Effect of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on Testosterone Production in Isolated Murine Testicular Cells

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is an endocrine disruptor that is known to have widespread effects on the reproductive system. We examined the effects of TCDD on testosterone production of primary cultured murine testicular cells. The cells, derived from the testes of ICR mice at age 65 days, were exposed to 10^{-2}–10^{4} pM TCDD for 3, 24, or 48 hr and treated with human chorionic gonadotropin for 6 hr to induce production of testosterone. At these concentrations of TCDD, the viability of testicular cells was not affected. No significant time- or TCDD concentration-dependent effects were observed on the secretion of testosterone. The data suggest that TCDD does not have a direct influence on testosterone production in the ICR mouse.

Key words —— 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, testosterone, primary culture, testis

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic of the polychlorinated dibenzo-*p*-dioxins, is an endocrine disruptor that affects the reproductive system of many species at low doses. Many effects are reported in the male rat, for example, decreased size of the accessory sex organs,[1] decreased daily sperm production,[2] and demasculinization.[3] In female rats, TCDD is known to result in endometriosis,[4] fewer pregnancies,[2] and teratogenicity[5] of offspring exposed via the maternal body. A decrease in plasma testosterone has been reported in many studies,[1,6] but no change[2,7,8] and even an increase[9] has been mentioned in some reports. The effects and outcomes vary widely according to TCDD dose, development status of the animals, exposure process, and animal strain. The mechanisms of TCDD toxicity are gradually being clarified. Mediation of the toxic effects of TCDD via aryl hydrocarbon receptors (AhR) and AhR nuclear translocators, in particular, have been clarified.[10] To investigate the effects of TCDD on the testis at the mRNA or protein level, experiments using testicular cells derived from the mouse have been carried out, and many interesting genes[11] and proteins[12] have been found. We investigated the direct effects of TCDD on the secretion of testosterone from mouse testicular cells and examined the viability of cells and concentrations of testosterone when primary cultured mouse testicular cells were exposed to TCDD.

MATERIALS AND METHODS

Animals —— Adult male specific pathogen-free ICR mice were purchased from Tokyo Laboratory Animals Science (Tokyo, Japan) and were delivered at 9 weeks of age. All mice were housed in clear plastic cages in groups of 4–5 per cage for 2 days. They were given food and water *ad libitum* and maintained on a 12-hr light-dark schedule.

Chemicals —— TCDD was purchased from GL Sciences (Tokyo, Japan). Dulbecco's modified Eagle medium and Ham's F12 (1 : 1) (DMEM/F12) were purchased from GIBCO BRL (Tokyo, Japan) and Earle's balanced salts (EBSS) from Sigma (St. Louis, MO, U.S.A.). Horse serum (HS) was purchased from Nacalai Tesque (Kyoto, Japan) and fetal bovine serum (FBS) from JRH Biosciences (Lenexa, KS, U.S.A.).

Preparation of Testicular Cells and Exposure to TCDD —— Twenty-three mice were killed by cervical dislocation. The testes were excised, decapsulated, and minced in DMEM/F12 containing gentamicin 20 μg/ml (Sigma). The mixture of testicular cells was enzymatically dispersed with gentle shaking in a medium containing collagenase 1.25 mg/ml, trypsin 125 μg/ml (DIFCO, Franklin Lakes, NJ, U.S.A.), and DNase I 10 μg/ml at 32°C for 30 min. The trypsin treatment was terminated by the addi-

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tion of DMEM/F12 supplemented with 5% HS and 2.5% FBS. The cells were centrifuged at 93 *g* for 5 min, and the supernatant was removed. The precipitate was resuspended in DMEM/F12 and allowed to settle for 5 min. The mixture of testicular cells was adjusted to 1.5×10^6 cells/ml with DMEM/F12 containing gentamicin 20 μg/ml. One milliliter of the cell solution was plated in a 24-well dish coated with collagen (Asahi Technoglass, Tokyo, Japan) and allowed to attach for approximately 20 hr at 32°C in a 95% air-5%CO_2 atmosphere. All procedures were performed under sterile conditions. After the medium was removed, cells were exposed for 3, 24, or 48 hr to 10^{-2}, 10^{-1}, 1, 10, 10^2, 10^3, or 10^4 pM TCDD dissolved in EBSS containing 0.02% dimethyl sulfoxide (DMSO). The same volumes of DMSO were added to groups of cells as controls for the same exposure times.

Measurement of Cell Viability —— L-lactose dehydrogenase (LDH) activity was measured (LDH release activity kit; Wako, Osaka, Japan) at 595 nm with a microplate reader in the supernatant that was collected from wells exposed to TCDD.

