# Effects of TCDD on the Growth of Endometriotic Sites Compared between Rats and Mice

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## 1. Introduction

Endometriosis is a disease in which endometrial tissue is found at sites outside the uterus[1]. Retrograde menstruation appears to permit the flow of endometrial tissue into the peritoneal cavity[2], and a predisposition toward altered immunocompetence may contribute to the development of the disease[3].

In previous work, exposure of rhesus monkeys to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in a dose-dependent increased incidence and severity of endometriosis over time[4]. Since TCDD exposure in animals produces a suppression of both cell mediated and humoral immunity[5], the effect of the chemical on immunocompetence is central to the proposed mechanism by which TCDD is likely to promote the incidence of endometriosis.

Other evidence suggests that the ovarian steroid hormones support the growth of endometriosis in primates and rodents. Ovariectomized monkeys that were seeded with endometrium had endometriosis after 12 to 16 weeks only if they had received either estrogen or estrogen plus progesterone therapy[6]. Induced endometriotic implants in rats regressed after ovariectomy, and treatment with 17$\beta$-estradiol led to the recurrence of the endometriotic sites[7].

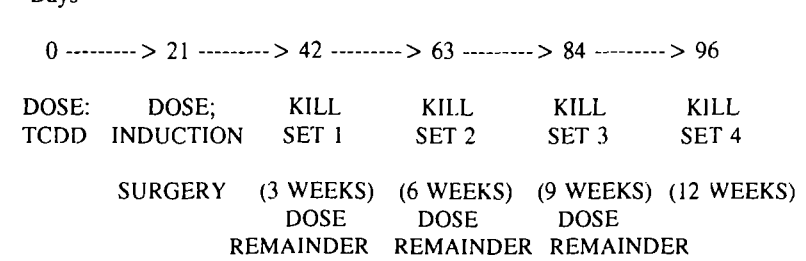
For many years the rat model of endometriosis has been used to investigate ameliorative effects of chemicals on endometriosis. Recently, a mouse model of induced endometriosis was developed[8]. The current study was designed to compare the effects of TCDD on surgically-induced endometriosis in rats and mice. These studies are based on the findings of Rier et al[4] and will aid in the understanding of the mechanisms of TCDD-induced endometriosis.

## 2. Materials and Methods

Animals. Female rats of the Sprague Dawley strain and B6C3F1 mice were purchased (Charles River, Raleigh, NC) at 60 days of age and allowed to acclimate for 1 week. Animals were caged in pairs (rats) or groups of 4 (mice) in clear polycarbonate cages measuring 20 x 25 x 47 cm. Food (Prolab rat, mouse, hamster 3000, Agway, Syracuse, NY) and water were provided ad libitum. Conditions in separate animal rooms were maintained at a temperature of 20 - 24 °C and 40 - 50% humidity with a photoperiod of 12:12 for mice and 14:10 for rats.

Experimental. A time course of the effect of TCDD on endometriosis in rats and mice was designed following range finding studies for dose level and time effects. Animals were divided into 4 sets (Fig. 1); within each set, 8 animals received 0, 3, or 10 μg TCDD/kg by gavage in corn oil. The first dose was given on day 0 and surgery for the induction of endometriosis[8,9] was performed on day 21 at which time animals received a second dose of TCDD. At 3 week intervals, animals were dosed again. Mice and rats in sets 1,2,3,and 4 were killed 3,6,9 and 12 weeks after surgery, respectively. At necropsy, blood was obtained for the measurement of hormones in the serum. Other evaluations included the diameter of the endometriotic sites (measured to the nearest 0.05 mm using calipers), and body, liver, thymus, ovarian, and uterine weights. 17β-estradiol and progesterone (rat) or progesterone (mouse) concentrations were measured by RIA (DPC, Los Angeles, CA) in serum previously frozen. Selected endometriotic sites and ovaries were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and evaluated histologically for pathology by Experimental Pathology Laboratories (Research Triangle Park, NC). Vaginal smears were recorded in rats for one week prior to the first TCDD dose (to eliminate non-cycling animals) and for 1 week prior to necropsy.

Figure 1. Time Course of TCDD Effect on Endometriosis: Design




Each set contains 3 dose groups: 0, 3, and 10 μg/kg TCDD.

