HYPERTENSION/PHYSIOLOGY

Abstracts 18 – 26

Moderators: Baha Sibai, MD; Cathy Spong, MD

18 Regulation of placental and renal hypoxia gene expression by VEGF121 therapy in a mouse model of preeclampsia induced by sFlt-1 overexpression

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OBJECTIVE: HIF-1α and TGFβ-3 expression is upregulated while CGM1 expression is downregulated under hypoxic conditions. We aimed to determine the effect of $VEGF_{121}$ therapy on the expression of these genes in the placenta and kidney in an animal model of preeclampsia induced by overexpression of sFlt-1.

STUDY DESIGN: At day 8 of gestation, CD-1 mice were randomly allocated to subcutaneous insertion of osmotic minipumps prepared with $VEGF_{121}$ (n=8) or phosphate buffered-saline solution (PBS) as a solvent-control (n=4). Pumps were calibrated to deliver 400μg/kg/day or equivalent PBS for 10 days. At day 9, $VEGF_{121}$ mice were randomly allocated to tail vein injections with Adv-sFlt-1 (10^9 PFU) or mFc (10^9 PFU) as virus-control (n=4/group). PBS-mice were treated with Adv-sFlt-1 (10^9 PFU). Animals were sacrificed on day 18. mRNA expression of HIF-1α, TGFβ-3, and GCM1 was measured by real time polymerase chain reaction (RT-PCR). Kruskal-Wallis test was used for statistical analysis (significance: $p<0.05$).

RESULTS: Placental HIF-1α expression was significantly higher in the PBS-sFlt-1 mice than in the VEGF-sFlt-1 and the VEGF-mFc mice (relative expression (RE) 2.38 $\pm$ 0.39 vs 0.88 $\pm$ 0.26 and 1.05 $\pm$ 0.09; $p=0.01$). Placental TGFβ-3 expression level was higher in the PBS-sFlt-1 mice as compared to the VEGF-sFlt-1 mice (RE 2.22 $\pm$ 0.58 vs 0.69 $\pm$ 0.18; $p=0.04$). Placental and renal GCM1 expression levels were significantly higher in the VEGF-mFc than in the PBS-sFlt-1 mice (RE 4.81 $\pm$ 0.74 and 2.49 $\pm$ 0.46 vs 2.14 $\pm$ 0.03 and 0.69 $\pm$ 0.22; $p<0.05$). Renal and placental GCM1 and renal HIF-1α expression did not differ significantly between the PBS-sFlt-1 and the VEGF-sFlt-1 mice.

CONCLUSIONS: $VEGF_{121}$ effectively reversed the changes in several hypoxia-related genes in the placenta. Our findings confirm that angiogenic imbalance plays a role in preeclampsia. Therapy with pro-angiogenic factors has the potential to improve placental function.

19 Maternal insulin resistance and preeclampsia

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OBJECTIVE: Insulin resistance (IR) is a hallmark of obesity and obesity is a consistent risk factor for preeclampsia. Our objective was to determine whether midtrimester maternal IR is associated with subsequent preeclampsia.

STUDY DESIGN: This is a secondary analysis of a randomized controlled trial in 10,154 low-risk nulliparous women administered vitamin C and E or placebo daily from 9-16 weeks' gestation until delivery. Of these, 1,187 women had fasting plasma glucose and insulin tested between 22 and 26 weeks' gestation. IR was calculated by the homeostasis model assessment (HOMA-IR) derived from fasting plasma insulin (I) and glucose (G) values ((IxG/22.5)). Univariate and multivariate analyses controlling for maternal body mass index, race, treatment group, enrollment blood pressure and gestational age at sampling are presented.

RESULTS: Eighty-five women developed preeclampsia and 592 remained normotensive without proteinuria. Fasting maternal G, I and HOMA-IR were significantly higher among those who subsequently developed preeclampsia compared with women who remained normotensive ($p \leq 0.01$). Women with a mid-gestation fasting G, I, or HOMA-IR $\geq$ the 75th percentile were 1.5 to 1.9 fold more likely to develop preeclampsia (Table). Multivariate analyses confirmed midtrimester fasting I and HOMA-IR at $\geq$ the 75th percentile to be associated with preeclampsia. A HOMA-IR at $\geq$ the 75th percentile had a sensitivity of 40% for subsequent preeclampsia with a 25% false positive rate in normotensive women without proteinuria.

CONCLUSIONS: Maternal IR is associated with a significantly increased risk of subsequent preeclampsia.

Table. Measures $\geq$ 75th %ile

Measure	PreE (%) N=85	Normal (%) N=592	OR	Adjusted OR
G	37.6	26.5	1.7 [1.0-2.7]	1.5 [0.9-2.5]
I	40.5	25.3	2.0 [1.3-3.2]	1.8 [1.0-3.1]
HOMA-IR	40.5	24.8	2.1 [1.3-3.3]	1.9 [1.1-3.2]

PreE = preeclampsia

20 Improvement of uterine artery resistive index and blood pressure in response to an Endothelin type A receptor antagonist in a rat model of preeclampsia

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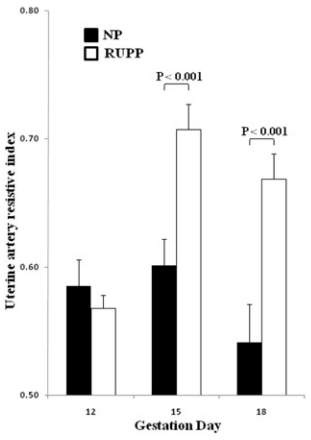
OBJECTIVE: To determine the effect of an Endothelin type A receptor antagonist (ETA) on uterine artery resistive index (Ut RI) and blood pressure in a rat model of placental ischemia produced by Reduction in Uterine Perfusion Pressure (RUPP).

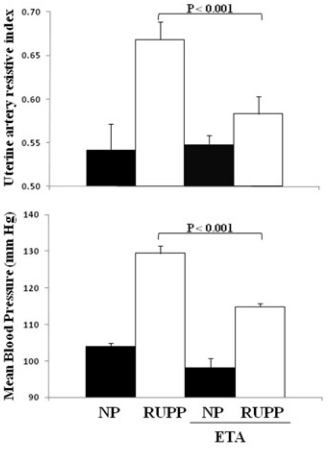
STUDY DESIGN: Power Doppler velocimetry measurements were performed on anesthetized pregnant Sprague Dawley rats with a Vevo 770 unit using a 30 MHz transducer and an insonating angle <30°. Ut RI was determined for the uterine artery bilaterally at three levels and the mean Ut RI was calculated. Ut RI was measured in the RUPP and normal pregnant controls (NP) on gestation days (GD) 12, 15 and 18. Ut RI was also determined on GD 18 in NP and RUPP dams after pretreatment with ETA. RUPP procedure was done on GD 14 with chronic constriction of the lower abdominal aorta above the iliac bifurcation (0.203 mm clip) and both ovarian arteries (0.100 mm clip). Pregnant dams treated with ETA received the agent in their drinking water (5mg/Kg/day) on GD 12 -19. The rats were instrumented with a carotid catheter for mean arterial pressure measurement (MAP) on GD 19.

RESULTS: Ut RI in NP and RUPP groups were 0.59+/−0.02 vs. 0.57+/−0.01 (P = 0.423), 0.60+/−0.02 vs. 0.71+/−0.02 (P = <0.001) and 0.54+/−0.03 vs. 0.67+/−0.02 (P = <0.001) on GD 12, 15 and 18 respectively. MAP in the NP and RUPP groups were 104+/−1 and 129+/−2 mm Hg, respectively (P = <0.001). Pretreatment with ETA attenuated both the MAP and GD 18 Ut RI in the RUPP group (115+/−1 mm Hg (P = <0.001); 0.58+/−0.02 (P = <0.001)) without affecting these parameters in the NP group (98+/−2 mm Hg (P = 0.054); 0.55+/−0.02 (P = 0.150)).

CONCLUSIONS: The improvement in uterine artery resistive index could be one potential mechanism for the reduction in blood pressure in response to an Endothelin type A receptor antagonist in placental ischemic pregnant rats.



Uterine artery resistive index variation by gestation day in NP and RUPP rats.



Uterine artery resistive index and mean arterial blood pressure in NP and RUPP with and without ETA pretreatment.

21 Novel insights into the mechanisms responsible for the up-regulation of the soluble receptor for advanced glycation end-products (sRAGE) in severe preeclampsia (sPE)

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OBJECTIVE: Activation of the pattern recognition cell surface receptor, RAGE, plays a key role in mediating oxidative and inflammatory cellular injury. sRAGE is a pleiotropic endogenous RAGE antagonist generated either by alternative splicing of RAGE mRNA (endogenous secretory RAGE: esRAGE) or by cleavage of the extracellular domain of the receptor. Our aim was to provide insight into the mechanisms responsible for the increased systemic and amniotic fluid (AF) levels of total sRAGE observed in sPE.

