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Introduction

- Pregnant women are occasionally prescribed opioid drugs like oxycodone, and abuse of this and other opioid drugs is common during pregnancy. There is evidence that exposure leads to low birthweight babies and other complications of pregnancy^{1,2}.
- An earlier experiment investigated the effects of oxycodone on pregnant mice and specifically their placentas. There was decreased maternal blood vessel area within the labyrinth region where nutrient and gas exchange occurs. This region corresponds to human syncytiotrophoblast³.
- This study also found reduced numbers of parietal trophoblast giant cells in the junctional zone, a region with possible homology to human extravillous trophoblast. Finally, there were many differences in gene expression in whole placentas³.
- To observe the effects of oxycodone on human placental cells we are using H1 Embryonic stem cells to generate the trophoblast lineage using BAP treatment⁴.

Hypothesis

Maternal treatment with oxycodone (OXY), a primary opioid at the center of the current crisis, deleteriously affects placental structure and gene expression patterns in the human as well as the mouse.

Materials and Methods

Cells- H1 human embryonic stem cells (hESC) were used for this experiment. They were driven along the trophoblast lineage by using BAP treatment. BAP treatment consists of using bone morphogenetic protein- 4 (BMP4) as well as inhibitors A83-01 (A83) and PD173074 (PD). The BMP4 is used to prime hESC into a self-renewing and totipotent state while A83 and PD increase expression of a marker for TB called HLA-G.

Treatments- Two concentrations of oxycodone (250 nM and 50 nM) were tested in this experiment. These concentrations bridge those used for animal studies. The oxycodone concentrations underwent a two-step dilution directly into the hESC medium that was used throughout the trials. BAP treatment components were added to the fresh medium before each medium change. Each trial had two wells dedicated to each concentration and three trials were run.

Hormone Assays- Medium was collected from each well on each day of the treatment. ELISA assays were run to assess human chorionic gonadotropin hormone. These were used to determine whether the cells were developing normally over time.

Cell Imaging- The cell colonies, with and without oxycodone, were imaged each day at 4x magnification to assess morphological differences between treatments.

DNA extraction- On day 7, the cells from each well were lysed and the DNA was collected using a Promega DNA purification kit. DNA content was used to normalize data

Effects of Oxycodone on Trophoblast Stem Cells

Results

Morphology- No obvious differences were present in the morphology of the cells without oxycodone compared to the cells with either concentration of oxycodone







Picture from day 7 (final day) of first trial. 9-23-20, 250nM well 5

Human chorionic gonadotropin hormone production- No significant differences were observed between the different oxycodone treatments and with controls. The figure below shows hCG concentration from day 4 of the experiment to day 7, where hCG production is usually maximal. There was an apparent slight increase in hCG from the cells that are treated with 250nM oxycodone, but the differences were not significant.

Effects of Oxycodone on **HCG** concentration





Picture form day 7 (final day) of first trial. 9-23-20, 50nM oxycodone, well 4



Control 50nM ZZ 250nM

Conclusions and Future Aims

- necessary.
- drug.

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No obvious changes in hCG concentration and morphology are consistent with a lack of effect of oxycodone on trophoblast differentiation. This outcome does to some extent contradict what we had found in the mouse study. Testing other parameters will be

Future experiment will be conducted to isolate RNA and permit an analysis in gene expression differences between treatments.

A second project will be to observe effects of oxycodone exposure on mouse trophoblast stem cells when they are driven to differentiate into trophoblast giant cells (TGC). TGC appear to be diminished in number when pregnant mice are exposed to this

My observation that oxycodone exposure has little observable effects on human trophoblast development may mean that the human placenta may not be a major target for opioids, although this does not rule out direct effects on the fetus.

Reterences