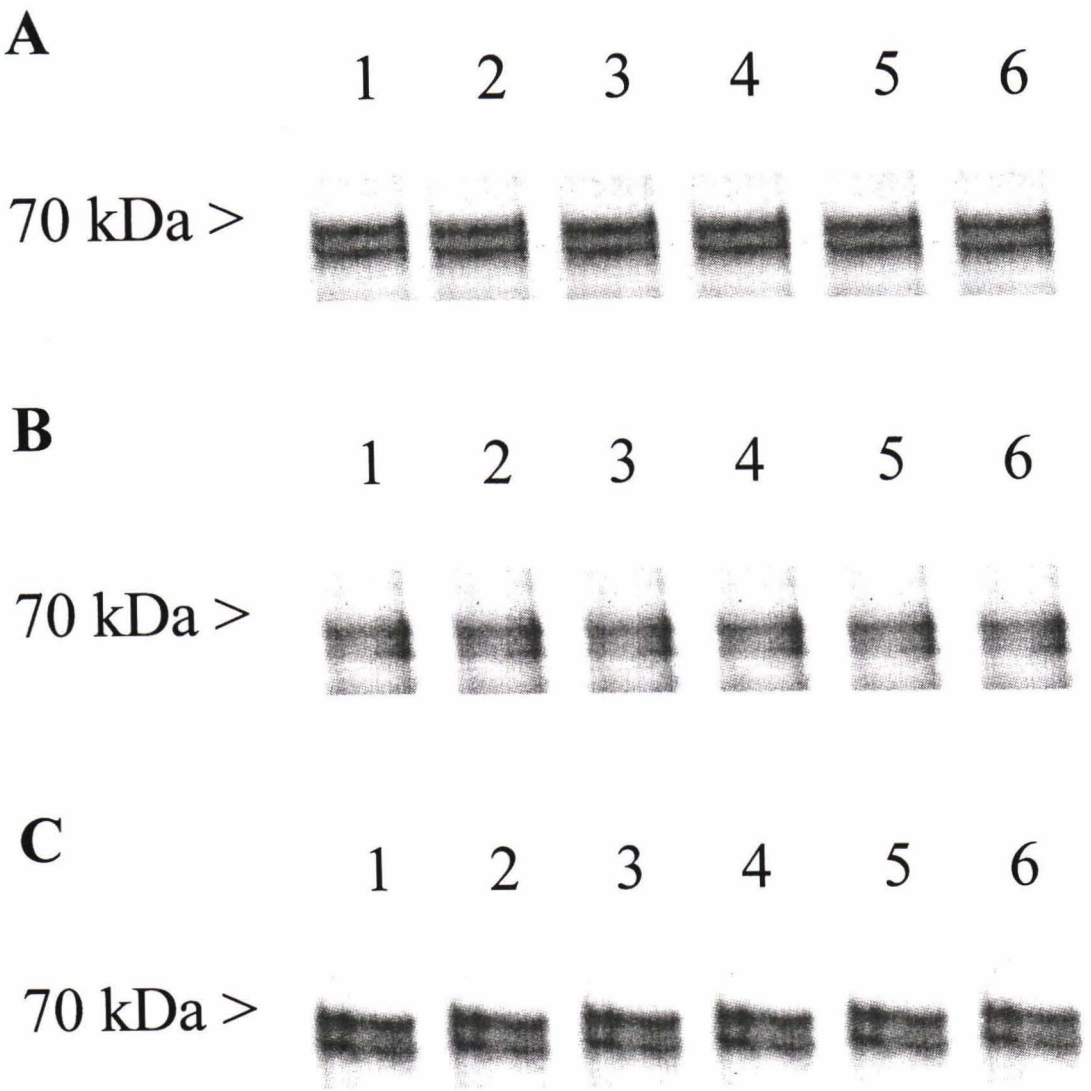

Fig. 9. **The effects of PGI_2 (panel A), $PGF_{2\alpha}$ (panel B) and U44069 (panel C) on COX-1 protein expressed in LPS (1 μg/ml) treated HUVEC. COX-1 protein were detected by Western blots using specific antibodies to COX-1. Equal amounts of protein (20 μg/lane) were loaded in each lane. Similar results were obtained with cell extracts from 3 separate batches of cells.**

of various EP receptor subtypes on COX-2 expressed in various mitogen activated endothelial cells should be elucidated.

ACKNOWLEDGEMENTS

This work was supported by a Grant from Siriraj China Medical Board to P. Akarasereenont.

