Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 6 (2016) 130–133

Contents lists available at ScienceDirect

Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

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

Short communication

Role of activin A in the pathogenesis of endothelial cell dysfunction in preeclampsia

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form 10 March 2016
Accepted 29 March 2016
Available online 31 March 2016

Keywords:
Preeclampsia
Activin
Follistatin
Endothelin
ICAM
VCAM

ABSTRACT

Circulating markers for endothelial activation such as endothelin-1 (ET-1), ICAM-1 and VCAM-1 are elevated in women with preeclampsia. Using human umbilical vein endothelial cells (HUVECs) as an *in vitro* model of the maternal vasculature, we show that activin A and preeclamptic serum upregulate ET-1, ICAM-1, and VCAM-1 in HUVECs. Further, we show that follistatin, a specific binding protein for activin, mitigates the upregulation of ET-1, ICAM-1 and VCAM-1 in HUVECs exposed to either activin A or preeclamptic serum. These data are consistent with activin A contributing to the pathophysiology of preeclampsia and suggest that therapies targeting activin signalling are worth exploring.

1. Introduction

Preeclampsia is a multisystem disorder unique to human pregnancy that remains a major cause of maternal and perinatal morbidity and mortality worldwide [1]. The mainstay of treatment of this incompletely understood condition still lies with temporary blood pressure control prior to inevitable, often premature, delivery. Over recent times, the anti-angiogenic factors sFlt-1 and sEng have received much interest as likely candidates underlying the hallmark maternal endothelial dysfunction of preeclampsia [2–4]. These insights have offered the prospects of new therapeutic approaches to the care of women with the disease [5].

One anti-angiogenic factor that has attracted less attention as a possible contributor to the pathogenesis of preeclampsia is activin A. Activin is a member of the TGFβ superfamily and is produced by the gonads, pituitary gland and other organs with diverse biological functions. Some of these include angiogenesis, wound healing, haematopoiesis, and organogenesis. Pertinent to pregnancy, activin is also secreted by the placenta across pregnancy with maximum levels at term [6–8]. Circulating levels of activin in women with preeclampsia are about 10-fold fold higher than those in women with a healthy pregnancy [9,10]. The increased circulating levels of activin in preeclampsia is thought to be secondary to oxidative stress-induced increased placental production [11]. Maternal serum levels of activin A are also elevated early in pregnancy in women who subsequently develop preeclampsia, months before the onset of clinical symptoms [12,13] and much earlier than the rises in levels of either sFlt-1 and sEng [3]. Recently it has been shown that the endothelial dysfunction induced by preeclamptic serum may be due, at least in part, to induction of oxidative stress by activin [14], quite separate from any effects of sFlt and sEng [15]. In this current study we wished to examine whether activin had any other effects on endothelial function, specifically exploring changes in endothelin (ET-1) and cell adhesion molecule expression.

2. Material and methods

Maternal blood was collected in serum separator tubes from 23 non-labouring women with a singleton pregnancy at 26–39 completed weeks gestation with preeclampsia, as defined by the Society of Obstetric Medicine of Australia & New Zealand [16], and from 20 women with a healthy singleton pregnancy, matched for gestation. Blood samples were centrifuged at 3500 rpm for

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http://dx.doi.org/10.1016/j.preghy.2016.03.001
2210-7789/

Table 1
Forward (F) and reverse (R) gene primer sequences and qPCR conditions.

mRNA of interest	Forward and reverse primer sequences
ET-1	F 5′-GAG AAA CCC ACT CCC AGT CC-3′ R 5′-GAT GTC CAG GTG GCA GAA GT-3′
ICAM-1	F 5′-CTG CAG ACA GTG ACC ATC-3′ R 5′-GTC CAG TTT CCC GGA CAA-3′
VCAM-1	F 5′-TGG ACC CCG GAT TGC TGC-3′ R 5′-AAA ACT CAC AGG GCT CAG GGT C-3′

mRNA of interest	Cycling condition profile	Cycles
ET	94 °C for 15 s, 60 °C for 30 s	40
ICAM	95 °C for 15 s, 60 °C for 10 s, 72 °C for 15 s	45
VCAM	95 °C for 15 s, 60 °C for 5 s, 72 °C for 10 s	40

Table 2
Patient characteristics.

Characteristic	Normal pregnant	Preeclamptic	Significance
Number of women	20	23	–
Maternal age (years)	32.5 ± 5.1	30.2 ± 5.3	p = 0.1
Gestational age (weeks)	31.5 ± 3.4	33.5 ± 4.3	p = 0.1
BMI (kg/m^2)	25.2 ± 3.8	26.4 ± 6.9	p = 1.0
Parity	0.6 ± 1.1	0.6 ± 1.1	p = 0.8
Systolic BP (mmHg)	107.3 ± 9.43	157.6 ± 18.0	p < 0.0001
Diastolic BP (mmHg)	62.4 ± 10.98	102.4 ± 8.2	p < 0.0001
Proteinuria (g/24 h)	0.0 ± 0.0	1.0 ± 1.2	p < 0.0001

(Results shown with mean ± standard deviation and compared using unpaired *t*-tests. BMI = body mass index, BP = blood pressure).

