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Effects of magnesium sulphate on placental expression of endothelin 1 and its receptors in preeclampsia

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Abstract

Objectives: To investigate the effects of magnesium sulphate ($MgSO_4$) on placental expression of endothelin 1 (ET-1) and its receptors in preeclampsia (PE).

Design and methods: Placentas were obtained from 10 normotensive (NT group) and 18 moderate preeclamptic (PE group) women. Among the PE group, 10 patients were treated with 0.9% NaCl solution (PES) and 8 women received $MgSO_4$ ($PEMgSO_4$). Placental mRNAs of ET-1, ET-1_A receptor (ET-1_AR) and ET-1_B receptor (ET-1_BR) were evaluated by Northern blot and quantified using densitometry.

Results: Placental ET-1_BR expression was lower ($P<0.05$) in the PES group without significant changes in the mRNAs of ET-1 and ET-1_AR when compared with the NT group. $MgSO_4$ treatment was associated with decreased ET-1 and increased ET-1_BR ($P<0.05$) expression, without significant changes in ET-1_AR.

Conclusions: The results of the present study showed that moderate PE is associated with low placental expression of ET-1_BR, and $MgSO_4$ treatment resulted in placental expression changes of the ET-1/receptors system.

Keywords: Magnesium sulphate; Endothelin 1; Placenta; Preeclampsia

Introduction

Preeclampsia (PE) affects 6–8% of pregnant women and it is characterized by the simultaneous presence of hypertension and proteinuria [1]. In addition, it is well known that PE is associated with decreased uteroplacental blood flux [2]. Although the aetiology of PE still remains unknown, it is generally thought that PE originates in the placenta [3]. The clinical syndrome of PE arises from secondary systemic circulatory disturbances that can be due to generalized endothelial dysfunction and/or results from an imbalance in the production of vasoactive factors [4]. In this regard, it is known that endothelin 1 (ET-1), a potent vasoconstrictor, can lead to paradoxical effects depending on its interaction with two different receptors, thus vasoconstriction results from interaction with the ET-1_A receptor (ET-1_AR) in the smooth muscle and vasodilatation through binding to ET-1_B receptor (ET-1_BR) in endothelium [5,6].

Expression studies of ET-1 in placental tissues from PE women are controversial. Whereas ET-1 expression has been shown to be higher in cultures obtained from trophoblast [7], it shows no changes in placental homogenates [8]. In addition, expression studies of ET-1 receptors in placental tissues from PE women have shown an increase in ET-1_AR without changes in ET-1_BR [8].

Preeclamptic placental tissue, which is under hypoxic conditions, due to impaired organ perfusion, is also under oxidative stress, since increased free radicals and lipid peroxidation has been documented in PE [9,10]. Furthermore, in a recent study, it has been shown that addition of ET-1 to both placental tissue and cell cultures increased the production of malondialdehyde (MDA), a marker of lipid peroxidation, suggesting a link between ET-1 and oxidative stress [11].

Eclampsia is a complication of PE characterized by the onset of generalized seizures, which can be prevented by treatment with magnesium sulphate ($MgSO_4$) [12,13]. In addition to its anticonvulsive effects, $MgSO_4$ decreases blood pressure [14,15] through a still unknown mechanism. $MgSO_4$ abolishes vaso-

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constriction induced by ET-1 and peroxides in placental vasculature [16,17]. Furthermore, our group has demonstrated that $MgSO_4$ is associated with changes in maternal serum levels of MDA and ET-1 in PE [15]. However, it is not known if $MgSO_4$ treatment modifies the expression of placental ET-1 system. Therefore, the aim of this study was to analyze *ET-1* gene expression, including ET-1_AR and ET-1_BR, in placentas obtained from normotensive and $MgSO_4$-treated preeclamptic women.

