

## **Prenatal Glucocorticoid Exposure for Preterm Birth: Investigating The Role Of Glucocorticoid Receptor Phosphorylation In The Development Of Neuropathology –**

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### **Introduction:**

Preterm birth is defined as birth before 37 weeks gestation. Several risk factors for preterm birth have been identified including pre-existing medical conditions, substance use, stress, and social factors<sup>1</sup>. Important social conditions to consider for at-risk mothers are women of lower socioeconomic status, African American women, and women on extremes of the maternal age spectrum. According to the CDC, in 2016 preterm birth rates were as high as 1 in 10 births. And preterm birth accounts for nearly 1/3 of all infant deaths in the US<sup>2</sup>.

A child born preterm is put at higher risk for complications because they have not completed full organ maturation in utero. Risks include intraventricular hemorrhage, periventricular leukomalacia, respiratory distress syndrome, and infections such as necrotizing enterocolitis. Synthetic corticosteroids (sGCs) such as betamethasone (Beta) and dexamethasone (Dex) have been most widely administered to women at risk of preterm delivery to accelerate fetal organ maturation. Both are widely preferred because they both cross the placenta in their fully active form, lack mineralocorticoid activity, and have weak immunosuppressive activity<sup>3</sup>. The American College of Obstetrics and Gynecology recommends a single course of glucocorticoids for women who are between 24 0/7 and 33 6/7 weeks gestation, and as early as 23 0/7 weeks gestation depending on the family's decisions about fetal resuscitation, who are at risk for preterm delivery within 7 days. A single course of corticosteroids can also be administered to women who are risk of preterm delivery within 7 days and who have not previously received a course of antenatal glucocorticoids, who are between 34 0/7 and 36 6/7 weeks gestation. A repeat course of corticosteroids can be administered for women who are at continued risk for preterm delivery before 34 0/7 weeks gestation, who had previously received a single course of glucocorticoids 14 days prior<sup>3</sup>.

Several studies outline the benefits of glucocorticoid use in preterm delivery. A study performed by the NICHD Neonatal Research Network observational cohort found that infants who received a single dose of antenatal corticosteroids between 23 0/7 and 25 6/7 weeks gestation had a reduction in death and neurodevelopmental impairment at 18-22 months of age. This study also revealed a reduced incidence of intraventricular hemorrhage, periventricular leukomalacia, and necrotizing enterocolitis<sup>4</sup>. However, there is increasing evidence against repeated courses of antenatal corticosteroid administration. The risks and benefits must be weighed but it is clear that sGC dosing must be tightly regulated. Studies have shown that serial courses of corticosteroid use increase the incidence of low birth weight infants<sup>5</sup>. Both human and animal studies indicate that there are deleterious effects on cerebral myelination, dendritic

morphology of neurons, function of the hypothalamus-pituitary-adrenal axis, and behavioral changes such as anxiety and depression in adulthood<sup>6</sup>. Our laboratory has shown that Dex administration to mouse neural stem cells from the cerebral cortex and hypothalamus lead to differential gene activation of over 200 genes within 4 hours<sup>7</sup> (s43). Our recent preliminary results indicate that a subset of these genes are activated when the glucocorticoid receptor (GR) is phosphorylated on one specific site, Serine 220. Changes in the phosphorylation status of this site has been implicated in human psychiatric diseases<sup>8</sup> (s50). The aim of this research is to identify how phosphorylation of the GR at this site impacts the neural stem cells behavior.

### **Hypothesis:**

Administration of antenatal glucocorticoids induces phosphorylation of GR on S220, which subsequently changes the transcriptional response of genes required to attenuate glucocorticoid responsiveness in mouse neural stem cells. This leads to changes in proliferation and differentiation and ultimately impact the anatomical organization of the postnatal brain.

### **Methodology:**

#### Animal model

To identify the role of phosphorylation of Gr on S200 we will use a unique knock-in mouse model where Serine 220 is replaced by alanine, inactivating this phosphorylation site. This S220A knock-in mouse will be used to decipher the role of phosphorylation in transcriptional response, neural stem cell proliferation and differentiation in response to Beta/Dex or control vehicle administration.

