RISK ASSESSMENT OF HEPATOTOXICITY OF TCDD DURING PREGNANCY

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Risk Assessment of Hepatotoxicity of TCDD During Pregnancy

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is an aryl hydrocarbon receptor (AhR) mediated combustion by-product to which humans are exposed mainly through ingestion and partially through industrial processes such as fossil fuel combustion. Previous mammalian studies have shown that dioxin exposure results in both hepatotoxic and nephrotoxic effects, which is especially concerning during maternal exposures[1]. The process of embryogenesis relies heavily on the maternal blood flow for nutrients and waste excretion. With increased waste in the bloodstream, pregnant mothers are more susceptible to liver injury when exposed to persistent environmental pollutants, such as dioxin. Our study aims to assess the implications of dioxin exposure to pregnant females in mice of varying backgrounds in order to determine how genetic background influences hepatotoxic susceptibility and identify highly susceptible phenotypes within the population. This will ultimately aid in the identification of genetic susceptibilities and provide information that will assist in the development and application of innovative methods in assessing human health risks.

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NOMENCLATURE

TCDD, dioxin	2,3,7,8-Tetrachlorodibenzodioxin
NOD/ShiLtJ	NOD
C57Bl/6J	B6
NZO/HiLt	NZO
A/J	A/J

CHAPTER I

INTRODUCTION

As a relentless environmental pollutant, TCDD has been shown to lead to a myriad of toxic effects ranging from skeletal abnormalities and developmental issues to organ failure and tumor promotion. TCDD is mediated by the aryl hydrocarbon receptor (AhR, dioxin receptor, TCDD receptor). This receptor is capable of a wide range of detrimental physiological effects as a result of binding this toxicant and others like it. AhR works in phases in order to control the expression of drug metabolizing enzymes, when induced by certain high affinity ligands –such as TCDD. The first phase (initiation) is the most relevant of these phases. In this phase, TCDD is able to induces transcription of drug-metabolizing enzymes[3], with the most common being cytochrome P450 1A1 (CYP1A1). In conjunction with other CYPS, these cytochromes are often what lead to many of the physiological abnormalities that are observed[4, 5]. Currently, there are many unknown components of the extent of the effects of TCDD. Previous studies have shown that histological analysis of organs and tissues exposed to TCDD are beneficial towards displaying empirical evidence that can be used to showcase the correlation between TCDD exposure and genetic background[1, 2]. This is what our study plans to do through histological analysis of TCDD-induced liver necrosis across 4 mouse strains. According to previous studies, when using a diverse mouse panel such as ours, we should be able to collect data that is capable of predicting the clinically relevant toxicities which are needed to identify response biomarkers[6]. This will ultimately play a role in providing more knowledge of the hepatotoxic effects of TCDD.

Objectives

In this study we aim to correlate dioxin susceptibility with the effects of genetic background by analyzing histological changes within maternal livers. We hypothesize that variation in genetic backgrounds has a significant effect on maternal hepatotoxic responses.

CHAPTER II

METHODOLOGY

We chose to evaluate 4 mouse strains: C57BL/6J, A/J, NOD/ShiLt, and NZO/HiLt, Pregnant mice were exposed to increasing doses of 0, and 100 ng/kg/day of TCDD for a period of 10 days following mating. For the first nine days each dose was administered via peanut butter aliquots and on the 10^{th} day, the dose was administered via gavage with laboratory grade olive oil as our vehicle. Halfway through the 10^{th} day, the mice were euthanized and livers collected. Livers were fixed in formalin and later underwent histological processing of H&E staining. Once staining was complete, the slides were imaged and assessed by a board certified veterinary pathologist, Dr. Amie Perry.

CHAPTER III

RESULTS

The responses to the TCDD were not universal, but rather varied in line with phenotypic variation. As shown in figure 1, NOD and A/J mice both showed no hepatotoxic differences between the control and high dose (100 ng/kg/day) groups. The NZO mice displayed an increase of glycogen in their hepatocytes at the high dose. This could possibly be the result of dioxin interfering with the livers ability to break down glycogen. B6 mice exhibited a significant amount of inflammation, often accompanied by signs of necrosis in their livers at the high dose. Necrosis was not seen in the control group of the same strain or any other strain.

