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Severely decreased activity of placental dimethylarginine dimethylaminohydrolase in pre-eclampsia

Maike Anderssohn [a,*], Laura M. Maaß [a,b], Anke Diemert [b], Nicole Lüneburg [a], Dorothee Atzler [a], Kurt Hecher [b], Rainer H. Böger [a]

[a] Department of Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[b] Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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ABSTRACT

Objectives: Asymmetric dimethylarginine (ADMA) is a key regulator of nitric oxide production. Elevations of ADMA have previously been associated with endothelial dysfunction in pre-eclamptic women. ADMA is degraded mainly by dimethylarginine dimethylaminohydrolase (DDAH), which is also expressed in placental tissue. Therefore, we measured placental DDAH expression and activity in pre-eclampsia and normal pregnancies in order to determine whether impairment of this enzyme in the pre-eclamptic placenta could contribute to elevations of ADMA levels in these women.

Study design: ADMA plasma levels were measured by LC–MS/MS in 18 pre-eclamptic patients and 28 controls. Placental DDAH activity was determined by measuring the degradation of [$^2H_6$]-labeled ADMA in tissue homogenates from placental biopsies in 15 women with pre-eclampsia and 16 controls. Placental mRNA expression of DDAH 1, DDAH 2, endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) and protein-arginine methyltransferase 1 (PRMT1) was determined in tissue biopsies by RT-PCR.

Results: Placental DDAH activity was almost undetectable in pre-eclampsia, and it was significantly higher in controls. ADMA plasma levels were higher in pre-eclampsia as compared to normal pregnancies (0.51 ± 0.15 μmol/l vs. 0.42 ± 0.07 μmol/l; $p = 0.005$), and the difference between maternal and fetal ADMA levels (feto-maternal ADMA gradient) was lower in pre-eclampsia (0.63 ± 0.20 μmol/l vs. 0.80 ± 0.18 μmol/l; $p = 0.02$). Furthermore, mRNA expression levels of DDAH 2 were significantly lower in pre-eclamptic women ($p = 0.04$), while PRMT1 expression levels were the same. In pre-eclampsia, we found only weak correlations between maternal ADMA levels and DDAH 1 ($r = -0.41$; $p = 0.22$) and DDAH 2 expressions ($r = -0.45$; $p = 0.17$) but a slightly stronger correlation between DDAH 2 expression and feto-maternal ADMA gradient ($r = 0.60$; $p = 0.07$).

Conclusion: Decreased DDAH activity in the pre-eclamptic placenta might contribute to elevated ADMA levels in these patients.

1. Introduction

Pre-eclampsia remains a major cause of preterm birth and perinatal mortality worldwide and delivery is still the only curative treatment for maternal symptoms [1,2]. The estimated incidence of pre-eclampsia in healthy women ranges from 2% to 8% and is even higher in women with pre-existing risk factors such as hypertension, diabetes, or previous preeclampsia [3,4]. By now, it is widely accepted that (a) the presence of a placenta is the key underlying factor for the development of pre-eclampsia and (b) the clinical symptoms arise from the development of endothelial dysfunction [1,3,5,6]. However, the pathophysiological link between the placenta and the development of maternal systemic endothelial dysfunction is still unclear. It has been suggested that soluble factors produced or set free by the placenta enter the maternal circulation and cause the clinical symptoms [7,8] and that these factors are produced in relevant amounts even before the onset of clinical symptoms [9].

Asymmetric dimethylarginine (ADMA) has been proposed to be one of these soluble factors for several reasons: (1) ADMA acts as a competitive inhibitor of endothelial nitric oxide synthase (eNOS) and by this causes endothelial dysfunction [10]. (2) ADMA is elevated in women with pre-eclampsia as compared to normal pregnancies [11–14]. (3) Infusion of synthetic inhibitors of NOS causes pre-eclampsia-like symptoms in rodents, which can be reversed by infusion of L-arginine [15–17].

* Corresponding author at: Department of Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. Tel.: +49 40 7410 53178; fax: +49 40 7410 59757.
E-mail address: m.anderssohn@uke.de (M. Anderssohn).

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ADMA plasma levels are mainly regulated by the expression and activity of its degrading enzyme, dimethylarginine dimethylaminohydrolase (DDAH), while only a minor portion is cleared via renal excretion [18]. Reduced expression of DDAH and pharmacological inhibition of DDAH result in elevated ADMA levels and endothelial dysfunction with consecutively increased systemic vascular resistance and elevated blood pressure [19]. Two isoforms of DDAH have been discovered so far with different tissue expression patterns. DDAH 1 is mainly found in the brain and in organs like pancreas, liver and kidneys, while DDAH 2 is highly expressed in the cardiovascular system, kidneys and placenta [20]. Therefore, it can be speculated that the placenta contributes to the clearance of ADMA during pregnancy via degradation of ADMA by placental DDAH.

In our study, we aimed to measure DDAH activity in placental biopsies from pre-eclamptic patients and healthy pregnancies to assess the functionality of the enzyme in the pre-eclamptic placenta. In order to analyze the relationship between placental DDAH and ADMA levels of pre-eclamptic women, we also measured L-arginine and ADMA plasma levels in these women and determined expression levels of genes relevant for ADMA metabolism like DDAH 1, DDAH 2, eNOS, protein-arginine methyltransferase 1 (PRMT1).