Subjects and methods

Placental samples

Placentas were collected from patients in accordance with the guidelines of the Declaration of Helsinki, and the study protocol was approved by the Human Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. All subjects signed a written informed consent. The study was performed cross-sectionally at delivery and included 28 patients: 10 normotensive (NT group) women and 18 moderate preeclamptic (PE group) women. Among the PE group, 10 patients were treated with 0.9% NaCl solution (PES group) and 8 women received $MgSO_4$ ($PEMgSO_4$ group). Magnesium sulphate 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. The NT group was chosen as control of the PES group, and the former as control of the $PEMgSO_4$ group. The diagnosis of moderate PE was based on the simultaneous presence of hypertension (systolic blood pressure $\geq$140 mm Hg and <160 mm Hg and/or diastolic blood pressure $\geq$90 mm Hg and <110 mm Hg) and proteinuria ($\geq$30 mg/dL) in at least two readings [1]. Only those women giving birth to a single newborn with Apgar scores of 7–10 were included in the study. Subjects with preexisting hypertension or previous PE, or liver, renal, heart or any other endocrine disorders, including those under nutritional supplements, diuretics, and hormonal treatments, were excluded from the study. Placentas were collected immediately after delivery. Placental tissue was removed only from the central part of the cotyledons and washed repeatedly in 0.9% NaCl to eliminate blood excess and frozen at −75 °C until assayed.

Northern blot

The cDNA probes were obtained from human normal placenta tissue by RT-PCR using the Invitrogen SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). The primers used for cDNA probes were for ET-1: sense 5′TTC CGT ATG GAC TTG GAA GC3′ antisense 5′ AAG CCA GTG AAG ATG GTT GG3′ [7]; for ET-1_AR: sense 5′TGG CCT TTT GAT CAC AAT GAC TTT3′ antisense 5′TTT GAT GTG GCA TTG AGC ATA CAG GTT3′ [18]; and for ET-1_BR: sense 5′ACT GGC CAT TTG GAG CTG AGA TGT3′ antisense 5′CTG CAT GCC ACT TTT CTT TCT CAA3′ [18]. The PCR products were electroeluted and labeled with [α-^{32}P] dCTP (110 TBq/mmol) using the Amersham-Biosciences Rediprime II DNA labeling Kit (Amersham-Biosciences, Buckingshamshire, UK). Total RNA from placental tissues was isolated by guanidinium isothiocyanate and the CsCl gradient centrifugation method [19]. For Northern analysis, 20 μg of RNA was size fractionated in 1.2% formaldehyde-agarose gels. After electrophoresis, RNA was transferred onto a nylon membrane filter (Millipore-N$^+$, Bedford, MA) and crosslinked using a UV crosslinker lamp (Amersham, Buckingshamshire, UK). Membranes were prehybridized with rapid-hyb buffer (Amersham-Biosciences, Buckingshamshire, UK) at 65 °C for 30 min, and then hybridized with the cDNA probes (53.3 MBq/L) for 2.5 h at 65 °C, and washed once with 2× SSC/0.1% SDS at room temperature for 20 min and then twice for 15 min with 0.1× SSC/0.1% SDS at 65 °C. Finally, membranes were exposed to Kodak X-OMAT AR film (Kodak, Rochester, NY) for 18–24 h at −75 °C with an intensifying screen. Loading was normalized against 28S RNA, and bands were quantified using an image analyzer (Eagle Eye system, Stratagene, USA) by densitometry.

Statistical analysis

All data are presented as mean±standard deviation. For clinical characteristics, statistical analysis was performed using analysis of variance (ANOVA) and significant differences among groups were determined by Fisher's protected last-square difference test. Unpaired Student's *t*-test was performed for placental expression comparisons. A value of $P<0.05$ was considered significant.

Results

Clinical characteristics

Table 1 summarizes the clinical characteristics of mothers and their newborns belonging to the NT, PES and $PEMgSO_4$ groups. Maternal and gestational ages were similar among groups. As expected, basal diastolic and systolic blood pressures were significantly higher ($P<0.05$) in the PE groups when compared to those in the NT group. As shown in this table, moderate PE was not associated with reduced newborn and placental weights.

Table 1
Clinical characteristics of the normotensive pregnant (NT), 0.9% NaCl- and $MgSO_4$-treated preeclamptic (PES and $PEMgSO_4$, respectively) women

	NT (n=10)	PES (n=10)	$PEMgSO_4$ (n=8)
Maternal age (years)	24.7±5.7	25.3±2.8	22.4±3.9
Gestational age (weeks)	39.2±0.6	39.1±1.3	38.8±0.71
Systolic blood pressure (mm Hg)	116±6	140±9[a]	144±6[a]
Diastolic blood pressure (mm Hg)	74±6	93±6[a]	98±6[a]
Proteinuria (mg/dL)	Trace	≥30	≥30
Newborn birth weight (kg)	3.22±0.35	3.31±0.98	3.07±0.60
Placental weight (g)	587±89	613±82	552±51

Values are given as the mean±standard deviation.
[a] $P<0.05$ vs. NT.