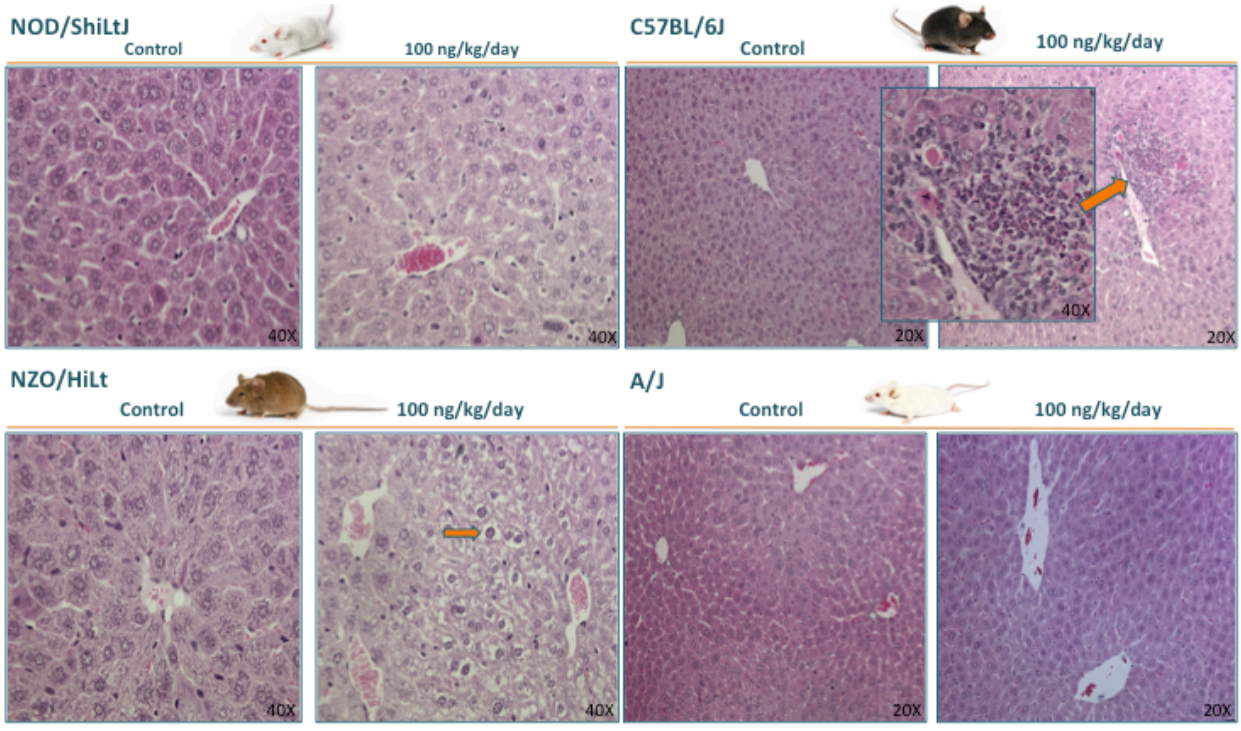

Figure 1. H&E staining of maternal livers in the control and high dose (100 ng/kg/day) group.

CHAPTER IV

CONCLUSION

The differences in hepatic cell response are due to both strain and dose differences. Control mice displayed nearly identical histological trends in their hepatocytes. When looking at the mice dosed with 100 ng/kg/day of TCDD, there clear differences in response to dioxin exposure. These differences were not universal, due to the effect of genetic variation among the strains. This data supports the premise of the study – being that genetic variability will have a significant impact on response to TCDD. In the case of B6 mice, the presence of necrosis and exaggerated inflammation in the dosed group indicates higher susceptibility to dioxin compared to NOD mice. In the future we will analyze serum LDL/VLDL levels and perform QTL mapping to potentially identify genomic variants associated with increased glycogen and inflammation within hepatocytes observed in susceptible strains.

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