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Short communication

Expression of aquaporin-3 (AQP3) in placentas from pregnancies complicated by preeclampsia

Natalia Szpilbarg [a], Alicia E. Damiano [a, b, *]

[a] Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO), CONICET, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Cátedra de Biología Celular y Molecular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

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ABSTRACT

Recently, we have reported that the blocking of AQP3 abrogates the apoptotic response of the trophoblast. Since trophoblast apoptosis is exacerbated in preeclampsia, we hypothesized that placental AQP3 is increased in these placentas in order to trigger the programmed cell death. Here, we examined mRNA levels, protein expression and localization of AQP3 in placentas from pregnancies complicated by preeclampsia and against what we expected, we found that AQP3 expression was significantly reduced, both at protein and mRNA levels, compared to normal placentas. In the light of our results, further studies are required to evaluate whether the decreased expression of AQP3 might be an adaptive response of the placenta to reduce the trophoblast apoptosis, which is related to the clinical manifestations of preeclampsia.

Preeclampsia is a multisystem disorder of unknown etiology unique to human pregnancy. It affects 2–5% of all pregnancies and is responsible for approximately 12% of maternal deaths worldwide [1].

Since the placenta has a crucial role in the development of this syndrome, alterations in placental functions may contribute to the pathogenesis of preeclampsia [2].

Water transport across the placenta represents one of the most important processes during pregnancy and increases throughout gestation accompanying fetal growth. Aquaporins (AQPs) are a family of water-channel cell membrane proteins widely distributed and their expression has been found in both placenta and fetal membranes. However, the function of AQPs in these tissues has not been fully determined. It was reported that increased expression of AQP3 in murine placenta enhanced placental permeability facilitating maternal to fetal water flow [3]. However, in human placenta the role of AQPs is still speculative. Several studies suggest that, in fetal membranes, AQPs may be involved in the regulation of amniotic fluid homeostasis [3,4]. Likewise, we proposed that AQP3 and AQP9 may take part in the transport of water and solutes between mother and fetus in human trophoblast [5]. In subsequent experiments, we found an increase in AQP9 expression and a reduced water uptake mediated by AQPs in placentas from pregnancies complicated by preeclampsia [6]. Since preeclampsia is not known to be associated with an altered water flux between the mother and the fetus, we propose that AQPs might not have a key role in water transport across human placenta [7,8].

In this context, recent studies have revealed unexpected cellular roles of AQPs, such as the participation in the physiology of organelles, proliferation, apoptosis and cell migration [9,10]. All these processes require transient changes in cell volume and the activity of certain ion transporters, many of which are altered in placentas from pregnancies complicated by preeclampsia [6,11–15].

Recently, we have explored the role of AQPs in placental programmed cell death. We observed that inhibition of these proteins, particularly AQP3, abrogates the apoptotic response in human placenta [16]. However, up to now, the expression of AQP3 in placentas from pregnancies complicated by preeclampsia was not studied.

Here, we have examined the mRNA levels, protein expression and localization of AQP3 in placentas from pregnancies complicated by preeclampsia. We hypothesized that an increase in the expression of AQP3 in these placentas, exacerbates trophoblast apoptosis triggering the clinical manifestations of this gestational hypertensive disorder.

* Corresponding author. Cátedra de Biología Celular y Molecular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 1st Floor, C1113AAD, Buenos Aires, Argentina.
E-mail address: adamiano@ffyb.uba.ar (A.E. Damiano).

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1. Methods

This study was approved by the local ethics committee of the Hospital Nacional Dr. Prof. Alejandro Posadas, Buenos Aires, Argentina, and a written consent was obtained from the patients before the collection of samples. Full-term normal placental tissues (n = 13) and placentas from pregnancies complicated by preeclampsia (n = 13) were obtained after cesarean section. Clinical data are shown in Table 1.

Total mRNA was isolated using an SV Total RNA isolation system (Promega Co) and reverse transcription was performed as previously described [5]. Semiquantitative RT-PCR was carried out using 5 μM of a specific oligonucleotide primer for human AQP3 (sense 5′- CCTGAACCCTGCGGTGACC-3′ and antisense 5′- GGCATAGCCGGAGTTGAAGC-3′). β-actin and human ribosomal protein L30 (L30) [17] primers were used as internal standard. Densitometry of the bands was performed by the ImageJ 1.44 software package.