Surgery. The surgical techniques employed were identical to those described by Vernon and Wilson[9] for the rat and by Cummings and Metcalf[8] for the mouse. Briefly, a 1 cm portion (rat) or the entire left horn (mouse) of each animal's uterus was ligated and removed to warm medium (Hams F-12). The uterine slice was opened longitudinally and cut into 6 (rat) or 3 (mouse) pieces of 2 - 3 mm square each. Each square was sutured, using 4-0 nylon suture, to a vessel of the intestinal mesentery of the same animal from which it originated. The muscle layers were closed with gut suture, and wound clips closed the skin.

Statistics. Data for endometrial site diameter, organ weights, and serum hormones were analyzed by Two-way ANOVA (GLM; SAS[10]) followed by individual preplanned comparisons using Least Squares Means (SAS[10]). Vaginal smear data were analyzed across time and across dose by Chi Square followed by post hoc evaluations using Fisher's Exact Test.

3. Results

TCDD promoted the growth of endometriotic sites in both rats and mice (Table 1). In

rats, a subtle increase in diameter was seen at 3 weeks (3 $\mu$g TCDD/kg; $P = 0.08$) and at 12 weeks (10 $\mu$g TCDD/kg; $P < 0.07$). When all data were analyzed for effect of dose, the increase in endometriotic site diameter was statistically significant ($P < 0.02$). Values for least squares means $\pm$ S.E. for 0, 3, and 10 $\mu$g TCDD/kg doses were $4.26 \pm 0.23$, $4.38 \pm 0.23$, and $5.16 \pm 0.276$. Dramatic increases in endometriotic site diameter were seen in mice at 9 weeks (10 $\mu$g TCDD/kg; $P < 0.01$) and at 12 weeks (3 and 10 $\mu$g TCDD/kg; $P < 0.0001$).

Data on hormone-dependent parameters differed between rats and mice. At 9 and 12 weeks in rats, TCDD produced a marked reduction in ovarian weight and an increase in the number of rats exhibiting vaginal persistent estrus. At 12 weeks in TCDD-treated rats, measurements indicated a significant increase in uterine weight, elevated 17$\beta$-estradiol, and histological evidence of ovulatory arrest indicated by reduced numbers of corpora lutea and the presence of secondary and tertiary follicles. In mice there was no effect of TCDD on ovarian or uterine weight across dose, although uterine weight increased with time. Evaluations of mouse ovaries were consistent with the lack of effect of TCDD on the measured hormonal values in the mouse. Histopathology of endometrial sites revealed fibrosis of the endometrial stroma in controls and either hemorrhage or the infiltration of the sites with polymorphonuclear cells in TCDD-treated rats.

Table 1. Diameter of Endometriotic Sites

| | | Dose of TCDD ($\mu$g/kg) | | |
|---|---|---|---|---|
| | | 0 | 3 | 10 |
| 3 weeks | rats | $3.98 \pm 0.29$ | $5.11 \pm 0.48$* | $4.89 \pm 0.33$ |
| | mice | $4.58 \pm 0.20$ | $4.41 \pm 0.20$ | $4.94 \pm 0.13$ |
| 6 weeks | rats | $3.95 \pm 0.22$ | $3.83 \pm 0.50$ | $4.62 \pm 0.51$ |
| | mice | $5.31 \pm 0.34$ | $5.55 \pm 0.37$ | $5.74 \pm 0.37$ |
| 9 weeks | rats | $4.82 \pm 0.45$ | $4.51 \pm 0.40$ | $5.25 \pm 0.87$ |
| | mice | $5.92 \pm 0.48$ | $6.89 \pm 0.36$ | $7.42 \pm 0.33$** |
| 12 weeks | rats | $4.30 \pm 0.37$ | $4.05 \pm 0.46$ | $5.88 \pm 0.82$* |
| | mice | $5.52 \pm 0.20$ | $7.88 \pm 0.37$** | $8.51 \pm 0.41$** |

* Significantly different from vehicle control, $P < 0.08$.
** Significantly different from vehicle control $P < 0.01$.

4. Discussion

Following repeated exposure to TCDD, both rats and mice exhibit increases in the diameters of surgically-induced endometriotic sites. However, the increases in diameter seen in the mouse are clearly greater than those seen in the rat and are clearly dose dependent. Potential differences in the range of responses to TCDD between rats and mice could contribute to the observed differences in the diameter of the endometriotic sites following

treatment with TCDD in these two species.

A specific difference between the rats and mice observed in this study was the constellation of hormonal effects seen at 9 and 12 weeks in the rat but not in the mouse. Estrogen is known to be important in the growth of endometriotic sites in monkeys[6] and rats[7]. Observations reported here on reduced ovarian weight, increased serum estrogen, and ovulatory arrest in rats in response to TCDD are consistent with other data[11]. Similar data from mice are unavailable. The TCDD-induced alteration in hormonal status of the rats at 12 weeks coincided with an increase in site diameter, suggesting that the changes in hormonal status may mediate the increase in site diameter in this species. In the mouse, however, such a correlation between endometriotic site size and altered hormonal status was not evident.

An alternate mechanism that may be important in the mouse is a TCDD-induced alteration in immunocompetence. Previous work has indicated that the size of endometriotic sites induced in rats can be regulated by an immunosuppressive agent[12]. However, TCDD produced a dose-related suppression of humoral immunity in mice but failed to reduce immunocompetence in rats even at 30 $\mu$g/kg TCDD[13]. TCDD also produces a suppression of cytotoxic T-lymphocyte activity (cell-mediated immunity) in mice via an Ah receptor-dependent mechanism[14]. These data and the pattern of observations from the current study suggest that, in the mouse, the immune altering effects of TCDD may be important in the promotion of endometriotic growth.

In summary, data from rats and mice treated with TCDD suggest a difference in the mechanism by which the growth of the surgically-induced endometriotic sites in each species is promoted. Hormonal effects appear predominant in the response of the rat to TCDD, suggesting that the major mechanism for promoting endometriosis in that species is hormonal. In the mouse, hormonal effects of TCDD appear minimal, but, in light of the dramatic growth of the endometriotic sites in treated mice, it is possible that a TCDD-induced alteration in immunocompetence mediates endometriotic growth in this species. Finally, TCDD appears to promote the growth of endometriotic tissue in both species, a phenomenon that is in agreement with the results reported for TCDD in monkeys[4].

5. References


1) Olive D.L. and L.B. Schwartz (1993) : Endometriosis. N. Engl. J. Med. 328, 1759-1769.
2) Grosskinsky C.L. and J. Halme (1993) : Endometriosis: The host response. Baill. Clin. Obst. Gynaecol. 7 (4), 701-713.
3) Dmowski W.P., D. Braun, and H. Gebel (1991) : The immune system in endometriosis. In: Thomas, E.J. and J.A. Rock, eds. Modern Approaches to Endometriosis. Boston: Kluwer Academic Publications, 97 - 111.
4) Rier S.E., D.C. Martin, R.E. Bowman, W.P. Dmowski, and J.L. Becker (1993) : Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fund. Appl. Toxicol. 21, 433-441.
5) Holsapple M.P., D.L. Morris, S.C. Wood, and N.K. Snyder (1991) : 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: possible mechanisms. Annu. Rev. Pharmacol. Toxicol. 31, 73-100.
6) Dizerega G.S., D.L. Barber, and G.D. Hodgen (1980) : Endometriosis: role of ovarian steroids in initiation, maintenance, and suppression. Fertil. Steril. 33, 649-53.

7) Rajkumar K., P.W. Schott, and C.W. Simpson (1990) : The rat as an animal model for endometriosis to examine recurrence of ectopic endometrial tissue after regression. Fertil. Steril. 53, 921-925.
8) Cummings A.M. and J.L. Metcalf (1995) : Induction of endometriosis in mice: a new model sensitive to estrogen. Reprod. Toxicol. Vol. 9, In press.
9) Vernon, M.W. and E.A. Wilson (1985) : Studies on the surgical induction of endometriosis in the rat. Fertil. Steril. 44, 684-694.
10) SAS Institute Inc. (1985) : SAS User's Guide: Statistics, 5th edition. Cary, NC: SAS Institute.
11) Li X., D.C. Johnson, and K.K. Rozman (1995) : Reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in female rats: Ovulation, hormonal regulation and possible mechanism(s). Toxicol. Appl. Pharmacol. In press.
12) Nothnick W.B., T.E. Curry, and M.W. Vernon (1994) : Immunomodulation of rat endometriotic implant growth and protein production. Am. J. Reprod. Immunol. 31, 151-162.
13) Smialowicz R.J., M.M. Riddle, W.C. Williams, and J.J. Diliberto (1994) : Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: differences between mice and rats. Toxicol. Appl. Pharmacol. 124, 248-256.
14) Kerkvliet N.I., L. Baecher-Steppan, B.B. Smith, J.A. Youngberg, M.C. Henderson, and D.R. Buhler (1990) : Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): Structure-activity relationships and effects of C57B1/6 mice congenic at the Ah locus. Fund. Appl. Toxicol. 14, 532-541.