POPULATION VARIABILITY AND THE TERATOGENIC EFFECTS OF EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN DURING CARDIOGENESIS

An Undergraduate Research Scholars Thesis

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ABSTRACT

Population Variability and the Teratogenic Effects of Exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin during Cardiogenesis

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2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, also known as dioxin, is a powerful, environmental teratogen that researchers have failed to properly assess. Current maternal exposure studies do not consider genetic background as a factor in response variation. We aim to incorporate genetic variability found within the population in assessing exposure risks to dioxin during pregnancy. We will focus particularly on cardiogenesis by investigating early expression of cardiogenic markers. By using 5 different strains of pregnant female mice, we will mimic individual genetic types found in the human population. Over a 10-day period, pregnant mice will be exposed to different doses of TCDD (0, 1, 100 ng/kg). The embryos will then be removed and the heart excised to analyze the molecular and histological changes in development. Ultimately, the results of this study will elucidate the importance of including population heterogeneity in assessing toxicant exposure risks during cardiac development and potentially aiding in the underlying mechanism and treatment.

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CHAPTER I

INTRODUCTION

Dioxin and dioxin-like compounds are found in the environment, leading to multiple biological consequences upon exposure. The most common and potent dioxin is 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, also known as TCDD. The general population is exposed to small amounts of this dioxin on a daily basis through everyday products and common fatty foods. The exposure of TCDD is of great concern during pregnancy as it increases the risk of fetal abnormalities, particularly heart malformations. A study was conducted that looked at the cardiovascular teratogenicity of TCDD in the chick embryo. It was found that TCDD caused a dose-related increase in the incidence of cardiovascular malformations (Cheung et. al. 1981). Some particular types of malformations were ventricular septal defects, aortic arch anomalies, and conotruncal malformations (Cheung et. al. 1981). Another study used zebra fish to better understand embryonic heart malformations resulting from *in* utero dioxin exposure. After 72 hours post fertilization, the embryos exhibited pericardial edema and reduced blood flow. The embryos also exhibited altered looping with the atria positioned distinctly posterior to the ventricles (Antkiewicz et. al. 2005). There is not much mammalian data that links dioxin-induced embryonic malformations with genetic variation in populations. Our study will identify how TCDD expression contributes to mammalian malformations of cardiac development. We will do this by assessing the changes in expression of two master transcriptional regulators: (1) Gata4, important for cardiomyocyte regulation and septal development, and (2) Nkx2.5, required for heart tube formation.

CHAPTER II

METHODS

We used the following strains of mice in this study: A/J, Balb/cJ, BXD40, C57BL/6J. and FVB/NJ. The mice are kept in the L.A.R.R. animal facility (TAMU) and arranged in trio matings. Each female is checked for a copulation plug daily. All the positive-plugged females are separated and placed in a disposable cage on a separate rack. These mice are then weighed, and body composition is analyzed using EchoMRI scans. Mice are dosed with dioxin for a period of 9 days with peanut butter as the vehicle. On the 10^{th} day, the mice were dose via oral gavage with an olive oil vehicle. On day 10.5, the mice were euthanized and embryos were extracted. The embryos were flash frozen in liquid nitrogen and stored in -80°C freezer. Embryonic hearts were excised from the embryo and underwent RNA isolation. The fold activation of *Gata4* and *Nkx2.5* was quantified using qPCR. This will later be compared to the morphological measurements to determine the consequences of expression changes.

