Pregnancy Hypertension 12 (2018) 58–64

Contents lists available at ScienceDirect

Pregnancy Hypertension

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

Changes in endothelial cell specific molecule 1 plasma levels during preeclamptic pregnancies compared to healthy pregnancies

Joost H.N. Schuitemaker[a,b,*], Thomas I.F.H. Cremers[c], Maria G. Van Pampus[d], Sicco A. Scherjon[e], Marijke M. Faas[a,e]

[a] Div. of Medical Biology, Dept. of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[b] Research & Development, IQ Products BV, Groningen, The Netherlands
[c] Dept. Pharmaceutical Analysis, University of Groningen, Groningen, The Netherlands
[d] Onze Lieve Vrouwe Gasthuis, Dept. of Obstetrics and Gynecology, Amsterdam, The Netherlands
[e] Dept. of Obstetrics and Gynecology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ARTICLE INFO

Keywords:
ESM-1
Endothelial cell specific molecule 1
Endocan
Preeclampsia
Pregnancy

ABSTRACT

Objective: We aimed to assess the levels of endothelial cell specific molecule 1 (ESM-1) during pregnancy and preeclampsia.
Methods: Plasma and placental samples were collected from women with a control pregnancy, early- or late-onset preeclamptic women and non-pregnant women (experiment 1). Plasma samples were collected between weeks 12 and birth from pregnant women at high risk for developing preeclampsia (experiment 2). ESM-1 plasma levels were measured by ELISA and in the placenta mRNA and protein were detected by immunohistochemistry and qPCR.
Results: In the first experiment we observed lower concentrations of ESM-1 in pregnant women as compared to non-pregnant women and higher concentrations during early- and late-onset preeclampsia as compared to control pregnancies of the same gestational age. Early- and late-onset preeclamptic pregnancies were not different from their subsequent controls in ESM-1 mRNA or protein levels in placental tissue. The second experiment showed that in women who had an control pregnancy, plasma ESM-1 levels were decreased as compared to non-pregnant women, from week 16 ± 2 until the end of pregnancy and returned to non-pregnant levels postpartum. In women who developed early- or late-onset preeclampsia, plasma ESM-1 was also decreased as compared to non-pregnant women from week 20 ± 2 until week 28 ± 2 of pregnancy. Then ESM-1 levels increased and were no longer different from levels in non-pregnant women on weeks 32 and 36.
Conclusions: Plasma ESM-1 levels are decreased during pregnancy and increased in early- and late-onset preeclampsia. The source of ESM-1 is probably not the placenta, but most likely maternal endothelial cells.

1. Introduction

Preeclampsia is one of the most serious complications that can occur during pregnancy, resulting in maternal and perinatal morbidity and mortality. The definition of preeclampsia was recently updated to the specification of a de novo hypertension (> 140/90 mm Hg) after 20 weeks of gestation accompanied by one or more of the following new onset conditions: proteinuria, other maternal dysfunction (renal insufficiency (creatinine > 90 μmol/L), liver involvement (elevated transaminases), neurological complications (eclampsia, visual disturbance/blindness and, or headaches accompanied by hyperreflexia) or haematological complications (thrombocytopenia, DIC, haemolysis)), or uteroplacental dysfunction (foetal growth restriction) [1]. Preeclampsia can occur as early-onset (birth before 34 weeks) or late-onset (birth after 34 weeks) preeclampsia [2] and risk factors, like diabetes mellitus, chronic hypertension, obesity and multiple gestation, increase the chance of developing preeclampsia [3].

