Title of Research:

20_PT03-05

Establishment of an adverse outcome pathway for the evaluation of developmental neurotoxicity in chemical-induced hypothyroidism

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Summary of Research:

Recently, the concern over the toxicity of environmental chemicals that induce hypothyroidism has been increased, since hypothyroidism during pregnancy can have a significant impact on the development of the fetal brain in human. However, the results of thyroid function disruption detected by toxicity testing have not been sufficiently utilized for risk assessment of chemical substances because the correlation between the degree of hypothyroidism during pregnancy and adverse effects on the development of the fetal brain remains unclear. In order to solve the problem, we focused on differentiation markers of mature neurons and produced reporter transgenic mice (Tg mice) carrying luciferase (Luc2) and LacZ genes downstream of a neuronal differentiation marker promoter. In the past year, we have confirmed the usefulness of Tg mice in assessing developmental neurotoxicity and found the possibility of evaluating the effects of hypothyroidism from early pregnancy on the infant brain using the antithyroid drug propylthiouracil (PTU). In this fiscal year, we analyzed the correlation between the expression profile of *in vivo* imaging in the infant brain and maternal thyroid-related parameters when hypothyroidism was induced by exposure to various doses of PTU from early pregnancy.

PTU induced a dose-dependent decrease in triiodothyronine (T3)/thyroxine (T4) and an increase in thyroid-stimulating hormone (TSH) in maternal blood. In histological analysis of the thyroid gland, symptoms of hypothyroidism such as thickening of follicular cells were observed even at low-dose PTU exposure prior to significant changes in serum T3/T4/TSH levels. In addition, the expression of reporter molecules in the brain of the infants was also increased in a dose-dependent manner of PTU. Furthermore, similar to the results of thyroid histological analysis in the mother, the effects were observed even at low-dose without significant changes in the serum levels of thyroid-related hormones.

These results suggest that in chemical-induced hypothyroidism, thyroid histology is a more sensitive indicator for detecting their effects than serum thyroid-related hormones, and that some adverse effects on brain development in the child may occur prior to changes in maternal serum thyroid-related hormones.

Timeline: April, 2022 – March, 2023

Topics:

1) Oral presentation at JCIA LRI Annual Workshop 2020 “Establishment of an adverse outcome pathway for the evaluation of developmental neurotoxicity in chemical-induced hypothyroidism” (On-line, August 26th, 2022)
2) Report in the Yomiuri Shimbun Morning Edition “Environmental Chemicals and the Brain:A New Approach Methodology Using "Glowing Mice" - Gifu Pharmaceutical University, etc., Verify Effects on Children” (February 7th, 2023)

Publications:

1) Ishida K, Tatsumi K, Minamigawa Y, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Neuronal differentiation reporter mice as a new methodology for detecting in vivo developmental neurotoxicity. ***Biochem. Pharmacol.***, 206:115332 (2022)
2) Ishida K, Matsumaru D, Shimizu S, Hiromori Y, Hisamitsu Nagase H, Nakanishi T, Evaluation of the estrogenic action potential of royal jelly by genomic signaling pathway *in vitro* and *in vivo*. ***Biol. Pharm. Bull.***, 45:1510–1517 (2022)
3) Ishida K, Furukawa M, Kunitani M, Yamagiwa R, Hiromori Y, Matsumaru D, Hu J, Nagase H, Nakanishi T, Novel, highly sensitive, *in vivo* screening method detects estrogenic activity at low doses of bisphenol A. ***J. Hazard. Mater.*** 445:130461 (2023).

Conference Presentations:

1) Ishida K, Minamigawa Y, Tatsumi K, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Validation of brain neuronal differentiation reporter mice for improved developmental neurotoxicity evaluation, 2022 ICCA-LRI & NITE Workshop, Yokohama/Japan, June 2022
2) Tatsumi K, Ishida K, Minamigawa Y, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Validation of brain neuronal differentiation tracer mice for improvement developmental neurotoxicity evaluation, The 49th Annual Meeting of the Japanese Society of Toxicology (Sapporo), 2022.
3) Mori K, Ishida K, Minamigawa Y, Tatsumi K, Matsumaru D, Murashima A, Nagase H, Kanda Y, Nakanishi T, Imaging analysis of the offspring brain in a model of maternal hypothyroidism, The 49th Annual Meeting of the Japanese Society of Toxicology (Sapporo), 2022.
4) Ishida K, Minamigawa Y, Tatsumi K, Mori K, Matsumaru D, Nagase H, Kanda Y, Takuma K, Nakanishi T, Establishment of *in vivo* neuronal differentiation tracing method for improved developmental neurotoxicity evaluation, The 62nd Annual Meeting of the Japanese Teratology Society (Kanazawa), 2022.
5) Ishida K, Matsumaru D, Nakanishi T, Perinatal hypothyroidism and DOHaD: Aiming for the risk assessment of neurodevelopmental disorders in offspring, Forum 2022 Pharmaceutical Health Sciences/Environmental Toxicology (Kumamoto), 2022.
6) Ishida K, Tatsumi K, Minamigawa Y, Mori K, Matsumaru D, Murashima A, Nagase H, Kanda Y, Takuma K, Nakanishi T, Neuronal differentiation reporter mice as a new methodology for detecting *in vivo* developmental neurotoxicity, *In vivo* imaging forum 2022 (Tokyo), 2022.