Pregnancy Hypertension 11 (2018) 1–6

Contents lists available at ScienceDirect

Pregnancy Hypertension

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

Aberrant pro-atrial natriuretic peptide/corin/natriuretic peptide receptor signaling is present in maternal vascular endothelium in preeclampsia

Yang Gu[a], Donna Thompson[a], Jie Xu[a,b], David F. Lewis[a], John A. Morgan[a], Danielle B. Cooper[a], Charles E. McCathran[a], Yuping Wang[a,*]

[a] Department of Obstetrics and Gynecology, Louisiana State University Health Sciences Center – Shreveport, LA, USA
[b] Department of Physiology, Harbin Medical University, Harbin, Heilongjiang, China

ARTICLE INFO

Keywords:
Corin
ANP
NPR
Pregnancy
Preeclampsia

ABSTRACT

Objective: Corin is a serine protease that converts pro-atrial natriuretic peptide (pro-ANP) to atrial natriuretic peptide (ANP), a cardiac hormone that regulates salt-water balance and blood pressure. ANP is degraded by natriuretic peptide receptor (NPR). This study was to determine if aberrant pro-ANP/corin/NPR signaling is present in maternal vascular system in preeclampsia.

Study design: Maternal venous blood was obtained from 197 pregnant women (84 normotensive, 16 complicated with chronic hypertension (CHT), 11 mild and 86 severe preeclampsia). Plasma corin and pro-ANP concentrations were measured by enzyme-linked immunosorbent assay. Maternal subcutaneous fat tissue was obtained from 12 pregnant women with cesarean section delivery (6 normotensive and 6 preeclampsia). Vascular ANP and its receptors NPR-A, NPR-B, and NPR-C expression were examined by immunostaining of paraffin embedded subcutaneous fat tissue sections.

Results: Corin concentrations were significantly higher in mild (2.78 ± 0.67 ng/ml, $p < .05$) and severe (2.53 ± 0.18 ng/ml, $p < .01$) preeclampsia than in normotensive (1.58 ± 0.08 ng/ml) and CHT (1.55 ± 0.20 ng/ml) groups. Pro-ANP concentrations were significantly higher in CHT (1.59 ± 0.53 ng/ml, $p < .05$) and severe preeclampsia (1.42 ± 0.24 ng/ml, $p < .01$) than in normotensive (0.48 ± 0.06 ng/ml) and mild preeclampsia (0.52 ± 0.09 ng/ml) groups. ANP and NPR-B expression was undetectable in maternal vessels from normotensive and preeclamptic pregnancies, but reduced NPR-A expression and increased NPR-C expression was found in maternal vessel endothelium in preeclampsia.

Conclusions: ANP is a vasodilator and NPR-C is a clearance receptor for ANP. The finding of upregulation of NPR-C expression suggests that circulating ANP clearance or degradation is increased in preeclampsia. These results also suggest that pro-ANP/corin/NPR signaling is dominant in the vascular system in preeclampsia.

1. Introduction

Corin is a serine protease of the trypsin superfamily. It is originally identified as a cardiac enzyme with abundant expression in atrial and ventricular myocytes in the heart [1]. Corin converts atrial natriuretic peptide (ANP) precursor (pro-ANP) to mature ANP, a circulating cardiac hormone that regulates salt balance, intravascular blood volume, and subsequently vascular tone. Therefore, corin and ANP signaling pathway molecules play an important compensative role in the cardiovascular system because of its diuretic, natriuretic, and vasodilating actions and also its inhibitory effects on renin and aldosterone secretion [2]. In humans, altered corin expression and production have been found in several cardiovascular and renal diseases. For example, plasma corin levels are reduced significantly in patients with heart failure [3]. Kidney corin expression is downregulated in proteinuric kidney diseases associated with increased sodium retention [4]. In mice, corin deficiency prevents pro-ANP processing and causes salt-sensitive hypertension and the hypertensive phenotype is exacerbated by high-salt diet when mice become pregnant [5]. Therefore, altered corin and ANP production and activity have a significant impact on vascular and renal functional homeostasis.