#### Molecular studies

##### (A) Affymetrix Expressed Genome Chip Analysis

In preliminary studies we identified a subset of genes that are altered in response to 4 hours of Dex exposure in S200A knock-in neural stem cells. These experiments were performed on two animals. I propose to repeat these experiments using Beta instead of Dex because Beta is the preferred sGC for prenatal use clinically. I will also increase the number of animals to achieve statistical significance. Neural stem cells will be derived from the cerebral cortical of E14.5 embryos from wildtype and S200A knock in mice. RNA will be isolated using Trizol and a miRNA kit per the manufacturer's instructions (Qiagen). RNA will be converted to cDNA, labelled and hybridized to the Affymetrix Clarius S Gene Chip containing all known coding genes and some long, non-coding genes using the Affymetrix RNA to cDNA labelling kit and hybridization reagents per the manufacturer's instructions. I will compare 3 samples from each of the following groups: wildtype plus Beta, wildtype plus vehicle, S200A plus Beta, S200A plus vehicle. Hybridization signals will be loaded

into TAC software and statistically analyzed using incorporated software to identify genes that are uniquely regulated by S200 phosphorylation after exposure to Beta. Genes will be divided into categories based on biological functions using pathway analysis software embedded in the TAC analysis suite.

(B) Validation by Quantitative Polymerase Chain Reaction.

Results will be validated by quantitative PCR on 3 independent samples, using QIAGEN RNA to cDNA synthesis kit and Sybr Green qPCR reagents. Samples will be compared by the delta delta CT method and fold change calculated and compared statistically using an ANOVA.

(C) Neural stem cell Biological Assays.

Dex has previously been shown to inhibit neural stem cell proliferation and alter cell differentiation. To determine if S220 phosphorylation is required for these cellular processes, neural stem cells will be cultured and exposed to Dex and Beta for 24 hours. Cell assays will be performed using a K2 Cellometer to measure cell proliferation (Propidium Iodide), cell death (Trypan Blue) and autophagy. We will generate using growth curves to plot the cell count at day 3, 5, and 7 of mouse neural stem cells post-exposure to Beta/Dex.

To examine cell differentiation, neural stem cells will be exposed to Dex or Beta for 24 hours and subsequently dissociated and grown on Laminin in differentiation media. Cells will be grown for 5 days and subsequently immunohistochemistry staining will be used to identify changes in the proportion of neurons, oligodendrocytes, or glia or changes in their morphology.

Once a biological function is identified that is impacted by S200 phosphorylation (outlined in aims B and C), the expression of each S200 regulated gene in that pathway will be examined using immunohistochemical staining in-vitro in proliferating, or differentiating cultures, or in sections of brains from embryos exposed to Beta for 24 or 48 hours in-vivo.

**Expected outcomes:**

Antenatal exposure to glucocorticoids induces site specific phosphorylation of GR, which directs the transcriptional response. This is important because GR phosphorylation is implicated in regulating promoter specificity, interactions between co-factors, the length and magnitude of transcription, and stability of receptors. Furthermore, site specific phosphorylation of GR is associated with specific psychiatric disease states including development of major depressive disorder, negative affect, and neuroticism. This project will identify genes, pathways and biological pathways that are sensitive to S200 phosphorylation. It will be a first step toward understanding how prenatal sGC exposure leads to anatomical and behavioral deficits in humans.

**Conclusion:**

Administration of antenatal glucocorticoids induce stem cell differentiation in the developing prenatal brain, predisposing to the development of psychiatric diseases later in childhood and adolescence.

The benefits of increased survival and reduced complications from preterm birth that a single course of corticosteroids provide, outweigh the risks. However, depending on the results of this study and additional studies, there may be recommendation against unnecessary serial courses of antenatal corticosteroids. Furthermore, with the identification of specific genes that are targeted with glucocorticoid use and their role in the development of psychiatric diseases, areas for early intervention in childhood or adolescence may be possible.

**Budget:****Total = \$2,100**

\$300 - All of the qPCR, proliferation and differentiation experiments will be performed by me with the assistance of a laboratory technician. Guidance and Affymetrix training will be provided by a faculty member. Reagents used for Affymetrix and qPCR studies. QPCR molecular biology reagents, SYBR green kits and oligo nucleotides.

**Reagents for cell culture experiments**

\$1,000 – antibodies for neurons, oligodendrocytes and glial subtypes.

\$200 – cell culture reagents, media, propidium iodide, laminin, glass cover slips & plates.

**Professional development**

\$100 Poster and publication costs.

\$500 Travel by student to a national meeting or conference registration to present findings.

## **References:**

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6. Wapner RJ, Sorokin Y, Mele L, Johnson F, Dudley DJ, Spong CY, et al. Long-term outcomes after repeat doses of antenatal corticosteroids. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *N Engl J Med* 2007;357:1190–8.