Effects of Maternal Oxycodone Exposure on Mouse Placental Development

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Background

Opioid Crisis

- In, 1996, oxycodone was first prescribed for the treatment of pain in the United States (1).
- Oxycodone belongs to a class of drugs referred to as opioids.
- In 2016, prescription opioids were abused by approximately four percent of the United States population (2).
- It has been found that health care costs for those that abuse oxycodone may increase to as much as $16,000 more than those who do not abuse oxycodone (2).

The Role of the Placenta in Fetal Development

- The placenta allows for the exchange of gases, nutrients, and waste (3). Gross and microanatomical structure of the mouse placenta is shown below (**Figure 1**).
- The proximity of the fetal placenta to the maternal blood in hemochorial placentation renders it vulnerable to pharmaceutical agents circulating in the mother's bloodstream (3).

Hypothesis

The overarching hypothesis tested in the current studies is that exposure of the mouse placenta to oxycodone through the dam leads to detrimental microanatomical and molecular changes, and these disturbances in the placenta can lead to long term health effects in resulting offspring.

Methods

Dosing

Twenty-four CD1 female mice were used in these ongoing studies. The female mice were randomly assigned into two groups of twelve. The treatment group received daily injections of oxycodone, while the control group received comparable doses of saline. The dose of oxycodone was determined through the analysis of doses used in previous, similar experiments (4,5). The mice were bred fourteen days after the onset of dosing and monitored for a vaginal plug, which indicates when the male bred to the female. The placentas and fetal samples were collected 12.5 days after a plug was observed.

Analysis of Structural Changes

In order to examine the structural effects of oxycodone on the placenta, placental tissue was fixed in 4% paraformaldehyde and H & E sections were prepared for histopathological analyses. This was done through the analysis of the vascularization of the spongiotrophoblast (SpongioTB) region. This was determined by quantifying the percentage of the SpongioTB comprised of blood vessels in each sample. The three main placental regions (labyrinth (LA), SpongioTB, and trophoblastic giant cells (GC) were compared against each other.

Analysis of Functional Changes

The effects of maternal exposure to oxycodone on the function of the placenta has been analyzed with RNA-seq analyses.

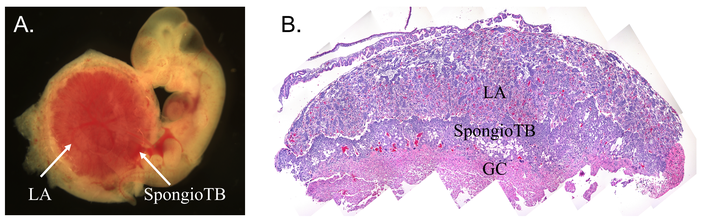

Figure 1: Mouse placenta. A.) A sub-gross view of the placenta is shown above. B.) A histological view of the placenta is shown. This depicts the labyrinth region (LB), the spongiotrophoblast region (SpongioTB), and the giant cell region (GC).

Results

PLACENTAL HISTOLOGY RESULTS

Oxycodone Reduces the GC Area in the Mouse Placenta

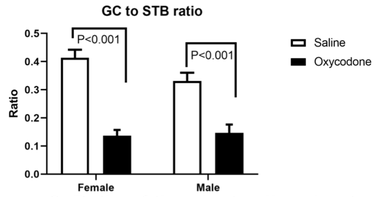

Figure 2: Ratio of the giant cell region to SpongioTB region. The data are presented as mean values ± standard errors. The reported ratios were 0.14 ± 0.02 vs 0.37 ± 0.02 respectively. P<0.001

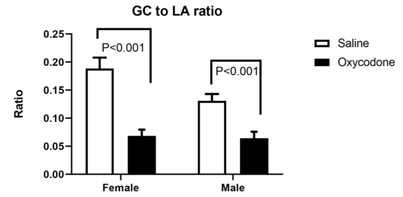

Figure 3: Ratio of the giant cell region to labyrinth region. The data are presented as mean values ± standard errors. These reported ratios were 0.07 ± 0.01 vs 0.16 ± 0.01 respectively. P<0.001

Oxycodone Induces Gene Expression Changes in Mouse Placenta

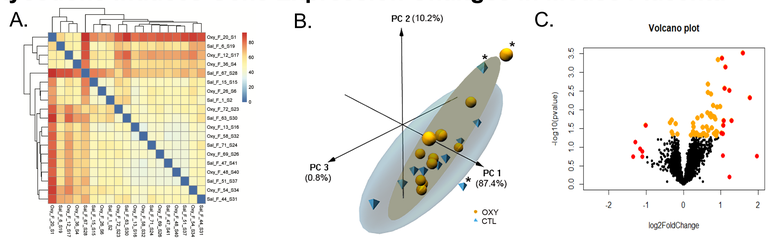

Figure 4: Gene Expression in the Placenta of Female Offspring. A.) This heat map depicts all of the genes and shows how the various individual replicates for each group (OXY-oxycodone and CTL-saline control cluster together). B.) 3D PCA plot reveals a few outliers (delineated with asterisks) that were removed in follow-up analyses. The percentages after each PC axis indicate amount of variation accounted for by a given PC axis with PC1 predictably accounting for the greatest percentage of the variation. C.) After removing the outliers, Volcano plot analyses reveals those genes that were differentially expressed. Genes represented in orange and red indicate a p=0.05. Genes represented in red indicate a log2 fold change>1 and p < 0.05.

