IMPAIRMENT OF VENTRAL PROSTATE BY MID-GESTATIONAL BUT NOT BY LATE-GESTATIONAL OR POSTNATAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DOXIN (TCDD)

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Introduction

Maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-doxin (TCDD) has been reported to cause a variety of disorders in reproductive organs of male offspring even at a low dose (1 - 4). Among them, the decreased size of ventral prostate was reported to be one of the most sensitive landrnarks (5, 6). Recently we also reported the decreased size of ventral prostate and the reduction of anogenital distance in the male offspring of Holtzman rats exposed to relatively low dose (> 50 ng/kg) of TCDD on gestational days 15 (GD15) (7). However, testicular weight or daily sperm production were not affected. This suggested that dioxin shows overt toxicity in the development of male organs, which are known to respond to 5alpha-dihydrotestosterone. Recent reports by Peterson and coworkers demonstrated that these impaired prostates showed decrease in androgen responsiveness without inhibiting prostatic 5alpha-dihydrotestosterone formation or testicular androgen production (5, 6). However, the mechanism of this phenomenon has not been clear yet. Our previous data using gas chromatograph-mass spectrometry revealed that TCDD-body burden of pups by TCDD administration on GD15 was much higher than that in fetuses, suggesting that total amount of TCDD transferred to pups from mammary gland of mother via lactation was much higher than *in utero* transfer (8) (Figure 1). This data made us to speculate that exposure to TCDD from mother's milk might be a major factor which causes male reproductive disorder by *in utero* and lactational dioxin-exposure and that it is considered as an important phenomenon in terms of health risk due to lactation in man.

We here compared the effects of GD15-, GD18-TCDD administrations (by oral, mother), and postnatal days 2 injection (s.c., pup), in order to understand mechanism of a relatively low dose of TCDD-action, which causes male rat reproductive organ disorders.

Materials and Methods

Animals: Pregnant Sprague Dawley rats (Crj:CD(SD)IGS) on gestational days (GD) 6 were purchased from Charles River Co., Japan and maintained in an air-conditioned isolated rack in SPF area of Panapharm Laboratory Co. (Kurizaki, Kumamoto, Japan). On GD 15 or GD18, pregnant rats (n=5) were given a single dose of TCDD (1 μg/kg mother bw; 5 ml/kg; p.o.) or an equivalent volume of vehicle (corn oil). For another treatment group, male pups on postnatal days (PND) 2 born from non-treated mothers (n=5) were given a single dose of TCDD (1 μg/kg

pup bw; 5 ml/kg; s.c.) or an equivalent volume of vehicle. After weanling, three males were housed per cage and two of them were randomly sacrificed on PND70.

Sample collection and processing: Length between the base of genital tubercle and the anterior edge of anus was measured by caliper as anogenital distance. Testis and epididymis of both sides were excised from abdomen and the surrounding adipose tissues were removed carefully. After weighing the testes and caput and cauda epididymis, portions of testis and cauda epididymis were homogenized by polytoron homogenizer. Spermatid and sperm head were counted by hemocytometer to determine testicular daily sperm production and cauda epididymal sperm reserve, respectively. The urine in bladder was removed and then, deferent ducts were cut at the base of bladder and anterior end of urethra was cut to excise the urogenital complex. After measurement of urogenital complex weight, ventral prostate was dissected and weighed.

TCDD measurement: The content of TCDD in kidneys gathered from 3 animals from each treated group was measured by gas chromatograph-mass spectrometry (GC-MS) as described previously (7).

Statistical analysis: All data were expressed by relative values to means of each control group. Statistical difference between means of control group and those of treated group was analyzed by one-tailed Student's t - test. Significance was set at $p < 0.05$.

Results and Discussion

We have examined if the reduction of ventral prostate weight or other male reproductive disorders were induced by TCDD administrations at other developmental stages than GD15. Testicular weight and daily sperm production were not affected by TCDD exposures at any stages. However, paired epididymal weight and cauda epididymal sperm numbers were significantly reduced to 85% and 53% of control levels by TCDD-exposure on GD15, respectively. No significant difference was detected in GD18- and PND2-exposed groups. Urogenital distance and ventral prostate weights were also significantly decreased to 81% and 64%, respectively, of control levels by TCDD-exposure only on GD15 (Figure 2). Anogenital distance was reduced in GD15- and GD18-TCDD exposed groups.

