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Effect of magnesium sulfate on the calcium-stimulated adenosine triphosphatase activity and lipid peroxidation of red blood cell membranes from preeclamptic women

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Abstract

The effect of the treatment with magnesium sulfate ($MgSO_4$) on Ca-ATPase activity and level of lipid peroxidation of red blood cells from preeclamptic pregnant women was examined because it is known that these parameters are affected with preeclampsia. Red cell ghosts from 11 normotensive and 11 preeclamptic pregnant women, before and after treatment with $MgSO_4$, were assayed for Ca-ATPase activity and level of lipid peroxidation, determined as TBARS or conjugated dienes. It was found that the Ca-ATPase activity is significantly lower and the level of lipid peroxidation is significantly higher in the preeclamptic women with no treatment, as compared to normotensive pregnant women. Both parameters return to normal values after the $MgSO_4$ therapy. These results can be mimicked by in vitro preincubation with $MgSO_4$ of intact red blood cells from preeclamptic pregnant women, without any treatment. Our data indicate that $MgSO_4$ treatment of preeclamptic pregnant women modifies both the Ca-ATPase activity and the level of lipid peroxidation of their red blood cell membranes, reaching values similar to those of normotensive pregnant women. The diminution of the level of lipid peroxidation by $MgSO_4$, can account for the increase in Ca-ATPase activity.

Keywords: Magnesium sulfate; Preeclampsia; Inside-out vesicles; Ca-ATPase; Lipid peroxidation; Human red blood cells

1. Introduction

Preeclampsia is an important complication affecting approximately 7–10% of pregnant women that appears after the 20th week of pregnancy, being far more common in the third trimester [1]. This disorder is characterized by vascular endothelial damage, abnormalities in plasma volume, hypertension, proteinuria, edema and generalized arteriolar vasospasm [2]. It can progress to eclampsia (seizures), renal failure, stroke, liver failure, pulmonary edema, and coagulopathy. Parenteral $MgSO_4$ is widely used to treat severe preeclampsia in order to prevent the recurrent seizures of eclampsia and for tocolysis in preterm labor [3]. In addition to this effect, the treatment with $MgSO_4$ is known to decrease blood pressure [4], the level of serum MDA, a marker of lipid peroxidation [5], as well as to have a protective effect on endothelial cells [6]. Despite $MgSO_4$ use for a long time, its mechanism of action at a molecular level in preeclamptic/eclamptic pregnant women remains an enigma. When initially administered, $MgSO_4$ has a mild vasodilatory effect in the maternal circulation. This vasodilation may improve feto-placental perfusion, known to be diminished in preeclampsia. Antenatal use of $MgSO_4$ decreases the incidence of intraventricular hemorrhage in neonates of preeclamptic pregnancies [7], protecting in this way the fetus. It has also been associated with a decreased incidence of cerebral palsy [8] and mortality [9] in premature and very low birth weight neonates. Other studies have also shown a decreased neonatal mortality rates when magnesium is

Abbreviations: Ca-ATPase, calcium-stimulated adenosine triphosphatase; MDA, malondialdehyde; Na,K-ATPase, sodium and potassium-stimulated adenosine triphosphatase; TBARS, thiobarbituric acid-reactive substances

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used for preeclampsia or preterm labor [10]. It has been suggested that $MgSO_4$ may interfere with calcium channels in the vascular smooth muscle, as well as in the endothelial cells [11].

In previous works, we have shown that the Ca-ATPase activity of maternal and neonatal red cell ghosts [12,13], myometrium [14] and syncytiotrophoblast basal (fetal facing) plasma membranes [15,16] of pregnant women with preeclampsia, is diminished by about 50% as compared to the ATPase activity of the same tissues from normotensive pregnant women. In addition, red cell membranes from preeclamptic women show higher levels of lipid peroxidation than those from normotensive pregnant women [13]. It has been suggested that there is a close relationship between the level of lipid peroxidation of the plasma membranes and the activity of the Ca-ATPase [17]. In the present work, we evaluated the effect of the treatment of preeclamptic pregnant women with $MgSO_4$ on both the Ca-ATPase and the lipid peroxidation level of their red cell membranes.

