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Advanced oxidation protein products enhances soluble Fms-like tyrosine kinase 1 expression in trophoblasts: A possible link between oxidative stress and preeclampsia☆

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ABSTRACT

Accumulation of advanced oxidation protein products (AOPPs) is prevalent in obesity, advanced maternal age, diabetes mellitus, and polycystic ovary syndrome. Alterations in the regulation and signaling of angiogenic pathways have been recognized as a link between these conditions and pre-eclampsia. To investigate the possible impact of AOPPs on soluble Fms-like tyrosine kinase 1 (sFlt-1) expression in trophoblasts. A trophoblast cell line (HRT-8/SVneo) was treated with various concentrations of AOPPs. The mRNA expression of sFlt-1, vascular endothelial growth factor (VEGF), and placental growth factor (PlGF) in trophoblasts were measured with the use of real-time polymerase chain reaction; and the secretion of sFlt-1, VEGF, and PlGF protein from trophoblasts were detected with the use of ELISA. Exposure of HRT-8/SVneo cells to AOPPs induced overexpression of sFlt-1 at mRNA and protein levels in a dose dependent manner. These effects could be inhibited by apocynin, an inhibitors of NADPH oxidase. Our data identified AOPPs as a class of important mediator in the regulation and signaling of angiogenic pathways of trophoblasts. Accumulation of AOPPs might contributes to the pathogenesis of preeclampsia by promoting sFlt-1 production in trophoblasts.

1. Introduction

Pre-eclampsia is a complex multi-system obstetric syndrome affecting about 5–10% of pregnant women and is characterized by hypertension and significant proteinuria at or after 20 weeks of pregnancy [1]. Alterations in the regulation and signaling of angiogenic pathways have been showed to contribute to the inadequate blood supply of the placenta in patients with pre-eclampsia [2,3].

Numerous studies have shown that trophoblast cells play important roles during placental development [4,5]. Placental trophoblasts are not only structural and biochemical barriers between the maternal and the fetal compartment during pregnancy, but also important for its capacity to generate angiogenesis and growth factors to promote vascularization of the placenta during its development.

Soluble Fms-like tyrosine kinase 1 (sFlt-1, also referred to as sVEGFR-1) is a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1 that lacks the transmembrane and cytoplasmic domains [6]. It is prominently produced by placental trophoblasts and is secreted into the maternal circulation [7]. sFlt-1 is an antagonist of VEGF and placental growth factor (PlGF) and may promote maternal endothelial damage and restriction of placental growth [8]. Abundant evidence suggests that increased trophoblast release of sFlt-1 causes the development of pre-eclampsia [9–11]. Maynard et al. [12] demonstrated hypertension, proteinuria, and edema in pregnant rats as a result of the administration of sFlt-1, suggesting that excess circulating sFlt-1 contributes to the pathogenesis of preeclampsia.

Advanced oxidation protein products (AOPPs) are a family of oxidized, dityrosine-containing protein products generated during excessive production of oxidants and often carried by albumin

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in vivo. The accumulation of plasma AOPPs is prevalent in diverse disorders such as diabetes [13], obesity [14], metabolic syndromes [15], and polycystic ovary syndrome [16]. Mounting evidences suggested that these population, when in pregnancy, have a higher risk of developing for preeclampsia. Most recently, we demonstrated that circulatory AOPPs are higher in patients with preeclampsia and associated with the severity of the disease [17].

Given its strong involvement in the pathogenesis of preeclampsia, attempts to identify factors that regulate sFlt-1 expression in trophoblasts have been made with the aim to investigate possible novel strategies for managing this condition. In the present study, we hypothesis that AOPPs may enhances sFlt-1 expression in trophoblasts and contributes to the pathogenesis of pre-eclampsia. The aim of the present study was to determine the effect of AOPPs on the expression of sFlt-1, VEGF and PlGF in trophoblasts.

2. Materials and methods

The Institutional Review Board at the Nanfang Hospital, Southern Medical University approved the study protocol and all procedures.

2.1. Cell culture

First trimester extravillous trophoblast cell line (HTR-8/SVneo cells) was a kind gift from Dr Charles H. Graham (Queen's University, Ontario, Canada). The cells were cultured in RPMI-1640 (HyClone, South Logan, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (HyClone), at 37 °C plus continuous supplement of 5% CO_2. Before experiments, HTR-8/SVneo cells were cultured in RPMI-1640 supplemented with 2.5% FBS for 18–24 h.

2.2. AOPPs preparation

AOPPs–human serum albumin (HSA) was prepared as described previously [18,19]. Briefly, HSA (100 mg/ml, Sigma, St. Louis, MO) was exposed to 200 mmol/L of HOCl (Fluke, Buchs, Switzerland) for 30 min and dialyzed overnight against PBS to remove free HOCl. The ratio of HSA to HOCL has been tested previously and the optimal ratio (HSA to HOCL = 1:134) was selected. The AOPPs preparation was passed through a Detoxi-Gel column (Pierce, Rockford, IL, USA) to remove any contaminated endotoxin. Endotoxin levels in the preparation were determined with the amebocyte lysate assay kit (Sigma) and were found to be below 0.025 EU/ml. AOPPs content in the preparation was determined as described previously. The content of AOPPs was 72.4 ± 9.8 nmol/mg protein in prepared AOPPs–HSA and 0.2 ± 0.02 nmol/mg protein in native HSA.

