Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 3 (2013) 39–43

Contents lists available at SciVerse ScienceDirect

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

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

Original Article

Placental angiogenin inhibitor (ribonuclease inhibitor), a novel gene in pre-eclampsia

V.L. Karanam [a,b,*], N. Anim-Nyame [a,b], N.M. Page [a]

[a] School of Life Sciences, Kingston University London, United Kingdom
[b] Department of Obstetrics and Gynaecology, Kingston Hospital, United Kingdom

ARTICLE INFO

Article history:
Received 13 October 2012
Accepted 22 October 2012
Available online 11 November 2012

Keywords:
Pre-eclampsia
Placental genes
Angiogenesis
Angiogenic factors
Angiogenin
Inhibitor

ABSTRACT

There is convincing evidence that imbalance between angiogenic and anti-angiogenic factors play an important role in the pathophysiology of pre-eclampsia. Angiogenin, a member of the RNase A super family, is a potent inducer of angiogenesis and serum levels are shown to be elevated in pre-eclampsia. We hypothesize that placental expression of angiogenin inhibitor which binds and blocks the activity of angiogenin is altered in pre-eclampsia and may play a role in its pathophysiology.

Placental expression of angiogenin inhibitor was measured in term placentae of 15 women with preeclampsia and 16 normal pregnant controls. The women were matched for age, parity and gestational age. Placental tissue was collected immediately after delivery and stored at −80°C until studied. Angiogenin inhibitor gene expression was measured using real-time quantitative polymerase chain reaction (rt-QPCR). The results were standardized using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as reference gene.

The mRNA expression of angiogenin inhibitor gene was significantly increased in pre-eclamptic placentae compared to normal pregnant controls [0.44 (0.174–1.048) versus 0.091 (0.029–0.301), median and interquatile range, $p = 0.027$ for pre-eclampsia and normal controls respectively]. There was no correlation between angiogenin inhibitor gene expression and maternal age, blood pressure, platelet count, gestation age, birth weight of the baby in pre-eclampsia and normal pregnancy.

This study showed that placental expression of the angiogenin inhibitor gene is significantly increased in pre-eclampsia and may play a role in its pathophysiology.

Introduction

Pre-eclampsia is a pregnancy specific multisystem disorder associated with maternal and perinatal mortality and morbidity [1]. The primary pathology of the disease remains unexplained though there is convincing evidence that the placenta plays a crucial role in the development of this disorder [2]. Normal placental development involves the invasion of extravillous trophoblast cell and remodelling of the uteroplacental vessels. Human placentation, therefore, represents a remarkable balance between concurrent degradation of the maternal decidual vasculature with highly coordinated angiogenesis [3]. The factors in the adult which regulate angiogenesis may also regulate embryonic and placental vascular development. Imbalance between pro-angiogenic and anti angiogenic factors (of which there are many), has been proposed to be central to the the pathophysiology of pre-eclampsia [4–8].

Angiogenin is one of the potent in vivo inducers of angiogenesis [9]. Increased mRNA expression and secretion of angiogenin at term over the first trimester chorionic villi

* Corresponding author. Present address: Specialist registrar, Department of Obstetrics and Gynaecology, Royal Derby hospital, Uttoxeter road, Derby DE22 3NE.
E-mail address: docvijuk@hotmail.com (V.L. Karanam).

2210-7789/$ - see front matter
http://dx.doi.org/10.1016/j.preghy.2012.10.002

were noted [10]. There are studies showing altered levels of maternal serum angiogenin in pregnancies complicated by pre-eclampsia [11,12].

Angiogenin inhibitor is a cytosolic protein found in all mammalian tissues which binds to mammalian ribonucleases with extraordinary affinity. Binding to angiogenin inhibitor blocks the active site of the enzyme angiogenin and abolishes ribonucleolytic activity. Dickson et al. reported that angiogenin inhibitor regulates the angiogenic activity of angiogenin in vivo [13]. Based on the facts that pro-angiogenic and anti-angiogenic factors play a role in the pathophysiology of pre-eclampsia and the functions of angiogenin inhibitor, we hypothesized that there could be altered levels of placental mRNA in pregnancies complicated by pre-eclampsia compared to normal pregnancy and this may contribute to the abnormal placental development in pregnancies complicated by this disease.