2. Materials and methods

2.1. Blood donors

Eleven normotensive and 11 severe preeclamptic pregnant women of the Maternity Hospital "Concepción Palacios" in Caracas, participated in this study in accordance with the ethical standards established by the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of the Maternity "Concepción Palacios" and by the Bioethics Committee of IVIC, and all women gave informed signed consent. This study was performed at admission and before delivery. All the pregnant women enrolled in the study were nulliparous, had similar demographic backgrounds, and belonged to urban population of Caracas. Gestational age was estimated from the date of the last menstrual period and confirmed by ultrasonography. Normotensive pregnant women had no history of hypertension and no evidence of hypertension or proteinuria during their pregnancy. The blood pressure was measured twice, 6 h apart at bed rest; the diastolic level was measured at Korotkoff phase V. Any woman 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 clinical data of normotensive and preeclamptic pregnant women are presented in Table 1.

Blood samples were obtained by venipuncture, with the patients in lateral decubitus position. Only women giving birth a single newborn with Apgar scores of 7–10 were included. All pregnant women with severe preeclampsia received $MgSO_4$ therapy, and paired blood samples were obtained before (at admission) and 24 h after the onset of the therapy. $MgSO_4$ treatment consisted of a loading dose of 4 g administered intravenously over a period of 30 min followed by a maintenance dose of 1 g/h [18]. After this treatment, the mean blood pressure was significantly lowered (Table 1). Maternal blood samples were obtained and immediately transported to our laboratory on ice. An amount of 10 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 preeclamptic pregnant women, 24 h after the onset of the $MgSO_4$ therapy. Each blood sample was centrifuged at 12,000 $\times$ g for 1 min at 4 °C 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 [19]. The ghosts were stored in a solution containing 17 mM Tris–HCl and 0.1 mM EDTA (pH 7.5 at 0 °C) and kept at −70 °C until use.

Table 1
Clinical data from 11 healthy pregnant women (normotensives) and 11 pregnant women with severe preeclampsia

	Normotensive	Preeclamptic
Age (yr)	21.2 ± 3.2	19.43 ± 2.9
Number of deliveries	0	0
Mean blood pressure before $MgSO_4$ (mm Hg)	82.8 ± 2.7	133.4 ± 3.2*
Mean blood pressure after $MgSO_4$ (mm Hg)	–	100.2 ± 3.7†
Protein excretion (g/24 h)	0.12 ± 0.09	5.48 ± 0.26*
Pathologic edema (number of women)	0	11
Preexistent renal disease	0	0
Diabetes mellitus	0	0
Pregnancy duration (wk)	38.9 ± 0.7	37.3 ± 0.5

* $P < 0.001$ vs. normotensive pregnant women.
† $P < 0.001$ vs. preeclamptic pregnant women before $MgSO_4$ therapy.

2.2. Inside-out membrane vesicles preparation and calcium uptake determination

The preparation of inside-out membrane vesicles from red cell ghosts and the measurement of their calcium uptake were carried out following the method described elsewhere [20].

2.3. ATPase assays

The Ca-ATPase activity of the red blood cell ghosts was determined as described elsewhere [17]. The Ca-ATPase activity was calculated as the difference between the amount of phosphate liberated in the tubes containing Ca^{2+} minus that liberated in the tubes without Ca^{2+}. The results are expressed as nanomoles of inorganic phosphate liberated per milligram of protein per minute, after

subtraction of a blank run in parallel without the membrane suspension, which was added after stopping the reaction.

2.4. Ultraviolet irradiation of the red blood cell ghosts

A 250 μl aliquot of the red blood cell ghosts (1 mg/ml protein) from normotensive pregnant women was poured into a glass vial, placed on ice, and illuminated from approximately 4 cm distance by a mineral light (wavelength 254 nm maximum, specified strength 280 $\mu W/cm^2$ at 15 cm distance) for different lengths of time [17] to induce lipid peroxidation.

2.5. Lipid peroxidation measurements

The amount of lipid peroxidation of the red blood cell ghosts was estimated by measuring both TBARS and conjugated dienes. The TBARS were determined according to the method described by Feix et al. [21]. The TBARS are expressed as nanomoles of malondialdehyde per milligram of protein. To determine the conjugated dienes, total lipids were extracted from red blood cell ghosts according to the method of Folch et al. [22], and the dienes were estimated by reading the total lipid extracts in hexane at 233 nm in a spectrophotometer, and by using an extinction coefficient of 27,000 M^{-1} cm^{-1} [23]. The conjugated dienes are expressed as nmol hydroperoxides/mg lipids.

