

January 15th, 2009

Final report for the Preeclampsia Foundation Vision Grant 2007 by Frauke von Versen-Hoeynck

I would like to thank the Preeclampsia Foundation for giving me the opportunity to work on my research project "The role of the hypoxia-inducible signal adenosine in placental amino acid transport" by providing me with funding money. The following provides a description of the proposed work and the work that has been accomplished over the last year. The work resulted in a manuscript that was submitted to the journal "Placenta" and is under review now as well as in a poster presentation at the 16th Conference of the International Society for Hypertension in Pregnancy (Washington, D.C., 9/2008) and the Annual Meeting of the Society for Gynecologic Investigation (Glasgow, UK, 3/2009).

a) Scientific Report:

Abnormal placental bed vascular pathology with reduced nutrient and oxygen delivery to the intervillous space is hypothesized to contribute to suboptimal fetal growth in IUGR and preeclamptic pregnancies. Recent studies indicate that a variety of signals such as adenosine are produced in response to hypoxia in tissues and are higher in women with preeclampsia. Amino acids are an important nutrient during fetal development and their placental uptake by Na⁺ dependent amino acid transport systems depends on Na⁺/K⁺ ATPase enzyme activity, which can be modified by adenosine. To date there is little information about the role of adenosine in preeclamptic pregnancies and the placenta. Therefore, the hypothesis of the proposal was that the hypoxia-inducible signal adenosine is involved in the regulation of human placental System A amino acid transport. We proposed two specific aims:

1. We will determine the presence of adenosine receptor subtypes A₁, A_{2A}, A_{2B} and A₃ in placental tissue.
2. We will investigate the role of adenosine receptor activation and inhibition to analyze the effect of these mechanisms on placental amino acid transport and Na⁺/K⁺ ATPase activity during placental normoxia (8% oxygen), hypoxia (2% oxygen) and hyperoxia (21% oxygen).

The funding of the project began in January 2008. I have been working on specific aim 1 and determined if adenosine receptor subtypes A₁, A_{2A}, A_{2B} and A₃ are present in placental tissue. After standardization of the methods we got the following results.

Human placenta expresses all known adenosine receptors, A₁, A_{2A}, A_{2B} and A₃

The pattern of adenosine receptor distribution was examined by immunocytochemistry of placentas from uncomplicated pregnancies. A₁, A_{2A}, A_{2B} and A₃ immunostaining was observed at different intensities in trophoblast cells (localized by cytokeratin 18 staining), with high immunoreactivity for the A_{2A} and A_{2B} receptors, and low immunoreactivity for the A₁ and A₃ receptors. All four adenosine receptors were present on endothelial cells (localized by CD31 staining). The A₁, A_{2A} and A₃ receptors were also co-localized with fibroblasts and myofibroblast surrounding the blood vessels and filling the interstitial space of the placental villous tree (localized by α -actin staining). However, the A_{2B} receptor was not detected in fibroblasts or myofibroblasts.

Adenosine receptor A₁, A_{2A}, A_{2B} and A₃ protein expression is higher in preeclampsia

Adenosine receptor protein was analyzed by western blot analysis on placental biopsies. The A₁, A_{2A}, A_{2B} and A₃ adenosine receptors were present in placental homogenate as verified by immunoreactive protein bands at molecular weights of 36, 45, 52 and 36 kDa, respectively. All four adenosine receptor subtypes were significantly higher in the placentas of PE and PE & SGA when compared to uncomplicated pregnancies (NP), (Figure). The receptor protein concentration of A₁, A_{2A}, A_{2B} and A₃ were 1.52 fold (1.39-2.42, P=0.046), 1.71 fold (1.4-2.02, P=0.046), 2.52 fold (1.15-3.41, P=0.03) and 2.36 fold (1.81-3.42, P=0.03) higher in PE compared to NP, respectively. Subjects with PE & SGA had a 1.65 fold (0.8-2.15, P=0.17), 1.92 (1.24-2.49, P=0.03), 2.16 fold (1.32-4.8, P=0.046) and 2.13 fold (1.19-4.04, P=0.046) higher concentration of the A₁, A_{2A}, A_{2B} and A₃ adenosine receptors compared to NP, respectively. The amount of the adenosine receptors was not different in placentas of women with SGA infants without preeclampsia compared to uncomplicated pregnancies (P=0.35; 0.25; 0.42; 0.25).

