日本財団補助金による
２０００年度日中医学学術交流促進事業

⑤．在留中国人研究者研究助成

(4)ダイオキシンによる脳セロメニン異常に関する研究

In utero and lactational exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin decreases serotonin immunoreactivity in the raphe nuclei of mice

Shi-Bin Cheng

Lecturer, Department of Physiology, Faculty of Medicine, Sun Yat-sen University of Medical Sciences, Guangzhou, P. R. China;

PhD student, Department of Anatomy, Faculty of Medicine, Kagoshima University, Japan

要旨

メスマウスにダイオキシンを経口投与したあと交配し、その産子を母乳飼育した。ダイオキシン胎盤/母乳暴露動物を6週齢で殺し、脳内セロトニンを免疫組織学的に検出した。さらに、セロトニン産生細胞の数をコンピュータ解析法を用いて定量的に分析した。その結果、ダイオキシン胎盤/母乳暴露動物の脳内セロトニン産生細胞は対照群に比較して著しく減数していることが明らかとなった。以上を学会報告した。

Key Words: 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin; Raphe nuclei; Serotonin; Mouse; Brain

Purpose:

2, 3, 7, 8-tetrachlorodibendizo-p-dioxin (TCDD) is one of the most toxic members among a broad group of chemical environmental contaminants termed dioxin. It is formed during combustion of municipal waste and many industrial processes. Because of its lipophilicity and resistance to physical and biological breakdown, it wildly persists and accumulates in the environment and food chain. A body of data has indicated a wide spectrum of toxic effects of TCDD on growth, reproduction, immune system, endocrine, carcinogenesis and central nervous system in laboratory animals and human. Acute administration of a lethal dose of TCDD to adult animals induces a variety of severe intoxication including anorexia, progressive body weight loss, and reduced locomotor activity before death. Perinatal exposure to TCDD elicited low core body temperature in the rat. However, the mechanisms of these pathophysiological syndromes are still unknown. Serotonin is wildly localized in the central nervous system and involved in mediating many physiological functions, e.g., food intake, cognition, emotion and body temperature. Thus, the present study is to

examine the serotonin activity in the brain of the mouse by *in utero* and *lactation* exposure to TCDD.

Methods:

Twelve female adult ddY mice were orally exposed to olive oil or olive oil containing TCDD for 8 weeks prior to mating. The offspring were consequently exposed to TCDD in utero and via lactation. Offspring were weaned on postnatal day 28 and group-housed in plastic cages, and then allowed to survive for another 6 weeks. After anesthetized, all animals were transcardially perfused with 100 ml 0.1 M phosphate-buffered saline followed by a fixative containing 4% paraformaldehyde and 0.2% picric acid in 0.1M phosphate buffer (pH 7.4) at 4℃. The brains were removed, post-fixed in the same fixative, and then immersed in 30% phosphate-buffered sucrose at 4℃. Serial colonial sections were cut on freeze microtome and collected in 0.1M PBS and then processed for serotonin immunocytochemistry by PAP method. In briefly, after blocking endoperoxidase by using 0.15% H_2O_2, the floating sections were pre-incubated in a blocking solution of 0.3% Triton X-100, 1% normal goat serum, 0.05% sodium azide and 0.3% bovine serum albumin (BSA) in

phosphate buffer saline, pH 7.4 (PBS), and then incubated for 3 days at 4℃ in a solution containing rabbit anti-serotonin antibody (1:10000) diluted in the blocking solution. After several rinses with PBS, sections were immersed in a solution of goat anti-rabbit IgG (1:2000) overnight at 4℃, and following several rinses, sections were then incubated rabbit PAP (1: 2000) overnight at 4℃. The second and third antibodies were diluted in 0.1M PBS containing 0.3% BSA and 1% normal goat serum. Following several rinses with 0.1 M Tris-HCl buffer (pH 7.6), the sections were treated with 0.02% diaminobenzidine in 0.003% hydrogen peroxide. After final washes, the sections were mounted onto gelatin-chrome alum-coated glass slides, air dried, dehydrated with ethanol, cleared in xylene, and cover-slipped. Number of serotonin-immunostained cell bodies was counted with aid of a public domain NIH image system. For statistical analysis of the differences in serotonin-immunostained cell number between TCDD-exposed offsprings and control mice, Dannett's test was used. The specificity of serotonin antibody was tested.

Results:

The distribution of immunohistochemically stained serotonergic neurons

in the control and TCDD-treated mice was similar to that of other mammals. Serotonin-immunoreactive neurons were present in the caudal linear nucleus, the median raphe nucleus, the supralemniscal area, the pedunculopontine tegmental nuclei, the deep mesencephalic nucleus, the raphe magus, the raphe obscurus, the raphe pallidus, ventrolateral medulla, dorsal and medial to the facial nucleus and surrounding the central canal of the rostral end of the spinal cord. In the male offspring animals by in utero and lactational TCDD-exposure, a marked decrease in intensity of serotonin-immunostaining and number of serotonin-positive neurons was found in all raphe nuclei. Dunnett's test analysis showed that there were significant differences in cell number between all TCDD-exposed offspring and vehicle mice.

Discussion:

Our results indicate that in utero and lactational exposure to TCDD induces a great decrease in serotonin activity of ddY mouse brain. This suggests that TCDD may act as a neuroteratogen, which produces long-term alterations in serotonin synthesis. It was reported that serotonin and/or its metabolite concentration in the brain of the adult

TCDD-susceptible Long-Evans rat increased after a single lethal dose of TCDD. Similar results were also reported in the Sprague-Dawley rat and hamster. Other studies indicated that prenatal and early postnatal exposure to dioxins changed behavior and psychic function in human and animals. Our present results suggest that a great decrease in serotonin activity may be associated with these pathological changes. This study is the first morphological demonstration of TCDD-induced biochemical changes in the central nervous system.

References:

1. Bjerke D. L., Brown T. J. et al., 1994. Toxicology and Applied Pharmacology 127, 258-267
2. Unkila M., Pohjanvirta R. et al., 1995. Int. J. Biochem. Cell Biol. 27, 443-455
3. Pohjanvirta R., Unkila M., et al., 1993. Life Sci. 53, 1479-1487
4. Bestervelt L.L., Nolan C. J., et al., 1991, 495-497
5. Schantz S., Ferguson S. A., et al., 1992. Neurotoxicology and Teratology, 14, -433-446