BASIC SCIENCE: OBSTETRICS

Specific systemic antioxidant response to preeclampsia in late pregnancy: the study of intracellular glutathione peroxidases in maternal and fetal blood

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OBJECTIVE: The physiopathology of preeclampsia is still unclear, but an imbalance between reactive oxygen species (ROS) and antioxidants, also called oxidative stress, appears to be an important contributing factor. The ROS promote lipid oxidation and are known to induce stress proteins, such as hemeoxygenase 1 (HO-1) and heat-shock protein 70 (Hsp-70). We hypothesized that glutathione peroxidases (GPx), a major class of antioxidant enzymes that regulate cell homeostasis by neutralizing lipid peroxides, are altered in the blood of preeclamptic women and neonates (venous cord blood).

METHODS: Thirty-one preeclamptic and 30 normotensive pregnancies were recruited. The blood was fractionated using a discontinuous gradient to separate the different cell types. The messenger ribonucleic acid (mRNA) expression of GPx-1 and -4, HO-1, and Hsp-70 were analyzed by quantitative reverse transcriptase–polymerase chain reaction. GPx-1 and -4 protein level in blood cells was also detected by Western blot. The experiments were analyzed using the Student *t* test.

RESULTS: The HO-1 and Hsp-70 mRNA expression in whole blood was significantly higher in both fetal and maternal circulations ($P < .05$). We also discovered that GPx-4 mRNA was 1.6-fold higher in blood of women with preeclampsia than in control pregnancies ($P = .04$). The latter was associated with an increase of both GPx-1 and GPx-4 protein and mRNA levels in the lymphocyte/monocyte fraction of the blood. Significantly higher GPx-4 mRNA levels in the fetal circulation of the preeclamptic group than the control group were also detected ($P < .001$).

CONCLUSION: These data indicate that preeclampsia is associated with a specific antioxidant response in both maternal and fetal circulations, likely in response to the deleterious oxidative stress observed in this syndrome.

Key words: antioxidants, cord blood, glutathione peroxidases, heat shock protein-32, heat-shock protein-70, hemeoxygenase-1, phospholipid hydroperoxide glutathione peroxidase, preeclampsia, pregnancy, whole blood

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The exact cause of preeclampsia (PE) remains unclear despite many clinical studies. A 2-stage model has been proposed to explain this syndrome: first, a reduced placental perfusion yields to the release of unknown factors (stage 1) that leads to a generalized endothelial cell dysfunction, resulting in the maternal syndrome of hypertension and proteinuria (stage 2).[1] The unknown factors released by the placenta suspected to cause PE include reactive oxygen species (ROS), cytokines, eicosanoids, or a combination of these mediators.[2]

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Oxidative stress is defined as an imbalance between prooxidants like ROS and antioxidants.[3] Stress proteins, such as hemeoxygenase (HO-1) and heat shock protein-70 (Hsp-70), are induced by oxidative stress.[4,5] There is now sufficient evidence to consider ROS promoters of endothelial cell dysfunction leading to preeclampsia. A number of reports indicated that lipid peroxidation products, such as malondialdehyde and F_2-isoprostanes, are elevated in plasma and placenta of women with preeclampsia in comparison with normal pregnancies.[6-8] Moreover, the total plasma antioxidant capacity was decreased in preeclamptic patients when compared with normotensive pregnancies.[9]

To counteract the oxidative stress, numerous antioxidants exist to maintain cell and tissue homeostasis. Glutathione (GSH) and vitamins C and E are examples of nonenzymatic antioxi-

dants. Enzymatic antioxidants include superoxide dismutase, catalase, and the glutathione peroxidases family (GPx).[10] The GPx play a critical role in the control of lipid peroxidation, mainly in catalyzing the breakdown of H_2O_2, lipids, and other organic hydroperoxides. There are 2 main intracellular GPx: GPx-1 and GPx-4.[11] GPx-1, also called classical GPx, is abundant in most tissues and is widely distributed within the cells.[10] The phospholipid hydroperoxide peroxidase or GPx-4 is a unique antioxidant enzyme that does not require the action of phospholipases to detoxify lipid peroxides. Moreover, GPx-4 is also able to use various thiols aside from GSH as cofactors.[12]

The exact antioxidant alterations in preeclampsia remain to be determined. Various results were obtained for extracellular or total GPx activities. Total GPx enzymatic activity was found to be lower in placenta,[13,14] whole blood,[15] and plasma[16] of the women with preeclampsia. However, whole blood levels of GPx were also found to be higher in another study.[17] To our knowledge, no one has yet investigated separately each of the specific GPx in preeclampsia.

