The Expression of Cyclooxygenase-2 in Human Umbilical Vein Endothelial Cell Culture from Preeclampsia

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Abstract

We have shown that HUVEC from normal pregnancy contained COX-1 protein but not COX-2 protein and released 6-keto-$PGF_{1\alpha}$ 277 ± 5 ng/ml (for 24 h). In contrast, HUVEC from preeclampsia contained both COX-1 and COX-2 protein and released significantly lesser amounts of 6-keto-$PGF_{1\alpha}$ (159 ± 8 ng/ml for 24 h; $p < 0.05$). Thus, COX-2 is expressed in HUVEC from preeclampsia but not in normal pregnancy and affects the release of prostacyclin suggesting the involvement of COX-2 in the pathogenesis of preeclampsia. The development of selective inhibitors of COX-2 may have a potential role in prevention and treatment of preeclampsia.

Key word : Preeclampsia, Cyclooxygenase-2, Human Umbilical Vein Endothelial Cell Culture

The pathogenesis of preeclampsia is poorly understood. The causes of altered vascular reactivity in preeclampsia are obscure, but research in two areas shows promise. One popular hypothesis is that vasoconstriction is due to a relative or absolute deficiency of vasodilating prostaglandins (PGs). Consistent with this theory are reports that the renal excretion of prostacyclin metabolites or the production of these eicosanoids by blood vessels or by the placenta is lower than normal in preeclampsia, or that thromboxane levels are increased[1-5]. Another hypothesis is that preeclampsia is caused by vascular endothelial-cell dysfunction[2,6]. For example, circulating substances that are cytotoxic and mitogenic and that increase the transcription and production of growth factors have been identified in endothelial cells in culture and are present before the disease becomes overt[6-8]. The roles of natriuretic factors, various pump inhibitors, circulating lipid peroxides, endothelin, and serotonin are all under study, but the studies have not yet yielded consistent results[2,9-10]. Recently, endothelial injury was found to be involved in many common physiological disturbances in preeclampsia such as hypertension, proteinuria, edema and activation of the hemostatic system.

Cyclooxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is con-

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verted to PGs, prostacyclin (PGI_2) and thromboxane (TX) A_2[11,12]. COX exists in at least two isoforms. One is a constitutive enzyme (COX-1) producing regulatory prostanoids under physiological conditions, whereas the other (COX-2) is induced by mitogens and proinflammatory cytokines during pathological states such as inflammation[13-16]. The amounts of each PG synthesized by COX-1 and COX-2 were different[17]. COX-2 may alter the amount of each PG in preeclampsia to produce a relative or absolute deficiency of vasodilating PGs. We have, therefore, investigated whether *i*) COX-2 protein is expressed in human umbilical vein endothelial cells (HUVEC) from preeclampsia, *ii*) COX-1 protein is increased in HUVEC from preeclampsia and *iii*) if there is a different release of 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2 which is major COX metabolites from endothelial cells) from HUVEC between normal pregnancy and preeclampsia.

MATERIAL AND METHOD

Subjects

Studies included 12 normal pregnant and 12 preeclamptic women who were recruited at admission to the Department of Obstetrics and Gynecology, Siriraj Hospital. Gestational age at the time of study did not differ significantly, 35 weeks (range 33 to 36 weeks) in the normal pregnant group, 36 weeks (range 34 to 38 weeks) in the preeclamptic group.

Preeclampsia was defined on the basis of the following criteria: no prior history of hypertension or renal disease, a blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic (manifested on two readings at least 6 h apart) or a rise in blood pressure of at least 30 mmHg systolic or 15 mmHg diastolic, and proteinuria of $\geq 1^+$ urine protein[18].

Cell culture

Human umbilical vein endothelial cells (HUVEC) were obtained from babies born to 12 preeclamptic mothers (pHUVEC) and 12 normal pregnant women (nHUVEC). Then, endothelial cells were extracted and cultured in Human Endothelial-SFM Basal Growth Medium (Gibco) containing 10 per cent foetal calf serum in 6-well culture plates (2 ml each well) under standard conditions[19]. Cells were grown to confluence until use and replaced with fresh medium for 24 h. In each experiment, HUVEC were obtained from a new umbilical cord and the cell cultures maintained by standard techniques[19].

