Modeling the *in-utero* effects of reactivator and anticonvulsant treatments following acute organophosphate exposure in a novel humanized mouse model

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Introduction

Organophosphates (OPs) make up the most common class of pesticides in the world, and they are also used as nerve agents. OPs function by irreversibly binding to acetylcholinesterase (AChE), preventing it from hydrolyzing (breaking down) acetylcholine, an important neurotransmitter (Ferguson et al., 2019). As a result, acetylcholine accumulates in the synaptic cleft and continues to transmit messages between neurons and muscles, leading to seizures, paralysis, and death, if not treated. While this mechanism of action is well understood, it does not fully explain the consequences observed in exposed populations, including abnormal neural development (Guillette et al., 1998), decreased birth weights (Ferguson et al., 2019), and congenital malformations (Richardson & Chambers, 2004). Current fielded treatments for OP intoxication attempt to mitigate the primary cholinergic affects of acute exposure, but if there are secondary mechanisms in action, as the data would suggest, novel treatments are required to better treat those exposed. To this end, MRICD has developed a humanized mouse model that has proven effective for modeling OP intoxication and testing the efficacy of novel treatments. The project's objective was to determine the impact of both OP and treatment exposure in an atypical circumstance: pregnancy. Brain regions of baby mice exposed *in-utero* to varying OP and treatment conditions were histologically evaluated for myelin (the fatty protein insulating neurons) concentrations as well as tissue-specific gene expression between conditions. The findings from this project can be used to create an effective treatment regimen for pregnant animals.

Materials and Methods

Cohorts of KIKO mice were mated (N = 50) and monitored for pregnancy using weight gain greater than 2 grams as an indication of pregnancy. These mice were utilized because they had been genetically modified to be more similar to humans. Pregnant females were exposed to either an OP (paraoxon or sarin) or saline, followed by the standard treatment (a cocktail of atropam and midazolam) or a saline vehicle control 5 minutes later. Mice were grouped into every possible exposure and treatment condition, with a double saline treatment group as the overall control. Exposure to either OP or saline occurred on gestational day 14, a major timepoint in cholinergic development (Richardson & Chambers, 2004). Females were allowed to carry to term, and 8 weeks after birth, all offspring were sacrificed. Next, brain tissue from all remaining offspring was quickly perfused and sliced into sections of 10 µm thickness using a Leica CM1900 frozen slicer to be evaluated for morphological characteristics. Myelin was stained with Luxol Fast Blue (LFB) (Figure 1). After staining, an Olympus Virtual Slide Scanner took high-resolution photographs of the slides, which were imported into QuPath, a program that can count cells and analyze ratios.

Materials and Methods (cont.)

A micro-pixelated image was then quantified using black-white saturation in order to give a myelination ratio per region. The four brain regions of focus were the corpus callosum (1), hippocampus (2), thalamus (3), and cerebral peduncle (4). Tissues were then collected from five randomly selected offspring from each group in a sex-balanced manner to evaluate gene expression levels in the cerebellum, cortex, bone marrow, and gonads through fluorescent chip hybridization (see Figure 2). This process quantified expression of genes compared to the double saline control. All the known genes found to be differentially (abnormally) expressed were grouped into their respective pathways.

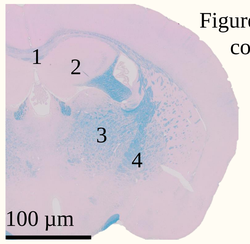


Figure 1 (left): Stained myelin in the cerebral cortex. Note the numbered brain regions of focus.

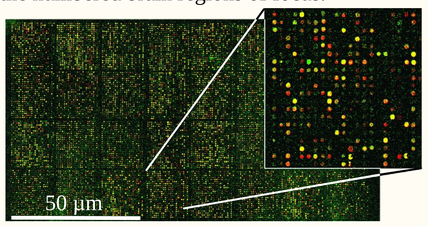
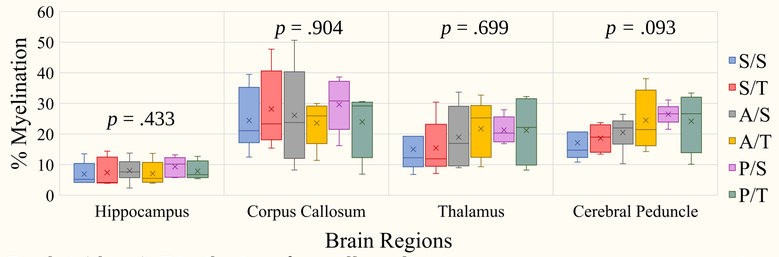


Figure 2 (right): Results from fluorescent gene chip hybridization. Bright colors indicate genes that are a differentially expressed (Deyholos & Galbraith, 2001).

Results

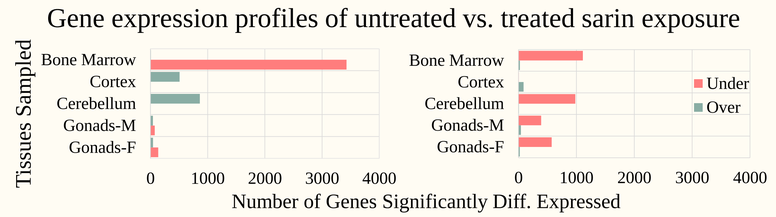
A one-way ANOVA, with a significance level of $p < .05$, was run for the two variables to compare experimental groups (n = 5 or 6, accounting for mice that died after exposure) to each other. In each brain region, there was no significant difference in percentage myelination across groups (Graph 1).



Graph 1 (above): Distribution of overall myelination rate across treatment groups. As for the treatment groups, S represents saline, A represents sarin, and P represents paraoxon. From left to right, $F(5,25)$ = 0.38, 0.22, 0.57, 1.39. It was expected that different exposure and treatment conditions would affect myelin coverage, but this was not observed in the samples.

In mice treated after paraoxon and sarin exposure, all tissues explored exhibited differential gene expression (Graphs 2 and 3).

Results (cont.)



Graphs 2 and 3 (above): Significant gene expression changes as a result of treatment ($p < .05$, ± 2-fold change). Tissue-specific gene expression changes were observed following sarin exposure. Bone marrow exhibited the highest impact of sarin, as well as the most benefit from the treatment. Conversely, gonads were adversely affected.

Conclusions

A humanized mouse model was successfully utilized to monitor both the effects of exposure and subsequent treatment of OP intoxication in pregnant mice, thus fulfilling the main objective. The results regarding myelination suggest that OP exposure has no effect on myelination coverage in the brain regions studied. This finding was unexpected, given the known susceptibility of these tissues to OP exposure. Alternatively, the gene expression profiles showed a major impact as a result of sarin exposure. This impact was then shown to be ameliorated by the currently fielded treatment option. However, changes in gene expression profiles as well as the efficacy of treatment were tissue-specific. For example, bone marrow was most affected by sarin and exhibited the greatest benefit from treatment, whereas gonads experienced a negative effect of treatment. Researching the mechanism by which OPs interfere with gene expression in this manner opens the door to the development of more targeted therapeutics. This pregnancy model can be used in future studies to document both neurodevelopment and genotoxicity as they relate to the developing cholinergic system.

References

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