2.6. Statistical analysis

Comparisons between treatment conditions were assessed by one-way ANOVA with the post hoc analysis with the Student–Newman–Keuls test. All results are expressed as means ± S.E. and (n) represents the number of experiments performed with different preparations. In all cases, the Ca-ATPase activity was calculated from paired data. A P-value ≤ 0.05 was accepted as statistically significant.

3. Results

Blood was drawn from pregnant women, either normotensive or with severe preeclampsia, during admission and after 24 h of treatment with $MgSO_4$ therapy. The Ca-ATPase activity of the red cell ghosts was assayed for each preparation, and the results are shown in Fig. 1. The Ca-ATPase activity of the red cell ghosts, as already stated [12,13], was lower for the preeclamptic women when compared with normotensive pregnant women. After 24 h of the $MgSO_4$ treatment, the Ca-ATPase activity of the red cell ghosts of the preeclamptic women returned to values similar to those of the normotensive pregnant women. This effect of the $MgSO_4$ therapy on the Ca-ATPase activity is not seen on the Mg-ATPase activity.

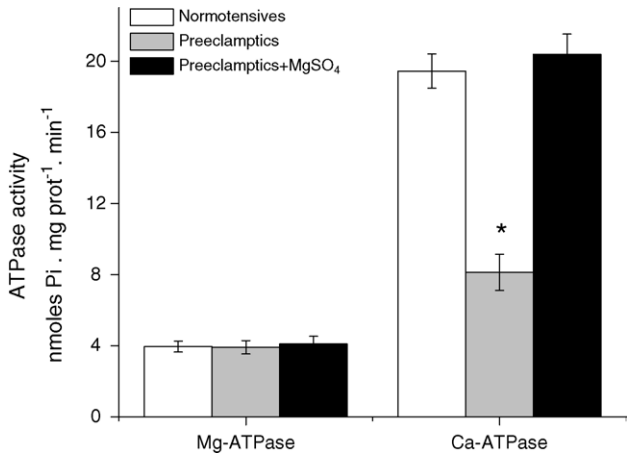

Fig. 1. Mg- and Ca-ATPase activities of red cell ghosts from normotensive and preeclamptic pregnant women, with and without $MgSO_4$ treatment. The Ca-ATPase activity was assayed in the presence of 0.5 μM calmodulin. Values are means ± S.E. of determinations carried out with 11 different preparations for each case. $^*P < 0.001$ preeclamptics vs. either normotensives or the same preeclamptics after $MgSO_4$ therapy.

This activity remains unchanged with preeclampsia, and also after the treatment with $MgSO_4$.

Inside-out vesicles of red cell ghosts were assayed for active calcium uptake. As shown in Fig. 2, the active calcium uptake of the red cell ghost inside-out vesicles from preeclamptic women before the $MgSO_4$ therapy, is clearly lower than that measured for the normotensive pregnant women (4.08 ± 0.10 nmol mg $prot^{-1}$ min^{-1} versus 8.06 ± 0.11 nmol mg $prot^{-1}$ min^{-1}, $p < 0.001$). Interestingly, the active calcium uptake by the vesicles from preeclamptic women after the $MgSO_4$ therapy shows higher values, similar to those of the normotensive women