The mechanisms underlying preeclampsia are still not well understood. Early onset preeclampsia is thought to develop following placental dysfunction, induced by impaired trophoblast invasion and abnormal spiral artery remodeling [4,5]. The dysfunctional placenta may release specific factors into the maternal circulation, such as proinflammatory cytokines, placental microvesicles, anti-angiogenic factors, such as sFlt-1 and sEndoglin [6,7] or ATP [8]. These factors further

* Corresponding author at: Div. of Medical Biology, Dept. of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
E-mail address: j.h.n.schuitemaker@umcg.nl (J.H.N. Schuitemaker).

https://doi.org/10.1016/j.preghy.2018.02.012
Received 6 November 2017; Accepted 28 February 2018
Available online 01 March 2018
2210-7789/ © 2018 Published by Elsevier B.V. on behalf of International Society for the Study of Hypertension in Pregnancy.

aggravate the normal occurring low grade systemic inflammatory response during pregnancy [2,9]. They also activate endothelial cells [7,10]. Finally, this proposed cascade of events may result in preeclampsia.

Late-onset preeclampsia is hypothesized to be associated with restricted villous perfusion, due to the increasing size of the placenta and villous constraints [11]. This results in abnormal placental perfusion and subsequent release of the same placental factors in the maternal circulation.

Endothelial cell specific molecule 1 (ESM-1) was described as an inflammatory factor that was increased in cardiovascular disease, sepsis and cancer [12–16]. This proteoglycan is produced by endothelial cells and is involved in a wide range of biological processes, such as proliferation, migration, cell adhesion and neovascularization. ESM-1 can be found in plasma [17–20] and the production is increased in the presence of pro-angiogenic molecules such as VEGF and FGF-2 [15]. The relation between these angiogenic factors and ESM-1 and the fact that ESM-1 is an inflammatory factor [13], suggest that ESM-1 levels might be increased during pregnancy and further increased in preeclampsia.

The aim of the present study was therefore to test the hypothesis that ESM-1 is increased during pregnancy and further increased during preeclampsia.

2. Material and Methods

2.1. Study population

This study has been approved by the medical ethics committee of the University Medical Center Groningen. Written informed consents were obtained from all patients.

Preeclampsia was defined according to the definition of the International Society for the Study of Hypertension in Pregnancy (ISSHP): diastolic blood pressure of ≥90 mmHg on two or more occasions, more than 4 h apart, and proteinuria of ≥300 mg/24 h published by Tranquilli et al. [1,21]. Early-onset preeclampsia was defined as giving birth before 34 weeks; late-onset preeclampsia was defined as giving birth in week 34 or later. In all our cases, preeclampsia was classified as severe (blood pressure higher or equal to 160 mmHg systolic or 110 mmHg diastolic). Experiment 1: For this experiment exclusion criteria for all groups were pre-existing hypertension, diabetes mellitus, vasculitis, renal disease, autoimmune disease, malignancies or women who had had recent trauma or surgery. Non-pregnant controls were recruited from the staff of the UMCG; the control pregnant women (with uncomplicated pregnancies) and preeclamptic patients were recruited during their visit or stay at the department of Obstetrics and Gynecology of the University Medical Center Groningen.

EDTA plasma samples were collected from non-pregnant women (N = 21), preeclamptic women (N = 38; n = 23 early onset and n = 15 late onset) and gestational age matched control pregnant women (24 controls to the early-onset preeclampsia; and 27 controls to the late-onset preeclampsia). Patient characteristics of these women are shown in Table 1. Blood samples were drawn from the antecubital vein in EDTA-plasma collection tubes (Venoject, Terumo Europe NV, Leuven, Belgium). Plasma samples were processed immediately by centrifugation (130*g*, 10 min. at 4 °C followed by 700*g*, 10 min. at 4 °C), split in aliquots and stored at −80 °C until the assay was performed. Samples were thawed just before dilution for use in the assay. To determine plasma ESM-1 concentration, the enzyme-linked immunosorbent assay (ELISA) specific for human ESM-1, was used according to the protocol supplied with the ELISA (Pregnostic®-PE *Ip*, IQ Products BV, Groningen, The Netherlands). The plasma samples were coded and evaluated blinded for outcome.

