Free Radical Biology and Medicine 57 (2013) 10–21

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

NADPH oxidase 2-derived superoxide downregulates endothelial $K_{Ca}3.1$ in preeclampsia

Shinkyu Choi [a], Ji Aee Kim [a], Hye-Young Na [a], Ji-Eun Kim [b], Seonghee Park [a], Ki-Hwan Han [b], Young Ju Kim [c], Suk Hyo Suh [a,*]

[a] Department of Physiology Medical School, Ewha Womans University, Seoul 158-710, Republic of Korea
[b] Department of Anatomy Medical School, Ewha Womans University, Seoul 158-710, Republic of Korea
[c] Department of Obstetrics and Gynecology, Medical School, Ewha Womans University, Seoul 158-710, Republic of Korea

ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form
24 October 2012
Accepted 9 December 2012
Available online 20 December 2012

Keywords:
Hypertension
Ca^{2+}-activated K^+ channel
Anti-lectin-like oxidized low-density lipoprotein receptor 1
NADPH oxidase
Oxygen radicals
Free radicals

ABSTRACT

Endothelial dysfunction is associated with $K_{Ca}3.1$ dysfunction and contributes to the development of hypertension in preeclampsia. However, evidence of endothelial $K_{Ca}3.1$ dysfunction in the vascular system from women with preeclampsia is still lacking. Therefore, we examined whether endothelial $K_{Ca}3.1$ dysfunction occurs in vessels from women with preeclampsia. We compared $K_{Ca}3.1$ and NADPH oxidase (NOX) expression in umbilical vessels and primary cultured human umbilical vein endothelial cells (HUVECs) from normal (NP; $n=17$) and preeclamptic pregnancy (PE; $n=19$) and examined the effects of plasma from NP or PE on $K_{Ca}3.1$ and NOX2 expression in primary cultured HUVECs from NP or human uterine microvascular endothelial cells. The endothelial $K_{Ca}3.1$ was downregulated, and NOX2 was upregulated, in umbilical vessels and HUVECs from PE, compared with those from NP. In addition, HUVECs from PE showed a significant decrease in $K_{Ca}3.1$ current. Plasma from PE induced $K_{Ca}3.1$ down regulation, NOX2 upregulation, phosphorylated-p38 mitogen-activated protein kinase downregulation, and superoxide generation, and these effects were prevented by antioxidants (tempol or tiron), NOX2 inhibition, or anti-lectin-like oxidized low-density lipoprotein (LDL) receptor 1 (LOX1) antibody. Oxidized LDL and the superoxide donor xanthine/xanthine oxidase mixture induced $K_{Ca}3.1$ down-regulation. In contrast, plasma from PE did not generate hydrogen peroxide, and the hydrogen peroxide donor *tert*-butylhydroperoxide induced $K_{Ca}3.1$ upregulation. These results provide the first evidence that plasma from PE generates superoxide via a LOX1–NOX2-mediated pathway and downregulates endothelial $K_{Ca}3.1$, which may contribute to endothelial dysfunction and vasculopathy in preeclampsia. This suggests $K_{Ca}3.1$as a novel target for patients with preeclampsia.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Preeclampsia, which is specified by high blood pressure and proteinuria, is a medical complication of pregnancy after 20 weeks of gestation that occurs in between 3 and 5% of pregnancies [1]. It is a leading cause of maternal mortality, especially in developing countries, and ordinarily results in significant morbidity and mortality for the neonate as a result of preterm delivery and fetal growth retardation. The only known remedy for this condition is the delivery of the fetus and placenta.

In preeclampsia, oxidative stress is demonstrable in the maternal circulation and the balance of oxidative status seems to be disrupted [2,3]. In women with preeclampsia, levels of substances that stimulate reactive oxygen species (ROS) production, such as cytokines [4], lipid peroxides [5], and oxidized low-density lipoprotein (LDL) [6], are increased in the maternal circulation, thereby increasing ROS production. In contrast, antioxidant capacity is markedly reduced in women with preeclampsia. A study of oxidative stress in maternal blood revealed notably reduced antioxidant levels [3]. Interestingly, another recent study reported that the altered lipid profile observed in preeclampsia is probably responsible for the low plasma level of antioxidative factors [7].

Maternal widespread endothelial dysfunction associated with increased oxidative stress is a central pathophysiological event in the maternal vascular system in preeclampsia and has been suggested as a primary issue in the development of preeclampsia [8].

Abbreviations: apocynin, 4′-hydroxy-3′-methoxyacetophenone; CM-DCFH-DA, 5-(and-6)-chloromethyl-2′,7′-dichlorofluorescin diacetate; DHE, dihydroethidine; HUtMEC, human uterine microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; KD, knockdown; LOX, anti-lectin-like oxidized LDL receptor; NOX, NADPH oxidase; NP, normal pregnancy; PE, preeclamptic pregnancy; ROS, reactive oxygen species; TBHP, *tert*-butylhydroperoxide; VEGF, vascular endothelial growth factor; X/XO, xanthine/xanthine oxidase mixture.

* Corresponding author. Fax: +82 2 2653 7891.
E-mail addresses: shsuh@ewha.ac.kr, shsuhilsan@naver.com (S.H. Suh).

0891-5849/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2012.12.009

Superoxide generation is increased in primary cultured human umbilical vein endothelial cells (HUVECs) from women with preeclampsia [9], and impaired endothelial function is reversed by antioxidant ascorbic acid administration [8]. Endothelial dysfunction in preeclampsia has been shown to be caused by an altered balance between pro- and antiangiogenic factors, such as vascular endothelial growth factor (VEGF) and the VEGF receptor [10–14]. Elevated maternal VEGF receptor concentration and decreased VEGF concentration cause increased superoxide production and, thereby, endothelial dysfunction [14]. Increased superoxide production by NADPH oxidases (NOXs), peroxynitrite degradation of the endothelial nitric oxide synthase cofactor tetrahydrobiopterin, and uncoupled endothelial nitric oxide synthase have been shown to contribute to endothelial dysfunction in a rat model of pregnancy-induced hypertension [15].

Cellular functions are very sensitive to ion channels, which regulate the flow of ions in and out of cells. The intermediate-conductance Ca^{2+}-activated K^+ channel ($K_{Ca}3.1$) is known to be a pivotal electrical trigger in vasorelaxation and to strongly contribute to endothelium-dependent relaxation [16]. Endothelial small- and intermediate-conductance Ca^{2+}-activated K^+ channels contribute to preserving endothelium-dependent relaxation under conditions of decreased NO bioavailability in resistant vessels from human patients with essential hypertension, suggesting that these Ca^{2+}-activated K^+ channels prohibit a further increase in blood pressure in human patients with essential hypertension [17]. $K_{Ca}3.1$ dysfunction might be involved in the endothelial dysfunction found in many kinds of cardiovascular diseases, including hypertension. In $K_{Ca}3.1$-deficient mice, endothelial $K_{Ca}3.1$ current was abolished, leading to a serious increase in arterial blood pressure and to mild left-ventricular hypertrophy [18]. We previously reported that endothelial dysfunction in α-galactosidase A knockout mice, an animal model of Fabry disease, is caused by $K_{Ca}3.1$ downregulation and dysfunction [19]. However, it is unknown whether $K_{Ca}3.1$ expression is reduced in vascular endothelial cells from patients with preeclampsia or whether the reduced $K_{Ca}3.1$ expression is associated with increased endothelial oxidative stress.

In this study, we sought to determine $K_{Ca}3.1$ expression in HUVECs from preeclamptic pregnancies (PE) and compare it with that from normal pregnancies (NP). We also determined whether increased oxidative stress was present in HUVECs from PE. We further examined the potential roles of oxidized LDL, anti-lectin-like oxidized LDL receptor 1 (LOX1), and NOX2 in ROS generation and $K_{Ca}3.1$ downregulation causing endothelial dysfunction associated with preeclampsia.

