The Effects of Choline Supplementation on Brain Structure in a Fetal Alcohol Model

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LAY SUMMARY:

Alcohol exposure prenatally may lead to Fetal Alcohol Spectrum Disorders (FASD). FASD is detrimental to the health of a fetus and may cause abnormalities in physical, behavioral, and neuronal development. In the brain, the hippocampus is responsible for many physiological functions, such as memory, which are important for normal development. In the hippocampus, alcohol has been shown to affect the dentate gyrus (DG) and region III of the hippocampus proper (CA3) areas. The neurons of the DG are formed by progenitor cells. Progenitor cells, like stem cells, have the tendency to differentiate into a specific type of neuron. Alcohol negatively affects hippocampal neurogenesis, the creation of new neurons within the brain. The disruption in the growth of neurons may lead to memory impairment. It could then translate to behaviors such as diminished spatial learning and delayed development.

In previous studies, supplementation with choline, a nutrient present in many foods, has led to the improvement in functions of hippocampal cells such as the granule cells in the DG of the hippocampus. Choline is a precursor molecule for acetylcholine, which is a neurotransmitter involved in physiological functions including creating memories and having muscle control. As found in previous studies, choline could be a compound that affects neurogenesis. Exposure to choline in early life may also be a precursor to improved performance in adulthood, specifically in cognitive function. Prenatal choline intake has a lasting positive effect on lifelong changes in cognitive function, all dependent on choline availability during gestation.

Alcohol has a negative effect on neuron formation and development, while choline has been shown to promote neurogenesis of granule cells located in the DG of the hippocampus. The hippocampus has a profound effect on cognitive performance. Thus, in this experiment, we will be testing the idea that choline can reverse the effects of FASD and increase the hippocampal progenitor pool, which will lead to an increase in the number of neurons in the DG of the hippocampus.
In this study, the effect of a choline diet is being tested in a fetal alcohol rodent model. Pregnant rat dams were administered a liquid diet with ethanol and choline supplementation, and an ethanol without choline liquid diet and an unadulterated liquid diet were used as control groups. Offspring pups were assessed for behavioral differences between groups, and brains from these pups will be used to assess the effects of ethanol exposure on the neuroanatomy of the hippocampus and choline’s ability to ameliorate these effects. Specifically, granule cells in the DG of the hippocampus will be quantified, and we expect to observe a decrease in granule cell number of ethanol exposed pups relative to control pups that is restored to control levels by choline supplementation.

This experiment will provide a better understanding of the effect of choline on hippocampal neurogenesis in an alcohol exposure model. The effect of choline on FASD could further serve as a basis for improving the lives of children who are prenatally exposed to alcohol via prevention of developmental deficits.

**HYPOTHESIS:**

In a rodent model of FASD, ethanol exposure during gestation will result in a decreased number of granule cells in the dentate gyrus (DG) of the hippocampus relative to control (no ethanol exposure), and maternal dietary choline supplementation will restore these neuronal populations to control levels in ethanol exposed rat dams.

**INTRODUCTION:**

A woman who consumes alcohol for the duration of her pregnancy may affect the health of her child in the womb, and this may result in Fetal Alcohol Spectrum Disorders (FASD). Alcohol readily passes through the placenta, and the fetus may experience the same blood alcohol level as the mother. Similarly, if the mother abstains from alcohol suddenly, she and her fetus will both have withdrawal symptoms. Children who are exposed to alcohol in utero face problems in physical and behavioral development or even prenatal mortality. The child may then suffer from microcephaly, executive function disorders, neuronal cell loss, and behavioral deficits. Neurobehavioral effects can manifest in poor memory and learning ability, attention deficits, and motor dysfunction.

Developmental deficits can be modeled in rodents to demonstrate the effect of ethanol on the development and function of the hippocampus. The hippocampus is located in the medial temporal lobe,
is part of the limbic system, and is involved in functions such as memory and spatial learning. The hippocampus houses the dentate gyrus (DG) and CA3, both of which are likely affected by alcohol exposure. The DG serves as the primary pathway of excitatory input into the hippocampus for its formation. This input is important for the formation and function of the hippocampus in memory and learning. Most histological abnormalities in pathological states are closely related to early development of the DG. The DG in a rodent is also one of the identified structures to have continuous neurogenesis throughout adulthood. Neurogenesis occurs by production of granule cells from precursor progenitor cells, which can be found on the border between the DG granule cell layer and the hilus of the DG. Previously, researchers have also demonstrated that an increase in physical activity and learning can promote neurogenesis of granule cells and be essential for learning.

Researchers have suggested that choline can affect hippocampal neurogenesis, which is important for memory and spatial learning. Choline, a compound present in many foods, has recently been identified as an essential nutrient that has been shown to influence cognitive and neural development prenatally. Choline has also been shown to be related to alterations to hippocampal pyramidal cells, however the cellular mechanisms have not been identified. Choline is a micronutrient that is integral in bodily processes such as nerve and liver function, brain development, energy, muscle movement, and metabolism. Choline can exist as phosphatidylcholine, a component of fat that is present in several types of foods. In the body, choline is the antecedent to the neurotransmitter acetylcholine, which in this case could have effects on brain function. Although it is present in many different foods, it could be insufficient during the time of pregnancy due to the high demands of the fetus, and as previously suggested, choline supplementation could improve physiological functions of hippocampal cells, but this has not been tested on a cellular level. Researchers have demonstrated choline’s ability to promote neurogenesis of hippocampal cells.

