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Short communication

TPRV-1 expression in human preeclamptic placenta

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ABSTRACT

Preeclampsia is a multisystem disorder unique to human pregnancy, characterized by abnormal placentation. Although its causes remain unclear, it is known that the expression of several transporters is altered. Transient receptor potential vanilloid 1 (TRPV-1) is a nonselective cation channel, present in human placenta. Here, we evaluated the expression of TRPV-1 in preeclamptic placentas. We observed a deregulation in TRPV-1 expression in these placentas which may explain the impaired Ca^{2+} homeostasis found in preeclampsia.

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1. Introduction

Preeclampsia is a multisystem syndrome unique to human pregnancy characterized by hypertension and proteinuria [1]. This gestational disorder represents a major factor for maternal and perinatal morbidity and mortality, and it affects 7–10% of pregnancies [2]. Although its causes remain unclear, preeclampsia is characterized by abnormal placentation. Accumulated evidence suggests that the expression of a variety of syncytiotrophoblast (STh) transporters is reduced or abnormal in preeclampsia [3–6].

One of the mechanisms by which Ca^{2+} diffuses from maternal blood to the STh is through the "transient receptor potential vanilloid" (TRPV) channels. TRPV channels are non-selective cation channels, which have preference for Ca^{2+}. Ca^{2+} is a second messenger involved in many biological processes. Thus, TRPV channels are proposed to have a crucial role in the proper placental and fetal development and alterations in transplacental Ca^{2+} exchange may seriously affect the normal function of the fetal-placenta unit [7].

TRPV-5 and TRPV-6 were identified in normal term human placentas and seem to regulate Ca^{2+} transport [8,9]. In addition, we also described TRPV-1 expression in rat placenta [10] and recently Costa and co-workers reported the expression of TRPV-1 in human trophoblast cells [11].

As regards preeclamptic placentas, it was found an abnormal transplacental Ca^{2+} exchange related to a reduced expression of TRPV-5 and TRPV-6 [12]. However, the expression of TRPV-1 in the setting of preeclampsia is still unknown.

Based on this background we hypothesize that the expression of TRPV-1 is altered in preeclampsia, showing a possible correlation between this syndrome and the expression of calcium transport genes. Therefore, we examined mRNA levels, protein expression and localization of TRPV-1 in preeclamptic placentas.

2. Methods

Following ethics approval, informed consent and based on clinical history samples were collected. Full-term normal ($n = 12$) and preeclamptic ($n = 12$) placentas were obtained after cesarean section. All placentas came from white Hispanic pregnant women with no diseases or previous history of disease who gave birth to a newborn without anomalies. Clinical data are shown in Table 1.

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Table 1
Clinical characteristics of severe preeclamptic and normotensive women. Values are mean ± SD. Severe preeclampsia was defined as systolic blood pressure ≥160 mmHg and/or diastolic pressure ≥110 mmHg, with proteinuria ≥0.3 g/day or 2 pluses on urine dipstick after the 20th week of gestation in a previously normotensive patient [1]. Patients with pre-existing hypertension or women that had experienced an adverse outcome were excluded of this study [3–5].

	Normotensive pregnant women	Severe preeclamptic pregnant women
Number of pregnant women	12	12
Parity		
Primiparous	7	8
Multiparous	5	4
Maternal age, *yr*	22.8 ± 1.2	24.1 ± 1.6
Gestational age, *wk*	38.8 ± 1.2	36.5 ± 0.9
Mean blood pressure, *mmHg*		
Systolic	113 ± 3.7[a]	168.0 ± 4.5[a]
Diastolic	64.1 ± 2.5[b]	115.0 ± 2.2[b]
Proteinuria	negative	+++
Body mass index (BM), kg/m^2	25 ± 4	23 ± 4
Birth weight, *g*	3110 ± 240	2760 ± 260
Fetal sex		
Male	6	5
Female	6	7

[a] $P < 0.05$.
[b] $P < 0.01$.

Fragments of cotyledons from normal and preeclamptic placentas were gently separated by dissection from different areas of each placenta, midway between the chorionic and basal plate. Afterwards, cotyledons were processed to exclude chorionic and basal plates and washed repeatedly with 0.9% NaCl to remove blood from the intervillous space. Villous tissue was further dissected into fragments of ~50 mg [4,24,25].