Material and methods

Human subjects

The study population consisted of Asian women who had either a NP or a PE (Table 1). Preeclampsia was defined by systolic blood pressure over 140 mm Hg and diastolic blood pressure over 90 mm Hg after 20 weeks gestation in a previously normotensive woman and new onset of proteinuria of over 300 mg of protein in a 24-h urine collection. Normotensive pregnant women who had completed their pregnancy without complications were included as controls. The study population was monitored at the Department of Obstetrics and Gynecology from the first trimester. Exclusion criteria included the following: altered renal function, diabetes or chronic diseases, twin pregnancies, recurrent miscarriages, fetal growth retardation, and *abruptio* placenta. All women with a history of essential hypertension and women who smoked were also excluded from this study. Gestational age was defined as the interval between the first day of the mother's last menstrual period and the date of delivery. Blood samples were drawn from the antecubital vein of each subject after obtaining informed consent.

Table 1
Clinical characteristics of subject groups.

	Normal ($n=17$)	Preeclampsia ($n=19$)
Maternal age (years)	33.7 ± 1.0	34.9 ± 1.0
Gestational age (weeks)	37.3 ± 1.0	36.1 ± 0.8
Systolic blood pressure (mm Hg)	119.6 ± 3.2	150.8 ± 3.1
Diastolic blood pressure (mm Hg)	76.6 ± 1.9	98.1 ± 3.3
Hematocrit (%)	34.9 ± 0.8	36.9 ± 1.1
Creatinine (mg/dl)	0.6 ± 0.02	0.7 ± 0.04
Birth weight (kg)	3.1 (2.6 to 3.8)	2.9 (1.8 to 4)

Values shown are means ± SEM and exclusively composed of plasma donors.

The investigation was approved by local ethics committee and the Institutional Review Board of the Ewha Women's University and was in accordance with the Declaration of Helsinki.

Cell culture and plasma treatment

Endothelial cells were isolated from human umbilical veins by collagenase treatment, as previously described [20]. HUVECs in suspension were plated into six-well cell culture plates and grown in complete M199 (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Life Technologies Corp., Carlsbad, CA, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, 15 μg/ml endothelial cell growth supplement (BD Biosciences, Rockville, MD, USA), 0.1 mM MEM nonessential amino acids (Life Technologies Corp.), and 10 U/ml heparin. Cultured cells were identified as endothelial cells in origin by their cobblestone appearance at confluence and positive staining with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acetylated low-density lipoprotein (Biomedical Technologies, Stoughton, MA, USA). HUVECs were used up to the second or third passage. Human uterine microvascular endothelial cells (HUtMECs) that were purchased from PromoCell GmbH (Heidelberg, Germany) were maintained in Endothelial Cell Growth Medium MV2 (PromoCell GmbH).

For plasma treatment, primary cultured HUVECs or HUtMECs were plated in six-well plates for 24 h. The concentration of FBS in the culture medium was then gradually decreased from 10 to 5, 2, and 0% for 30 min and HUVECs or HUtMECs were incubated in serum-free medium for 30 min. After that, culture medium was substituted with plasma from NP or PE and cells were incubated in plasma for 24 h.

Electrophysiology

The patch-clamp technique was used in whole-cell configurations at room temperature. Whole-cell currents were measured using ruptured patches and monitored in voltage-clamp modes with an EPC-9 (HEKA Elektronik, Lambrecht, Germany). The holding potential was 0 mV and currents were monitored by the repetitive application of voltage ramps from −100 to+100 mV with a 10-s interval (sampling interval 0.5 ms, 650-ms duration). The standard external solution contained (in mM) 150 NaCl, 6 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 10 glucose, with pH adjusted to 7.4 with NaOH. The pipette solution for whole-cell recording contained (in mM) 40 KCl, 100 K-aspartate, 2 $MgCl_2$, 0.1 EGTA, 4 Na_2ATP, 10 Hepes, with pH adjusted to7.2 with KOH. For buffering free Ca^{2+}, the appropriate amount of Ca^{2+} (calculated using CaBuf software; G. Droogmans, Leuven, Belgium; ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/cabuf.zip) was added in the presence of 5 mM EGTA.

Download English Version:

https://daneshyari.com/en/article/8271635

Download Persian Version:

https://daneshyari.com/article/8271635

Daneshyari.com