20 min at room temperature and the serum withdrawn and pooled into a normal pregnancy or preeclamptic group and stored at −20 °C until use within 6 months. Umbilical cords (n = 15) from healthy women with a singleton pregnancy undergoing elective caesarean section at term (37–40 weeks gestation) were collected.

Human umbilical vein endothelial cells (HUVECs) were isolated and expanded as previously described [17]. Experiments were conducted in 96-well plates using confluent HUVECs at passage 3. After establishing confluence, HUVECs were either pre-treated with activin A (40 ng/mL) (R&D Systems, Minneapolis, MN) or human serum (20% v/v) from either the normal or preeclamptic pools. Twenty-four hours after pre-treatment, HUVECs were treated with follistatin (600 ng/mL) (R&D Systems, Minneapolis, MN) for 24 h prior to the collection of cell lysate, using cell-to-signal lysis buffer (Ambion, Austin, TX) for subsequent RNA quantification. HUVEC experiments were replicated a total of eight times prior to analysis.

Total activin A and follistatin were measured as described previously [8,18], using an activin A two-site ELISA (Beckman Coulter/DSL, USA) and an in-house follistatin discontinuous radioimmunoassay. Total cell-associated RNA was isolated and reverse transcription performed with 12 μg of total RNA using Oligo(dt) primers (Life Technologies, Carlsbad, CA) and Superscript III (Invitrogen, Carlsbad, CA). Quantitative real-time PCR (RT-qPCR) was then performed by reconstituting 5 μL of sample cDNA with primers specific for either ET-1, ICAM-1 and VCAM-1 in SYBR Green reaction mix (Roche Diagnostics, Mannheim, Germany). Normalization of results was achieved by performing RT-qPCR runs for each gene of interest in conjugation with 2 μL of cDNA to detect 18s mRNA. The samples were held in a Rotor gene RG3000 (Roche Molecular Systems, Pleasanton, CA) at 95 °C for ten minutes for initial denaturation. This was followed by a cycling profile unique to each gene of interest, with 18s run at every profile for comparison and eventual quantification of results using the $2^{-\frac{\Delta\Delta C}{T}}$ method as described previously [19]. The primer sequence and q-PCR cycling conditions for each of the gene analysed is described in Table 1.

3. Results & discussion

Characteristics of both the normal pregnant and preeclamptic subjects are shown in Table 2.

Serum concentrations of activin A ($p < 0.0001$, Fig. 1A) and follistatin ($p < 0.0001$, Fig. 1B) were significantly higher in women with established preeclampsia compared to those with a normal pregnancy, confirming previous reports [9,11]. We also showed

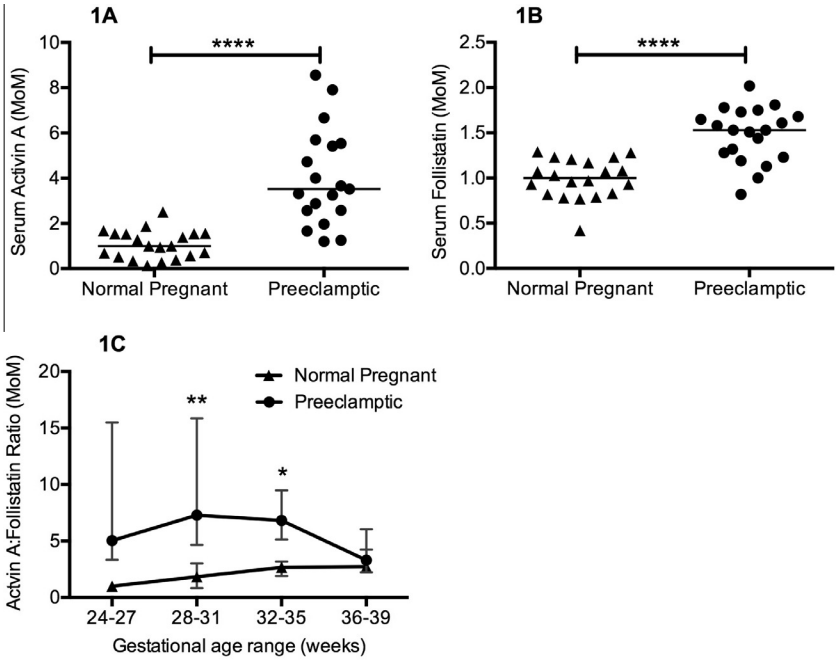

Fig. 1. A) Concentrations of activin A and follistatin in normal pregnant (n = 20) and preeclamptic (n = 23) serum. B) Concentrations of follistatin in normal pregnant (n = 20) and preeclamptic (n = 23) serum. C) Activin A:follistatin ratio in normal pregnant (n = 20) and preeclamptic (n = 23) serum grouped into four-week gestational ages. Individual values plotted (expressed as MoM) and horizontal bars represent group median (± IQR in 1C). Results analysed using Mann Whitney *U* test where $^{****}p < 0.0001$, $^{**}p = 0.008$ and $^{*}p = 0.02$.