(Received for publication on November 22, 2001)

REFERENCES

1. Vane JR, Botting RM. The mode of action of anti-inflammatory drugs. Postgrad Med J 1990; 66 (Suppl 4): S2-S17.
2. Vane JR, Botting RM. The prostaglandins. In: Vane JR, Botting RM. eds, Aspirin and Other Salicylates. London: Chapman & Hall Medical, 1992: 17-34.
3. Smith WL, Marnett IJ. Prostaglandin endoperoxide synthase: Structure and catalysis. Biochim Biophys Acta 1991; 1083: 1-17.
4. Mitchell JA, Akarasereenont P, Thiemermann C, Vane JR. Selectivity of nonsteriodal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 1993; 90: 11693-7.
5. Lee SH, Soyoola E, Chanmugam P, et al. Selective expression of mitogen-inducible cyclooxygenase in macro-phages stimulated with lipopoly-saccharide. J Biol Chem 1992; 267: 25934-8.
6. O'Banion MK, Winn VD, Young DA. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc Natl Acad Sci USA 1992; 89: 4888-92.
7. Maier JAM, Hla T, Maciag T. Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. J Biol Chem 1991; 265: 10805-8.
8. Akarasereenont P, Bakhle YS, Thiemermann C, Vane JR. Cytokines mediate the induction of cyclo-oxygenase-2 by activating tyrosine kinase in bovine aortic endothelial cells stimulated by bacterial lipopoly-saccharide. Br J Pharmacol 1995; 115: 401-8.
9. Akarasereenont P, Mitchell JA, Thiemermann C, Vane JR. Comparison of the induction of cyclo-oxygenase and nitric oxide synthase by endotoxin in endothelial cells and macrophages. Eur J Pharmacol 1995; 273: 121-8.
10. Fedyk ER, Phipps RP. Prostaglandins E_2 receptors of the EP_2 and EP_4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. Proc Natl Acad Sci USA 1996; 90: 10978-83.
11. Lo CJ, Cryer HG, Fu M, Lo FR. Regulation of macrophage eicosanoid generation is dependent on nuclear factor kappaB. J Trauma 1998; 45: 19-23.
12. Brown NL, Alvi SA, Elder MG, et al. Interleukin-1 β and bacterial endotoxin change the metabolism of prostaglandins E_2 and $F_{2\alpha}$ in intact term fetal membranes. Placenta 1998; 19: 625-30.
13. Ichikawa A, Sugimoto Y, Negishi M. Molecular aspects of the structures and functions of the prostaglandin E receptors. J Lipid Mediat Cell Signal 1996; 14: 83-7.
14. Akarasereenont P, Hide E, Ney P, et al. The induction of cyclooxygenase-2 elicited by endotoxin in endothelial cells and macrophages is inhibited by prostaglandin E_1 and 13, 14-dihydro prostaglandin E_1. Agent Action 1995; 45: 59-64.
15. Akarasereenont P, Techatrisak K, Chotewuttakorn S, Thaworn A. The induction of cyclooxygenase-2 in IL-1β-treated endothelial cells is inhibited by prostaglandin E_2 through cAMP. Med Inflam 1999; 8: 287-94.
16. Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins: Identification by morphologic and immunologic criteria. J Clin Invest 1973; 52: 2745-56.
17. Akarasereenont P, Mitchell JA, Appleton I, et al. Involvement of tyrosine kinase in the induction of cyclo-oxygenase and nitric oxide synthase by endotoxin in cultured cells. Br J Pharmacol 1994; 113: 1522-8.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Meth 1983; 65: 55-63.
19. Lim H, Dey SK. Prostaglandin E_2 receptor subtype EP2 gene expression in the mouse uterus coincides with differentiation of the luminal epithelium for implantation. Endocrinology 1997; 138: 4599-606.
20. Woodward DF, Regan JW, Lake S, Ocklind A. The molecular biology and ocular distribution of prostanoid receptors. Surv Ophthalmol 1997; 41 (Suppl 2): S15-S21.
21. Nishigaki N, Negishi M, Ichikawa A. Two Gs-coupled prostaglandin E receptor subtypes, EP2 and EP4, differ in desensitization and sensitivity to the metabolic inactivation of the agonist. Mol Pharmacol 1996; 50: 1031-7.
22. Akarasereenont P, Mitchell JA, Bakhle YS, et al. Comparison of the induction of cyclooxygenase and nitric oxide synthase by endotoxin in endothelial cells and macrophages. Eur J Pharmacol 1995; 273: 121-8.
23. Altstaedt HO, Berzewski B, Breddin HK, et al. Treatment of patients with peripheral arterial occlusive disease Fontaine stage IV with intravenous iloprost and PGE1: A randomized open controlled study. Prostaglandins Leukot Essent Fatty Acids 1993; 49: 573-8.
24. Ares JJ, Outt PE. Gastroprotective agents for the prevention of NSAID-induced gastropathy. Curr Pharm Des 1998; 4: 17-36.
25. Cabezas E. Medical *versus* surgical abortion. Int J Gynaecol Obstet 1998; 63 (Suppl 1): S141-6.
26. Becker AJ, Stief CG, Schultheiss D, et al. Pharmacological therapy of erectile dysfunction. Urologe A 1998; 37: 503-8.

