OBSTETRICS

Activin and NADPH-oxidase in preeclampsia: insights from in vitro and murine studies

Rebecca Lim, PhD; Rutu Acharya, BSc; Pavitra Delpachitra, MBBS; Sebastian Hobson, MBBS; Christopher G. Sobey, PhD; Grant R. Drummond, PhD; Euan M. Wallace, MD

OBJECTIVE: Clinical management of preeclampsia has remained unchanged for almost 5 decades. We now understand that maternal endothelial dysfunction likely arises because of placenta-derived vasoactive factors. Activin A is one such antiangiogenic factor that is released by the placenta and that is elevated in maternal serum in women with preeclampsia. Whether activin has a role in the pathogenesis of preeclampsia is not known.

STUDY DESIGN: To assess the effects of activin on endothelial cell function, we cultured human umbilical vein endothelial cells in the presence of activin or serum from normal pregnant women or pregnant women with preeclampsia, with or without follistatin, a functional activin antagonist or apocynin, a NADPH oxidase (Nox2) inhibitor. We also administered activin to pregnant C57Bl6 mice, with or without apocynin, and studied maternal and fetal outcomes. Last, we assessed endothelial cell Nox2 and nitric oxide synthase expression in normal pregnant women and pregnant women with preeclampsia.

RESULTS: Activin and preeclamptic serum induced endothelial cell oxidative stress by Nox2 up-regulation and endothelial cell dysfunction, which are effects that are mitigated by either follistatin or apocynin. The administration of activin to pregnant mice induced endothelial oxidative stress, hypertension, proteinuria, fetal growth restriction, and preterm littering. Apocynin prevented all of these effects. Compared with normal pregnant women, women with preeclampsia had increased endothelial Nox2 expression.

CONCLUSION: An activin-Nox2 pathway is a likely link between an injured placenta, endothelial dysfunction, and preeclampsia. This offers opportunities that are not novel therapeutic approaches to preeclampsia.

Key words: activin, endothelium, NADPH oxidase, oxidative stress, preeclampsia

Cite this article as: Lim R, Acharya R, Delpachitra P, et al. Activin and NADPH-oxidase in preeclampsia: insights from in vitro and murine studies. Am J Obstet Gynecol 2015;212:86.e1-12.

Preeclampsia remains a leading cause of maternal and perinatal death and morbidity worldwide.[1,2] The recognition that much of the maternal syndrome is due to systemic maternal endothelial dysfunction [1] and that this dysfunction likely arises, at least in part, from the excessive placental release of antiangiogenic factors[1,3-5] offers the potential for new directed therapies. Candidate targets include soluble fms-like tyrosine kinase 1 (sFlt),[6,7] a soluble splice variant of the vascular endothelial growth factor receptor, and soluble endoglin (sEng),[8] a circulating form of the transforming growth factor−β coreceptor endoglin. Women with preeclampsia, particularly those with early-onset severe disease, have greatly increased circulating levels of both sFlt and sEng,[6-8] which, when administered to pregnant rats, are able to induce a preeclampsia-like syndrome.[6,9] The recent demonstration that removal of sFlt-1 by apheresis in women with early-onset preeclampsia stabilized blood pressure and prolonged pregnancy provided the first proof-of-principle evidence that the mitigation of the effects of the antiangiogenic factor sFlt is therapeutically effective.[10]

Another vasoactive factor that is increased in the maternal circulation in women with preeclampsia is activin.[11] Activin is a member of the transforming growth factor−β family.[12] During pregnancy the majority of activin A in the maternal circulation is derived from the placenta.[13] Circulating levels of activin in women with preeclampsia are approximately 10-fold higher than those in women with a healthy pregnancy.[13,14] Indeed, elevated maternal levels of activin

From The Ritchie Centre, Monash Institute of Medical Research (Drs Lim and Wallace and Ms Acharya); Departments of Obstetrics and Gynecology (Drs Lim, Delpachitra, Hobson, and Wallace) and Pharmacology (Drs Sobey and Drummond), Monash University; and Women's and Children's Program, Monash Health, Monash Medical Centre (Drs Hobson and Wallace), Clayton, VIC, Australia.

Received Sept. 13, 2013; revised June 10, 2014; accepted July 15, 2014.

Supported by funding from the Patricia Penrose bequest, Monash University Faculty of Medicine, Nursing, and Health Sciences Strategic Grant Scheme; the Royal Australian and New Zealand College of Obstetricians and Gynaecologists Research Foundation; National Health and Medical Research Council (NHMRC) Project Grant number 1029148; and the Victorian Government's Operational Infrastructure Support Program.

The authors report no conflict of interest.

Corresponding author: E.M. Wallace, MD. euan.wallace@monash.edu

0002-9378/$36.00 • © 2015 Elsevier Inc. All rights reserved. • http://dx.doi.org/10.1016/j.ajog.2014.07.021

precede the clinical onset of preeclampsia by many months,[15] which suggests that activin may have a causative role in the pathogenesis of the disease. Accordingly, we hypothesized that the increased circulating level of activin that is observed in women with preeclampsia could contribute to systemic maternal endothelial dysfunction.