Total mRNA was isolated using an SV Total RNA isolation system (Promega Co.) and reverse transcription was performed as previously described [3,4]. Semiquantitative RT-PCR was carried out using specific oligonucleotide primer for human TRPV-1 (sense 5′-CAAGAACATCTGGAAGCTGC-3′ and antisense 5′-CTTCTCCCCGGAAGCGGCAGG-3′) [13]. β-actin primers were used as an internal standard. Densitometry of the bands was performed by the ImageJ 1.44 software package.

TRPV-1 protein was assessed by Western blot. 100 μg of protein were used for immunoblot studies. After blocking, membranes were incubated overnight with the primary antibody anti-TRPV-1 (Alomone Labs. Cat# ACC-030, 1:500) and then with a goat anti-rabbit immunoglobulin G ([IgG] Jackson ImmunoResearch Laboratories, Inc.; 1:10,000) conjugated to peroxidase. Densitometry was performed after normalization with β-actin.

In both cases values were plotted as TRPV-1/β actin relative ratio. The results were expressed as medians and ranges; $P < 0.05$ was considered statistically significant. Data were analyzed using GraphPad Prism (v6.0; San Diego, CA, USA) and represented as boxes and whisker plots.

For localization studies [3], the tissue sections were permeabilized with Triton X-100, and then samples were incubated overnight with the primary antibody (1:100). Later, samples were placed in prediluted link antibody, and incubated in a solution of streptavidin conjugated horse-radish peroxidase. Staining was conducted with Vectastain kit (Vector Laboratories). Labeling was visualized by reaction with DAB (diaminobenzidine tetrahydrochloride), and counterstained with hematoxylin. Control samples were performed by omitting the primary antibody.

3. Results and discussion

Previous reports proposed that TRPVs are modulators of Ca^{2+} intracellular levels in the STh [14]. Recently, the expression of TRPV-1 was described in cytotrophoblast and STh cells of normal term placenta, and it was associated with the regulation of the apoptotic process in the trophoblast [11].

Here, we showed that TRPV-1 gene transcription was increased in preeclamptic placentas *($P < 0.01$)* (Fig. 1A). As it was observed in other tissues, intermittent hypoxia due to abnormal placentation might up-regulate TRPV-1 mRNA levels [15,16].

However, TRPV-1 protein expression significantly decreased in preeclamptic placentas compared with normal ones *($P < 0.01$)*. Concerning its localization, we observed that TRPV-1 labeling was detected in the apical membrane of STh in normal placentas while it was detectable at very low levels in preeclamptic placentas (Fig. 1C). This discrepancy between mRNA and protein levels is not clear yet. Seyoung and co-workers suggested that TRPV-1 protein levels are regulated via an autophagy-dependent manner [17]. Autophagy is an intracellular degradation system associated to several physiological processes. Moreover, it was demonstrated that authophagy is exacerbated in trophoblast cells of preeclamptic placentas [18,19]. Therefore, the decrease of TRPV-1 expression may be induced by an increased degradation of the protein.

On the other hand, studies on STh demonstrated that the plasma membrane fluidity plays a key role in the modulation of placental transport function [20]. Changes in membrane lipid composition may affect fluidity and lipid–protein interaction [5,21,22]. In pathological conditions such as preeclampsia, we have recently found that the apical membranes of STh are more rigid related to an increase in sphingomyelin [23]. Consequently, we speculate that these changes may contribute to create an unfavorable environment for TRPV-1 insertion in the plasma membrane of STh leading to an abnormal expression of this protein.

In conclusion, the impaired Ca^{2+} homeostasis found in preeclampsia may also correlate with the reduced TRPV-1 expression in preeclamptic placentas. Further studies are needed to define whether these alterations play a direct role in the pathogenesis of this syndrome.