ผลของโพรสตาแกลนดินส์ต่อเอ็นซัยม์ COX-2 ที่ถูกกระตุ้นการสร้างโดยเอ็นโด-ทอกซินในเซลล์เยื่อบุผนังหลอดเลือด

ประวิทย์ อัครเสรีนนท์, พ.บ., ปร.ด., กิติรัตน์ เตชะไตรศักดิ์, พ.บ., ปร.ด.**, ศิริกุล โชติวุฑฒากร, วท.ม.*, อธิวัตน์ ถาวร, ประกาศนียบัตร (พ.ว.ก.)**

Cyclooxygenase (COX) ซึ่งมีอยู่ 2 ชนิดคือ COX-1 และ COX-2 เป็นเอ็นซัยม์ที่ใช้ในการสร้างโพรสตาแกลนดินส์ (PGs) หรืออาจเรียกว่า COX-metabolites ได้แก่ PGI_2, PGE_2, $PGF_{2\alpha}$ และ TXA_2 โดย COX-metabolites ถูกสร้างในปริมาณที่แตกต่างกันขึ้นอยู่กับชนิดของเซลล์และเอ็นซัยม์ COX ที่ใช้ในการสร้าง ซึ่งเชื่อว่าน่าจะมีขบวนการควบคุมการสร้าง COX-metabolites โดยตัวเองด้วย (auto feedback regulation) การศึกษานี้ จะทำการศึกษาผลของ COX-metabolites แต่ละชนิดได้แก่ PGI_2, PGE_2, $PGF_{2\alpha}$ และ U44069 (TXA2 analogue) ต่อการกระตุ้นการสร้าง COX-2 โดยเอ็นโดทอกซินในเซลล์เยื่อบุผนังหลอดเลือด การทำงานและโปรตีนของ COX ถูกวัดโดยใช้ enzyme immunoassay (EIA) และ immuno-blotting พบว่า PGI_2, PGE_2, $PGF_{2\alpha}$ และ U44069 ไม่มีผลต่อการทำงานของ COX-1 รวมไปถึงการปรากฏของโปรตีน COX-1 ที่น่าสนใจคือ PGI_2, $PGF_{2\alpha}$ และ U44069 ก็ไม่มีผลต่อการทำงานของ COX-2 รวมไปถึงการปรากฏของโปรตีน COX-2 แต่ PGE_2 (0.03, 0.3 หรือ 3 μM) เท่านั้นที่สามารถยับยั้งการทำงานของ COX-2 รวมไปถึงการปรากฏของโปรตีน COX-2 โดยสัมพันธ์กับขนาดที่ใช้ ผลการศึกษาบ่งชี้ว่า PGE_2 คือโพรสตาแกลนดินส์ตัวสำคัญที่มีบทบาทในการควบคุมย้อนกลับ (negative feedback regulation) ต่อการทำงานของ COX-2 รวมไปถึงการปรากฏของโปรตีน COX-2 ที่ถูกกระตุ้นการสร้างโดยเอ็นโดทอกซิน

คำสำคัญ : COX-2, โพรสตาแกลนดินส์, เอ็นโดทอกซิน, เซลล์เยื่อบุผนังหลอดเลือด

ประวิทย์ อัครเสรีนนท์, กิติรัตน์ เตชะไตรศักดิ์,
ศิริกุล โชติวุฑฒากร, อธิวัตน์ ถาวร
จดหมายเหตุทางแพทย์ ฯ 2544; 84 (ฉบับพิเศษ 3): S696-S709

* ภาควิชาเภสัชวิทยา,

** ภาควิชาสูติศาสตร์-นรีเวชวิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700