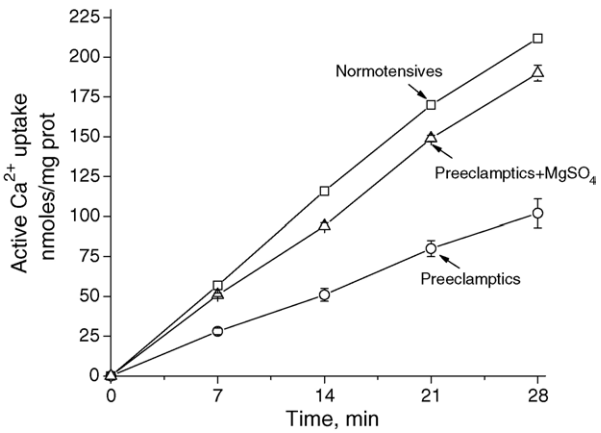

Fig. 2. ATP-dependent calcium uptake by red blood cell inside-out vesicles from normotensive and preeclamptic pregnant women, with and without $MgSO_4$ treatment. Experiments carried-out in the presence of 0.5 μM calmodulin. The active calcium uptake for each condition was: normotensives = 8.06 ± 0.11 nmol mg $prot^{-1}$ min^{-1} ($r = 0.999$, $n = 11$); preeclamptics (before $MgSO_4$ therapy) = 4.08 ± 0.10 nmol mg $prot^{-1}$ min^{-1} ($r = 0.997$, $n = 11$, $P < 0.001$ vs. normotensives); preeclamptics + $MgSO_4$ = 7.37 ± 0.15 nmol mg $prot^{-1}$ min^{-1} ($r = 0.998$, $n = 11$, $P < 0.001$ vs. preeclamptics before $MgSO_4$ therapy). Values are means ± S.E. of determinations carried out with 11 different preparations for each case.

(7.37 ± 0.15 nmol mg prot^{-1} min^{-1}). The passive calcium uptake by the vesicles is very low and similar in all the cases (data not shown).

In order to study a possible direct effect of $MgSO_4$ on the Ca-ATPase activity, we assayed the enzyme of red cell ghosts from either normotensive or preeclamptic pregnant women, in the presence of different concentrations of $MgSO_4$ in the incubation medium. Since the Ca-ATPase requires magnesium in the assay medium, the ATP concentration for this particular experiment was adjusted to 4 mM and the $MgCl_2$ was varied in order to keep a constant Mg:ATP ratio of 3:2. The maximal concentration of $MgSO_4$ assayed was 6 mM since, under normal circumstance, the $MgSO_4$ therapy never should reach values above 4 mM in the blood plasma of the preeclamptic women. The results of this experiment are shown in Fig. 3: notice that the presence of $MgSO_4$ in the assay medium does not affect the Ca-ATPase activity.

As already shown before [17,24], and again illustrated in Fig. 4, the level of lipid peroxidation of the red cell ghosts, determined by measuring their levels of TBARS or conjugated dienes, is higher for the preeclamptic women, as compared to the normotensive pregnant women. However, as also shown in Fig. 4, the level of lipid peroxidation of red cell ghosts from preeclamptic women is significantly reduced after the $MgSO_4$ therapy, reaching values similar to those of the normotensive pregnant women.

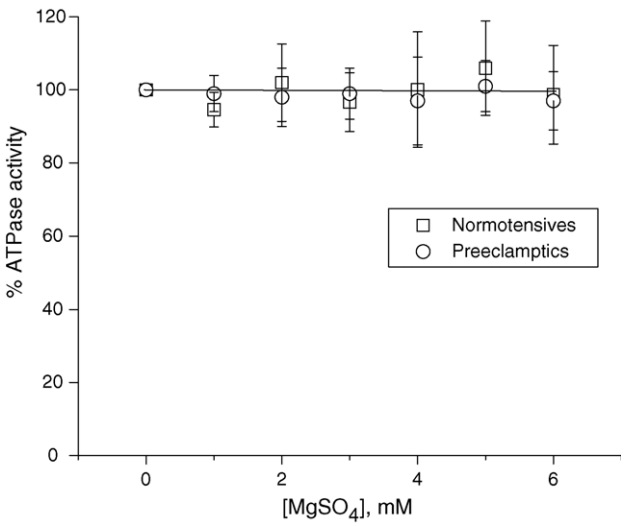

Fig. 3. Effect of different concentrations of $MgSO_4$ in the assay medium on the Ca-ATPase activity of red cell ghosts from either normotensive or preeclamptic pregnant women. Ca-ATPase activity was assayed in the presence of 0.5 μM calmodulin. The ATP concentration in the assay medium was adjusted to 4 mM and the $MgCl_2$ was varied in order to keep constant the Mg:ATP ratio (3:2). The Ca-ATPase activities in the absence of $MgSO_4$ (100%) were: 20.15 ± 0.91 nmol Pi mg prot^{-1} min^{-1}, normotensives ($n = 11$); 10.14 ± 0.55 nmol Pi mg prot^{-1} min^{-1}, preeclamptics ($n = 11$). In all the cases, the values are means ± S.E. of 11 determinations, carried out with different preparations. P = n.s. normotensives vs. preeclamptics.