In this study, brains extracted from offspring of dams exposed to ethanol during pregnancy will be used. The experimental dams received a choline supplemented liquid ethanol diet. The diet was administered before mating and from gestational day 5 through 20. The diet consisted of 6 treatment groups. Each of the treatment groups were fed via J-feeders using a novel choline-supplemented liquid diet. The treatment groups are broken down below:
1. Liquid 93G diet, *ad libitum*, consisting of 93G diet powder, Maltose-Dextrin, and Water
2. Choline 93G diet, *ad libitum*, consisting of 93G diet powder, Maltose-Dextrin, Choline, and Water
3. Ethanol 93G diet, *ad libitum*, consisting of 93G diet powder, Ethanol, and Water
4. Ethanol Pair-fed 93G diet, calorically restricted to match Diet 3 consumption, consisting of 93G diet powder, Maltose-Dextrin, and Water
5. Ethanol + Choline 93G diet, *ad libitum*, consisting of 93G diet powder, Ethanol, Choline, and Water
6. Ethanol + Choline Pair-fed, calorically restricted to match Diet 5 consumption, consisting of 93G diet powder, Maltose Dextrin, Choline, and Water

Offspring of dams receiving these dietary supplementations were tested for developmental milestones and spatial learning ability using a battery of tests. In newborns, development of physical appearances such as weight, unfurling of ears, opening of eyes, and growth of fur were assessed daily, beginning at postnatal day 2 (PD2). Developmental milestones, such as surface righting, negative geotaxis, and bar holding were assessed throughout development (PD2-21). During adolescence (PD45-50), spatial learning ability was assessed using a Morris Water Maze task. Pups were then perfused on PD51 using a buffer solution, phosphate buffered saline (PBS), and their tissues (brain, liver, testes, blood, and plasma) were collected. The brains were extracted and fixed in 4% formaldehyde overnight, then stored in 1x PBS. They were then cryoprotected in sucrose (15% for 24 hours, followed by 30% for 24 hours). The tissue was then frozen in isopentane and stored in a -80°C freezer.

Prenatal choline supplementation is expected to affect neurogenesis throughout gestation and in adulthood. The pups prenatally exposed to ethanol are expected to have a decline in physical and cognitive functions, while those supplemented with choline in addition to ethanol exposure should return to baseline control levels. The increase in cognitive and developmental function of those supplemented with choline should translate into neurogenesis of granule cells in the DG of the hippocampus. As a result, choline is expected to ameliorate the effects of FASD in children of pregnant mothers who do not abstain from alcohol use.
METHODS:

For my analyses in this proposal, I will only be examining a subset of treatment groups (offspring pups from Diets 1, 3, and 5). The objective sample size was 10 unique litters per treatment group each culled to 8 pups. Brains from offspring of these treatment groups (n=338) will be sectioned at -20°C into 60µm coronal slices using a cryostat. These frozen sections will be mounted onto slides. A standard protocol for immunolabeling will then be used on the sectioned brains as outlined in Niculescu et al. (2006). The sections will then be incubated in a primary antibody. A secondary antibody will be used to detect binding. The slides will then undergo nuclei counterstaining with DAPI, a nucleic acid die, which enables better orientation within the tissue to then be viewed by microscopy. The positive granule cells within the DG will be viewed and counted using confocal microscopy. Group means will then be analyzed for differences.

STATISTICAL PLAN:

To analyze the data collected, a t-test will be used to compare the difference between number of granule cells in the ethanol exposed only relative to control, and then in the ethanol and choline exposed treatment group relative to only ethanol exposed. The t-test serves as an indicator of statistical significance between the two groups and is an indication of the difference in between populations. Furthermore, analysis of variance (ANOVA) will be conducted to test the three groups - ethanol/choline, ethanol, and control - for statistically significant differences among the means. We expect that the supplementation with the choline will return the granule cells to the level of the controls, but it may only partially remediate the decrease in granule cells. We would then expect that the only significant difference would be between ethanol and control, because the ethanol/choline group would fall somewhere between the other two groups.

EXPECTED RESULTS AND SIGNIFICANCE:

We have tissue from 338 offspring from 46 unique litters. In my analyses, 31 litters with 248 pups will be analyzed. I expect the results to show an increase in the amount of granule cells in the DG of the hippocampus after exposure to an ethanol/choline diet as compared to only ethanol in a rodent model. Prenatal supplementation of choline is predicted to have a significant effect on hippocampal neurogenesis, which can translate to behavior and cognitive function, prenatally and throughout
adulthood. The significance of such findings could be a precursor to future essential diet supplementation for pregnant mothers, especially those who are unable to stop consuming alcohol. Choline may be able to reverse the effects of alcohol-derived health problems and improve the development of the baby.

REFERENCES:


