International Journal of Gynecology and Obstetrics (2006) **95**, 127–131

International Journal of GYNECOLOGY & OBSTETRICS

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CLINICAL ARTICLE

Placental tissue cyclo-oxygenase 1 and 2 in pre-eclamptic and normal pregnancy

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Received 28 April 2006; received in revised form 19 June 2006; accepted 20 June 2006

KEYWORDS
Cyclo-oxygenase;
Malondialdehyde;
Placenta;
Pre-eclampsia

Abstract

Objective: To investigate the activities of the 2 isoforms of prostaglandin synthetic enzyme cyclo-oxygenase (COX), COX-1 and COX-2, in the placental tissue of women with pre-eclampsia and healthy pregnant women. The relationship between placental lipid peroxidation and the activities of COX-1 and COX-2 was also investigated. *Methods:* Tissue specimens were obtained from pre-eclamptic women (20 had severe pre-eclampsia and 38 had mild pre-eclampsia) and 27 healthy pregnant women who underwent cesarean section before the onset of labor. Malondialdehyde (MDA) levels and COX-1 and COX-2 activities were measured in placental tissue homogenates. *Results:* Mean activities for COX-1 and COX-2 were significantly lower in women with severe pre-eclampsia than in healthy controls ($P<0.05$ and $P<0.01$, respectively). COX-1 and COX-2 activities were also lower in women with mild pre-eclampsia than in healthy controls, but the difference was of borderline significance ($P=0.049$ and $P=0.059$, respectively). The mean placental MDA level was significantly higher in pregnant women with severe and mild pre-eclampsia than in healthy pregnant women ($P<0.01$ for both). The correlation analysis showed significant negative correlations between MDA and COX-1 ($r=-0.44$, $P<0.001$) and MDA and COX-2 ($r=-0.45$, $P<0.001$) in the placental tissue of women with pre-eclampsia. *Conclusion:* These results suggest that COX-1 and COX-2 activities are decreased in the placental tissue of women with pre-eclampsia, probably by oxidative stress.

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doi:10.1016/j.ijgo.2006.06.016

1. Introduction

Pre-eclampsia is a pregnancy-specific, multisystem syndrome that affects between 0.4% and 2.8% of all pregnancies in developed countries [1], and is still one of the leading causes of maternal and perinatal morbidity and mortality. Severe pre-eclampsia can lead to eclampsia, which is characterized by maternal convulsions thought to be caused by cerebral vasoconstriction. It is believed that vascular changes affect not only the symptoms but also the genesis of pre-eclampsia. Normal pregnancy is characterized by a decrease in vascular tone, a decrease in systemic vascular resistance, and refractoriness to pressor agents. These maternal vascular adaptations are disturbed in pre-eclampsia. Several factors have been implicated in these abnormalities, including increased levels of reactive oxygen species (ROS), disturbances in prostaglandin function, and endothelial dysfunction.

Several studies have reported increased ROS levels and decreased antioxidant levels in placentas obtained from pre-eclamptic women [2,3]. Possible causes of oxidative stress in pre-eclampsia may be increased mitochondrial activity, reduced antioxidant scavenging potential, and the occurrence of ischemia and reperfusion events in the placenta [4].

Cyclo-oxygenase is the key enzyme in the biosynthesis of prostaglandins and thromboxane from arachidonic acid. Two isoenzymes, COX-1 and COX-2, have been identified, the former as a regulator of physiologic functions and the latter as a mediator in pathophysiologic reactions such as inflammation. A splice variant of COX-1 and its RNA, COX-3, was recently detected, especially in the central nervous system [5]. COX-1 and COX-2 are expressed in the placental tissue samples [6] and several groups of investigators have reported that placental levels of COX-1 and/or COX-2 were increased in the placentas of healthy pregnant women in labor at term [7,8]. But there are controversial data regarding COX levels or activities in the placentas of women with pre-eclampsia, which have been found increased [6], unchanged [9] or decreased [10].

The aim of this study was to investigate whether placental COX activities were different in the placentas of women with pre-eclampsia, and the relationship between placental COX activities and MDA levels, a marker of lipid peroxidation.

2. Patients and methods

The study included 85 pregnant women (20 had severe pre-eclampsia, 38 had mild pre-eclampsia, and 27 were healthy). Following the American College of Obstetricians and Gynecologists recommendations, pre-eclampsia was defined as a persisting elevated diastolic blood pressure ($\geq$90 mm Hg), a proteinuria (>300 mg in a 24-h urine sample) and the presence of edema [11]. Mild pre-eclampsia was diagnosed if a blood pressure of 140/90 mm Hg was observed at least on 2 occasions 6 h apart, with or without proteinuria. Severe pre-eclampsia was diagnosed when the following criteria were present: (A) a systolic blood pressure of 160 mm Hg or greater or a diastolic blood pressure of 110 mm Hg or greater on 2 occasions at least 6 h apart, with the patients resting in bed; and (B) a proteinuria of 5 g or greater in a 24-h urine collection or of 3+ or greater on dipstick in at least 2 random clean-catch samples at least 4 h apart.

Inclusion criteria were pre-eclampsia; normal response to glucose tolerance testing; no evidence of recent infections such as rubella, toxoplasma, hepatitis B or C, cytomegalovirus, or syphilis; absence of uterine contractions; nonsmoker status; singleton pregnancy; gestational age corroborated by ultrasonography before the 20th week of gestation; and no fetal structural anomaly.

Because experiments have shown an increase in placental levels of COX-1 and COX-2 during labor, placental tissue was obtained from women undergoing cesarean section before the onset of labor. The indications for cesarean section were breech presentation or previous cesarean section. Placental tissue samples from the middle zone were collected free of placental membranes and washed thoroughly with ice-cold Tris buffer, pH 7.4, containing 0.16 mg/mL of heparin to remove any red blood cells and clots, then stored at -80°C until assayed.

A sample of placental tissue was homogenized in 5 mL of cold buffer (0.1 M Tris—HCl, pH 7.8, containing 1 mM EDTA) per gram of tissue and centrifuged at 10,000$\times g$ for 15 min at 4°C. An assay kit (Cayman, Ann Arbor, MI, USA) was used for the measurement of placental COX activity. This kit measures the peroxidase activity of COX. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine at 590 nm. COX-2 activity was measured using the COX-1-specific inhibitor.

To assess MDA levels, placental tissue samples were first homogenized with ice-cold 1.15% potassium chloride. The MDA levels were then assayed by monitoring thiobarbituric acid reactive substance formation as described previously [12]. Total thiobarbituric acid reactive substances were expressed