Regulators of Complement System Activation Change with Placental Ischemia-induced Hypertension in Rat

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Background: Preeclampsia is characterized by new onset hypertension, reduced placental perfusion, and increased activation of the complement system, part of the innate immune system. It remains the leading cause of mortality and morbidity in mother and fetus, complicating 2-8% of pregnancies (Steegers et al, 2010). This condition results in decreased blood flow to the placenta due to abnormal remodeling of uterine arteries. This can lead to lower birth weight and preterm birth. Besides administering medication to reduce blood pressure with possible adverse effects on the fetus, there is no therapy except delivery of the placenta (Steegers et al, 2010).

Dr. Jean Regal's lab is currently conducting research on the role of the complement system, a part of the innate immune system, in the development of pregnancy-induced hypertension. The complement system is present in blood and can be activated via three pathways, but all pathways ultimately lead to activation through the protein C3, which is cleaved into activation products C3a and C3b. C3a can circulate in blood throughout the body, whereas C3b is able to covalently bind to tissue, i.e. C3 deposition (Regal et al, 2015). Cleavage of C3 can lead to cleavage of C5 into C5a and C5b, and so on. Continued activation of the pathway through C9 can lead to a C5b-9 membrane attack complex that can form a membrane pore and lead to cell lysis (Regal et al, 2015).

Placental ischemia-induced hypertension is simulated by reduced utero-placental perfusion pressure (RUPP) surgery in pregnant rats, which is performed on day 14 of a 21 day gestation and involves placement of silver clips on the aorta and ovarian arteries. This reduces

blood flow to placenta and increases blood pressure in the mother (Li et. al, 2012). Increased complement system activation occurs in women with preeclampsia and in RUPP rats, and blocking of complement system activation in RUPP rats prevents the development of high blood pressure (Lynch et. al, 2010; Lillegard et. al, 2013). In this model circulating C3a increases, suggesting either increased activation of complement through C3 and/or insufficient changes in endogenous regulators to limit complement activation.

The net complement activation occurring can be thought of as a balance between how much the pathway is activated by stimuli and dampening of the pathway by endogenous regulators. In human, complement regulators CD55 and CD59 have been shown to increase in placenta of preeclamptic pregnancies compared to normal pregnancies, indicating upregulation to control excessive complement activation (Buurma et al, 2012). In addition, excessive complement activation in kidney has been demonstrated in preeclampsia (Penning et al, 2015). **Thus, we hypothesized that increased complement activation following placental ischemia in rat leads to an increase in complement regulators in kidney and placenta.**

Methods: On gestation day (GD) 14, rats underwent either Sham surgery or clip placement on ovarian arteries and abdominal aorta to reduce uterine perfusion pressure (RUPP) resulting in increased maternal blood pressure on GD19. On GD19, mean arterial pressure (MAP) was measured via arterial catheter, and serum and plasma collected. Serum C3a and soluble C5b-9 were measured as indicators of systemic complement activation, and to what extent the activation of the pathway was occurring. Circulating C3a was measured in serum by Western Blot. C3a concentration was expressed relative to a pool of yeast activated complement used as

standard. Soluble C5b-9 was measured in plasma using an ELISA assay (Hycult Biotech). C3 deposition was also measured using immunohistochemistry, to quantify the amount of local complement activation occurring in each tissue. Frozen tissue sections of placenta and kidney were stained using goat anti-rat C3 (MP Biochem, 55713), followed by fluorescent secondary antibody Alexa Fluor 594 donkey anti-goat (Jackson Immunoresearch) Staining was graded by a blinded observer from 0-3, negative to strongly positive.

Membrane bound complement regulators investigated included: 1) CD55 and Crry which limit complement activation through C3 and C5, and 2) CD59 which limits formation of C5b-9 membrane attack complex. Kidney cortex and placenta were collected and mRNA was extracted using RNeasy Mini Kit (QIAGEN). Finally, quantitative RT-PCR was used to measure mRNA for complement regulators CD55, CD59, and Crry using β-actin as an internal control. Efficiency of the amplification of message for each primer was also tested to ensure it was similar to the efficiency of amplification for the control, β-actin. Lastly, products of the quantitative RT-PCR were purified and sequenced to ensure that Crry, CD55, and CD59 were indeed being measured.

Results: As expected, MAP significantly increased ($p < 0.05$) in RUPP (109±3 mmHg, n=9) vs. Sham (95±3 mmHg, n=7) animals, with a corresponding increase in C3a (0.49±0.14 units/ul, RUPP; 0.22±0.09 units/ul, Sham). Soluble C5b-9 did not significantly differ in plasma of RUPP vs. Sham animals. In placenta, mRNA for Crry and CD59 significantly decreased, and message for CD55 did not significantly change with placental ischemia (Figure 1). In placenta, C3 deposition significantly increased following placental ischemia (Figure 2). In kidney cortex CD59 mRNA significantly increased in RUPP vs. Sham with a 1.16±0.04 fold change ($p < 0.05$).

A slight increase in CD55 mRNA was noted (1.21±0.09 fold change; p= 0.07) in RUPP vs. Sham kidney cortex, and no change in Crry mRNA was detected (Figure 1). In kidney, no significant change in C3 deposition was seen following placental ischemia (Figure 2).
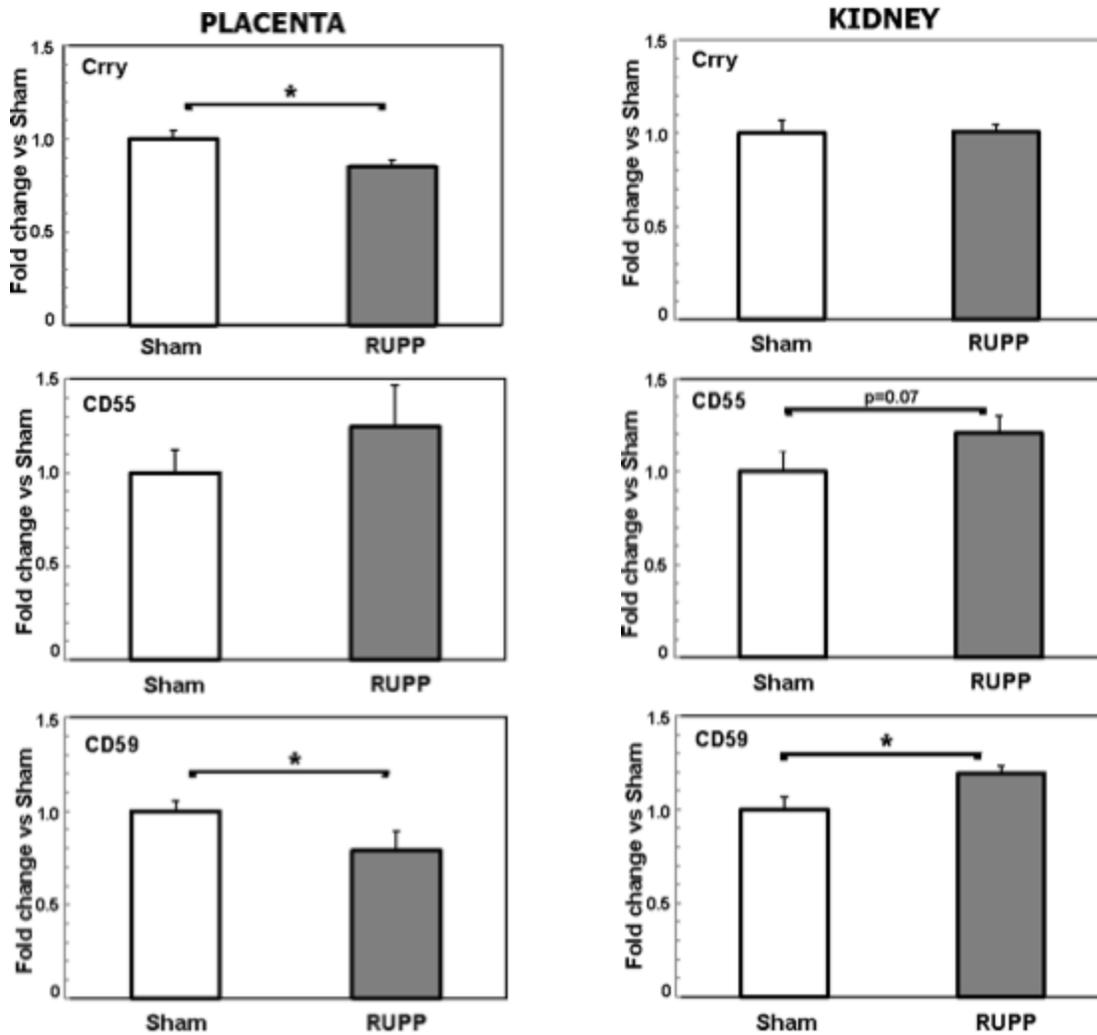