CHAPTER III

RESULTS

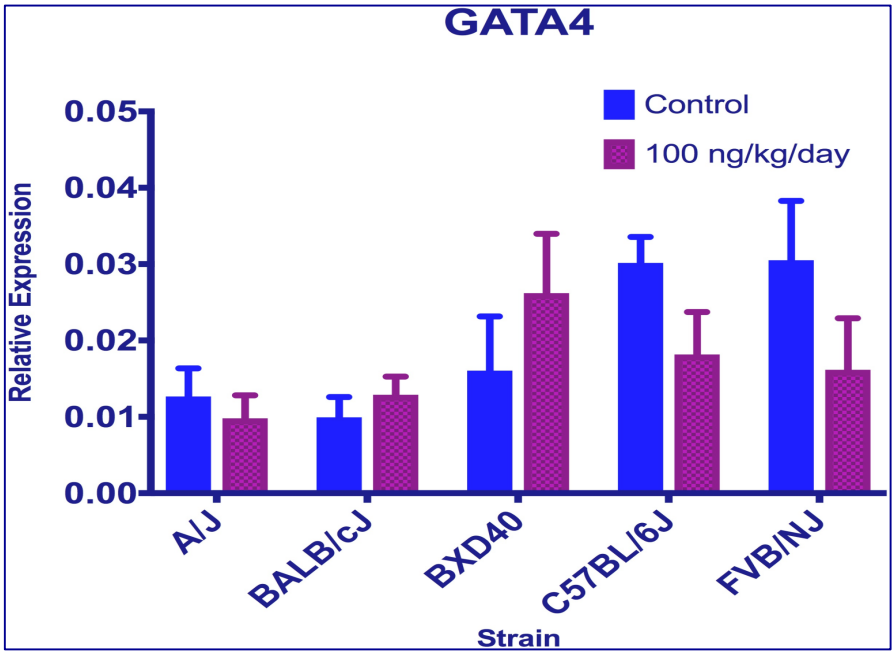

Figure 1: Gene Expression of Gata4 for five different strains at at dose of 0 and 100 ng/kg/day of TCDD

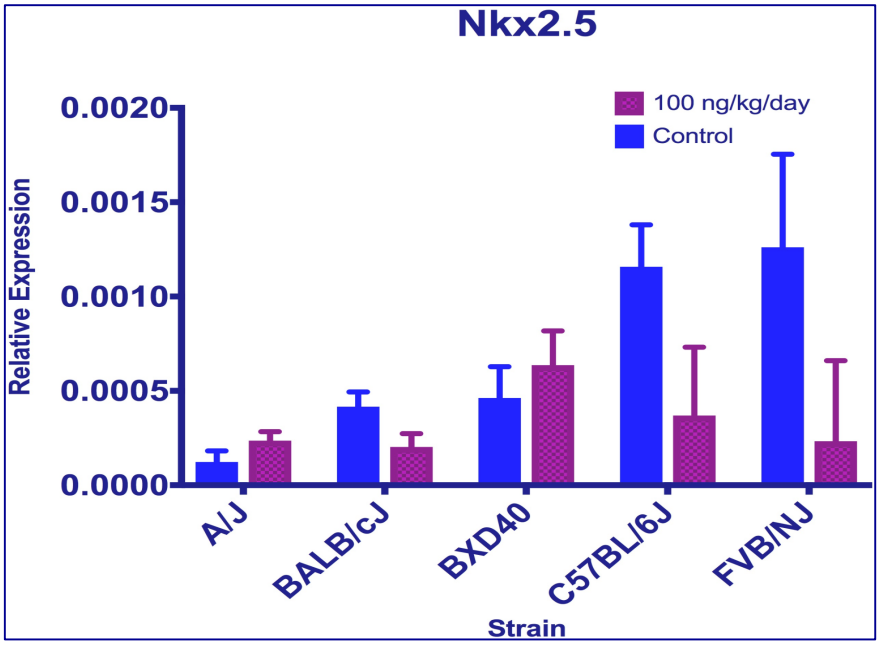

Figure 2: Gene Expression of Nkx2.5 for five different strains at a dose of 0 and 100 ng/kg/day of TCDD

CHAPTER IV

CONCLUSION

The data indicates that cardiogenesis might be impaired by dioxin. There are trends towards down regulation of *Nkx2.5* and *Gata4* expression levels in both C57BL/6J and FVB/NJ mice as shown in figure 1 and 2. There is also an upward trend of of *Nkx2.5* and *Gata4* expression levels in strain BXD40 mice as shown in figure 1 and 2. Increasing the sample size for each strain may yield more significant differences. Future work will include increasing the number of strains and expanding the dose range. More cardiogenic markers will also be tested.

REFERENCES

Antkiewic, D. S., Burns, G. C., Carney, S. A., Peterson, R. E., Heideman W. (2005). Heart Malformation Is an Early Response to TCDD in Embryonic Zebrafish. Toxicol. Sci., 82(2), 368-377. http://toxsci.oxfordjournals.org/content/84/2/368.short

Cheung, M. O., Gilbert, E. F., & Peterson, R. E. (1981). Cardiovascular teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the chick embryo. Toxicology and Applied Pharmacology, 61(2), 197-204. doi:http://dx.doi.org/10.1016/0041-008X(81)90409-9