Recent animal studies showed that corin is able to activate ANP in

Abbreviations: pro-ANP, pro-atrial natriuretic peptide; ANP, atrial natriuretic peptide; NPR, natriuretic peptide receptor; CHT, chronic hypertension; ELISA, enzyme-linked immunosorbent assay; BMI, body mass index; GTP, guanosine triphosphate; cyclic cGMP, guanosine monophosphate; VSMCs, vascular smooth muscle cells

* Corresponding author at: Department of Obstetrics and Gynecology, Louisiana State University Health Sciences Center – Shreveport, 1501 Kings Highway, Shreveport, LA 71130, USA.
E-mail address: ywang1@lsuhsc.edu (Y. Wang).

https://doi.org/10.1016/j.preghy.2017.12.001
Received 29 June 2017; Received in revised form 20 November 2017; Accepted 1 December 2017
2210-7789/ Published by Elsevier B.V. on behalf of International Society for the Study of Hypertension in Pregnancy.

the pregnant uterus to promote spiral artery remodeling and to prevent pregnancy-induced hypertension [6–8]. In humans, up-regulation of corin was found in the late secretory phase in endometrium and in first trimester decidua tissue [7]. While, in corin- or ANP-deficient mice, trophoblast invasion was markedly impaired as compared to the wild type animals [6]. Study also showed that maternal ANP levels were significantly increased in pregnant women, and further increased in women with preeclampsia, a hypertensive disorder in human pregnancy [9]. It is known that the action of ANP is regulated via binding to its receptor on cell membrane. However, it is not known if altered ANP receptor expression occurs in preeclampsia. In the present study, we measured maternal plasma corin and pro-ANP levels and examined maternal vascular ANP and natriuretic peptide receptor A (NPR-A), NPR-B, and NPR-C expression in normotensive and preeclamptic pregnant women to test our hypothesis that aberrant pro-ANP/corin/NPR signaling is present in maternal vascular system in preeclampsia. We also measured corin and pro-ANP levels in pregnant women complicated with chronic hypertension to determine if differences exist for corin and/or pro-ANP levels in pregnant women complicated between preeclampsia and chronic hypertension.

2. Materials and methods

2.1. Patient information and blood and subcutaneous fat tissue sample collection

Collections of maternal blood and subcutaneous fat tissue were approved by the Institutional Review Board for human research at Louisiana State University Health Sciences Center – Shreveport (LSUHSC-S), Louisiana and the study was conducted in the Department of Obstetrics and Gynecology, LSUHSC-S. Written consent was obtained from all study subjects. Maternal venous blood was obtained either at the Perinatal Clinics or at Labor and Delivery Unit before delivery from 197 pregnant women: 84 from normotensive, 16 from pregnancy complicated with chronic hypertension, 11 from mild preeclampsia and 86 from severe preeclampsia. For blood collection, ethylenediamine tetraacetic acid (EDTA) was used as anticoagulant. Plasma sample was then extracted after centrifugation, aliquot and stored at −70 C until analyzed. Maternal subcutaneous fat tissue was collected during cesarean section delivery from 12 pregnant women, 6 from normotensive and 6 from preeclampsia. Freshly obtained subcutaneous fat tissue was fixed with 10% formalin and then embedded with paraffin. Normotensive pregnancy is defined as pregnancy with maternal blood pressure < 140/90 mmHg, absence of proteinuria and medical complications. Diagnosis of chronic hypertension in pregnancy is defined as either a documented history of high blood pressure antedating pregnancy or persistent elevation of blood pressure (≥140/90 mmHg) on two occasions more than 24 h apart before the 20th week of gestation. Mild preeclampsia is defined as follows: sustained systolic blood pressure ≥140 mmHg or a sustained diastolic blood pressure of ≥90 mmHg on two separate readings; proteinuria measurement of 1+ or more on dipstick, or 24 h urine protein collection with ≥300 mg in the specimen (after 20 weeks of gestation). Severe preeclampsia is defined when one or more of the following criteria is present: maternal blood pressure ≥160 mmHg or a sustained diastolic blood pressure ≥110 mmHg on two separate readings at least six hours apart; proteinuria ≥3+ on dipstick or ≥2 gram/24 h after 20 weeks of gestation; persistent headache or other cerebral or visual disturbances; persistent epigastric pain; or serum creatinine > 1.2 mg/dl. Smokers were excluded. None of the study subjects had signs of infection. To avoid clinical phenotypic differences in preeclamptic patients, patients complicated with HELLP syndrome (hemolysis, elevated liver enzyme and low platelet count), diabetes and/or renal disease were excluded. Clinical characteristics of study subjects whose plasma sample was tested are presented in Table 1 and whose subcutaneous fat tissue was used are present in Table 2.