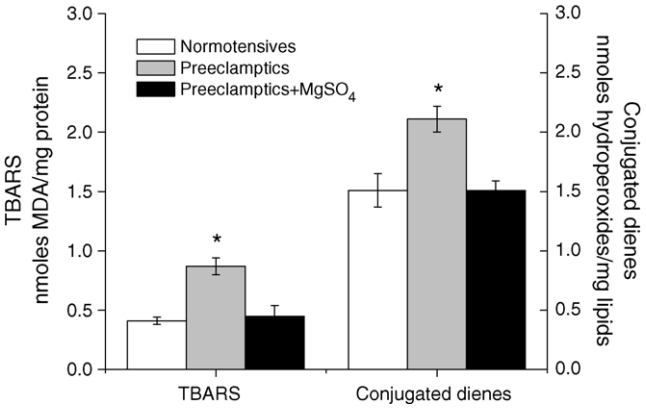

Fig. 4. TBARS and conjugated dienes of red cell ghosts from normotensive and preeclamptic pregnant women, with and without $MgSO_4$ treatment. Values are means ± S.E. of determinations carried out with 11 different preparations for each case. $^{*}P < 0.001$ vs. either normotensives or the same preeclamptics after $MgSO_4$ therapy.

Intact red blood cells from either normotensive or preeclamptic pregnant women not treated with $MgSO_4$, were preincubated in vitro with 4 mM $MgSO_4$ at 4 °C for 24 h, and then washed and used to prepare red cell ghosts. The membranes were then assayed for Ca-ATPase activity and lipid peroxidation. The results of these experiments are shown in Tables 2 and 3, respectively. The in vitro treatment with 4 mM $MgSO_4$ of red blood cells from normotensive pregnant women, does not affect either the Ca-ATPase activity (Table 2) or the level of lipid peroxidation (Table 3). On the other hand, preincubation with 4 mM $MgSO_4$ of red blood cells from untreated preeclamptic pregnant women, produces an important increase in their Ca-ATPase activity (Table 2) and a reduction in their level of lipid peroxidation (Table 3), reaching in both cases values similar to those of normotensive pregnant women. The observed effects are similar to those produced by the in vivo

Table 2
Effect of in vitro preincubation with 4 mM $MgSO_4$ for 24 h at 4 °C of intact red cells from preeclamptic and normotensive pregnant women on their Ca-ATPase activity

Preincubation	Normotensive	Preeclamptic
None	19.88 ± 1.02	9.58 ± 0.44
4 mM $MgSO_4$	21.36 ± 2.06	$17.15 \pm 1.11^{*}$

Intact red cells from either gestational controls or preeclamptic women without any treatment, were previously washed twice with a saline solution of 150 mM NaCl, 10 mM Tris–HCl pH 7.4 at 4 °C, and resuspended with the same solution (50% hematocrit) in the presence or absence of 4 mM $MgSO_4$. The cell suspensions were kept at 4 °C with gentle shaking during 24 h. After the preincubation, the red cells were again washed with the saline solution and utilized to prepare red cell ghosts, which were assayed for Ca-ATPase activity, as described under Section 2. ATPase activity is expressed as nmol Pi mg prot^{-1} min^{-1}. Values are means ± S.E. of determinations carried out with preparations from different women ($n = 11$ in each case).

* $P < 0.001$ vs. no preincubation.