2. Materials and methods

The study protocol was approved by the local ethics committee and written informed consent was obtained from all participants prior to inclusion in the study.

2.1. Participants

We collected blood samples from 18 women with pre-eclampsia (PE) including four women with the "hemolysis, elevated liver enzymes and low platelets" (HELLP) syndrome and 28 healthy controls (C) at the end of the third trimester of pregnancy. We included all pre-eclamptic patients referred to the University Medical Center Hamburg-Eppendorf, Hamburg, Germany for clinical monitoring during December 2008 and December 2009 who agreed to participate in the study. Controls were healthy pregnant women who came to the University Medical Center for delivery. Exclusion criteria were any pre-existing medical condition that demanded pharmacotherapy other than hypertension or diabetes mellitus. Placental biopsies and umbilical cord venous blood were obtained from 15 of the pre-eclamptic patients and 16 of the healthy controls after delivery to determine fetal plasma L-arginine, ADMA and SDMA and placental DDAH expression and activity. The majority of the women were delivered by caesarian sections, with the exception of three pre-eclamptic patients and two women of the control group who delivered vaginally. The caesarian sections in the control group were elective caesarian sections owing to breech presentation or cephalopelvic disproportion.

Pre-eclampsia was defined according to the National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Pregnancy [21] as systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg on repeated measurements with onset after 20 weeks of gestational age and proteinuria >300 mg/24 h or at least "++" on dipstick testing (≥30 mg/dl). Severe pre-eclampsia was defined as blood pressure above 160 mmHg systolic and/or 110 mmHg diastolic or proteinuria >5 g/24 h or oliguria (<500 ml urine/24 h).

All pregnancies were otherwise uncomplicated singleton pregnancies except for the development of pre-eclampsia. Three women had developed pre-eclampsia in previous pregnancies, four had a positive family history for pre-eclampsia and three women were diagnosed with arterial hypertension prior to the pregnancy. None of the women had diabetes prior to the pregnancy or developed gestational diabetes.

2.2. Laboratory measurements

Mass spectrometric determination of L-arginine, ADMA and SDMA from maternal and fetal plasma samples was performed as described elsewhere by using a validated high throughput LC–MS/MS assay [22–23]. In brief, 25-μl aliquots of plasma samples were precipitated with 2 μM [$^2H_6$]-ADMA and 50 μM L-[$^2H_7$]-L-arginine in 100 μl methanol using 96-well 0.20-μm microfiltration plates (Millipore, Schwalbach, Germany). After conversion to their butyl ester derivatives, analytes were analyzed on a Varian 1200L Triple Quadrupole MS (Varian, Walnut Creek, CA, USA) in the positive electrospray ionization (ESI+) mode. All other laboratory parameters were determined using routine laboratory methods.

2.3. Determination of placental gene expression levels

Placental biopsies were obtained from 2 to 3 different sites of the placenta that were upon inspection free from infarction after delivery and quickly frozen in liquid nitrogen. Tissue samples were stored at −80 °C until further analysis. RNA was extracted using the miRNeasy Mini Kit (Qiagen N.V., Venlo, The Netherlands) according to manufacturer's instructions. RNA quality was assessed by agarose gel electrophoresis: only samples with two clearly detectable ribosomal RNA bands (26 of 31) were used for reverse transcription. Reverse transcription of 1 μg total RNA was performed using the RevertAid H Minus First Strand cDNA Synthesis Kit (MBI Fermentas Inc., Burlington, Canada). Real-time PCR was performed on an ABI PRISM 7900 HT thermal cycler using predesigned, validated TaqMan assays for DDAH 1, DDAH 2, iNOS, eNOS, PRMT1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Applied Biosystems Inc., Carlsbad, USA) according to manufacturer's protocol.

2.4. DDAH activity assay

DDAH activity in placental biopsies was determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) as previously described in detail [24]. Deuterium labeled [$^2H_6$]-ADMA was used as a substrate for DDAH and C-13 and deuterium labeled [$^{13}C_5{}^2H_6$]-ADMA was used as internal standard for quantification of [$^2H_6$]-ADMA. Tissue samples were homogenized in PBS buffer and the homogenate was centrifuged in a pre-cooled (4 °C) centrifuge for 5 min at 12,000 × *g*. 80 μl of the resulting supernatant were added to 20 μl aliquots of PBS buffer containing 50 μM [$^2H_6$]-ADMA and incubated for 60 min at 37 °C. DDAH activity was calculated as the amount of [$^2H_6$]-ADMA degraded within this period. Reactions were stopped by addition of 100 μl internal standard dissolved in methanol to precipitate proteins. Samples were derivatized with 1 N butanolic HCl and subsequently analyzed by LC–MS/MS. All activities were normalized to tissue protein content.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, USA) and Graph Pad Prism 5.0 (GraphPad Software Inc., La Jolla, USA). Data are expressed as means ± SD or as median and interquartile range where appropriate. Comparisons of the groups were examined by Student's *t* test for parametric data, Mann–Whitney *U* test for nonparametric data, and Chi square test for categorical data. Spearman correlation coefficients