STUDY DESIGN: In a case-control study we analyzed maternal serum samples from 81 singleton pregnancies grouped as: **i)** sPE (n=37, GA median [IQR]: 30 [27-32] wks); **ii)** healthy pregnancies delivered at term (CRL, n=31, GA: 30 [26-32] wks); **iii)** chronic hypertension (crHTN, n=13, GA: 32 [28-35] wks). There was no significant GA difference among groups at enrollment. AF was retrieved from 24 sPE women at the time of surgical delivery. For comparison we used 24 GA-matched AF samples from women without AF infection who delivered at term. Total sRAGE and esRAGE were measured by specific ELISAs. Placental villous tissue and amniochorion from 10 sPE cases and 10 GA-matched women with idiopathic preterm birth were obtained at delivery. Real-time qPCR was used to evaluate mRNA expression of the full-length RAGE and esRAGE.

RESULTS: **1)** sPE women had significantly higher maternal serum total sRAGE and esRAGE compared to CRLs and crHTN even after correction for hematocrit ($P<0.001$); **2)** AF total sRAGE and esRAGE levels were elevated in sPE ($P<0.001$); **3)** In CRLs, the contribution of esRAGE to total sRAGE was higher in blood compared to AF (25% vs. 15%, $P<0.001$), a proportion that further increased in sPE; **4)** Relative to full-length RAGE receptor, sPE women had a significant elevation in esRAGE transcript in amniochorion ($P=0.032$) but not placental villous tissue ($P=0.975$).

CONCLUSIONS: High maternal serum and AF esRAGE levels paralleled by elevated esRAGE expression in amniochorion implies that transcription plays a critical role in modulating the activity of the RAGE system in sPE.

22 Effect of postnatal angiotensin-converting enzyme inhibition on fetal programming of adult vascular function in mice lacking endothelial nitric oxide synthase

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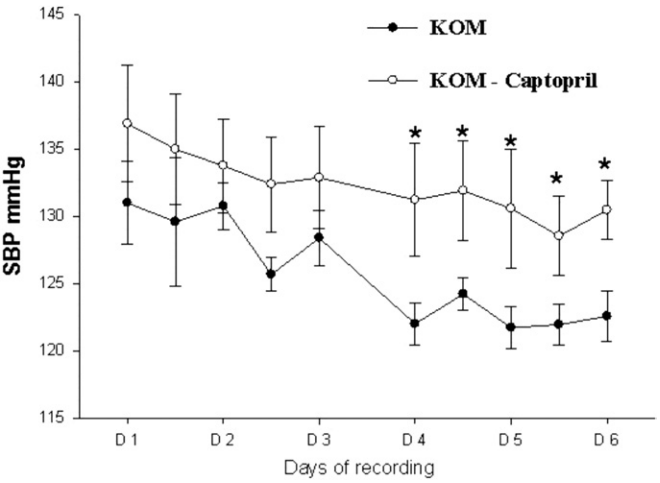
OBJECTIVE: Heterozygous offspring born to transgenic mice lacking endothelial nitric oxide synthase have altered fetal programming of vascular function, and develop hypertension later in life. Angiotensin converting enzyme inhibitors have been used to prevent progression of hypertension and vascular remodeling. Our objective was to determine whether captopril prevents the fetal programming of adult hypertension in the previously characterized animal model.

STUDY DESIGN: Homozygous NOS3 knockout (KO) female and wild type male mice (WT) were bred to produce heterozygous (KOM), male offspring were used. At 4 week of age, osmotic minipumps were inserted subcutaneously, and set to deliver captopril or vehicle at a rate of 0.25 μl/hr for 4 weeks. At 14 weeks of age blood pressure (BP) catheters were inserted through the left carotid artery into the aortic arch and BP was recorded continuously for 7 days in the conscious unrestrained offspring using a telemetry system. Mean BP (MBP), pulse pressure (PP), systolic BP (SBP) and diastolic BP (DBP) were analyzed. Student t-test was used for statistical analysis ($p<0.05$ denotes significance).

RESULTS: Offspring weight was similar between the 2 groups. KOM offspring treated with captopril had significantly higher MBP, SBP, and PP compared with KOM control from day 4 to day 6 of recording (Figure, $p<0.05$). DBP was increased only on the last day of recording. HR was not different between the 2 groups.

CONCLUSIONS: Captopril treatment in the early postnatal period worsens rather than prevents the hypertension in an animal model of utero-placental insufficiency. We speculate that angiotensin is important in early vascular and renal development.



23 Placenta macrophages of obese women originate from infiltrating maternal monocytes

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OBJECTIVE: Pregnancy is low-grade inflammatory condition manifested as metabolic dysfunction of maternal adipose tissue and placenta. In obesity, the accumulation of macrophages in the placenta contributes to amplification of the inflammation at the maternal-fetal