Placenta 30 (2009) 642–648

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

CFTR May Modulate AQP9 Functionality in Preeclamptic Placentas

M. Castro-Parodi [a,1], L. Levi [a,1], V. Dietrich [a], E. Zotta [b], A.E. Damiano [a,*]

[a] Cátedra de Biología Celular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Junín 956 1er piso, CP 1113 Buenos Aires, Argentina
[b] Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 7mo piso, CP 1121 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Accepted 29 April 2009

Keywords:
AQP9
CFTR
Syncytiotrophoblast
Preeclampsia
Human placenta

ABSTRACT

Preeclampsia (PE) is a hypertensive disorder unique to human pregnancy. Although its causes remain unclear, it is known that altered placental villous angiogenesis and a poorly developed fetoplacental vasculature can affect the transport functions of the syncytiotrophoblast (hST).

We have previously observed that in preeclamptic placentas there is an increase in AQP9 protein expression, with a lack of functionality. Up to now, the mechanisms for AQP9 regulation and the role of AQP9 in the human placenta remain unknown. However, there is strong evidence that the cystic fibrosis transmembrane conductance regulator (CFTR) regulates AQP9 functionality.

Objective: Here, we studied CFTR expression and localization in hST from preeclamptic placentas in order to investigate if alterations in CFTR may be associated with the lack of activity of AQP9 observed in PE.

Methods: The expression of CFTR in normal and preeclamptic placentas was determined by Western Blot and immunohistochemistry, and CFTR-AQP9 co-localization was determined by immunoflurescence. Water uptake experiments were performed using explants from human normal term and preeclamptic placentas treated with CFTR inhibitors.

Results: We found that CFTR expression significantly decreased in preeclamptic placentas, and that the hST apical labeling almost disappeared, losing its co-localization with AQP9. Functional experiments demonstrated that water uptake diminished in normal term explants incubated with CFTR inhibitors.

Conclusions: These results suggest that CFTR expression decreases in preeclampsia and may thus be implicated in the regulation of AQP9 activity.

1. Introduction

Nutrients, antibodies, infectious agents and other substances passing from maternal to fetal circulation within the villous placenta have to cross the continuous, mitotically inactive, multinucleated syncytiotrophoblast (hST) cells. This tissue that results from the fusion of the underlying cytotrophoblast cells forms a syncytium with minimal tight junctions. Consequently, the transport from mother to fetus should take place primarily via transcellular routes [1–3]. Nevertheless, physiological data indicate that both a transcellular and a paracellular pathway are available for transfer across the human placenta, but the morphological correlate of the latter is uncertain [4] and the possibility exists that wide, non-specific, paracellular channels, allowing the passage of large hydrophilic molecules, may also be present [5,6]. However, the molecular mechanisms of these processes are little known.

Transcellular water flux may be facilitated by aquaporins (AQPs). AQPs are a family of small integral membrane proteins (30 kDa monomers) that transport either water alone or water and small solute(s) such as glycerol. AQPs increase cell plasma membrane water permeability 5- to 50-fold as compared with membranes where water moves primarily through the lipid bilayer [7–9].

There are at least 13 AQPs in mammals which show a wide range of distribution in organs that are actively involved in water movement. According to their structural and functional properties, AQPs are divided into two subgroups: "classical aquaporins" (AQP0, 1, 2, 4, 5, 6 and 8, selective for water), and "aquaglyceroporins" (AQP3, 7, 9 and 10, permeable to both water and neutral solutes). AQP11 and AQP12 have been recently identified and are more distantly related [10,11].

Aquaporin 9 (AQP9) is a member of the aquaglyceroporin subfamily of AQPs and shares the highest amino acid sequence homology with AQP3, AQP7, and AQP10 [12,13]. In addition to water, AQP9 transports small uncharged molecules like glycerol,

* Corresponding author. Tel.: +54 11 9648200.
E-mail address: adamiano@ffyb.uba.ar (A.E. Damiano).
[1] Castro-Parodi M. and Levi L. contributed equally to this work.

0143-4004/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2009.04.012

urea, purines, and pyrimidines [14,15], but its physiological function(s) remains unknown. AQP9 expression has been reported in many tissues [16]. We have previously reported the expression of aquaglyceroporins permselective to urea and glycerol (AQP3) as well as to a broad range of small solutes (AQP9) in hST from normal human placenta [17]. We proposed that these aquaglyceroporins may participate not only in the water transport between mother and fetus but may also play a role in the rapid movement of solutes across cell membranes with minimal osmotic perturbation. Recently, we have described an increase in AQP9 expression in hST from preeclamptic placentas with a lack of functionality for water and mannitol [18].

Little is known about AQP9 regulation. However, the presence of numerous sites of regulation in the gene and on the protein has been described.

Studies in liver and brain suggested that a negative insulin response element (IRE) in the promoter region participates in this regulation of levels of AQP9 protein [19,20], Tsukaguchi et al. also described a putative hypertonicity response element in the promoter region of AQP9 [14]. In addition, several pathways leading to regulation of AQP9 expression have been identified including protein kinases A (PKA) and C (PKC) [21,22]. Despite the presence of consensus sites for phosphorylation by PKC on the AQP9 protein, direct regulation of the channel by phosphorylation has not yet been observed [22].

Mitogen activated protein kinase (MAP-kinase) pathways, P38 MAP-kinase, were also shown to be implicated in an increase of AQP9 expression after an osmotic stress and ischemic infarct [23,24].

However, up to now, the functional regulation of AQP9 has not been clarified.