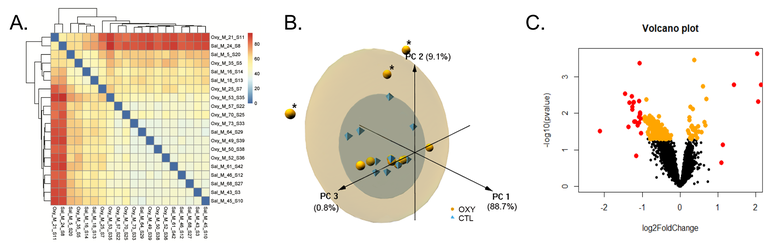

Figure 5: Gene Expression in the Placenta of Male Offspring. A.) This heat map depicts all of the genes and shows how the various individual replicates for each group (OXY-oxycodone and CTL-saline control cluster together). B.) 3D PCA plot reveals a few outliers (delineated with asterisks) that were removed in follow-up analyses. The percentages after each PC axis indicate amount of variation accounted for by a given PC axis with PC1 predictably accounting for the greatest percentage of the variation. C.) After removing the outliers, Volcano plot analyses reveals those genes that were differentially expressed. Genes represented in orange and red indicate a p=0.05. Genes represented In red indicate a log2 fold change>1 and p < 0.05.

Table 1: Top Differentially Expressed Genes Based on P Value in Female Placental Samples. The data presented in this table are significant. All p values are less than p < 0.005.

Gene ID	Gene Name	pvalue	Fold Change	Up/Down Regulated in Oxycodone Treatment Group Compared to Saline Control
Prl7b1	prolactin family 7, subfamily b, member 1(Prl7b1)	0.000305	3.0151	⬆
Ipp	IAP promoted placental gene(Ipp)	0.000411	2.0419	⬆
Prl8a6	prolactin family 8, subfamily a, member 6(Prl8a6)	0.000452	1.9002	⬆
Prl8a8	prolactin family 8, subfamily a, member 81(Prl8a8)	0.00071	2.1904	⬆
Psg-ps1	pregnancy specific glycoprotein pseudogene 1(Psg-ps1)	0.002067	1.5759	⬆
Prl7a2	prolactin family 7, subfamily a, member 2(Prl7a2)	0.003038	2.3445	⬆

Table 2: Top Differentially Expressed Genes Based on P Value in Male Placental Samples The data presented in this table are significant. All p values are less than p < 0.005. Even though, *Prl7a2* is not amongst the top 5 genes by this criterion, it is included to show how the directionality in terms of expression differs from female placenta.

Gene ID	Gene Name	pvalue	Fold Change	Up/Down Regulated in Oxycodone Treatment Group Compared to Saline Control
Cubn	cubilin (intrinsic factor-cobalamin receptor)(Cubn)	0.000239	4.100143588	⬆
Apob	apolipoprotein B(Apob)	0.0002853	5.17224172	⬆
Tpm3	tropomyosin 3, gamma(Tpm3)	0.0003518	1.29247749	⬆
Tfpi2	tissue factor pathway inhibitor 2(Tfpi2)	0.0004237	0.476090528	⬇
Apoa4	apolipoprotein A-IV(Apoa4)	0.0004486	5.861951127	⬆
Prl7a2	prolactin family 7, subfamily a, member 2(Prl7a2)	0.0041401	0.540139325	⬇

Conclusions & Future Aims

- Maternal oxycodone exposure reduces GC area of the placenta, and effects seem to be more prominent in the placenta of females.
- Maternal oxycodone exposure induces sex-dependent gene expression changes in the placenta with many genes, including those in the prolactin family, being placental-specific.
- For some genes, such as *Prl7a2*, the expression patterns diverged between females and males with placenta of female conceptuses exposed to oxycodone showing enhanced expression of this gene; whereas, in their male siblings, it was downregulated.
- Future studies will further explore how maternal use of oxycodone may induce epigenetic changes, including DNA methylation alterations.

Acknowledgements

We would like to thank Robert Schmidt, Sarabjit Kaur, Camryn Long, Tess Willemse, and Alaina Baumgart for assisting with animal husbandry throughout this experiment.

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