Table 3
Effect of in vitro preincubation with 4 mM $MgSO_4$ for 24 h at 4 °C of intact red cells from normotensive and preeclamptic pregnant women on their level of lipid peroxidation

Preincubation	Normotensive		Preeclamptic	
	TBARS	Dienes	TBARS	Dienes
None	0.39 ± 0.08	1.51 ± 0.14	0.88 ± 0.14	2.11 ± 0.11
4 mM $MgSO_4$	0.31 ± 0.12	1.55 ± 0.15	0.47 ± 0.05*	1.67 ± 0.10†

Intact red cells from either gestational controls or preeclamptic women without any treatment, were previously washed twice with a saline solution of 150 mM NaCl, 10 mM Tris–HCl pH 7.4 at 4 °C, and resuspended with the same solution (50% hematocrit), in the presence or absence of 4 mM $MgSO_4$. The cell suspensions were kept at 4 °C with gentle shaking during 24 h. After the preincubation, the red cells were again washed with the saline solution and utilized to prepare red cell ghosts, which were assayed for TBARS and conjugated dienes as described under Section 2. TBARS are expressed as nmol malondialdehyde per milligram of protein. Conjugated dienes are expressed as nmol of hydroperoxides per milligram of lipids. Values are means ± S.E. of determinations carried out with preparations from different women (in each case, $n = 11$).

* $P < 0.05$ vs. no preincubation.

† $P < 0.01$ vs. no preincubation.

administration of $MgSO_4$ to the preeclamptic women (Figs. 1, 2 and 4).

Considering that UV irradiation of red cell ghosts promotes the release of oxygen free radicals, mainly in the form of hydroxyl radicals [25], we irradiated red cell ghosts from normotensive pregnant women with UV in the presence of different concentrations of $MgSO_4$ and determined the amount of TBARS as a way to test the possible interaction of this salt with oxygen radicals. The results of this experiment are presented in Fig. 5. While 0.25–0.75 mM concentrations of $MgSO_4$ enhance the UV-induced lipid peroxidation, higher concentrations (1–4 mM) of this salt produce a significant inhibition of red cell membrane lipid peroxidation.

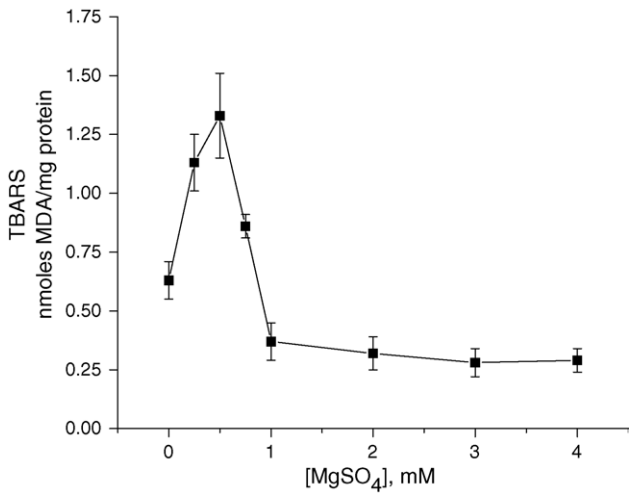

Fig. 5. Effect of $MgSO_4$ on the level of TBARS of red cell ghosts from normotensive pregnant women, irradiated during 30 min at 4 °C with UV light (254 nm). Different concentrations of $MgSO_4$ were mixed with the ghosts before irradiation with UV light. Values are means ± S.E. of determinations carried out with 11 different preparations for each case.

4. Discussion

The activity of the Ca-ATPase of red blood cell ghosts and the active calcium uptake of inside-out vesicles prepared with them, are about 50% lower for preeclamptic women, as compared to normotensive pregnant women (Figs. 1 and 2). Increased membrane lipid peroxidation has been proposed to be responsible for the inhibition of the Ca-ATPase activity of red blood cell ghosts from preeclamptic women [17]. In this regard, both TBARS and conjugated dienes, two different ways to assess the level of lipid peroxidation of plasma membranes [25], are significantly increased in preeclampsia (Fig. 4), in total agreement with previous findings [12,13,17,20]. On the other hand, both Ca-ATPase activity and TBARS return to normal values in the postpartum [24], paralleling the disappearance of all the symptoms of preeclampsia.

Parenteral $MgSO_4$ administration is the medical treatment for the preeclamptic women to prevent the recurrent seizures of eclampsia and for tocolysis in preterm labor [3]. Twenty-four hour after the onset of the $MgSO_4$ therapy, both Ca-ATPase activity and level of lipid peroxidation of the red blood cell membranes from preeclamptic women, show values similar to those of the normotensive pregnant women (Figs. 1 and 4). This effect is also seen when red blood cells from untreated preeclamptic women, are incubated in vitro with $MgSO_4$ for 24 h at 0 °C (Tables 2 and 3).

