Effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the next generation: mechanisms and prevention of developmental disorders and sexual immaturity

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Effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the next generation: mechanisms and prevention of developmental disorders and sexual immaturity （2,3,7,8-Tetrachlorodibenzo-*p*-dioxin 母体曝露による次世代影響：発育障害と性未成熟のメカニズムと予防）

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【Objectives】

Maternal exposure to dioxins causes growth and developmental disorders in the offspring. The mechanisms underlying these damages and approaches to their management remain poorly understood. Therefore, future studies should focus on elucidating the mechanisms underlying dioxin toxicity and developing convenient countermeasures to protect the health of the next generation. Previous research in our laboratory revealed that the sexual immaturity of the next generation due to dioxins is caused by perturbation of the endocrine system in the fetal pituitary gland. Dioxin-induced toxicity in the next generation has been studied mainly through three mechanisms (Fig. 1): reduction of luteinizing hormone (LH) and growth hormone (GH) in infant pituitary glands, and reduction of pituitary prolactin in maternal rats. Previous research in our laboratory revealed that a decrease in LH in offspring during the critical period suppresses sexual behavior, such as mating, after growth [1–3], and a decrease in GH in offspring during the critical period causes growth retardation [4, 5]. It was also confirmed that a decrease in maternal pituitary prolactin, which is a parenting hormone that regulates maternal behavior, leads to decreased maternal behavior, and causes a decline in infant growth [6]. Further elucidation of these detailed mechanisms and the development of methods to prevent maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are warranted. Our previous studies have provided a deeper understanding of the mechanisms underlying the post-generational toxicity of TCDD.

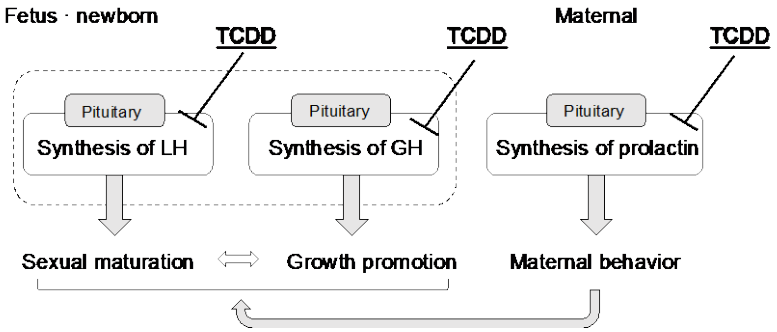

Fig. 1. Presumed mechanism of dioxin next-generational toxicity based on pituitary hormones.

【Methods】

In the experiment described in Section 1, Wistar rats at gestational day (GD) 15 were orally administered with TCDD (1 μg/kg) or corn oil (control group) and then allowed spontaneous parturition. Simultaneously, the rats from the control and TCDD groups were further subdivided into two groups, and one group each of the control and TCDD subgroups were subjected to α-lipoic acid (LA) intervention until PD21. At postnatal day (PND) 4, the number of offspring was adjusted to 4 males and 4 females and weaned at PND21. The SDN-POA volumes in males were detected at PND28. The saccharin preference experiment was carried out until females reached 7 weeks old.

In the experiment described in Section 2, female rats were treated with TCDD or corn oil (control) in the same manner as described above and allowed spontaneous parturition. At seven weeks of age, the F1 females were pregnant. Breast milk was collected on PD5, and mammary gland tissue

was collected on PD21. Similarly, at seven weeks of age, F2 females became pregnant, and their breast milk was collected on PD5.

In the experiment described in Section 3, female rats were treated with TCDD or corn oil (control) in the same manner as described above and allowed spontaneous parturition. Rats from the control and TCDD groups were further subdivided into two groups, and one group each of the control and TCDD subgroups was subjected to aripiprazole (ARI) intervention. For both groups, the GD20 diet was replaced with a diet containing ARI until PD7. The concentrations of ARI in the diet were set at 0.003% and 0.009% for low and high doses, respectively. Maternal behavior was evaluated using PDs 2, 4, and 7. Dam tissues were collected on PD7. Offspring body weights and body lengths were measured on PNDs 2, 4, and 7.