Abundance of AQP3 protein was assessed by Western blot. 100 μg of membrane protein were used for immunoblotting studies. After blocking, membranes were incubated overnight with the primary antibody anti-AQP3 (Alpha Diagnostic International Inc, 1:1000) and then with a goat anti-rabbit immunoglobulin G ([IgG] Jackson ImmunoResearch Laboratories, Inc.; 1:10,000) conjugated to peroxidase. To confirm equal loading, each membrane was also analyzed for β-actin protein expression and stained with Ponceau S as general protein marker [18]. Densitometry was performed and the values were plotted as AQP3/β-actin or Ponceau S relative ratios.

Localization of AQP3 was studied by immunohistochemistry. Samples were incubated overnight with the primary antibody (1:100). Later, the 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), the labeling was visualized by reaction with DAB (diaminobenzidine tetrahydrochloride), and counterstained with hematoxylin. Control samples were performed by omitting or blocking the primary antibody with the specific peptide [5,6].

2. Results and conclusion

In the present study, we demonstrated that AQP3 expression was significantly reduced in placentas from pregnancies complicated by preeclampsia compared to normal ones (Fig. 1A and B). Regarding its localization in normal placentas, as we previously reported, AQP3 was located into the apical membrane of syncytiotrophobast cells [5]. However, in placentas from pregnancies complicated by preeclampsia, AQP3 labeling was almost undetectable (Fig. 1C).

One of the most common features in placentas from pregnancies complicated by preeclampsia, is the diminished endovascular invasion by cytotrophoblasts and the inadequate remodeling of the uterine spiral arteries. Consequently, the perfusion of these placentas is impaired, and oxygen concentration within the intervillous space is more variable than in normal placentas, resulting in an ischemia/reperfusion type injury [19–22]. Thus, fluctuations in oxygen tensions increase of the oxidative stress and the apoptosis of the trophoblast.

We have previously reported that AQP3 is involved in the physiological apoptosis of the trophoblast [16]. Trophoblast apoptosis is exacerbated in preeclampsia [23], but AQP3 expression unexpectedly decreased. Therefore, the participation of AQP3 in placental apoptosis in preeclampsia is not clear.

Moreover, the reduced expression of AQP3 in placentas from pregnancies complicated by preeclampsia is consistent with our previous findings in explants exposed to oxygen changes [16]. We found that AQP3 protein expression decreased after oxygen deprivation, and the subsequent reoxygenation failed to restore AQP3 to basal levels, possibly due to the oxidative damage of the plasma membrane of syncytiotrophoblast. Along with this idea, recently we found that the apical membranes of syncytiotrophoblast from pregnancies complicated by preeclampsia are more rigid than normal ones because of an increase of long and unsaturated sphingomyelin molecular species which alters the membrane order of the trophoblastic cells [24].

In conclusion, our results showed a reduced expression of AQP3 in placentas from pregnancies complicated by preeclampsia. Further studies are needed to elucidate whether the decreased expression of AQP3 might be an adaptive response of the placenta to reduce the trophoblast apoptosis, which is related to the clinical manifestations of preeclampsia.

Declaration of interest

The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Table 1
Clinical characteristics of normal and severe preeclamptic pregnant women.
Normal pregnant women had maternal blood pressures ≤110/70 mmHg, no proteinuria, and no other complications. Severe preeclampsia was defined as high maternal blood pressure (140/90 mmHg) and proteinuria (300 mg/24 h), after 20 weeks of gestation.
Proteinuria was analyzed by Test Urine Labstix Strip. The strips were read visually comparing the test areas with the color scale on the label. Readings are reported in terms of negative, trace, 1+, 2+, 3+ and 4+ corresponding to each color change.

	Normotensive Pregnant Women	Severe Preeclamptic Pregnant Women	*p-values*
Number of pregnant women	13	13	–
Parity			
Primiparous	7	9	–
Multiparous	6	4	–
Maternal age, *(years)*	24.6 ± 5.5	26.7 ± 6.6	*NS*
Gestational age, *(weeks)*	38.9 ± 1.2	36.6 ± 2.5	0.01
Mean blood pressure, *mmHg*			
Systolic	110.0 ± 4.0	160.0 ± 5.0	0.01
Diastolic	63.0 ± 2.5	112.0 ± 1.8	0.01
Proteinuria	negative	3+	–
Body Mass index (BMI), kg/m^2	25 ± 3	23 ± 4	*NS*
Birth weight, *g*	3110 ± 240	2760 ± 260	0.01
Fetal sex			
Male	7	6	–
Female	6	7	–

LMP: last menstrual period. Values are mean ± SD. *p-values* reflect comparison with the “normotensive pregnant women” column. *NS, non-significant.*