Adenosine receptor A₁, A_{2A}, A_{2B} and A₃ mRNA expression is higher in preeclampsia

To determine whether the increases in A₁, A_{2A}, A_{2B} and A₃ receptor protein were associated with an increase in mRNA encoding the A₁, A_{2A}, A_{2B} and A₃ receptor, mRNA content was determined with quantitative real-time RT-PCR of mRNA from placental biopsies. The expression of the adenosine receptors was normalized to the expression of two endogenous references (18S, TBP) in each sample. Results were similar whether 18S or TBP was used as endogenous control. In placentas of preeclamptic women, the expression of the A₁, A_{2A}, A_{2B} and A₃ receptors was elevated 1.25 fold (1.08-1.47, P=0.046), 1.98 fold (1.68-2.55, P=0.03), 1.77 fold (1.34-2.07, P=0.03) and 2.24 fold (1.57-5.74, P=0.046) compared to uncomplicated pregnancies, respectively. In preeclampsia with SGA, A₁, A_{2A}, A_{2B} and A₃ receptor expression was elevated 1.14 fold (1.05-1.37, P=0.03), 2.38 fold (1.73-3.33, P=0.046), 1.89 fold (1.32-2.59, P=0.03) and 1.23 fold (1.15-1.58, P=0.046). By contrast, in placentas of women with SGA infants without preeclampsia receptor expression was not significantly different from uncomplicated pregnancies (P=0.91; 0.12; 0.17; 0.75).

Hypoxia increases placental expression of the A_{2A} receptor

To determine whether adenosine receptor protein and mRNA expression are influenced by hypoxia, we incubated placental villous explants for 24 h at 21% or 2% oxygen. In vitro hypoxia increased the protein amount of the A_{2A} receptor 1.46 fold (1.33-1.48, P=0.046) and mRNA expression 1.32 fold (1.25-1.61, P=0.046) in explants from uncomplicated pregnancies compared to paired samples exposed to 21% oxygen. Elevations in HIF-1 α were measured to confirm the effect of hypoxia on placental explants. HIF-1 α protein increased 2.3 fold in villous explants from uncomplicated pregnancies exposed to 2% oxygen compared to paired samples exposed to 21% oxygen. Protein and mRNA for the A_{2A} receptor were 1.25 fold (1.11-1.35, P=0.17) and 1.1 fold (1.02-1.32, P=0.07) higher in preeclamptic placentas, after exposure to 2% oxygen compared to paired explant samples exposed to 21% oxygen. The expression of the A₁, A_{2B} and A₃ adenosine receptors was not significantly affected by exposure to hypoxia.

Implications: Our localization findings suggest possible roles for adenosine in the regulation of placental functions such as nutrient transport and placental hemodynamic control. As the placenta relies entirely upon circulating and locally produced vasoactive substances for vascular control the detection of vasoactive adenosine and its receptors within placental blood vessels is likely of physiological significance. Further functional studies are warranted to increase our understanding of the complex molecular regulation underlying the actions of adenosine in the placenta.

Future goals: After working as a postdoctoral fellow at Magee Womens Research Institute of the University of Pittsburgh I accepted a position at the Department of Obstetrics and Gynecology, Medizinische Hochschule Hannover, Germany, starting March 1st, 2009. There I will continue my clinical training and keep working on research projects. I am planning on continuing to work on Specific Aim 2. I will submit a grant application to the German Research Foundation to acquire funding for future projects.

b) Non-Technical report:

Changes during the development of the placenta, the tissue that delivers oxygen and nutrients to the baby, can lead to reduced growth of the baby or the development of preeclampsia, a disorder with high blood pressure and excretion of protein in the urine of the mother. The transport of amino acids, nutrients necessary for the production of protein, from the mother to the baby is very important for baby's growth. Reduced oxygen concentrations and the production of several factors such as adenosine might influence the transport of amino acids. Therefore the focus of this proposal was to investigate the role of adenosine in the transport of amino acids in the placenta.

The first aim was to determine if adenosine receptor subtypes A₁, A_{2A}, A_{2B} and A₃ are present in placental tissue. After standardization of the method we used 3 different types of placental preparations: whole placental tissues, placental villous explants (2mm² big pieces of trophoblast containing tissue) and microvillous membranes (MVM, cell membranes of the trophoblast). We found all four adenosine receptors to be present in the 3 placental preparations when we used Western Blotting. Since we found all adenosine receptors to be expressed on MVM this suggests that they are important in regulating functions of the syncytiotrophoblast the cell type in the placenta that is involved in nutrient and oxygen transport. We also used immunofluorescent staining to detect adenosine receptors on placental sections and found them to be present on trophoblast, endothelial and fibroblast cells.

In addition to the proposed work for aim 1 we looked at the amount of receptors in placentas of women with uncomplicated pregnancies, preeclamptic pregnancies with and without small for gestational age babies and women with growth restricted babies without preeclampsia. Our results show an increased amount of all four adenosine receptor subtypes in placentas of women with preeclampsia with and without small babies.

Moreover we were also interested in the effect of reduced oxygen on the amount of adenosine receptors since reduced oxygen delivery to the placenta is proposed to be involved in the pathophysiology of preeclampsia. We incubated placental villous explants under normal oxygen (20%) and reduced oxygen (2%). Here we found a higher expression of the A_{2A} receptor after exposure to hypoxia.

