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Gelsolin is an endogenous inhibitor of syncytiotrophoblast extracellular vesicle shedding in pregnancy

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ABSTRACT

Background: Preeclampsia, a pregnancy-specific inflammatory disorder, is characterized by high levels of anti-angiogenic protein, soluble fms-like tyrosine kinase 1 (sFlt1), in the maternal circulation. sFlt1 producing molecular machinery is present in syncytiotrophoblast extracellular vesicles that are released by the placenta into maternal plasma during normal pregnancy, a process greatly accelerated in preeclampsia. We hypothesized that syncytiotrophoblast extracellular vesicles exposes cytoplasmic actin to plasma resulting in depletion of plasma gelsolin (pGSN), an abundant plasma protein that scavenges circulating actin and other pro-inflammatory mediators.

Objective: To test whether pGSN levels would be lower in preeclampsia and to assess whether recombinant human plasma gelsolin (rhpGSN) may promote placental health by decreasing shedding of syncytiotrophoblast extracellular vesicles.

Methods: We tested pGSN levels in third trimester plasma samples from women with preeclampsia and non-hypertensive pregnancies. We then assessed whether rhpGSN may act as a negative regulator of syncytial shedding in placental explant culture and dynamic mechanical stretch studies.

Results: pGSN levels fall in late pregnancy and decline further in preeclampsia patients. Recombinant human pGSN (rhpGSN) at 100 μg/ml limits spontaneous syncytiotrophoblast vesicle release and sFlt1 protein dissemination by normal placental explants. Higher rhpGSN doses (500 μg/ml) also limit syncytiotrophoblast vesicle and sFlt1 dissemination from preeclamptic placental explants. rhpGSN also mitigates syncytiotrophoblast vesicle during dynamic mechanical stretch.

Conclusions: 1) pGSN, an anti-inflammatory factor of maternal origin is reduced in preeclampsia and may contribute to disease progression and 2) exogenous rhpGSN supplementation can limit the dissemination of toxic syncytiotrophoblast vesicle that characterizes the disease state.

1. Introduction

Preeclampsia is a hypertensive disease of pregnancy that complicates 3–5% of pregnancies, characterized by endothelial dysfunction and widespread multi-organ dysfunction [1–3]. The disease is a leading cause of morbidity and mortality during pregnancy for both mother and fetus [4,5]. Preeclampsia arises from a stressed placenta that releases soluble factors into the maternal vasculature that in turn induces endothelial dysfunction and signs/symptoms of the disease [2]. Maternal constitutional factors, such as obesity and chronic hypertension are risk factors for preeclampsia that appear to enhance host susceptibility to toxic factors released by the placenta.

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The molecular nature of the placental toxic factors has been extensively studied. Recent evidence suggests high levels of anti-angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt1) of placental origin, contribute to the disease process [6–9]. sFlt1, a soluble factor acting by neutralizing endothelial growth factors, is progressively and continuously higher throughout the course of many preeclamptic pregnancies compared to normal pregnancies [8,10,11]. Animal studies have shown that exogenous administration of sFlt1 leads to preeclampsia-like features [12–14].

Along with sFlt1 release, preeclampsia is also characterized by the systemic release of necrotic, apoptotic or aponecrotic syncytiotrophoblastic material (STBM) (also referred to as syncytiotrophoblast extracellular vesicles) from the placenta in pathologically high volumes and rates compared to normal pregnancy [15–21]. Notably, the size and the profile of proteins present within these extracellular vesicles in preeclampsia are also significantly altered as compared to normal pregnancy [20,22–24]. Interestingly, STBM has recently been characterized to contain Flt1 protein [25] and mRNA machinery capable of *de novo* gene transcription and translation [26]. Limiting STBM release and its cargo of active anti-angiogenic proteins may therefore be a potential mechanism to limit the progression of preeclampsia.

Plasma Gelsolin (pGSN) is a 84 kDa secreted isoform of a cytoplasmic actin-binding protein involved with apoptosis, cytoskeletal rearrangement, cell motility and cell shape changes [27]. Many cells secrete pGSN, and it circulates in high concentrations in human plasma (190–300 mg/L) [27–29]. pGSN blood levels fall in numerous acute and chronic inflammatory states, including trauma, burns, sepsis, rheumatoid arthritis and chronic kidney disease. The magnitude of its reduction parallels the extent of tissue damage [30–32]. A critical degree of pGSN depletion precedes and predicts adverse clinical outcomes including death [31,33]. Repleting pGSN in many rodent injury models prevents such outcomes [34–36]. Of relevance to preeclampsia are reports suggesting a physiologic role for pGSN in human syncytiotrophoblast and placental health [37–39], including one that documented decreased pGSN levels in preeclamptic patients compared to control pregnancies [39].