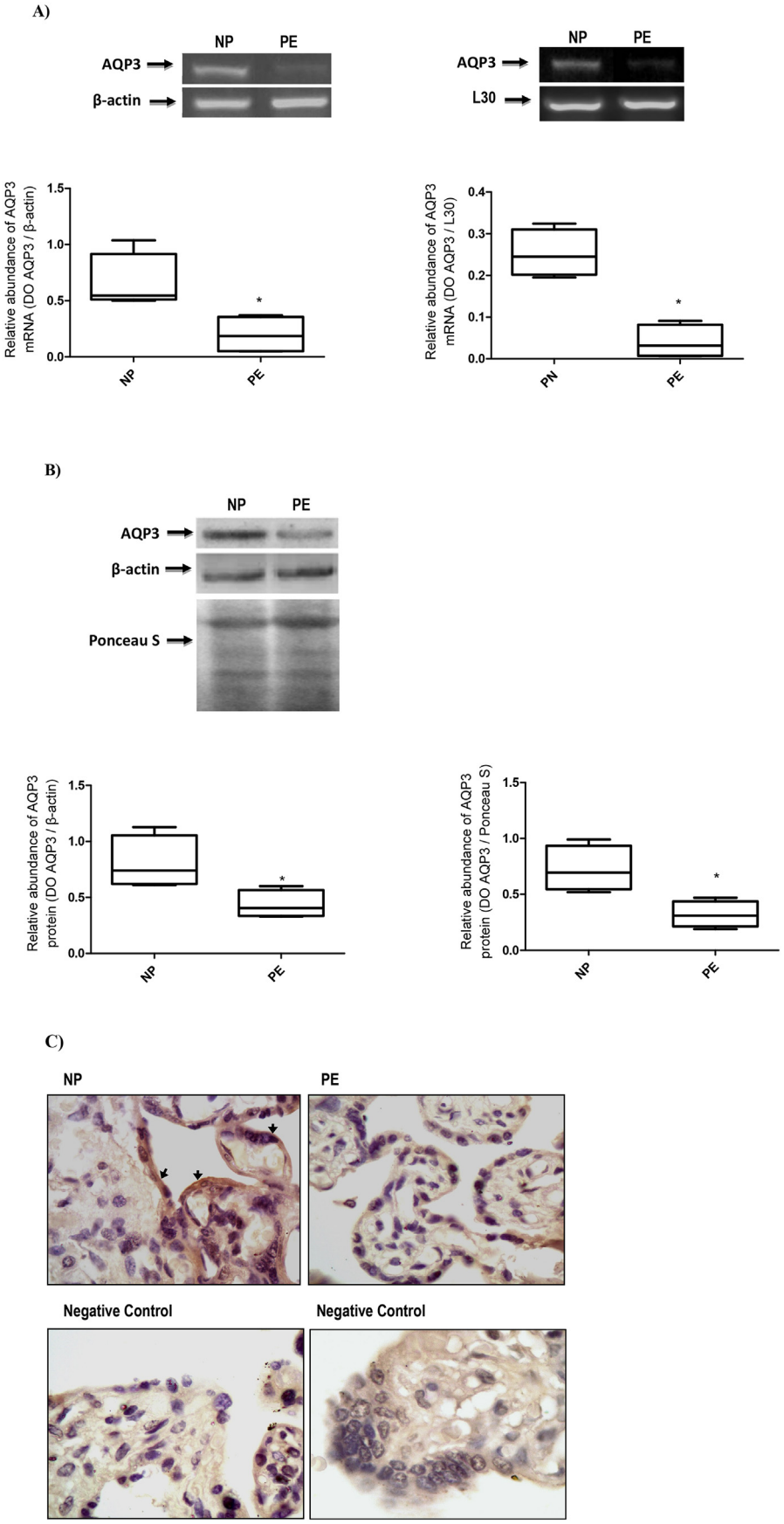

Fig. 1. A) Semiquantitative RT-PCR and B) semiquantitative Western blot analysis of AQP3 abundance in normal placentas (NP) and placentas from pregnancies complicated by preeclampsia (PE). The expression of AQP3 mRNA and protein are decreased in PE in comparison to normal NP. Each plotted value corresponds to the mean ± SEM *($n = 13$, $^{*}p < 0.05$).* C) Immunostaining with an anti-AQP3 antibody revealed specific labeling in the apical membrane of syncytiotrophoblast (arrows) in control placentas. However, in explants from preeclamptic placentas the label was weakly detected. Negative controls were performed by replacing the primary antibody by a non-immune rabbit serum or by adsorbing the primary antibody with the specific peptide. Magnification: *×1000.*

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