Declaration of interest

The author declares that there is no conflict of interest that

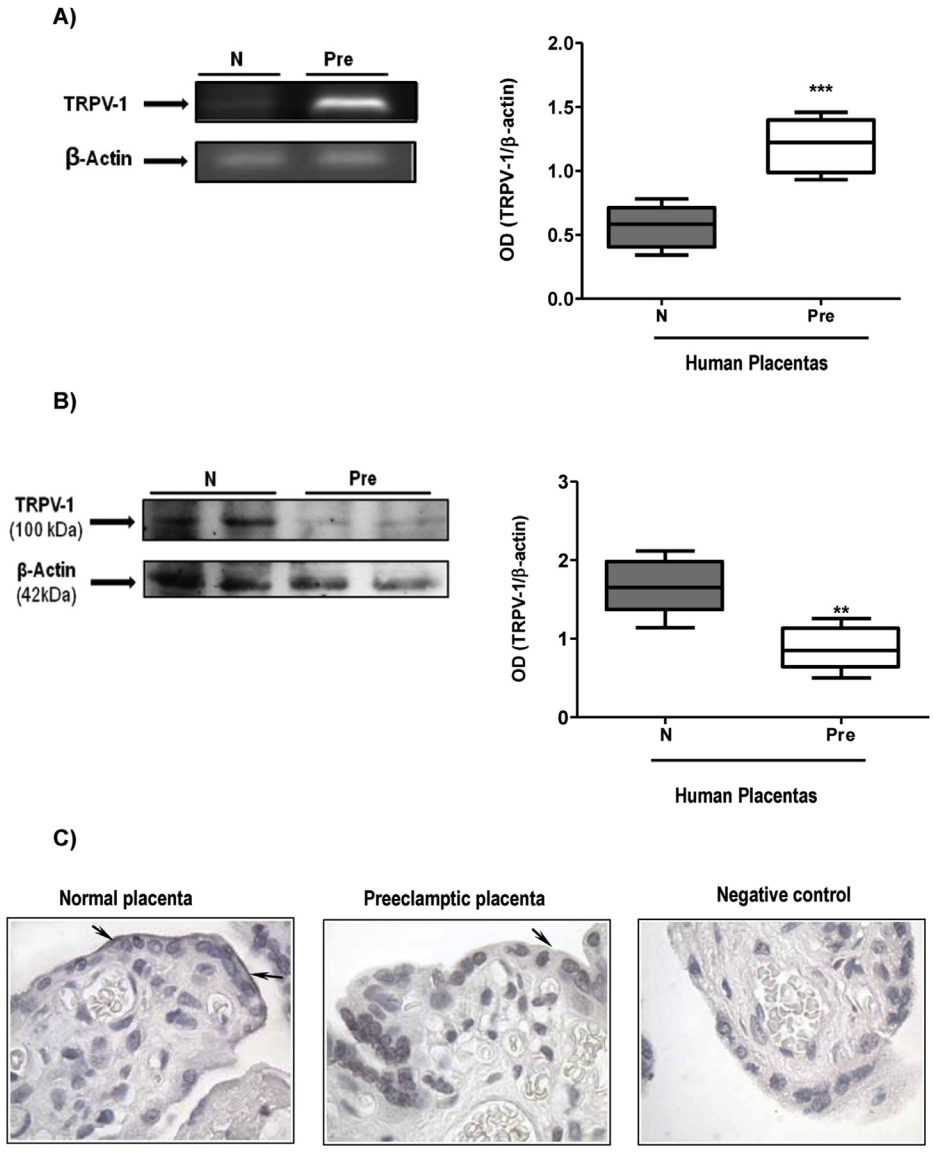

Fig. 1. Placental TRPV-1 expression in normal and preeclamptic placentas. A) Semicuantitative RT-PCR of TRPV-1 gene expression in normal (N) and preeclamptic (Pre) placentas. Densitometry analysis showed that TRPV-1 was significantly increased in preeclamptic placenta (***$P < 0.001$, $n = 12$). B) Representative Western blot for TRPV-1 in normal (N) and preeclamptic (Pre) placentas. Densitometry of immunoblots containing TRPV-1 protein level expression was performed, and after normalization for β-actin, the values were plotted as TRPV-1/β actin relative ratio. TRPV-1 protein of preeclamptic placenta showed a significantly decreased compared with normal placenta (**$P < 0.01$, $n = 12$). The results were expressed as medians and ranges; $P < 0.05$ was considered statistically significant. Data were represented as boxes and whisker plots. C) Immunolocalization of TRPV-1 in representative sections of placental villous from normal and preeclamptic pregnant women. Arrows indicate the labeling in the apical membrane of STh from normal placentas. This labeling was detectable at very low levels in preeclamptic placentas. Negative controls were performed by omitting the primary antibody and replaced by a non-immune rabbit serum. Magnification: 1000 ×.

would prejudice the impartiality of this scientific work.

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