Calcium-ATPase Activity of Red Blood Cell Ghosts from Preeclamptic Women, Antepartum and Postpartum

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ABSTRACT

Objective: We determined the calcium activated adenosine triphosphatase (Ca-ATPase) activity and level of lipid peroxidation thiobarbituric acid-reactive substances (TBARS) of red blood cell ghosts in the antepartum and postpartum of normotensive and preeclamptic pregnant women. Methods: Samples of venous blood were obtained by venipuncture of nulliparous normotensive and preeclamptic pregnant women antepartum and two, four, six, and 20 weeks postpartum. The red blood cell ghosts were prepared and assayed for Ca-ATPase activity and TBARS. Main Outcome Measure(s): We expected to find a return to normal values of both Ca-ATPase activity and TBARS level of the red blood cell ghosts, modified in the preeclamptic pregnant women, during their puerperium. Results: The Ca-ATPase activity of red cell ghosts from preeclamptic women, antepartum, is lower than that of normotensive pregnant women. The ATPase activity returns to normal values during the first six weeks of postpartum. The level of TBARS of red cell ghosts from preeclamptic women follows a pattern that is inversely proportional to the one of the Ca-ATPase activity. Conclusions: Preeclampsia produces a significant diminution of the Ca-ATPase activity and an increase in the levels of

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TBARS in the erythrocytes. As soon as all the symptoms of preeclampsia disappear in
the postpartum, both Ca-ATPase activity and TBARS return to normal values.

Key Words: Preeclampsia; Ca-ATPase; Red blood cell ghosts; TBARS; Postpartum.

INTRODUCTION

In 1990 we reported that the calcium adenosine phosphatase (Ca-ATPase) activity
of red blood cell ghosts is diminished in preeclamptic pregnant women as compared
to normotensive pregnant women (1). These results have been confirmed by other
laboratories (2,3). Furthermore, Sowers et al. (3) claimed that a diminution of the Ca-
ATPase activity of erythrocytes is already seen in the first trimester of pregnancy of
women that developed preeclampsia at a late stage. We have recently demonstrated (4)
that the reduction of Ca-ATPase activity is not restricted to the maternal red blood
cells, but can also be seen in the red cell ghosts of umbilical cord blood of pre-
eclamptic mothers, indicating a direct effect of preeclampsia on the neonates.

It has been reported that there is a significant rise in the intracellular free calcium
concentration in erythrocytes (5), platelets (6), lymphocytes (7), and placental tissue (8)
in preeclamptic women. The cause of this increase in intracellular free Ca\textsuperscript{2+} is
unknown. Since the activity of the plasma membrane Ca-ATPase is responsible for the
fine control of the cytosolic concentration of calcium (9), a reduction in its activity may
be associated, at least in part, with the elevation in intracellular free calcium
concentration of the above mentioned cells.

Although the overall pathogenesis of preeclampsia is unclear, it is generally viewed as
a multisystem disorder with vascular dysfunction at its center. It has been proposed that
there is an important participation of arteriolar vasoconstriction with increased total
peripheral vascular resistance (10) in the developing of preeclampsia. This increased
peripheral resistance associated with vasoconstriction seems to be dependent upon an
enhanced Ca\textsuperscript{2+} influx across the vascular myocytic membrane and, in fact, may be
affected by calcium antagonists (11). If the preeclamptic condition leads to an increased
intracellular free Ca\textsuperscript{2+} concentration then the vascular smooth muscle tension might also
increase (12). Accordingly, we could also expect, at least in theory, to find a link between
the plasma membrane Ca-ATPase activity, which is responsible for the fine control of
the intracellular free Ca\textsuperscript{2+} concentration, and the arterial blood pressure.

The activity of the Ca-ATPase is sensitive to the level of lipid peroxidation of the
plasma membrane (13), which has been found to be increased in preeclampsia (4,13).
This condition could be the result of the combination of different mechanisms. A
lowered level of beta-carotene (14) and antioxidant activity (15) have been found in
serum of preeclamptic women. Also, increased levels of both lipid peroxides and
thromboxane in placentae from preeclamptic women have been demonstrated (16).
Increased thromboxane production is an indication of increased cyclooxygenase
activity, which leads to increased reactive oxygen species formation. These radicals
interact with lipids, proteins, and carbohydrates, either those circulating in the blood or
those forming the plasma membranes of the placenta and nearby tissues (17).
If there is a close relationship between the level of lipid peroxidation, the Ca-ATPase activity, and preeclampsia, one could expect that as soon as hypertension, proteinuria, and edema recede in the puerperium of the preeclamptic women, both plasma membrane lipid peroxidation and Ca-ATPase activity would return to normal values. In the present work, we evaluated the Ca-ATPase activity and the level of lipid peroxidation of the red blood cell ghosts of pregnant women, either normotensive or preeclamptic, from the last days of pregnancy until 20 weeks postpartum.