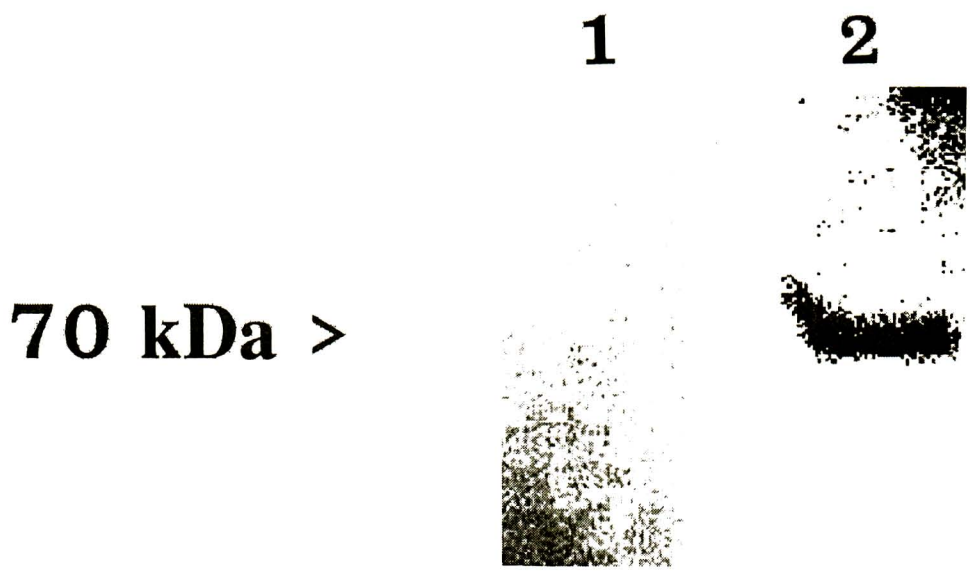

Fig. 1. The figure shows Western blots using polyclonal antibodies to COX-2 of cell extracted from HUVEC of normal pregnancy (nHUVEC) and preeclampsia (pHUVEC). Control nHUVEC at 24 h contained no COX-2 protein (lane 1). In contrast, pHUVEC at 24 h contained COX-2 protein (lane 2). Equal amounts of protein (20 μg/lane) were loaded in each lanes. Similar results were obtained using cell extracts from 12 separate batches of cells.

Measurement of the release of 6-keto-$PGF_{1\alpha}$ in the supernatant medium and the isoform of COX protein from pHUVEC and nHUVEC

After HUVEC were incubated with fresh medium for 24 h, the supernatant from pHUVEC and nHUVEC was measured for 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2 which is the major COX metabolite from endothelial cells) by EIA kit (Amersham, U.S.A.). The remaining cells were extracted as previously described[20] for detection of COX-1 and COX-2 protein by immunoblot (Western blot) analysis using polyclonal antibody for COX-1 and specific antibody for COX-2 (Cayman, U.S.A.).

Statistical analysis

Student's paired or unpaired *t*-tests, as appropriate, were used to determine the significance of differences between means and a p-value of less than 0.05 was taken as statistically significant.

RESULTS

The expression of the isoform of COX protein in pHUVEC and nHUVEC

The study showed that COX-2 protein is expressed in human umbilical vein endothelial cells (HUVEC) from preeclampsia but not in HUVEC from normal pregnancy (Fig. 1). Moreover, the amount of COX-1 protein expressed in HUVEC from preeclampsia was not increased when compared to HUVEC from normal pregnancy (Fig. 2).

The release of 6-keto-$PGF_{1\alpha}$ in the supernatant medium from pHUVEC and nHUVEC

The release of 6-keto-$PGF_{1\alpha}$, the major COX metabolite in endothelial cells, in supernatant medium at 24 h was measured before extracting cells for immunoblotting. The results showed that 6-keto-$PGF_{1\alpha}$, levels in pHUVEC were less than in nHUVEC (159 ± 8 and 277 ± 5 ng/ml; $n = 12$, respectively), see Fig. 3. These different levels were significant at $p < 0.05$ using the unpaired *t*-test.

DISCUSSION

We have shown that there are COX-2 proteins expressed in pHUVEC but not in nHUVEC (Fig. 1). Moreover, the amount of COX-1 protein expressed in pHUVEC was not increased when compared to nHUVEC (Fig. 2). Interestingly, the release of 6-keto-$PGF_{1\alpha}$ at 24 h in pHUVEC was significantly less than in nHUVEC (Fig. 3). The results clearly demonstrated that COX-2 had been involved in the pathogenesis of preeclampsia and affected the release of 6-keto-$PGF_{1\alpha}$ in pHUVEC.

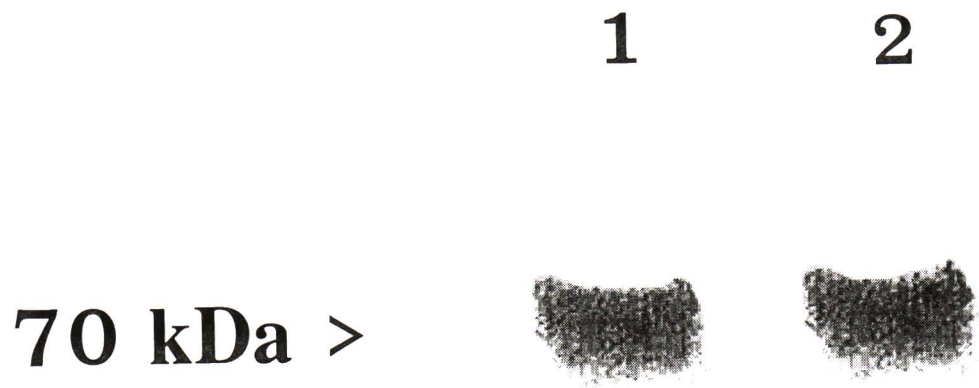

Fig. 2. The figure shows Western blots using polyclonal antibodies to COX-1 of cell extracted from HUVEC of normal pregnancy (nHUVEC) and preeclampsia (pHUVEC). Control nHUVEC (lane 1) and pHUVEC (lane 2) at 24 h contained COX-1 protein. The amount of COX-1 protein expressed in pHUVEC is not increased when compared to nHUVEC. Equal amounts of protein (20 μg/lane) were loaded in each lane. Similar results were obtained using cell extracts from 12 separate batches of cells.

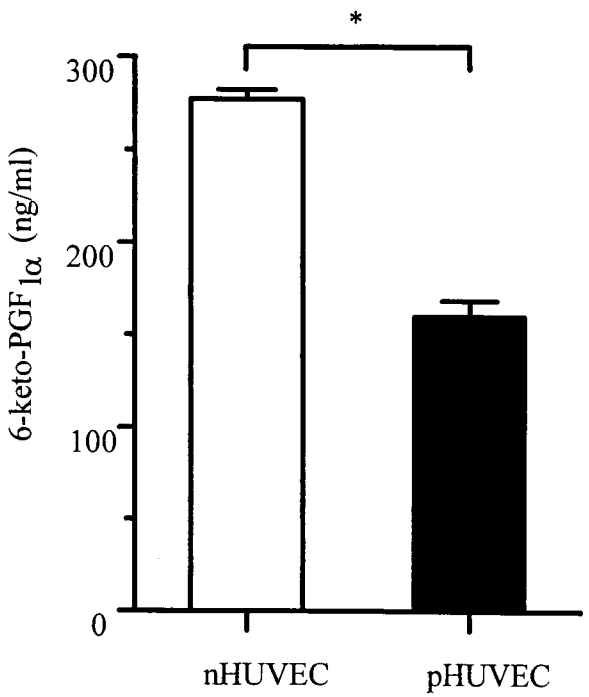

Fig. 3. The figure shows COX activity measured by the accumulation of 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2 which is major COX metabolite in endothelial cells) at 24 h in the supernatant medium from HUVEC of normal pregnancy (nHUVEC; white column) and preeclampsia (pHUVEC; black column). Data are expressed as mean ± s.e. mean from 12 determination. * $p < 0.05$ when compared to control nHUVEC at 24 h.