Figure 1. Message for Crry, CD55, and CD59 was measured in both placenta and kidney using quantitative RT-PCR.
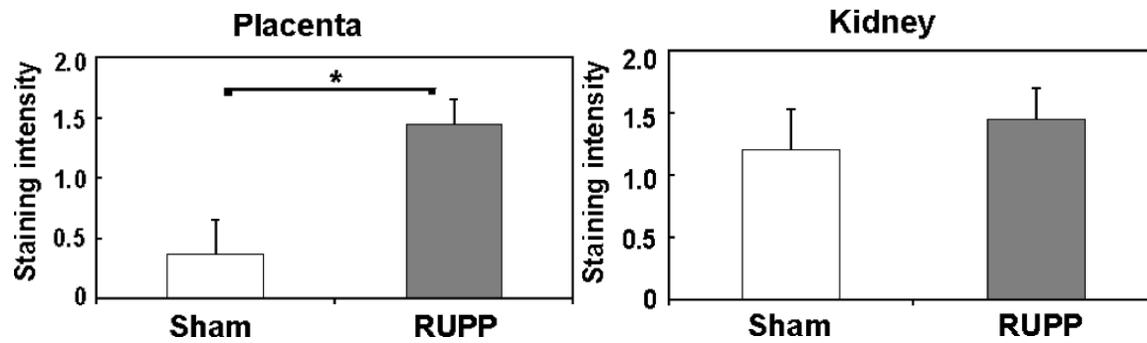
Figure 2. C3 deposition was measured in both placenta and kidney using immunohistochemistry.

Conclusion: These data demonstrate that placental ischemia-induced changes in complement regulators are dependent on the tissue type. Contrary to our hypothesis, regulators were decreased in placenta. This suggests that following placental ischemia, the decrease in endogenous regulators in placenta contributes to the net increase in complement activation as demonstrated by increased C3 deposition and circulating C3a (Figure 1, 2). A reason for the difference between the human studies and our rat model may be that our model is only able to mimic from placental ischemia on, rather than preeclampsia as a whole. In kidney, the increase in complement regulators is sufficient to limit C3 deposition, suggesting that complement activation in kidney does not contribute to increased circulating C3a following placental ischemia (Figure 1, 2).

One future direction could be manipulating regulation of the complement system in the placenta as a potential target for therapy, however at the moment we have no way of increasing regulators. We know that the complement system is overactivated in preeclamptic pregnancies, and that by inhibiting complement activation hypertension is attenuated. However, we cannot

target an entire portion of the immune system without leaving the mother vulnerable to other diseases. If we can understand how the complement system is activated we can find a more targeted approach to manipulate the complement system to help treat preeclampsia and retain the positive effects of complement activation in defending against infection.

Works Cited

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