METHODS

Donors

Pregnant women (11 normotensive and 11 preeclamptic), at the Maternity Hospital ‘‘Concepción Palacios’’ in Caracas, Venezuela, participated in this study. The normotensive patients were 22.4±1.9 (SE) years of age at delivery and 39.5±0.6 (SE) weeks of gestational age. The preeclamptic patients were 19.6±1.8 (SE) years of age at delivery and 38.0±0.7 (SE) weeks of gestational age. All normotensive and preeclamptic pregnant women enrolled in the study were nulliparous. We reviewed their medical records to confirm diagnosis and obtain other relevant data. Normotensive pregnant women showed no history of hypertension and no evidence of hypertension or proteinuria during their pregnancy. The preeclamptic pregnant women were identified after detection of proteinuria (>300 mg prot/24 h), a blood pressure ≥140/90 mm Hg or a rise of 30 mm Hg in systolic pressure or of 15 mm Hg in diastolic pressure (measured twice, six hours apart at bed rest), and edema. Any patient that, according to her medical history, was under medical treatment to control blood pressure, or if she was taking >1 g of elemental calcium per day during pregnancy, or if she had a history of chronic hypertension, diabetes, calcium metabolism disorders, or any other chronic medical illness, was not considered for this study. The study was approved by the Institutional Review Board of the Maternity ‘‘Concepción Palacios’’ and by the Bioethics Committee of Instituto Venezolano de Investigaciones Científicas (IVIC), and all women gave informed signed consent. Three out of 11 preeclamptic women that participated in the study before delivery decided to withdraw from the study during their postpartum period.

Red Blood Cell Ghosts Preparation

Ten mL of venous blood were collected into heparinized collection tubes from either normotensive or preeclamptic pregnant women before delivery (antepartum). Blood samples were also taken from the same women, two, four, six, and 20 weeks after delivery (postpartum). The maximum evaluated postpartum time (20 weeks) was chosen because at this time there would have been a turnover of the entire erythrocyte population. Each blood sample was centrifuged at 12,000 × g for 1 min and the buffy coat and the plasma were discarded.

Hemoglobin-free red blood cell ghosts were prepared from the packed red cells following the method of Heinz and Hoffman (18). The ghosts were stored at -20°C in
a Tris-EDTA solution containing 17 mM Tris-HCl and 0.1 mM ethylenediamine
tetraacetic acid (EDTA) (pH 7.5 at 0°C), and were always assayed within seven days of preparation.

ATPase Activities

The Ca-ATPase activity was determined by measuring the quantity of inorganic phosphate liberated from the hydrolysis of ATP, according to the method described elsewhere (19). Briefly, the Ca-ATPase activity assay was carried out as follows: 100 µL of ghosts (1 mg prot/mL) were incubated for 30 min in 400 µL of a medium containing (final concentrations): 3 mM MgCl₂; 2 mM ATP; 80 mM NaCl; 15 mM KCl; 0.1 mM ethylene glycol bis(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA); and 50 mM Tris-HCl (pH 7.4 at 37°C), in the presence and absence of 20 µM free Ca²⁺. Calmodulin from bovine brain, at a concentration of 3 µg/mL, was present in all the assays. The reaction was stopped by the addition of 300 µL of a cold solution containing: 2.86% ascorbic acid; 1.76% HCl; 0.48% molybdic acid; and 2.86% sodium dodecyl sulfate. The samples were shaken and kept at 0°C for 10 min. Then, 200 µL of a solution of 5% sodium citrate, 5% sodium arsenite and 5% acetic acid were added to each tube, which were rewarmed after shaking for 10 min at 37°C. The absorbance of each tube was determined in a Milton Roy spectrophotometer at 705 nm. The ATPase activity was expressed as nmol Pi/mg prot . min, after subtraction of a blank run in parallel under the same conditions except for the membrane suspension, which was added only after the addition of the 300 µL of solution to stop the reaction. Each sample was run in triplicate or quadruplicate. The Ca-ATPase activity was calculated as the difference in the phosphate liberated in a medium containing Mg²⁺ +Ca²⁺ minus the one liberated in the same medium but in the absence of calcium. The Ca-ATPase activity was linear up to 90 min of incubation and 300 µg of total protein in the assay medium. The protein concentration, in all the cases, was determined by the Coomassie blue dye binding assay (Bio-Rad Laboratories, Richmond, CA).