Our preliminary data suggest a role of adenosine receptors in pregnancy and especially in pregnancies where reduced oxygen concentrations are involved in the pathophysiology.

Figure

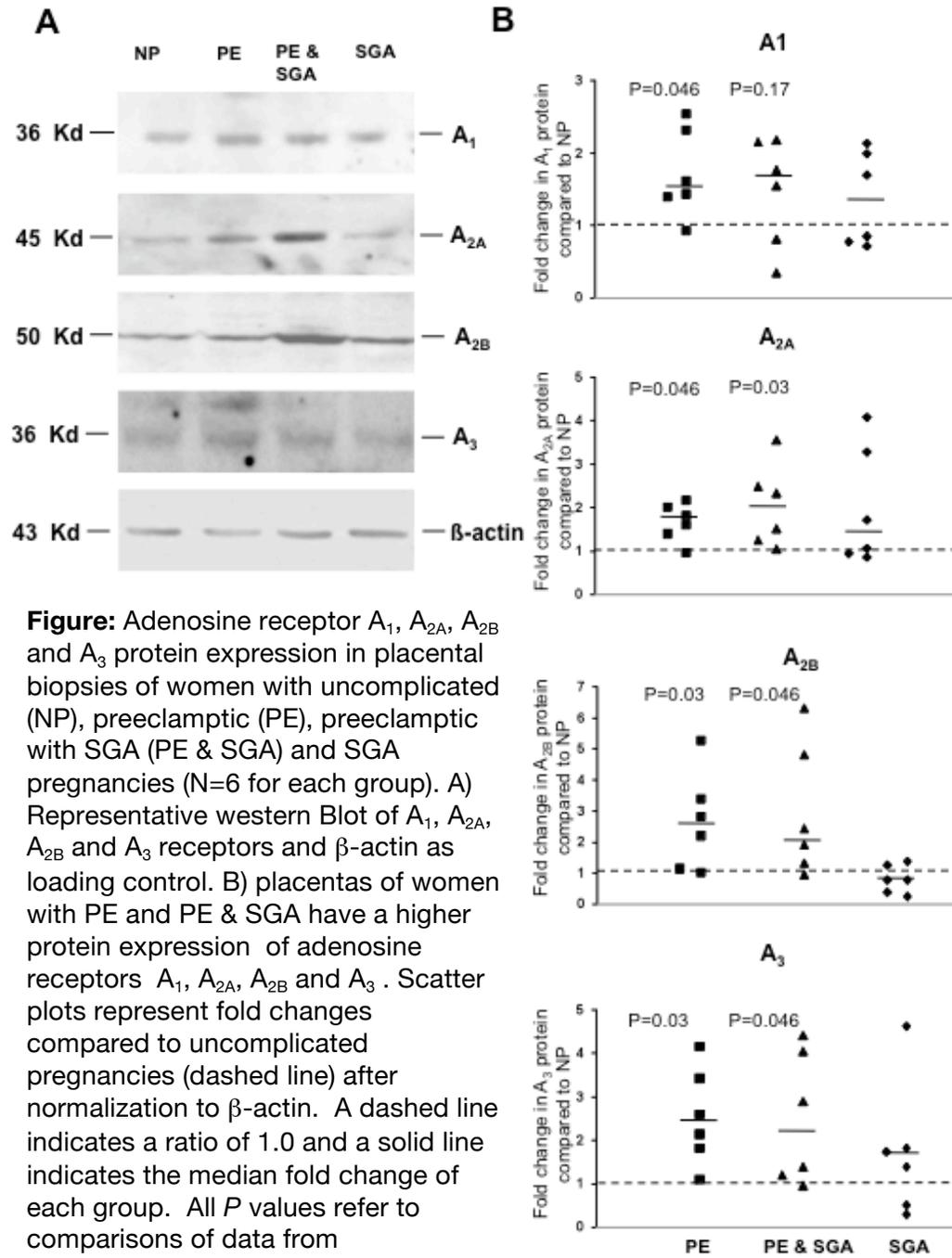


Figure: Adenosine receptor A₁, A_{2A}, A_{2B} and A₃ protein expression in placental biopsies of women with uncomplicated (NP), preeclamptic (PE), preeclamptic with SGA (PE & SGA) and SGA pregnancies (N=6 for each group). A) Representative western Blot of A₁, A_{2A}, A_{2B} and A₃ receptors and β-actin as loading control. B) placentas of women with PE and PE & SGA have a higher protein expression of adenosine receptors A₁, A_{2A}, A_{2B} and A₃. Scatter plots represent fold changes compared to uncomplicated pregnancies (dashed line) after normalization to β-actin. A dashed line indicates a ratio of 1.0 and a solid line indicates the median fold change of each group. All P values refer to comparisons of data from uncomplicated pregnancies.