Methods

Blood and tissue collection

Blood and tissues were collected from women after written, informed consent was obtained and with the approval of the Southern Health Human Research Ethics Committee. Maternal blood was collected from the antecubital vein of women with a singleton pregnancy in absence of labor. Samples were obtained from women with established preeclampsia, as defined by the Society of Obstetric Medicine of Australia and New Zealand,[16] who were receiving labetalol and/or nifedipine and from women with a healthy singleton pregnancy at matched gestations (26-39 weeks). Serum was separated and pooled into 2 gestational age-matched pools: normal pregnancy pool and preeclampsia pool.

Human umbilical vein endothelial cell (HUVEC) isolation, culture, and treatment

HUVECs were isolated from umbilical cords that were obtained from women with a healthy singleton pregnancy and were cultured, as previously described.[17] Cells were stimulated with 50 ng/mL recombinant human activin A (R&D Systems, Minneapolis, MN), 20% (volume/volume) preeclampsia serum, or 20% (volume/volume) normal pregnancy serum in M199 media. After 24 hours of stimulation with activin A or pooled pregnancy serum, either 600 ng/mL follistatin 288 (R&D Systems) or apocynin (1, 10, or 100 μmol/L; Sigma Aldrich, Castle Hill, NSW, Australia) was added.

Endothelial integrity

Integrity of an endothelial monolayer was determined with the use of a horseradish peroxidase–based permeability assay, as previously described.[18] Briefly, HUVECs were grown to confluence on gelatinized 8.0 μmol/L pore size polycarbonate transwell inserts (BD Australia, North Ryde, NSW, Australia). Cells were treated with activin A or pooled pregnancy serum for 24 hours before exposure to inhibitors for a further 24 hours. Culture media were then removed from both upper and lower chambers, and 400 μL of 0.5 μg/mL horseradish peroxidase (Sigma-Aldrich) in fresh media was added to the upper chamber. Fresh media (800 μL) was added to the lower chamber and incubated for 60 minutes at 37°C. Media (30 μL) was removed and added to 860 μL reaction buffer (50 mmol/L NaH_2PO_4, 5 mmol/L guaiacol; Sigma-Aldrich). H_2O_2 (100 μL) was added and incubated at room temperature for 30 minutes before absorbance was read at 470 nmol/L.

Transendothelial resistance was determined with the Millicell electrical resistance system (Millipore, Billerica, MA) as previously described.[19] Symmetrically apposing electrodes were placed in the upper and lower chambers that allowed a uniform current to flow across the transwell insert. The electrodes were immersed so that the shorter electrode was in the upper chamber and the longer electrode was in the lower chamber. The resistance was recorded when the meter indicated a stable resistance.

Quantitative real-time polymerase chain reaction on HUVECs

RNA was isolated from the cells with Trizol reagent (Life Technologies, Mulgrave, Victoria, Australia); 1 μg DNase-treated RNA was used for the synthesis of complementary DNA (cDNA) with the Thermoscript real-time polymerase chain reaction (PCR) system (Life Technologies). Messenger RNA (mRNA) expression was determined with quantitative PCR performed on Rotorgene (Qiagen Pty Ltd, Chadstone, Victoria, Australia) with prevalidated primers for NADPH oxidase (Nox2), endothelin-1, and GAPDH (SABiosciences, Qiagen Pty Ltd) at the following cycling conditions: 95°C for 10 minutes then for 40 cycles of 95°C for 20 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Data were compiled using 3 cell isolates and 4 technical replicates.

Reactive oxygen species measurement

HUVEC were allowed to grow to confluence in 96-well view plates (PerkinElmer, Glen Waverley, Victoria, Australia). Cells were stimulated for 12 hours with either activin A or 20% pooled pregnancy serum with or without inhibitors. The plate was then dark-adapted for 20 minutes in a Chameleon chemiluminescence plate reader (Hidex, Turku, Finland) before the addition of the 1 μmol/L reactive oxygen species

TABLE 1
Primers used for quantitative polymerase chain reaction

Gene	Primer
Human Nox2 forward	5′-TGG CAC CCT TTT ACA CTG-3′
Human Nox2 reverse	5′-CCA CTA ACA TCA CCA CCT CA-3′
Human eNOS forward	5′-GTG ATG GCG AAG CGA GTG AA-3′
Human eNOS reverse	5′-CCG AGC CCG AAC ACA CAG AA-3′
Mouse eNOS forward	5′-CAA CGC TAC CAC GAG GAC A-3′
Mouse eNOS reverse	5′-CTC CTG CAA AGA AAA GCT CTG G-3′
18s forward	5′- GTC TGT GAT GCC CTT AGA TGT C-3′
18s reverse	5′- AAG CTT ATG ACC CGC ACT TAC-3′

eNOS, endothelial nitric oxide synthase; *Nox2*, NADPH oxidase.

Lim. Activin and NOX in preeclampsia. Am J Obstet Gynecol 2015.

Download English Version:

https://daneshyari.com/en/article/6144578

Download Persian Version:

https://daneshyari.com/article/6144578

Daneshyari.com