Lipid Peroxidation Measurements

The amount of lipid peroxidation of the plasma membranes was estimated by measuring the thiobarbituric acid-reactive substances (TBARS). The TBARS measurements were carried out following the method of Feix et al. (20). The absorbance was measured at 532 nm and the TBARS values were calculated by using a malondialdehyde standard curve, prepared by acid hydrolysis of 1,1,3,3-tetramethoxypropane. The values are expressed as nmol malondialdehyde/mg prot.

Statistical Analysis

Statistical analysis was performed by the Student’s t-test. All results are expressed as mean±S.E. and (n) represents the number of experiments performed with different membrane preparations. In all the cases, the Ca-ATPase activity was calculated by paired data.
RESULTS

Ca-ATPase Activity of Red Blood Cell Ghosts from Normotensive and Preeclamptic Pregnant Women, Antepartum and Postpartum

Figure 1 shows the Ca-ATPase activity of red cell ghosts from normotensive and preeclamptic women, antepartum and postpartum. It can be seen that the Ca-ATPase activity of the red blood cell ghosts from preeclamptic women, antepartum, is about 50% lower than that of normotensive pregnant women. It can also be seen that the ATPase activity of the preeclamptic women begins to rise in the puerperium, reaching normal values after six weeks postpartum. The Ca-ATPase activity of red blood cell ghosts from normotensive women is the same, antepartum and postpartum. It is also important to notice that the recovery of the Ca-ATPase activity in the postpartum period of the preeclamptic women, is independent of the turnover of the entire erythrocyte population, since the ATPase activity is the same at six weeks and 20 weeks postpartum.

The TBARS of Red Blood Cell Ghosts of Normotensive and Preeclamptic Pregnant Women, Antepartum and Postpartum

Figure 2 shows the level of TBARS of red cell ghosts from normotensive and preeclamptic women, antepartum and postpartum. It is to be noted that the level of TBARS of the red blood cell ghosts from preeclamptic women, antepartum, is significantly higher than that of normotensive pregnant women. In the postpartum period, the level of TBARS of the red cell ghosts from preeclamptic women diminished.
progressively, reaching normotensive values near the sixth week postpartum. Similarly to the rise of the Ca-ATPase activity during postpartum, the reduction of the TBARS of the red cell ghosts in the postpartum of the preeclamptic women was independent of the turnover of the entire erythrocyte population. When the Ca-ATPase activity is plotted as a function of the TBARS level in the red blood cell ghosts, as shown in Figure 3, it can be seen there is a good correlation between these two parameters.

**Figure 2.** TBARS of red blood cell ghosts from normotensive (●) and preeclamptic mothers (○), 1 day before delivery (antepartum) and until 20 weeks postpartum. Values are means ± SE of different preparations, (normotensives, n=11, preeclamptics, n=11 antepartum and n=8 postpartum).

**Figure 3.** Ca-ATPase activity as a function of the TBARS level of the red blood cell ghosts. Each value corresponds to determinations carried out with different preparations, antepartum and postpartum, from normotensive and preeclamptic women. Values are means ± SE of different preparations, (n=61).
Table 1. Ca-ATPase activity of red blood cell ghosts, mean arterial blood pressure, presence of proteinuria and edema from either normotensive or preeclamptic pregnant women, antepartum (1 day) and postpartum (20 weeks).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Antepartum</th>
<th>Postpartum (20 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATPase activity</td>
<td>18.5±1.0 (11)</td>
<td>19.2±0.8 (11)</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.4±0.08 (11)</td>
<td>0.37±0.04 (11)</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>84±3 (11)</td>
<td>85±2 (11)</td>
</tr>
<tr>
<td>Urinary protein</td>
<td>&lt;300</td>
<td>&lt;300</td>
</tr>
<tr>
<td>Edema</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Preeclamptic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATPase activity</td>
<td>7.1±0.5 (11)</td>
<td>18.0±0.9 (8)</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.97±0.04 (11)</td>
<td>0.43±0.04 (8)</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>127±6 (11)</td>
<td>85±5 (8)</td>
</tr>
<tr>
<td>Urinary protein</td>
<td>&gt;800</td>
<td>&lt;300</td>
</tr>
<tr>
<td>Edema</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

Values are means ± SE of determinations carried out with different samples (numbers given between parenthesis represent the number of determinations with different women).

(a) nmol Pi/mg prot . min.
(b) nmol MDA/mg prot.
(c) mm Hg.
(d) mg prot/24 h.

