36 TOLL LIKE RECEPTOR 4 (TLR-4) IS ESSENTIAL FOR FETAL NEURONAL INJURY IN INFLAMMATION-INDUCED PRETERM BIRTH

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OBJECTIVE: Inflammation-induced preterm birth (IIPB) is a major cause of adverse neurological outcome (ANO). ANO has been linked to white matter and glial damage. Recently, we have demonstrated that IIPB results specifically in neuronal injury. The activation of TLR-4 is implicated in inflammation-induced brain injury in both neonatal and adult animal models but has not been investigated in fetal injury from preterm birth. This study investigates whether TLR-4 is essential for fetal neuronal injury in IIPB.

STUDY DESIGN: TLR-4 mutant mice, C3H/HEJ, were mated and HEJ embryos transferred to pseudopregnant CD-1 mice. OUJ and CD-1 embryos were used as controls. CD-1 dams pregnant with CD, OUJ or HEJ embryos were randomized to intrauterine LPS or saline using our mouse model of preterm birth on E15. The presence of LPS in amniotic fluid and maternal serum was assessed with LAL assay. Neuronal morphology from exposed and non-exposed fetal brains from HEJ and controls was investigated using cortical culture technique with confocal microscopy at division day 3 (DD3). TLR4, GFAP, and PLP expression in wild type neuronal and glial cultures was assessed by QPCR.

RESULTS: LPS was present in amniotic fluid and maternal serum within 3 hrs after treatment. QPCR revealed that TLR-4 was present on astrocytes, but not in neurons in wild type on E15. Wild type fetuses exposed to in vivo LPS demonstrated significant disruptions in neuronal morphology with decreased aggregation and number of dendritic processes. In contrast, cortical cultures from LPS exposed HEJ brains did not demonstrate any of these morphological differences and had similar number of dendritic processes on DD3 ($P>0.05$) as saline exposed CD and HEJ fetuses.

CONCLUSION: Activation of TLR-4 is a critical mechanism by which neuronal injury occurs in preterm births. Neuronal injury in II PTB appears to be secondary to LPS activating TLR-4 on astrocytes and subsequent astrocyte-neuronal interaction. Targeting TLR-4 may serve as a potential therapeutic option to prevent neuronal injury in the setting of preterm birth.

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37 ADMINISTRATION OF 17OH PROGESTERONE ATTENUATES TNF ALPHA-INDUCED HYPERTENSION DURING PREGNANCY

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OBJECTIVE: Increases in inflammatory cytokines may be an important link between placental ischemia, endothelial dysfunction, and hypertension in women with preeclampsia. We have previously demonstrated that a 2-fold increase in circulating TNFα increases mean arterial pressure (MAP) in the pregnant rat. The objective of this study was to examine the role of 17-OHP, a known anti-inflammatory agent, in attenuating TNFα-induced hypertension in gravid rats.

STUDY DESIGN: Sprague-Dawley rats were anesthetized on Day 14 of pregnancy and underwent either 1) anesthesia; 2) injection of intraperitoneal progesterone (.83mg); 3) mini-osmotic pump infusing TNFα (50 ng/day); or 4) injection of intraperitoneal progesterone and mini-osmotic pump infusing TNFα. On day 18, carotid artery catheters were inserted; on day 19 MAP, maternal and pup weights were measured. Blood, kidneys and placentas were collected. Plasma was analyzed for circulating progesterone and TNFα.

RESULTS: TNFα administered to normal pregnant rats significantly increased MAP from 103+/−2 to 115 +/−3 mmHg ($p<0.01$) with a two-fold increase in circulating TNFα (23+/−8 vs. 47+/− 7pg/ml). However, MAP did not increase in response to a 2-fold increase in TNFα in pregnant rats receiving 17-OHP (98 +/−3 17-OHP vs. 100 +/− 4 mmHg 17-OHP+TNFα). There was no significant difference in weight gain, litter size or placenta weight among any of the groups.

CONCLUSION: Administration of 17-OHP attenuates TNFα-induced hypertension in the pregnant rat model without significantly affecting maternal weight gain, litter size, pup size or placental size. These results suggest that 17-OHP may be a viable option for treatment of hypertension associated with elevated cytokine levels in preeclamptic women.