2.3. Intervention with cell cultures

HTR-8/SVneo cells were rinsed with phosphate-buffered saline solution, replenished with fresh serum-free media, and cultured for an additional 24 h. To evaluate the effect of AOPPs, the cells were incubated with serum-free medium and different concentrations of AOPPs-HSA (50, 100, and 200 μg/ml) and HSA (200 μg/ml). Moreover, previous studies showed that plasma AOPPs can induce cellular dysfunctions through NADPH-dependent mechanisms [18,20]. So in the present study, we further observed the effect of apocynin, an inhibitors of NADPH oxidase, on trophoblast in vitro and to explore whether apocynin may regulated the effect of AOPPs on the expression of sFlt-1, VEGF and PlGF in trophoblasts.

2.4. RNA extraction, reverse transcription, and real-time quantitative polymerase chain reaction

Total RNA was extracted from HTR-8/SVneo cells with the use of an RNeasy mini kit (Qiagen). One mg total RNA was reverse transcribed in a 20 ml volume with the use of Rever Tra Ace a (Toyobo) according to the manufacturer's instructions. Real-time quantitative polymerase chain reaction (PCR) and data analysis were performed using Lightcycler (Roche Diagnostic) according to the manufacturer's instructions. 2 μL cDNA in 20 μL volume was amplified with the use of oligonucleotide primers based on human sFlt-1, VEGF, and PlGF sequence. sFlt-1Primers (sense 5′-GCACCTTGGTTGTGGCTGACT-3′, antisense 5′-GGGCCCGGGGTCTCATTATT-3′) were used to amplify a 643-bp product; VEGF primers (sense 5′-CCCTGATGAGATCGAGTACATCT-3′, antisense, 5′-GCCTCGGCTTGTCACATTTT-3′) were used to amplify a 245-bp product; PlGF primers (sense 5′-GGGGAAGAGGAGGAGAGAGA-3′, anti-sense 5′-CTCTCACGTTGTTGAAGGCA-3′) were used to amplify a product of 268 bp. The PCR conditions for each mRNA were as follows. sFlt-1:45 cycles at 95 °C for 10 s, 63 °C for 10 s, and 72 °C for 27 s; VEGF:45 cycles at 95 °C for 10 s, 65 °C for 10 s, and 72 °C for 12 s; PlGF: 40 cycles at 95 °C for 10 s and 64 °C for 10 s. All PCRs were followed with melting curve analysis. Human glycerinaldehyde dehydrogenase (GAPDH) primers (Toyobo) were used to ensure RNA quality and amounts. The PCR conditions for GAPDH were 30 cycles at 98 °C for 10 s, 60 °C for 2 s, and 72 °C for 20 s. The Ct value of GAPDH is stable throughout the experiment. The expression level of each mRNA was normalized according to GAPDH levels in each case.

2.5. Measurement of protein secretions in culture supernatants

Conditioned culture media were centrifuged and stored at −80 °C until assay. Concentrations of sFlt-1, VEGF, and PlGF in supernatants were measured with the use of their specific ELISA kits (Quantikine; R&D Systems) according to the manufacturer's instructions.

2.6. Statistical analysis

All experiments were performed 3 times or more independently and all the values were expressed as the mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) with the application of the Dunnett's test, the least significant difference test, and a three-factor ANOVA classification. Differences were considered as statistically significant at $P < 0.05$.

3. Results

3.1. Effect of AOPPs on sFlt-1, VEGF and PlGF mRNA expression in HTR-8/SVneo cells

As shown in Fig. 1, exposure of HTR-8/SVneo cells to AOPPs induced significantly overexpression of sFlt-1 and VEGF at mRNA level in a dose dependent manner, while no significant increases of PlGF at mRNA level were observed (Fig. 1C).

3.2. Effect of AOPPs on sFlt-1, VEGF and PlGF secretion in HTR-8/SVneo cells

As concentrations increased, AOPPs significantly increased sFlt-1 protein level (Fig. 2A). Both VEGF and PlGF protein secretion levels were slightly higher, but not significantly increased by AOPPs (Fig. 2B, C).

3.3. Effect of apocynin on the expression of sFlt-1 in HTR-8/SVneo cells treated with AOPPs

Previous studies showed that plasma AOPPs exerts its wide-range cytotoxic effect through NADPH-dependent mechanisms [18,20]. So we further investigated whether apocynin (100 μmol/L), an inhibitors of NADPH oxidase, can regulate the angiogenic factors secreted by trophoblasts treated with AOPPs (200 μg/ml). Both sFlt-1 and VEGF mRNA expression and protein secretion in HTR-8/SVneo cells treated with AOPPs could be inhibited by apocynin, while no significant changes of PlGF at mRNA and protein level were observed.

4. Discussion

Normal placentation requires trophoblast invasion of maternal spiral arteries, in which trophoblast replaced the arterial media and transformed the uteroplacental circulation into a high-flow, low-resistance system [21]. Vascular remodeling occurs under the influence of several angiogenic factors, including VEGF and PlGF [3,9].

Mounting evidences suggested excess circulating sFlt-1, a potent binder and inhibitor of VEGF and PlGF, could impaired the placental angiogenesis, is increased in women destined to develop pre-eclampsia [3,7–9]. These findings suggest an important association between angiogenic factors and the pathogenesis of preeclampsia.

Increased recognition of trophoblasts dysfunction as a link between obesity [22], advanced maternal age [23], diabetes mellitus [24], polycystic ovary syndrome [25] and preeclampsia has highlighted the importance of determining mechanisms underlying the pathophysiological abnormalities in these populations. Here we