Most studies on oxidative status in preeclampsia have been focused on maternal and placental parameters. Only a few studies have been carried out on the fetus.[18-21] Impacts are not limited to the reduced placental perfusion and iatrogenic prematurity. Davidge et al[22] found evidence of an endothelial activation in fetuses of women with preeclampsia. Their erythrocyte GPx activity and their umbilical venous plasma antioxidant capacity (total peroxyl radical-trapping antioxidative parameter) are significantly lower than control pregnancies.[18,21]

In light of previous studies,[1,23] the assessment of the role and importance of the oxidant imbalance in the physiopathology of preeclampsia is of the utmost importance. The main purpose of this study was to determine which intracellular GPx enzyme (GPx-1 and/or GPx-4) is affected in the fetal and maternal blood by preeclampsia. Furthermore, we have investigated in which cell type the change of GPx status was observed in the maternal blood.

MATERIALS AND METHODS

Patient recruitment

This study was performed at the Centre Mère-Enfant du Centre Hospitalier Universitaire de Québec between December 2004 and December 2005. The institutional ethical committee approved the study, and all participants gave a written informed consent. Gestational hypertension with proteinuria (preeclampsia) was defined according to the Canadian Hypertension Society Consensus[24] as a diastolic blood pressure of 90 mm Hg or more on 2 separate readings at least 4 hours apart with proteinuria of $\geq$ 0.3 g/day (plus $\geq$ 1 on dipsticks).

Sixty-one pregnant women (31 preeclamptic and 30 normotensive women) and their newborns were recruited; all were singleton pregnancies. The normotensive group consisted of women with a normal antenatal period. Patients with extreme age ($<$ 20 years and $>$ 35 years), high body mass index ($>$ 30 kg/m^2), preexisting medical conditions (diabetes mellitus, chronic hypertension, renal disease, and any coagulation disorder), women in labor, multiple pregnancy, or any concurrent medical or obstetric complications, such as premature rupture of membranes, intrauterine growth restriction, and gestational diabetes, were excluded from the study.

Blood collection

Twenty milliliters of blood were collected in heparinized tubes before the active phase of labor. Immediately after clamping of the umbilical cord, 10 mL of venous cord blood was also collected. The processing of the blood sample was made within 2 hours. Five hundred microliters of whole blood was frozen in dry ice and kept at -80°C for further messenger ribonucleic acid (mRNA) analysis. The remaining blood was centrifuged at 180 $\times$ *g* for 10 minutes at 20°C. The supernatant (plasma) was centrifuged again at 1300 $\times$ *g* for 25 minutes to remove platelets from plasma. The plasma and platelets were frozen separately and kept at -80°C. The pellet of the first centrifugation was completed to the initial volume with Hanks' balanced salt solution (HBSS; Invitrogen, Carlsbad, CA) N-2-hydroxyethylpipera-zine-N′-2-ethane sulfonic acid (HEPES) 10 mM (pH 7.4), 1.6 mM $CaCl_2$, and 5 mL of 3% dextran were added.

After dextran sedimentation, leukocytes were separated by centrifugation on lymphocyte separation medium (Wisent; St Bruno, Québec, Canada).[25] The lymphocytes and monocytes, located at the interface, were purified by centrifugation at 425 $\times$ *g* in HBSS/HEPES 10 mM (pH 7.4) and 1.6 mM $CaCl_2$. A pellet of lymphocytes/monocytes was obtained and then frozen for mRNA and Western blot analyses. The pellet obtained after the use of lymphocyte separation medium is constituted by a majority of neutrophils and a minority of erythrocytes. The erythrocytes were removed by hypotonic lysis in water. HBSS 10 $\times$ was added, and the solution was centrifuged at 425 $\times$ *g* for 5 minutes to obtain a pellet of neutrophils (frozen in dry ice and kept at -80°C for mRNA and Western blot analysis).

RNA isolation from whole blood and preparation of complementary cDNA

The RNA was extracted from whole blood using RiboPure blood kit according to the manufacturer's instructions (Ambion; Streetsville, ON, Canada). Five hundred microliters of a blood sample was processed in 300 μL of acid-phenol-chloroform solution. Four micrograms of total RNA was reverse transcribed with random hexamer primers and the Superscript II reverse transcriptase (Invitrogen) as previously described.[26] The first-strand cDNA was diluted 20 times in sterile water and used as the template in the quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) mixture.

Quantitative RT-PCR

Sets of primers were designed according to known human sequences to amplify specific products for GPx-1 (forward: 5′-gactacacccagatgaacgagc-3′; reverse: 5′-cccaccaggaacttctcaaag-3′), GPx-4 (forward: 5′-tttccgccaaggacatcg-3′; reverse: 5′-accacgcagccgttcttgtc-3′), HO-1 (forward: 5′-acccatgacaccaaggac-3′; reverse: