EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN ON SURGICALLY INDUCED ENDOMETRIOSIS IN MICE AND THE ROLE OF AH RECEPTOR

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Introduction

Endometriosis is a disease of women, characterized by the growth of endometrial tissue outside the uterus, usually in the peritoneal cavity. This condition may cause infertility and a high degree of pain. Precise etiology and pathogenesis of endometriosis remains unknown. While retrograde menstruation is faulted for providing endometrial tissue to the peritoneal cavity, immunological defects may play a role in the development of endometriosis[1]. It is also well recognized that endometriotic tissues are estrogen dependent[2]. Environmental toxicants could affect the development and growth of endometriosis through either hormone mimicry, immunosuppression, or both mechanisms[3]. Recently, an increased incidence and severity of endometriosis was reported in rhesus monkeys treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[4]. The effects of TCDD on the female reproductive tract have also been investigated using the recently developed rodent model of endometriosis. Subchronic exposure to TCDD provoked an increase in the growth of surgically induced endometriotic lesions in both rats and mice[5]. By contrast, subacute exposure to TCDD resulted in inhibition of the endometriotic lesions in ovariectomized mice replaced with exogenous estrogen[6]. Moreover, a recent study by Cummings et al.[7] reported a promotive effect in prenatal plus adult exposure but not in adult exposure only. Hence, the effects of TCDD on the development of endometriosis are still controversial.

Many of the well-studied effects of TCDD are mediated by the aryl hydrocarbon receptor (AhR)[8]. Using the structure activity relationship study of some polyhalogenated aromatic hydrocarbons, Johnson et al.[9] suggested that the proliferative effect of TCDD on endometriotic lesions may be AhR-mediated. However, there is no direct evidence supporting this hypothesis. Recently, we clearly showed the involvement of AhR-mediated mechanism in TCDD teratogenesis by the experiment with AhR-deficient mice[10].

The present study aimed at evaluating the effects of TCDD on the growth of endometriosis, and at examining the role of AhR using AhR-deficient mice.

Materials and Methods

Dosing. Sexually mature C57BL/6 female mice from CLEA Japan, Inc. (Tokyo) were used. Ten days before induction surgery, TCDD (Cambridge Isotope Laboratories, originally solved in nonane at a concentration of 50 μg/1.2 ml, and diluted with corn oil) was orally given by gavage. In the first series of experiment, the dose levels applied were 10 and 40 μg/kg body weight and the dose volume was 5 ml/kg body weight. Control mice received the vehicle. In one subgroup the dosing was single, and in another repeat dosing was applied a total of four times with three weeks interval. In the second

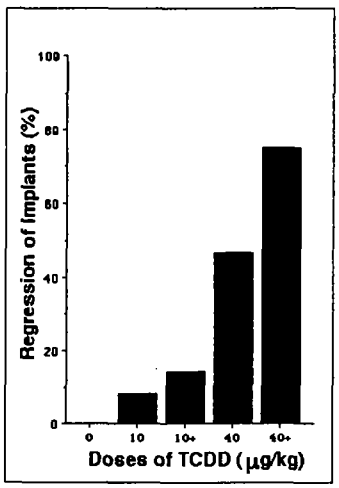
series, lower levels (0.156, 0.625 and 2.5 μg/kg) with repeat dosing were applied. In the experiment using AhR-deficient mice, the dose levels were 2.5 and 40 μg/kg. The generation of *Ahr*$^{-/-}$ mice and checking of genotypes were as described before[10].

Surgery. Since mice have an estrous cycle instead of a menstrual cycle and do not develop endometriosis naturally, endometriosis must be induced through surgical methods. Induction surgery was performed 10 days after the first dosing using a modified method described previously[5,11]. Right uterine horn was excised, dissected longitudinally, and cut into several pieces of full thickness tissues (2 x 2 mm). The uterine pieces (3 or 4 pieces) were implanted into the peritoneal cavity by suturing onto alternating mesenteric blood vessels.

Tissue Analysis. Eight weeks after surgery the animals were killed by cervical dislocation. Endometrial implants diameters and weights were measured as indicators of endometriotic development. Weights of left uterine horn, ovaries, liver, and thymus were also measured. The tissues were fixed in Bouin's solution and processed for histopathology.

Results and Discussion

By 8 weeks after surgery, surgically-induced endometriotic sites were fluid-filled, cyst-like structure of spherical or ovoid shape (3-5 mm in diameter). Histologically, the sites consisted of outer smooth muscle, middle stromal layer with endometrial glands, and inner layer of single columnar epithelial cells. The lumen of the cystic sites were filled with amorphous material in which inflammatory cells were seen. These results of morphological changes are consistent with the previous studies[5,6]. Bilateral ovariectomy at the same time of induction surgery resulted in the complete regression of endometrial implants, although tissue architecture was well preserved histologically, indicating the dependence on estrogen and/or other factor(s) from ovary of growth of endometrial implants.