The mechanisms by which 6-keto-$PGF_{1\alpha}$ release is decreased in pHUVEC are not known. Several PGs such as PGI_2 (6-keto-$PGF_{1\alpha}$), TXA_2, and $PGF_{1\alpha}$ can be synthesised by COX which exists in at least 2 isoforms namely COX-1 and COX-2. Thus, there are two posibilities to explain the mechanism. Firstly, the different PGs which were released from pHUVEC by both COX-1 and COX-2 may produce autoinhibition as a previous report showed that PGE_1 can inhibit COX-2 induction in endothelial cells[21]. Secondly, there may be some plasma or serum factors which are responsible for altered prostacyclin production (6-keto-$PGF_{1\alpha}$) in preeclamptic patients. This hypothesis was supported by Baker who found that chronic exposure (72 h) to plasma from preeclamptic women alters endothelial cells and results in decreased prostacyclin production[22]. Therefore, the decrease in PGI_2 level from pHUVEC can refer to events in the circulation of the preeclamptic mother. These cause imbalance of vasodilating and vasoconstricting PGs. So, the significance to date of our results supported the hypothesis that the imbalance of PGs by decreased PGI_2 production from the endothelium causes the increase of blood vessel tone in the preeclamptic patient. The mechanism of this imbalance is by the induction of COX-2.

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REFERENCES

1. National High Blood Pressure Education Program Working Group report on high blood pressure during pregnancy. Am J Obstet Gynecol 1990; 163: 1689-712.
2. Lindheimer MD, Katz AI. Physiology and pathophysiology. In : The kidney. Seldin DW, Giebisch G, eds. 2nd ed. Raven Press, New York, 1992: 3371-431.
3. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol 1985; 152: 335-40.
4. Fitzgerald DJ, Fitz Gerald GA. Pathophysiology, diagnosis, and management. In : Hypertension. Laragh JH, Brenner BM, eds. Vol. 2. Raven Press, New York, 1990: 1789-807.
5. Remuzzi G, Ruggenenti P. Prevention and treatment of pregnancy-associated hypertension: what have we learned in the last 10 years? Am J Kidney Dis 1991; 18: 285-305.
6. Roberts JM, Taylor RM, Goldfein A. Clinical and biochemical evidence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia. Am J Hypertens 1991; 4: 700-8.
7. Taylor RN, Heilbron DC, Roberts JM. Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy. Am J Obstet Gynecol 1990; 163: 1839-44.
8. Taylor RN, Musci TJ, Rodgers GM, Roberts JM. Preeclamptic sera stimulate increased platelet-derived growth factor mRNA and protein expression by cultured human endothelial cells. Am J Reprod Immunol 1991; 25: 105-8.
9. Rubin PC, ed. Handbook of hypertension. Vol. 10. Elsevier, Amsterdam, 1988.
10. Cunningham FG, Mac Donald PC, Gant NF, Leveno KJ, Gilstrap IIILC, eds. Williams obstetrics. 19th ed. Appleton & Lange, Norwalk, 1993: 763-807.
11. Vane JR, Botting RM. The mode of action of anti-inflammatory drugs. Postgrad Med J 1990; 66 (suppl 4): S2-S17.
12. Smith WL, Marnett LJ. Prostaglandin endoperoxide synthase: structure and catalysis. Biochem Biophys Acta 1991; 1083: 1-17.
13. Lee SH, Soyoola E, Chanmugam P, et al. Selective expression of mitogen inducible cyclo-oxygenase in macrophages stimulated with lipopolysaccharide. J Biol Chem 1992; 267: 25934-8.
14. Maier JAM, Hla T, Maciag T. Cyclo-oxygenase is an immediate early gene induced by interleukin-1 in human endothelial cells. J Biol Chem 1990; 265: 10805-8.
15. 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.
16. Xie WL, Robertson DL, Simmons DL. Mitogen-inducible prostaglandin G/H synthase: A new target for nonsteroidal anti-inflammatory drugs. Drug Develop Res 1992; 25: 249-65.
17. 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.
18. Hughes EL. ed. Obstetric-gynecologic terminology. FA Davis, Philadelphia, 1991: 422-3.
19. 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.
20. Akarasereenont P, Mitchell JA, Appleton I, Thiemermann C, Vane JR. 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.
21. Akarasereenont P, Hide E, Ney P, Thiemermann C, Vane JR. 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. Agents Actions 1995; 45(Suppl): 59-64.
22. Baker PN, Davidge ST, Barankiewicz J, Roberts JM. Plasma of preeclamptic women stimulates and then inhibits endothelial prostacyclin. Hypertension 1996; 27: 56-61.