【Results】

1. Maternal administration of LA restores genital atrophy in males and sexual dimorphism in both male and female offspring. TCDD-induced LA insufficiency during sexual differentiation is likely a direct cause of TCDD reproductive toxicity. This study demonstrated that LA treatment can prevent and recover next-generation dioxin reproductive toxicity, suggesting its potential as an effective protective measure against dioxin toxicity (Fig. 2).

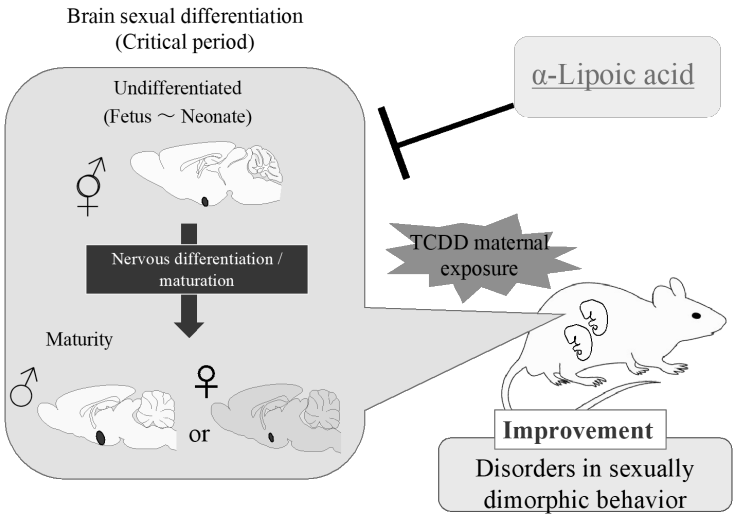

Fig. 2. Presumed mechanisms of TCDD maternal exposure-induced reproductive toxicity in offspring and the recovery effects of LA supplementation.

2. Maternal exposure of F0 dams to TCDD reduced milk production in F1 and F2 offspring during lactation. This reduction is consistent with a decreased prolactin constitution [7]. Breast developmental disorders and changes in the milk nutrient composition during lactation have also been observed in F1 females. Maternal exposure to TCDD attenuated prolactin levels in F1 and F2 females, which inhibited mammary gland development and milk secretion, and decreased milk nutrient protein levels. These inhibitory effects due to decreased prolactin levels may explain the suppression of infant maturity and low prolactin levels in female offspring. These findings suggested a novel mechanism for the generational inheritance of TCDD-induced developmental disorders (Fig. 3).

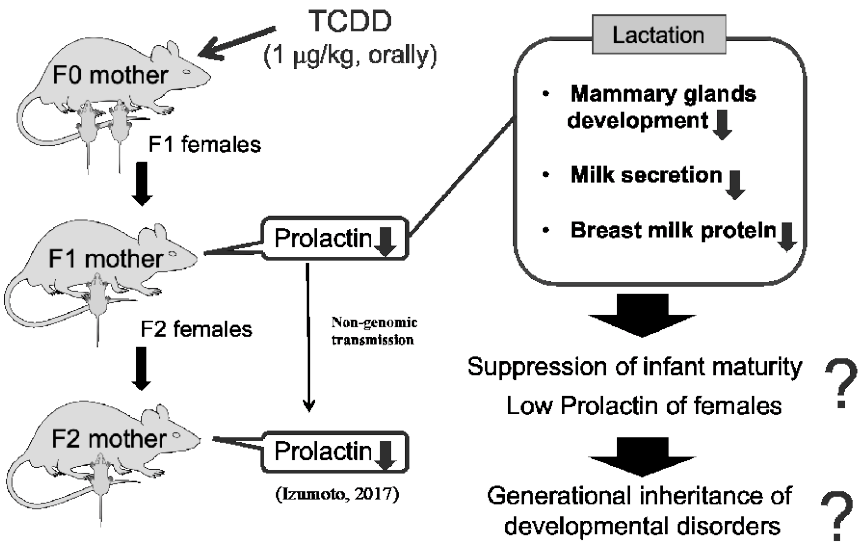

Fig. 3. Presumed mechanisms of TCDD maternal exposure-induced transgenerational toxicity.