Materials and methods

Subjects and sample acquisition

To investigate the hypothesis, we compared the placental expression of the angiogenin inhibitor gene in pre-clampsia to normal pregnant controls.

Placenta samples were obtained from 15 women with pre-eclampsia and 16 normal pregnant controls recruited from Kingston Hospital maternity unit. The women were matched for age, body mass index (BMI), parity and racial background. Pre-eclampsia was defined as new onset hypertension of at least 140/90 mm of Hg on two separate occasions at least 4 h apart and significant proteinuria after 20 weeks of gestation and reversal of both by six weeks of delivery (National High Blood Pressure Education Program Working Group on High Pressure in Pregnancy, 2000). Proteinuria was defined as spot urinary protein creatinine ratio of more than 30 mg/mmol or a 24-h urinary protein excretion greater than 300 mg protein (International Society for the Study of Hypertension in Pregnancy, 2001). Women with medical or surgical conditions and who smoked were excluded from the study. Gestational age was assigned by a first trimester ultrasound scan. The study was approved by the London and Surrey Borders Research ethics committee.

Placental samples were collected immediately after delivery. A total of eight biopsies were taken from all segments of placenta avoiding membranes, each measuring around two cubic centimetres, washed in PBS and immersed in Trireagent. The placental samples were homogenized with power Gen homogenizer immediately and stored at −80°C until use.

RNA isolation and cDNA synthesis

The RNA was extracted from the placental samples using TriReagent (Sigma–Aldrich, Poole, UK) according to the manufacturer's instructions. The quality and quantity of RNA were measured using Nanodrop ND-1000 (Labtech International Ltd., Lewes, East Sussex, UK). The RNA quality was assessed by using denaturing agarose gels and ethidium bromide staining to check for degradation. The integrity was also checked by Agilent 2100 Bioanalyser using the RNA 6000 LabChip™ kit. Total placental RNA (400 ng) was reverse transcribed to complimentary DNA (cDNA) in 20 μL volume reaction using Superscript TM || reverse transcriptase kit and Oligo (dT). (Invitrogen life technologies, Paisley, UK).

Real time quantitative polymerase chain reaction

In this study SYBR green technology was used for Quantitative PCR and results were analysed with relative expression to GAPDH as a reference gene. Primer sequences and annealing temperature for the target gene (angiogenin inhibitor) and the reference gene (GAPDH) are presented in Table 1. The specificity of each primer pair was confirmed by melting curve analysis which resulted in single product specific melting temperatures. We conducted standard nucleotide BLAST to confirm the specificity of the primers and checked for the absence of single nucleotide variations. Possible primer pairs were run through Sigma Genosys DNA Calculator (http://www.sigma-genosys.com/calc/DNACalc.asp) to second check T_ms, as well as the likelihood for primer dimers and secondary structure formation. All primers were obtained from Sigma Genosys, Haverhill, UK.

Samples were run in duplicate in a 96-well plate (Biorad CFX96) for the target gene and reference genes. Real-time PCR mastermix of the following reaction components (Quantace, Ltd, UK) was prepared: 25 μl of 2× Sensimix dT, 1 μl of 50x SYBR green, 2 μl each of the forward and reverse primers (0.5 μM). Two microlitres of cDNA and master mix were pipetted into the well and sterile water was added up to a total volume of 50 μl. The following optimized thermal profile was used: denaturation programme (95 °C for 10 min), amplification and quantification programme repeated 40 times (95 °C for 10 s, 60 °C for 30 s, with a single fluorescence measurement), melting curve programme (55–95 °C with a heating rate of 0.1 °C per second and a continuous fluorescence measurement). The fluorescence data were collected and the mRNA quantified with pbase^{plus} software which is included in the CFX platform. The normalized expression of angiogenin inhibitor gene against GAPDH was estimated according to the method of Schmittgen and Livak [14].

Statistical analysis

Using the Shapiro–Wilk normality test, the data were found to be nonparametric and therefore the Mann–Whitney U-test was used to compare the difference in angiogenic inhibitor gene expression between the two groups. Spearman correlation coefficients were used to determine the correlation between the gene expression and clinical and biochemical variables in the two groups of women. *p*-Value of less than 0.05 was considered statistically significant. The software Graph Pad Prism (version 5) was used for statistical analysis.