

## 論文の内容の要旨

論文題目           Molecular and morphological analysis of developmental toxicity in the cerebral cortex upon *in utero* exposure to environmental chemicals  
(胎仔期環境化学物質曝露による大脳皮質発生毒性の分子・組織学的解析)

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Pregnant women are exposed to a variety of environmental chemicals, and some of the chemicals will pass through the placental barrier and enter into the fetal bloodstream. The prevalence of developmental disorders has been suggested to be partly associated with exposure to environmental chemicals. The developing brain is highly sensitive to environmental chemicals. It has been revealed that the low-dose environmental chemical exposure that does not induce manifest toxicity in dams may induce adverse outcomes in offspring resulting in irreversible changes in brain functions. The cerebral cortex plays a key role in higher brain function, such as learning, memory and emotion. It is composed of highly organized cellular layers that are formed by neuronal migration. Orchestrated formation of the cerebral cortex architecture is crucial for normal brain function. Among various kinds of environmental chemicals, bisphenol A (BPA) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have been shown to influence the higher brain function. Thus, the objectives of this study were to investigate whether prenatal exposure to low doses of BPA or TCDD affected the cerebral cortex development using neuronal migration as a representative endpoint of developmental neurotoxicity, and explored the molecular mechanisms underlying the developmental abnormalities in the brain.

Bisphenol A, a high volume chemical used to produce plastics, has been suspected to affect infants and children's health including brain development. This study was attempted to elucidate the possible effects of maternal low-dose BPA exposure on the process of brain development. Pregnant

mice were exposed to BPA by implanting osmotic pump at a daily dose equivalent to 0 (control), 40 (BPA40), 400 (BPA400)  $\mu\text{g/kg}$  b.w. from embryonic day (E) 14.5 to E18.5. To investigate whether *in utero* BPA exposure affects neuronal migration, fetuses were subjected to an *in utero* electroporation (IUE) at E14.5 to introduce fluorescent protein expression vectors (pCAG-mCherry) into neural progenitor cells to visualize the migration processes, and brains were analyzed at E18.5. Neuronal migration was significantly perturbed in the BPA40 group compared with the control group and the BPA400 group. This result was consistent with those obtained in our previous study, in which adult male mice born to BPA-exposed dams (40 $\mu\text{g/kg}$  b.w./day) significantly exhibited impulsive behavior compared with the control group, but there was no significant difference between BPA400 group and the control group, suggesting BPA affects the cerebral cortex development in a non-monotonic dose response manner. To study the molecular mechanism basis of this phenomenon, several genes required for neuronal migration were examined. TrkB, a receptor for neurotrophins, was significantly increased in the BPA400 group compared with the control group. Robo1, a neuronal guidance receptor, was significantly increased in the BPA40 group compared with BPA400 group. In addition, dopamine and its metabolites in the forebrain of male pups were significantly increased in BPA400 group compared with BPA40 group. Serotonin turnover, i.e., ratio of 5-HIAA to 5-HT, in the mid-hindbrain was significantly decreased in BPA400 group compared with the control group. This study shows that prenatal BPA exposure significantly affected cerebral cortex development, supporting the hypothesis that abnormal behaviors in adulthood have fetal origins due to abnormal neuronal migration and metabolic alteration in monoamine levels.

Dioxins and related compounds are a group of persistent organic pollutants. 2,3,7,8-Tetrachlordibenzo-*p*-dioxin (TCDD), the most toxic congener of dioxin compounds, has been shown to exhibit developmental neurotoxicity even at a low dose. In previous studies, perinatal exposure to low doses of TCDD was found to affect behavioral flexibility, sociality, fear memory, paired associate learning and memory with its mechanisms being elusive. To test the hypothesis that

there may be morphological abnormal alteration in cerebral cortex during fetal period upon *in utero* TCDD exposure, the pregnant B6 mice were orally administered TCDD at a dose of 0 (control), 0.6 (TCDD0.6) or 3.0 (TCDD3.0)  $\mu\text{g/kg}$  b.w. at E12.5, and neuronal migration was analyzed at postnatal day (P) 0. A significant reduction in neuronal migration was observed in both TCDD0.6 and TCDD3.0 groups compared with the control group, suggesting that cerebral cortex development was interrupted by prenatal TCDD exposure. To study the gene expression with regard to possible TCDD effects on cerebral cortex development, 44 genes were analyzed by quantitative real-time PCR. The mRNA expression of Cyp1a1, Cyp1b1, AhRR, Cxcl4, Pitx3, and Pisd-ps3 was found to be significantly altered in TCDD-exposed brains compared with the control group. Next, B6 and B6.D2N-Ahrd/J (B6D2) mice that possess high affinity b-type AhR allele and low affinity d-type AhR allele, respectively, were used to study the role of AhR in TCDD induced toxicity in the developing brain. The pregnant B6 and B6D2 mice were orally administered TCDD at a dose of 0 or 20  $\mu\text{g/kg}$  b.w. at E12.5, and the brains of pups were examined at P0. The expression of Cyp1a1 and Cyp1b1 in the brain of TCDD-exposed B6 mice was significantly enhanced compared with that of B6D2 mice, and the cortical thickness was significantly decreased in TCDD-exposed B6 mice but that was not found in B6D2 mice. These data suggest that developing toxicity of TCDD is mediated through AhR. Furthermore, similar to the effects of prenatal TCDD exposure (0.6, 3.0  $\mu\text{g/kg}$  b.w.) on neuronal migration in B6 mice, a subtle but significant difference was found in B6D2 mice after exposed to a relatively high dose of TCDD (20  $\mu\text{g/kg}$  b.w.). To test the hypothesis that AhR plays a role in neuronal migration, conditional AhR knockout (AhR-KO) mice were generated by Cre-loxP system. AhR<sup>flx</sup> mice having loxP sites on either side of exon 2 of the AhR gene were subjected to IUE at E14.5 to introduce Cre expressing vectors into the progenitor cells for the purpose of deletion the AhR gene, and the neuronal migration was analyzed at E17.5 or E18.5. There was a significant difference in neuronal migration at E18.5 in AhR-KO group compared with the control group, indicating that AhR may play a role in neuronal migration.

The present studies show that prenatal exposure to low doses of BPA or TCDD affected cerebral cortex formation, which possibly results in abnormal higher brain function later in adulthood, suggesting the association between chemical exposure and abnormal behavior may have its origins at the early stage of brain development. Future studies are needed to answer the key question how the abnormal morphological phenotype of neuronal migration affects the higher brain function, such as learning, memory, and emotion in adulthood.