3. TCDD treatment significantly reduced prolactin expression and maternal behavior, and ARI treatment significantly restored this suppression. In addition, measurements of body height and weight, which are used as indicators of infant growth and development, revealed that the height and weight of pups from TCDD-exposed dams were significantly suppressed. These results were consistent with the decreased maternal prolactin expression, and ARI intervention significantly restored this suppression. These findings suggest that ARI intervention can ameliorate developmental delay in the next generation by restoring maternal prolactin levels and behaviors (Fig. 4).

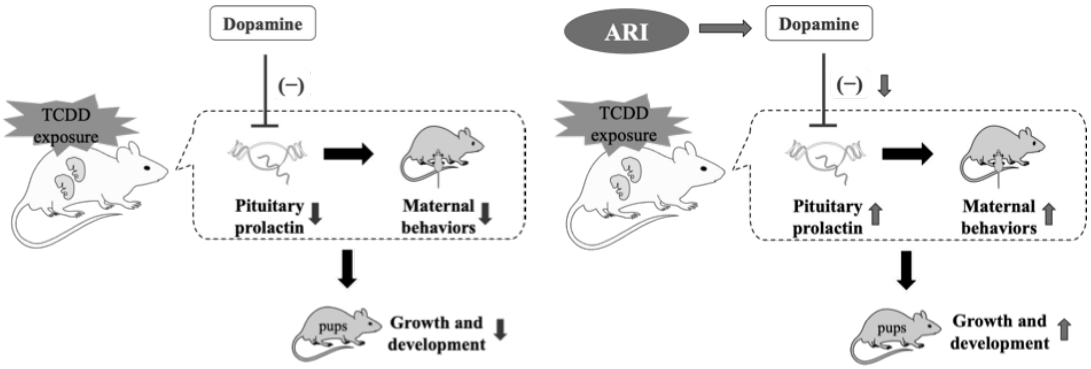

Fig. 4. Presumed mechanisms of TCDD maternal exposure-induced growth retardation in offspring and the recovery effects of ARI supplementation.

【Discussion】

This study investigated the mechanisms and prevention of developmental disorders and sexual immaturity in offspring caused by maternal exposure to TCDD. Dioxins exert toxicity in offspring via three mechanisms (Fig. 1): reduction of LH and GH in the infant pituitary, and reduction of prolactin in the maternal pituitary. To explore the mechanisms by which maternal TCDD exposure causes sexual immaturity in offspring, we previously found that part of the pituitary LH suppression is caused by decreased hypothalamic LA, and that maternal LA supplementation exerts a recovery

effect on LH. In the present study, we confirmed that maternal LA supplementation had a recovery effect on the sexual differentiation of the offspring (Fig. 2). In addition, female offspring from dams maternally exposed to TCDD exhibited breast development and milk secretion disorders during lactation because of low prolactin levels. These findings provide novel insights into mechanisms underlying next-generation toxicity. Alterations in F1 milk nutrients during early lactation were also confirmed. Simultaneously, insufficient nutrients obtained from the offspring may also cause developmental toxicity in TCDD; however, subsequent experiments are necessary for confirmation. Nevertheless, the present results confirm that decreased prolactin levels are one of the mechanisms underlying TCDD-induced developmental impairment in offspring (Fig. 3). Therefore, we explored an intervention method to restore prolactin levels during pregnancy. Results showed that maternal supplementation with ARI significantly restored prolactin mRNA expression and parenting behavior, indirectly attenuating growth impairment in the offspring (Fig. 4). The administration of LA and ARI during pregnancy and lactation can improve reproductive toxicity and some developmental disorders in the offspring by restoring the secretion of LH in the offspring and prolactin in dams. However, neither of these methods can fundamentally restore GH secretion in the offspring. Therefore, future experiments are warranted to develop intervention methods to restore GH levels in the offspring and attenuate or eliminate offspring toxicity induced by TCDD through a combination of drugs.

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【Publication】

Yuan M*, Sano H*, Nishino T, Chen H, Li RS, Matsuo Y, Nishida K, Koga T, Takeda T, Tanaka Y, Ishii Y. α-Lipoic acid eliminates dioxin-induced offspring sexual immaturity by improving abnormalities in folic acid metabolism. *Biochem Pharmacol.* 2023; 210:115490. *Equally contributed