Similar findings have been reported for the Ca-ATPase activity of red cell membranes of asphyxiated newborns [26]. Asphyxia produces an important diminution of the Ca-ATPase activity of red cell ghosts from newborns during their initial first 48 h of life. Post-asphyxia treatment with $MgSO_4$ of the asphyxiated newborns shows an important level of protection of the Ca-ATPase of the red cell membranes since its activity remains unchanged in the asphyxiated newborns. Additionally, Maulik et al. [27], have shown that fetal guinea-pig brain hypoxia increases the level of lipid peroxidation of the plasma membranes of the tissue, and decreases the activity of their Na,K-ATPase. Post-hypoxia treatment with magnesium, reversed the effect of hypoxia and returned to normal values the level of lipid peroxidation and the Na,K-ATPase activity.

It has been observed that magnesium deficiency is associated with an enhanced injury and production of reactive oxygen species [27–29], as well as with an important reduction in the level of cellular glutathione [30]. It has also been observed that magnesium supplementation reverses these effects [27,28]. These results have been explained considering that Mg, by binding to the NMDA receptor-ion channels, would decrease the cellular Ca influx, affecting in this way the Ca-stimulated oxygen free radicals generating pathways, such as phospholipases, lipoxygenases and cyclooxygenases [31,32]. On the other hand, the synthesis of glutathione, an important antioxidant of the cells and substrate of the glutathione peroxidase, is Mg-dependent. In this regard, the anti-peroxidant effect of

intravenous $MgSO_4$ has been previously seen in patients with acute aluminium phosphide poisoning [33], an effect probably mediated through its capacity to increase the level of glutathione. Considering the fact that serum magnesium concentration seems to be reduced in the preeclamptic women [34], the $MgSO_4$ administration, by increasing the magnesium serum concentration, could increase the synthesis and hence the serum level of glutathione, contributing in this way to increase the antioxidant status of the body.

The effect of in vitro $MgSO_4$ incubation of red blood cells from preeclamptic women on the Ca-ATPase activity (Tables 2 and 3), requires further explanation. Thus, considering the fact that: (a) there is not a direct effect of $MgSO_4$ in the assay medium on the activity of the Ca-ATPase (Fig. 3), (b) the $MgSO_4$ treatment of the cells, induces an important reduction on the level of lipid peroxidation of their membranes (Table 3) and (c) it has been shown a close inverse relationship between the activity of the Ca-ATPase and the level of lipid peroxidation of the red blood cell membranes [24], it may be concluded that the reduction in the level of lipid peroxidation of the plasma membranes produced by the treatment, could account for the elevation of the Ca-ATPase activity. The inhibitory effect of $MgSO_4$, at concentrations above 1 mM, on the lipid peroxidation process induced by UV light (Fig. 5), indicates an antioxidant effect of this chemical, but it does not explain the finding that the $MgSO_4$ effect on the red blood cells from preeclamptics, either in vivo or in vitro, returns the level of lipid peroxidation of the red cell membrane to values similar to those of the normotensive pregnant women. Obviously, the 24 h treatment with $MgSO_4$, either in vivo or in vitro, cannot induce de novo synthesis of new phospholipids and modify the lipid profile of the red cell membrane. Magnesium could be doing two different effects at the membrane level: on one hand, scavenging free radicals in the fatty acid residues, avoiding in this way further lipid peroxidation and, on the other hand, inducing a reorganization of the plasma membranes, resulting in a reduction in the level of their lipid peroxidation. In this regard, it has been previously suggested that magnesium appears to be involved in free radicals bioavailability [35–37] by means of different mechanisms, including the possibility of direct complexation by magnesium of hydroxyl radicals and lipid peroxides in the hydrophobic microenvironment of the cell membrane. Even more, it has been shown that magnesium is able to bind negative charges of lipid and protein molecules, reducing in this way their electrostatic repulsion and stabilizing the red cell membrane [38].