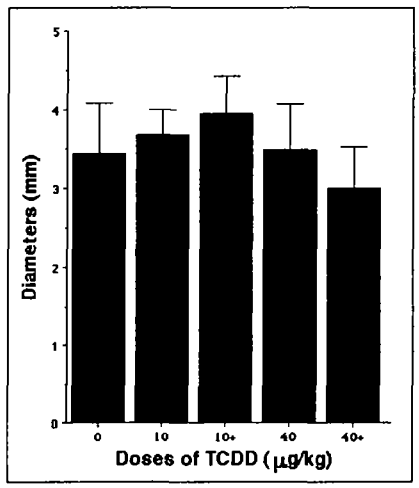


Fig. 1 (left) Regression of endometrial implants of TCDD-treated mice.
+ letter in dose level means repeat dosing with three weeks interval.
Fig. 2 (right) Diameters in long-axis of endometriotic lesions.

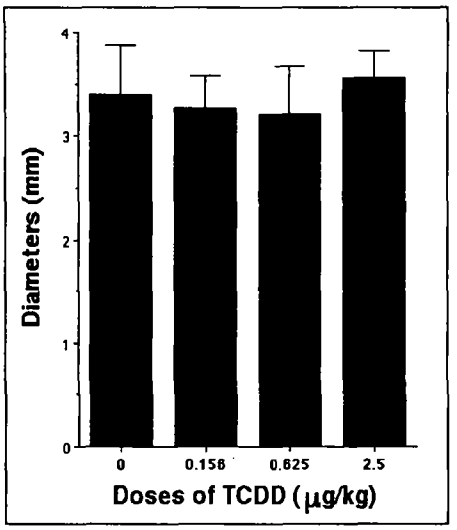


Fig. 3 Diameters in long-axis of endometriotic lesions at lower levels of dosing.

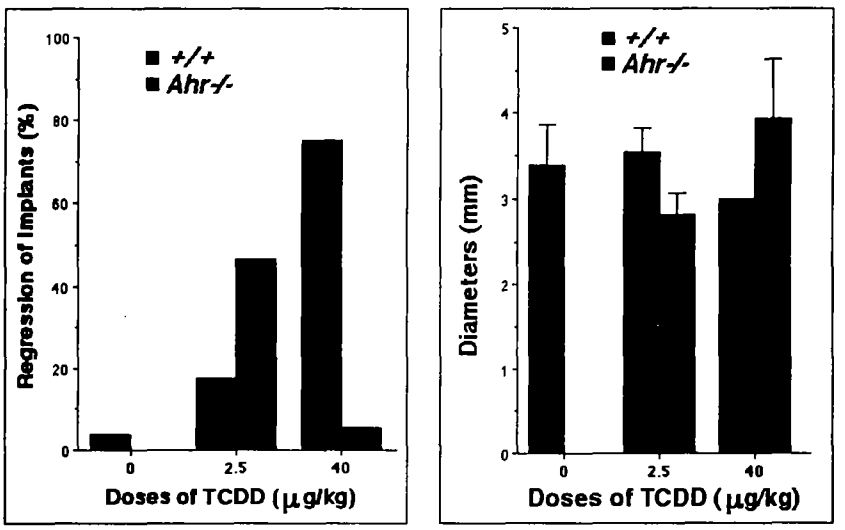


Fig. 4 The response of AhR-deficient mice to TCDD.
(Left) Regression of endometrial implants.
(Right) Diameters in long-axis of endometriotic lesions.

One of the prominent effects of TCDD in this study was the regression of implants, as shown in Fig. 1. No regression occurred in controls. At repeat dosing of 40 μg/kg TCDD regression was remarkable. This is probably due to the decrease in estrogen production since in these animals

ovarian as well as thymic atrophy was severe, or alternatively, may reflect the antiestrogenic effect of TCDD.

Figures 2 and 3 show the diameters in long-axis of endometriotic sites. Note regressed implants were omitted from the measurements. Differences in the growth of endometriotic sites were not significant. Even at the lower levels of dosing no significant differences in the growth were obtained, although Johnson et al.[9] reported the enhanced growth at the dose levels of 1 and 3 μg/kg TCDD.

The response of AhR-deficient mice to TCDD exposure is shown in Fig. 4. No differences in the growth (diameters) were observed, however, the incidence of regression at 40 μg/kg of TCDD was clearly lowered in AhR-deficient mice, indicating the involvement of AhR-mediated mechanism in this process. The incidence of regression at 2.5 μg/kg of TCDD was rather high. Underlying variability in this curiosity was uncertain.

In summary, we have shown that TCDD exerts an AhR-mediated inhibitory or regressive effect on the growth of surgically induced endometriotic lesions at a dose of 40 μg/kg. TCDD had no effect at the lower dose levels.

Acknowledgment
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