2.2. Measurement of plasma concentrations of corin and pro-ANP

Maternal plasma concentrations of corin and stable N-terminal prohormone forms of ANP (pro-ANP) were determined by enzyme-linked immunosorbent assay (ELISA). DuoSet ELISA development kits for human corin (DY2209) and human pro-ANP (DY8247) were purchased from R&D Systems, Inc. (Minneapolis, MN). All assays were carried out according to the manufacturer's instruction. The range of the standard curve for corin was 15.6 to 4000 pg/ml and the range of the standard curve for pro-ANP was 15.6 to 2000 pg/ml. Plasma sample was 1:2 diluted for corin assay and assayed directly for pro-ANP. All samples were tested in duplicate and blinded to outcome. Within assay variations were < 7% for both assays.

2.3. Immunohistochemistry

Expression of ANP and its receptors NPR-A, NPR-B and NPR-C were examined using paraffin embedded subcutaneous fat tissue sections and placental villous tissue sections by a standard immunohistochemistry staining procedure. Antibody for ANP (AF3366) was obtained from R&D system (Minneapolis, MN), antibodies for NPR-A (sc-137041) and NPR-B (sc-293451) were obtained from Santa Cruz Biotechnology (San Diego, CA), and antibody for NPR-C (ab37617) was purchased from Abcam (Cambridge, MA), respectively. The antibody concentration used for the experiment was recommended by the manufacturers: 2 μg/ml for ANP, 4 μg/ml for both NPR-A and NPR-B, and 20 μg/ml NPR-C, respectively. Stained slides were counterstained with Gill's formulation hematoxylin. Tissue sections stained with isotype IgG or secondary antibody only served as negative control. Slides stained with the same antibody were processed at the same time. Stained slides were reviewed under an Olympus microscope (Olympus IX71, Japan), and images were captured by a digital camera and recorded into a microscope-linked PC computer.

2.4. Data analysis

Clinical demographic data is presented as mean ± SD. Data for plasma corin and pro-ANP concentrations were presented as mean ± SD ng/ml. Statistical analysis was performed with ANOVA or un-paired test by computer software Prism 5 (GraphPad Software, Inc. La Jolla, CA). Tukey's test was used as a post hoc test. A probability level < .05 was considered statistically significant.

3. Results

3.1. Patient clinical data

Patient clinical information including maternal age, racial status, gravida, body mass index (BMI), blood pressure, and gestational age at blood draw and delivery and delivery mode was obtained by chart review. The clinical characteristics for subjects whose plasma sample was assayed for corin and pro-ANP are shown in Table 1. Maternal age was higher in chronic hypertensive group than in normotensive pregnant group, but not different between mild or severe preeclamptic group vs. normotensive group. BMI was significantly higher in the chronic hypertensive and mild preeclamptic groups vs. normotensive and severe preeclamptic groups, but not different between severe preeclamptic vs. normotensive groups. Blood pressure was significantly higher in chronic hypertension, mild and severe preeclamptic groups vs. normotensive pregnant group. The demographic data for subjects whose subcutaneous fat tissue was used to determine ANP, NPR-A, NPR-B, and NPR-C expression are shown in Table 2.