How magnesium exerts its antioxidative effect is yet unclear and it could be related to inhibition of the iron driven lipid peroxidation [39,40]. Membrane lipid peroxides can be decomposed by iron, yielding hydroxyl radicals ($^{\bullet}OH$). The $^{\bullet}OH$ is highly unstable and reacts rapidly after formation, leading to site-specific oxidation reactions [25,41]. Even when the hydrophilic iron molecules are largely excluded from the hydrophobic interior of the cell membranes, they can bind rapidly to membrane proteins and to the polar head groups of phospholipids [42,43]. Metal cations, such as magnesium, may compete with iron for these binding sites, inhibiting, consequently, an increased lipid peroxidation [43,44].

The biphasic and concentration-dependent effect of $MgSO_4$ on the TBARS response to UV light irradiation of red cell membranes (Fig. 5) needs to be analyzed. Lipid peroxidation was induced by UV irradiation which promotes the release of oxygen free radicals under our experimental conditions [25]. The inhibitory effect of $MgSO_4$ on the TBARS of the red cell membrane under UV light irradiation, when 1–4 mM concentrations of this salt were used, might be related to a direct complexation by magnesium of oxygen free radicals avoiding their interactions with membrane components. Interestingly, the $MgSO_4$ therapy raised the serum Mg^{2+} concentration from 0.63 ± 0.04 to 1.41 ± 0.11 mmol/l ($n = 11$, $P < 0.001$), concentration under which it causes an inhibitory effect on the TBARS of the red cell membrane (Fig. 5). On the other hand, 0.25–0.75 mM concentrations of $MgSO_4$ enhanced the TBARS of the red cell membrane under UV light irradiation, an effect that has been previously observed with phosphatidylethanolamine liposomes [45]. It can be hypothesized that Mg^{2+}, at 0.25–0.75 mM concentrations, can replace iron to induce lipid peroxidation. However, this possibility can be ruled out since Mg^{2+} has a fixed oxidation state and cannot substitute for transition metals in redox reactions that yield free radicals. Another interesting possibility should be that Mg^{2+} binds to membranes and subtly alter lipid composition in a manner that promotes peroxidation [25]. This possibility deserves further research. The results of Fig. 5 support the idea that the net effect of Mg^{2+} on lipid peroxidation appears to be the sum of both inhibition and stimulation of lipid peroxidation, being this controlled by the Mg^{2+} concentration. This might have potentially important clinical implications for diseases associated with oscillations in either intracellular or extracellular Mg^{2+} concentrations, since this could change the lipid peroxidation status of the plasma membranes.

It is known that the plasma membrane Ca-ATPase exerts a role in maintaining basal levels of intracellular calcium, participates in dynamic Ca^{2+} regulation and is a crucial player of Ca^{2+} export during normal and pathological conditions [46]. Therefore, inhibition of the plasma membrane Ca-ATPase in preeclamptic women could cause the cells to gain calcium [47]. In fact, a high intracellular free calcium concentration has been found in erythrocytes [48], lymphocytes [49], granulocytes [50], monocytes [50], platelets [51], and placental tissue [52] from patients with preeclampsia. An increased intracellular free Ca^{2+} concentration of the vascular smooth muscle could contribute to the development of vasoconstriction, increasing in this way the total peripheral vascular resistance [53]. Since the lipid peroxidation-dependent diminution of the Ca-ATPase

activity in preeclampsia has been also shown in the smooth muscle of myometrium [14], we might consider the possibility that the $MgSO_4$ therapy can enhance the Ca-ATPase activity of the vascular smooth muscle. This would diminish the cytosolic free calcium concentration of these cells, promoting vasodilator effects and therefore decreasing the total peripheral vascular resistance. This hypothesis can explain both cerebral and peripheral vasodilator effects of $MgSO_4$ therapy [54].

On the other hand, it has been proposed that the intracellular calcium concentration level may play a dominant role in both the initiation and propagation of seizure discharge [55] as well as in the development and maintenance of acquired epilepsy [56]. The treatment with $MgSO_4$ may allow the neurons to maintain "normal" Ca^{2+} levels by increasing the plasma membrane Ca-ATPase activity. This might be critical to block the beginning and propagation of a seizure discharge. It is clear that this subject requires further research in order to understand the cellular mechanisms underlying the beneficial effects of the $MgSO_4$ therapy, particularly its role in the prophylaxis of eclampsia-related seizures.

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