(c)oefficient of determination, $r^2=0.76$, which can be taken as an indication that the oxidation status of the plasma membrane might regulate the activity of this membrane ATPase.

Resolution of hypertension, proteinuria, and edema occurs normally in the puerperium of the preeclamptic patients. Circulating lipid peroxides, which have been shown to be increased in the blood plasma of preeclamptic women in comparison with that of normotensive pregnant women (21), return to normal values as soon as 48 hours postpartum (22). The diminution of the TBARS level of the red blood cell ghosts of the preeclamptic women, in the postpartum period, might be the result of the resolution of the oxidative stress during the puerperium.

Table 1 presents a summary of the Ca-ATPase activity and the TBARS level of red blood cell ghosts, the mean blood pressure, and the presence or not of proteinuria and edema, antepartum and after 20 weeks postpartum, for both normotensive and preeclamptic women. In contrast to the normotensive patients, who did not show any variation in the measured parameters, the preeclamptic patients showed resolution of all of them, including the Ca-ATPase activity and the TBARS level of their erythrocytes.

**DISCUSSION**

The Ca-ATPase activity of erythrocytes in pregnant women is significantly diminished in preeclampsia (1–3). The lower Ca-ATPase activity of the red blood cell ghosts from pregnant women with preeclampsia may be explained as due to a reduced
turnover rate of the enzyme (1,4). Considering that modifications in the level of lipid peroxidation of cell membranes, through changes in membrane lipid fluidity, could affect directly the turnover rate of the Ca-ATPase (17); and that this enzyme is particularly sensitive to membrane lipid peroxidation (13), it has been proposed that the diminution of the Ca-ATPase activity associated with preeclampsia could be the result of the increased level of membrane lipid peroxidation also observed in this disease (4,13). This effect has been explained considering that lipid peroxidation directly affects the membrane fluidity (17), which in turn affects the activity of the Ca-ATPase since this enzyme is strongly dependent on the fluidity status of the membranes (13). In the present work, we demonstrate that, similarly to the triad of symptoms of preeclampsia, the lowered Ca-ATPase activity and the higher level of membrane TBARS of the red cell ghosts of preeclamptic women, return to normal values during the postpartum of these women (Figures 1,2, Table 1).

It is reasonable to conclude that the Ca-ATPase activity of the red blood cell ghosts of the preeclamptic women is affected by the high level of lipid peroxidation of these membranes. Although the pathophysiologic changes of preeclampsia abate with delivery, the changes in the plasma membranes produced by this disease, i.e., increased lipid peroxidation and decreased Ca-ATPase activity, last for several weeks. The longer delay recovery of the biochemical characteristics of the plasma membranes is compatible with recovery of the cellular antioxidant mechanisms, which have been reported to be affected by preeclampsia (14,15).

The recovery of the Ca-ATPase activity in the postpartum of the preeclamptic women could also be due to the appearance of new erythrocytes formed without the influence of preeclampsia. However, this possibility can be discarded since the Ca-ATPase activity is completely restored before the complete turnover of the entire erythrocyte population in postpartum. In preliminary experiments, through the determination of the specific phosphorylated intermediate of the Ca-ATPase, we estimated the number of Ca-ATPase molecules in red blood cell ghosts from normotensive and preeclamptic patients. It was found that the amount of the phosphorylated intermediate of the Ca-ATPase in the red blood cell ghosts does not change with preeclampsia (data not shown). This may be taken as an indication that the diminution of the Ca-ATPase activity seen in preeclampsia does not seem to be associated with changes in the number of Ca-ATPase molecules, but in the turnover rate of the enzyme.

The rise in membrane TBARS and the concomitant reduction in plasma membrane Ca-ATPase activity with preeclampsia are unlikely to account for the primary etiology of the disease. However, they may be responsible for some of the secondary events of preeclampsia. The relevance of our observations to the pathophysiologic changes of preeclampsia, may be related to the fact that a reduction in the activity of the Ca-ATPase of the plasma membranes, could result in an increased cytosolic cell calcium concentration of the tissues. An increased cytosolic calcium concentration in the vascular smooth muscle cells could result in increased muscle tension. This vasoconstrictive effect could lead to a rise in blood pressure (22). As soon as the plasma membrane Ca-ATPase and, consequently, the cytosolic free calcium concentration return to normal values after delivery, there is a decreased muscle tension in the vascular smooth muscle cells and, hence, the arterial blood pressure could return to normal values. Even when there must be several factors involved in the developing of the high arterial blood pressure of the
Ca-ATPase Activity, Preeclampsia, and Puerperium

preeclamptic patient, the present results must be taken into account when trying to explain the pathophysiologic changes of preeclampsia.

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