การปรากฏของซัยโคลอ๊อกซีย์เจเนส-2 ในเซลล์เพาะเลี้ยงจากผนังหลอดเลือดของสายรกเด็กในหญิงตั้งครรภ์เป็นพิษ (preeclampsia)

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เซลล์จากผนังหลอดเลือดของสายรกเด็กที่คลอดจากหญิงตั้งครรภ์ปกติ (nHUVEC) และครรภ์เป็นพิษ (pre-eclampsia; pHUVEC) ถูกนำมาเพาะเลี้ยง เมื่อเซลล์โตเต็มที่จึงสกัดเซลล์มาวิเคราะห์หาการปรากฏของโปรตีน COX-1 และ COX-2 โดยวิธี Western blot หน้าที่ของโปรตีน COX (COX activity) จะถูกวัดโดยดูจากปริมาณ 6-keto-$PGF_{1\alpha}$ ซึ่งเป็น stable metabolite ของโปรสตาไซคลิน (prostacyclin; PGI_2; major COX metabolites ใน HUVEC) โดยวิธี enzyme immunoassay (EIA) พบว่า nHUVEC (12 ราย) หลั่ง 6-keto-$PGF_{1\alpha}$ ในปริมาณ 277 $\pm$ 5 ng/ml ที่ 24 ชั่วโมง และมีการปรากฏของโปรตีน COX-1 แต่ไม่พบการปรากฏของโปรตีน COX-2 ในขณะที่ pHUVEC (12 ราย) หลั่ง 6-keto-$PGF_{1\alpha}$ ในปริมาณที่น้อยกว่า nHUVEC ที่ 24 ชั่วโมง (159 $\pm$ 8 ng/ml) และมีโปรตีนของ COX-1 และ COX-2 ปรากฏอยู่ จากการศึกษาวิจัยบ่งชี้ว่า COX-2 มีบทบาทเกี่ยวข้องกับกลไกการเกิดโรคความดันเลือดสูงในหญิงตั้งครรภ์เป็นพิษ การศึกษาเพิ่มเติมในแง่ของหน้าที่ (activity) และกลไกการสร้าง (mechanism of induction) ของ COX-2 จะช่วยทำให้เข้าใจพยาธิสรีระและพยาธิวิทยาของโรคความดันเลือดสูงในหญิงตั้งครรภ์เป็นพิษมากขึ้น และการใช้สารยับยั้งหน้าที่หรือการสร้างโปรตีนของ COX-2 อาจจะมีส่วนช่วยในการป้องกันและรักษาโรคความดันเลือดสูงในหญิงตั้งครรภ์เป็นพิษได้

คำสำคัญ : หญิงตั้งครรภ์เป็นพิษ, ซัยโคลอ๊อกซีย์เจเนส-2, เซลล์เพาะเลี้ยงจากผนังหลอดเลือดของสายรกเด็ก

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