For the analysis of placental ESM-1 protein and mRNA expression, biopsies were collected from early-onset preeclamptic (N = 27) and late-onset preeclamptic women (N = 12). Since all early-onset preeclamptic women delivered by caesarean section, control placentas were collected from women (N = 15) who delivered by caesarean section for other reasons than preeclampsia, for instance breech presentation. Women with late-onset preeclampsia delivered vaginally, therefore control placentas were collected from control pregnant women (N = 14) who delivered vaginally. Patient characteristics are shown in Table 2. Placental biopsies were randomly collected by taking 1 by 1 cm tissue samples from the chorionic villi (after removal of the decidua), avoiding infarcted areas, and snap frozen in liquid nitrogen and stored at −80 °C until further processing. For mRNA isolation 5 small biopsies of the chorionic villi (after removal of the decidua) were taken randomly (avoiding infarcted areas), pooled, snap frozen and stored at −80 °C until further processing.

Immunohistochemical staining of placental sections: Placental cryostat sections (4 μm) were cut. After drying, 10 min acetone fixation and drying again, the sections were stained with the primary antibody mouse anti-human ESM-1 monoclonal MEP14 (1 μg/ml) (IQ Products BV, Groningen, The Netherlands). Control sections were stained without the use of a primary antibody. After washing with phosphate buffered saline (PBS), exogenous peroxidase was blocked by incubation for 30 min in 0.25% H_2O_2 in PBS. After washing, the slides were incubated with biotin-conjugated goat-anti-mouse (Southern Biotech, Birmingham, AL, USA) for 60 min, washed again and incubated with horseradish peroxidase conjugated streptavidin (Dako, Heverlee, Belgium) in PBS for 30 min. The presence of ESM-1 was visualized with 3-amino-9-ethyl-carbazole, followed by hematoxylin staining. All of the incubation steps were carried out at room temperature and all sections were stained in 1 procedure. After staining, all slides were scanned with the Aperio TMAscanner (Aperio, Vista, USA). The control sections were consistently negative. Quantification of the ESM-1 protein expression in placenta was done using Aperio ImageScope (Aperio, Vista, CA, USA). We determined the amount of positivity using the 'positive pixel count v9'. This was done in at least 3–5 tissue areas per section. Of each placental section the mean number of positive pixels per tissue area was used for evaluation.

Placental ESM-1 mRNA expression was analyzed using real-time RT PCR from a random selection of the placentas used for immunohistochemistry. This set consisted of nine early-onset preeclamptic placentas and of seven control placentas from women who delivered by caesarean section and six late-onset preeclamptic placentas and six control placentas from women who delivered vaginally. mRNA isolation was done using TRIzol (Life Technologies Europe, Bleiswijk, the Netherlands) followed by the determination of the 260/280 ratio with use of the Nanodrop spectrophotometer (Thermo Fisher Scientific, DE, USA), to determine the purity and quality of the mRNA. All samples had an optimal 260/280 ratio of about 2.0, indicating the purity of the mRNA. Reverse transcription to cDNA was done using Superscript II reverse transcriptase (Eurogentec, Maastricht, The Netherlands). ESM-1 and proteasome non-ATPase regulatory subunit 4 (PSMD-4), used as a reference gene, analysis was done using predesigned gene expression assay Taqman primer-probe combinations (Hs00199831_m1 and Hs00356654_m1, Life Technologies). PCRs were performed in a reaction volume of 25 μl with ABsolute QPCR ROX mix (Life Technologies) and 40 cycles on a ABI Prism 7900HT Sequence Detection System (Life Technologies). The analysis of the qPCR data was performed using the software of the ABI Prism 7900HT platform. ESM-1 mRNA levels were corrected for PSMD-4 expression by calculating the ΔCt (Ct ESM-1 – Ct PSDM4) value and plotted as $2^{-\Delta Ct}$ values.

Experiment 2: In the second set of experiments, we tested the course of ESM-1 concentration in plasma during pregnancy and preeclampsia. Therefore, a different set of plasma samples was collected between week 12 ± 2 until birth from pregnant women at high risk for developing preeclampsia. Samples were used from the cohort described by Wong et al [22]. In this cohort a total of 103 pregnant women were included. They were in their first trimester and presented with one of

Download English Version:

https://daneshyari.com/en/article/8675009

Download Persian Version:

https://daneshyari.com/article/8675009

Daneshyari.com