The GC-MS analysis showed that TCDD contents in the kidneys were 1.60 pg/g from GD15-group, 3.34 pg/g from GD18-group, 2.32 pg/g from PND2-group, indicating that retained TCDD amount in GD15-group were lower than those of GD18- and PND2-groups. Nevertheless, the fact that reduction of epididymal and ventral prostate weights were observed only in GD15-group suggests that these effects could be derived from temporal alteration in fetus around GD15 by TCDD-exposure, but not by TCDD-exposure around GD18 or postnatal period. These results also strongly suggest that there is a critical window to cause impairments of male reproductive systems, including reduction of ventral prostate weight, by *in utero* and lactational TCDD exposure. In the previous reports by Peterson and coworkers using maternally TCDD-treated Holtzman rats and cross-fostering method, both *in utero* (IU) and lactational (L) exposures affected on ventral prostate weight of male pups (9). However, in the most recent study using mice, they found out there was the most sensitive period between GD13 and GD16 for impairing ventral prostate development by TCDD exposure (10). Our results seem to be consistent with the latter reports using mice.

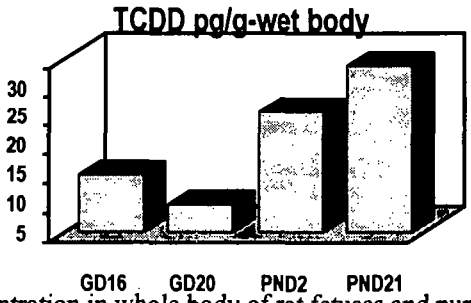

Figure 1. TCDD concentration in whole body of rat fetuses and pups maternally exposed to TCDD (800 ng/kg mother bw) on GD15. The TCDD concentration was measured by GC-MS and expressed in pg per wet body weight.

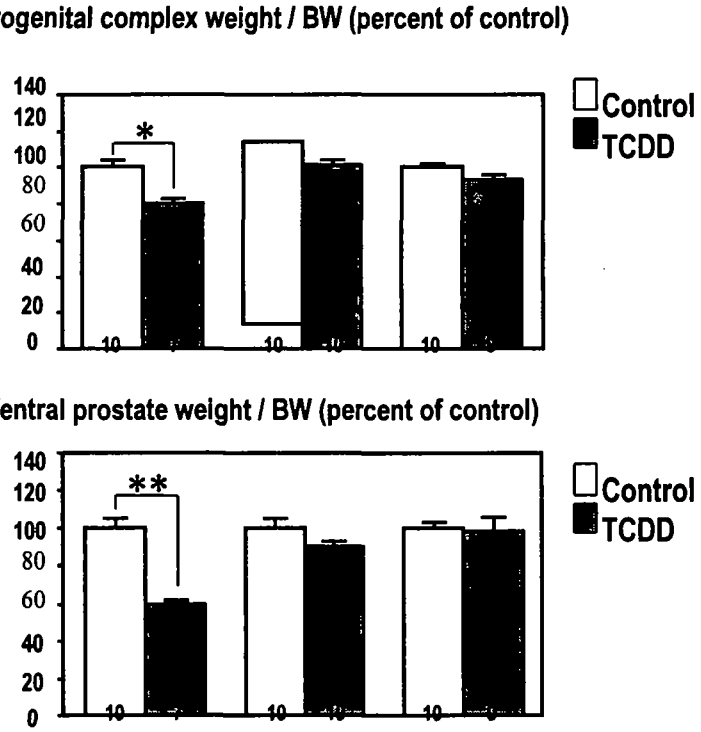

Figure 2. Effects of maternal and postnatal exposures to TCDD on urogenital complex and ventral prostate weights of male rats on PND 70. Pregnant rats were orally administered 1 μgTCDD/kg mother body weight on GD15 and GD18, or male pups on PND2 were subcutaneously injected 1 μgTCDD/kg pup body weight. The values expressed are the mean ± SE of relative weight to the average of each control group. The numbers of animals examined in each group in indicated above the x-axis. Statistically significant difference between means from control was analyzed by Student's *t*-test (* : $P<0.05$, ** : $P<0.01$). Note that significant reductions of urogenital complex and ventral prostate weights were detected only in rats treated on GD15.

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