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38 THE RELATIONSHIP BETWEEN POLYMORPHISMS IN THE HUMAN PROGESTERONE RECEPTOR AND CLINICAL RESPONSE TO 17 ALPHA-HYDROXYPROGESTERONE CAPROATE FOR THE PREVENTION OF RECURRENT SPONTANEOUS PRETERM BIRTH

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OBJECTIVE: 17 alpha-hydroxyprogesterone caproate (17OHPC) has been shown to reduce the recurrence risk of spontaneous preterm birth (SPTB). Our aim was to assess if women with single nucleotide polymorphisms (SNPs) in the human progesterone receptor (hPR) are more or less likely to respond to 17OHPC for the prevention of recurrent SPTB.

STUDY DESIGN: Secondary analysis of 463 women enrolled in a multicenter, prospective, double-blind study of 17OHPC vs. placebo for the prevention of recurrent SPTB. Individuals were genotyped with 20 SNPs in the hPR gene. Allele and genotype frequencies were calculated and evaluated for evidence of genetic predisposition to 17OHPC response.

RESULTS: DNA was extracted and genotyped from the saliva of 335 patients; 231 (69%) received 17OHPC (cases), and 104 (31%) received placebo (controls). All SNPs were in Hardy-Weinberg equilibrium. SPTB rates in each group were similar to the original cohort. Clinical characteristics, racial distribution, and allele frequencies were not significantly different between cases and controls. The majority (60.3%) of patients in our cohort were African-American.

SPTB was less common among women who received 17OHPC compared to controls regardless of allele status for all SNPs. However, among African-American women who received 17OHPC, those who were carriers of the minor allele (G) in SNP rs471767 (A/G) were more likely to have SPTB compared to those carrying the major allele (38% preterm <37 weeks gestation vs. 22% respectively, $p=0.007$, odds ratio 2.2, 95% CI: 1.2-3.9). SPTB <32 weeks gestation was also more likely in non-African-American women who received 17OHPC for SNPs rs503362 (G allele), rs471767 (A allele), and rs578029 (T allele); $p=0.009$, 0.029, & 0.048 respectively.

CONCLUSION: The clinical efficacy of 17OHPC for prevention of recurrent SPTB may be altered by polymorphisms in the hPR gene. Further analysis of interactions between the hPR SNPs (haplotypes) is ongoing and could reveal a more specific 17OHPC response profile.

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39 MAGNETIC RESONANCE IMAGING OF MURINE PLACENTAL INJURY

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OBJECTIVE: To elucidate mechanisms underlying placental hypoxic injury associated with intrauterine growth restriction, we hypothesized that magnetic resonance (MR) technology can define hypoxia-induced placental dysfunction.

STUDY DESIGN: Using an enclosed hypoxia chamber we exposed pregnant C57BL/6 mice to FiO2=12% (Hpx, n=15) between embryonic day 15.5 (E15.5) to E18.5 (term=E19.5). We included two controls: normoxia with food restriction similar to the intake of hypoxic mice (N-FR, n=12), or food ad libitum (N-AL, n=15). On E18.5 we utilized dynamic contrast-enhanced (DCE) MRI (4.7 tesla) to define the uptake and distribution of Gd-based contrast agent, with quantitative parameter analysis of signal intensities from the placenta and fetal kidneys. After imaging we assessed feto-placental weight, placental histology and gene expression (using RT-qPCR).

RESULTS: When compared to N-AL and N-FR controls, we observed significant IUGR (weight reduction by 28% and 14% respectively, $p<0.05$) in Hpx dams. This was accompanied by reduced placental weight and increased placenta/fetus ratio when compared to the N-AL and N-FR groups (27% and 18% respectively, $p<0.05$). Using MRI-based assessment of placental contrast-agent kinetics, normalized to maternal arterial input, we detected a higher initial uptake of agent into the N-FR placentas. Importantly, we found decreased placental clearance of contrast media (by 61%, $p<0.05$) in Hpx mice, compared to either control group (61%, $p<0.05$). This was accompanied by diminished enhancement of the hypoxic fetal kidneys (23%, $p<0.05$), indicating reduced trans-placental transport of gadolinium. These changes were associated with altered expression of placental transcripts, including Phlda2, Sgk2, Gcm1, Tpbpa, and Plf, reflecting abnormal placental growth and injury.

CONCLUSION: Exposure to hypoxia during the end of mouse pregnancy reduces placental perfusion and clearance of contrast. MRI-based DCE imaging provides a novel tool for dynamic assessment of placental function in vivo. (Supported by ACOG/Ortho-McNeil Training grant).

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