We report here confirmation that pGSN is reduced in preeclampsia compared to pregnant and non-pregnant states. We then tested whether exogenous administration of recombinant human pGSN (rhpGSN) would promote placental health by decreasing the shedding of STBM and subsequent release of sFlt1 protein in both normal placental and preeclamptic tissue explants. Having documented that dynamic stretching of various organs stimulates cell microparticle release [40], we also examined the effect of periodic dynamic stretch on placental explants in culture and whether rhpGSN influences syncytiotrophoblast vesicle shedding.

2. Materials and methods

2.1. Materials

Recombinant human plasma gelsolin (rhpGSN) was manufactured by fermentation in *Escherichia coli* and was provided by BioAegis Therapeutics, Inc. This recombinant protein has been used in prior animal studies [34].

2.2. Human studies

Plasma samples were collected from normal and preeclamptic patients who delivered at the Beth Israel Deaconess Medical Center. The institutional review boards at the Beth Israel Deaconess Medical Center approved these studies and subjects gave informed consent. Preeclampsia was defined by criteria published by American College of Obstetrics and Gynecology [41]. Approximately 5 mL of EDTA blood were collected from subjects prior to delivery via venipuncture, centrifuged at 3500*g* for 10 min, and the plasma was collected and stored at −80 °C without thaw before analysis. Clinical information for the subjects in the plasma study is included in Table 1. For placental studies, discarded placental tissue was obtained following C-section. Biopsies were obtained from discarded placental tissue within 30 min of delivery. Following removal of decidual tissue, several villous biopsies (2 cm^3 samples from the central cotyledon of the placenta) were excised from the maternal surface midway between the chorionic and basal plates. Villous tissue was cut to 0.5 cm and rinsed twice in 50.0 mL of ice-cold PBS for 2 min. After rinsing, villous tissue was used in explant cultures. Clinical characteristics for the subjects that contributed placenta are included in Table 2.

2.3. STBM preparation

Using a 12-well Netwell culture system as described previously [26], placental villous explant culture was conducted in 3 mL M199 culture for 72 h on a rotational rocker. Following culture, explants were weighed and flash frozen and media was collected for STBM isolation. Conditioned medium was centrifuged for 10 min at 800*g* at room temperature. The top 1 mL was saved for further analysis (high-speed STBM), the middle 1 mL was discarded, and the bottom 1 mL (low-speed STBM) was centrifuged at 800*g* and pellet

Table 1
Characteristics of study subjects (plasma study).

	Non-Preeclampsia	Preeclampsia
	N = 17	N = 17
Maternal age (years)	30.6 ± 6.4	30.4 ± 6.0
Maternal Body Mass Index	24.4 ± 5.0	25.8 ± 5.8
Race	47% Caucasian 29% African American 12% Asian 12% others	47% Caucasian 35% African American 12% Asian 6% others
Gestational Age at Delivery (weeks)	36.0 ± 4	33.4 ± 4
Highest Blood Pressure		
Systolic in mmHg	120.5 ± 12.2	158.2 ± 18.6**
Diastolic in mmHg	75.3 ± 7.7	100.8 ± 6.3**
Birth weight (g)	2501.5 ± 758.3	1950.9 ± 771.2*

Data presented as Mean ± SD.
* $p < 0.05$.
** $p < 0.0001$.

Table 2
Characteristics of study subjects (placenta study).

	Normal pregnancy N = 18	Preeclampsia N = 9
Maternal age (yrs)	33.6 ± 5.5	31.0 ± 4.9
Maternal Body Mass Index	25.2 ± 4.2	27.3 ± 8.1
Race	56% Caucasian 6% African American 12% Asian 26% others	88% Caucasian 12% African American
Gestational age at delivery (weeks)	39.0 ± 1.0	34.6 ± 3.5*
Highest Blood Pressure		
Systolic in mmHg	122.3 ± 10.3	168.3 ± 16.8**
Diastolic in mmHg	77.7 ± 10.0	102.4 ± 8.0**
Birth weight (g)	3258.3 ± 439.8	2195.9 ± 878.8*

Data presented as Mean ± SD.
* $p < 0.05$.
** $p < 0.0001$.

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