Several previous reports indicate that cystic fibrosis transmembrane conductance regulator (CFTR) is able to interact with various membrane proteins by regulating their transport activity as well as by functioning as a cAMP-regulated chloride channel [25]. Among them are the epithelial Na^+ channels [26,27] and the outwardly rectifying Cl^- channels [28]. CFTR is also involved in the regulation of water flux by AQPs [29–31]. So far, there is no evidence that CFTR could affect their molecular expression. This channel provides an exit pathway for secondary active chloride transport from blood to lumen, followed passively by sodium. The resulting accumulation of NaCl in the lumen generates an osmotic gradient for water secretion via AQPs.

Cheung et al. reported an interaction between epididymal CFTR and AQP9 and found a significant increase in water permeability, consistent with a synergistic effect of the two proteins in conferring water permeability in *Xenopus* oocytes. Therefore, they concluded that CFTR activation is required for AQP9 to increase its activity [30].

CFTR has been found on the apical membrane of hST from human normal term placenta [32] and serves as a conductive pathway for anions, whereas AQPs are related with water transport. These proteins are therefore essential for normal fetal growth and development.

In this study, we focused on the hypothesis that, in human placenta, CFTR interacts with AQP9, modulating its activity. Hence, the aim of our study was to establish whether the AQP9 functionality correlates with the molecular expression of CFTR in normal and preeclamptic placentas.

2. Materials and methods

2.1. Tissue collection

This study was approved by the local ethics committees of the "Hospital Nacional Dr. Prof. Alejandro Posadas", Buenos Aires, Argentina, and written consent was obtained from patients before the collection of samples.

Table 1
Patient characteristics (mean ± SEM).

	Term control	Severe preeclampsia
Number of pregnant women	6	6
Maternal age, year	23.6 ± 1.3	26.4 ± 2.1
Gestational age, week	39.7 ± 0.8	37.1 ± 0.4
Mean blood pressure, mmHg		
Systolic	110 ± 4.2*	160.5 ± 4.5*
Diastolic	67 ± 3.5**	110.0 ± 2.8**
Birth weight, g	3070 ± 250	2670 ± 294

*$P < 0.01$; **$P < 0.01$.

Full-term normal and preeclamptic placental tissues were obtained after cesarean section. Normal pregnant patients ($n = 6$) had maternal blood pressures ≤ 110/70 mmHg, no proteinuria, and no other complications. Preeclamptic patients ($n = 6$) had gestational hypertension > 140/90 mmHg, with proteinuria that developed for the first time during pregnancy. The gestational age was 37–42 weeks and the maternal age had a range between 20–30 years old in both groups of patients. No differences were observed between the newborn weight from normal and preeclamptic mothers. (Table 1)

2.2. Immunoblotting

Human placenta villi from normal term and preeclamptic placentas were processed according to the method previously described [17]. Briefly, human chorionic villi were fragmented, and washed with unbuffered 150 mM NaCl. The tissue was then shaken for 1 h with 1.5 volumes of HES buffer (10 mM HEPES-KOH, 0.1 mM EGTA, 250 mM sucrose) pH 7.4, with protease inhibitors (0.2 mM PMSF, 25 μg/mL *p*-aminobenzamidine, 20 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin), followed by filtration and centrifugation at 3100 *g* for 10 min. The supernatant was then further centrifuged for 10 min at 11,000 *g* and the resulting supernatant centrifuged for 70 min at 16,000 *g*.

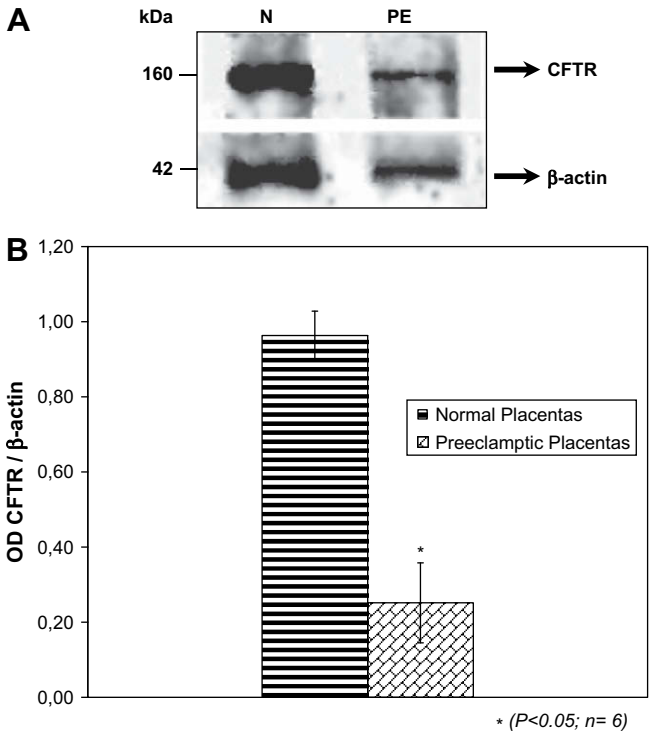

Fig. 1. Semiquantitative immunoblotting analysis of CFTR abundance in hST. (A) A representative immunoblot shows that CFTR protein level expression was weakly detectable in preeclamptic placentas (PE) as compared to normal ones (N). β-actin expression was determined to control for unequal loading. (B) Densitometry of immunoblots containing AQP9 protein level expression was performed, and after normalization for β-actin, the values were plotted as the AQP9/β-actin relative ratio. Each plotted value corresponds to the mean ± SEM obtained from six placentas. *$P < 0.05$.