Title of Research: ***25-1-12***

Elucidation of a New AOP Mechanism for Developmental Neurotoxicity Mediated by Oxidative Stress and Neuroinflammation

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Summary of Research:

To assess the effects of chemical exposure on children's neurodevelopment (developmental neurotoxicity, DNT), the OECD established new guidelines (No. 377) in 2023, combining 17 types of in vitro tests. These in vitro tests are expected to contribute significantly to elucidating molecular initiating events, such as receptor inhibition, and key events at the cellular level, such as neural differentiation, within Adverse Outcome Pathways (AOPs). However, it is increasingly recognized that existing test methods alone have limitations in elucidating AOPs. For example, the morphology and function of neurons and glial cells that make up the brain vary depending on the region and environment, but it is not easy to evaluate these changes in in vitro tests. On the other hand, while these aspects can be evaluated using mammalian models such as rats, issues arise regarding cost, effort, and animal welfare. Developing new approach methods that complement the weaknesses of existing methods and accelerating the elucidation of the AOP of DNT can contribute to accurately assessing the impact of chemicals on children's neurodevelopment and to realizing a sustainable society through the appropriate use of chemicals. In recent years, it has been established that oxidative stress and neuroinflammation are key events in the AOP of DNT, and that the oxidative stress and neuroinflammation induced by chemical exposure during development vary depending on the brain region. Furthermore, zebrafish, a vertebrate, are recognized as a useful model for chemical toxicity assessment because they are not considered animals during the larval stage (up to approximately 5 days post-fertilization) due to their inability to feed independently, thus raising few ethical concerns, and because the development of various organs is similar to that in mammals. The objective of this study is to elucidate the AOP by creating zebrafish that allow for the evaluation of the morphology and function of microglia, which are closely involved in oxidative stress and neuroinflammation in the brain,in each brain region, and by establishing a new DNT test using these zebrafish larvae. This year, we established a method for quantifying the morphology of microglia in the brain regions of zebrafish larvae by performing live imaging using two-photon excitation microscopy on transgenic zebrafish that express a fluorescent protein under the control of the mpeg1 promoter, a microglia- and macrophage-specific gene. We also used this method to evaluate the DNT of ethanol and valproic acid exposure. Furthermore, we generated transgenic zebrafish to visualize oxidized glutathione and hydrogen peroxide, markers of oxidative stress, in microglia. Next fiscal year, we will expose these zebrafish to compounds that are positive and negative for DNT and evaluate changes in oxidized glutathione and hydrogen peroxide in microglia for each brain region.

Timeline:

March 2025 ~ February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

Mizuki Yuge, Junko Koiwa, Takashi Shiromizu, Eri Wakai, Akira Migoguchi, Yuhei Nishimura. **Application to developmental toxicity testing of a novel method for whole-brain imaging of microglia in zebrafish.** *Current Research in Toxicology* 10:100276 (2025)