Measurement of Secreted Testosterone —— EBSS containing TCDD was removed, and the testicular cells were treated with human chorionic gonadotropin (hCG) 1 IU/ml (Biogenesis, Technology Road, Poole, UK) dissolved in EBSS at 32°C for 6 hr. The testosterone concentration was determined with a radioimmunoassay (RIA) kit (DPC, Los Angeles, CA, U.S.A.) according to the manufacturer's instructions.

Statistical Analysis —— Viable cell numbers are shown as the mean ± S.E. counts of four determinations; testosterone concentration is shown as the mean ± S.E. concentration of three determinations. Statistical significance was determined using Student's *t* test, and a *p*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Dose–Dependent Effect of TCDD on Cell Viability of Primary Cultured Mouse Testicular Cells

Primary cultured cells excised from testis of 65-day-old mice were exposed to several concentrations of TCDD, and cell viability was determined using the LDH releasing assay (Fig. 1). No significant differences in LDH release activity were observed at 3 or 24 hr (data not shown). At 48 hr, no significant alterations were observed in comparison with the viability of control cells. These results correspond with results obtained in experiments carried out in cell strains derived from BALB/c mice.[11,12]

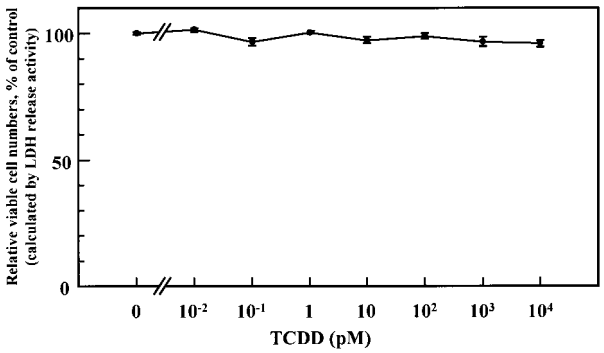

Fig. 1. LDH Release from Mouse Testicular Cells Exposed to TCDD

Testicular cells were isolated from ICR mice and cultured on collagen-coated dishes for 19 hr. After attachment to the dishes, cells were exposed to TCDD for 48 hr. Each point represents mean ± S.E., $n = 4$.

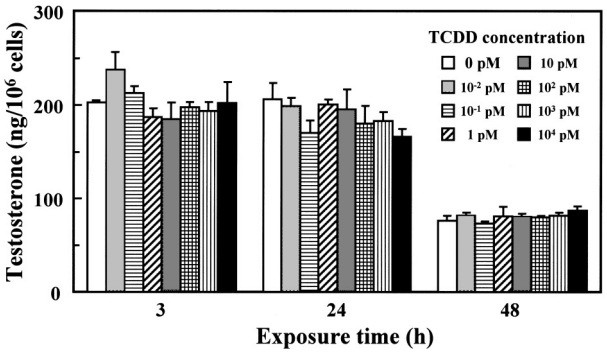

Fig. 2. Dose– and Time–Dependent Effect of TCDD on Secretion of Testosterone

Cells were exposed to 10^{-2}–10^4 pM TCDD for 3, 24, or 48 hr in the culture medium. After exposure, the medium was exchanged with medium containing human chorionic gonadotropin 1 IU/ml and cells were cultured for 6 hr. The concentration of testosterone was determined with an RIA kit. All concentrations shown are mean ± S.E., $n = 3$.

Dose– and Time–Dependent Effects of TCDD on Testosterone Secretion

Dose– and time–dependent effects of TCDD on testosterone secretion into medium were examined (Fig. 2). We measured testosterone concentrations at 3, 6, 12, 18, 24, 30, 36, 42, and 48 hr in the absence of TCDD (data not shown). They did not change until 24 hr and gradually decreased to approximately 1/3 at 48 hr. In general it is difficult to maintain long-term potent testosterone synthesis in cultured cells. In the cells exposed to TCDD for 3, 24, and 48 hr, the amount of testosterone secretion

was almost same as in the control groups, and no statistically significant difference was observed even at the highest concentration of TCDD. We performed this experiment independently three times. Dose–dependent alterations were not observed in any of the experiments.

Theobald *et al*. compared ICR mice with Long-Evans rats and Syrian hamsters to determine the effects of *in utero* and lactational TCDD exposure on male reproductive system development.[13] They reported that the mice were less sensitive than the other two animals to most of the effects studied, including serum testosterone concentration, except for a decrease in epididymal sperm numbers. The present study results support their findings and suggest that the constancy of serum testosterone levels may be due to an intrinsic high resistance of mouse testicular cells against TCDD.

We previously carried out proteomic[12] and RNA arbitrarily primed polymerase chain reaction analyses[11] of mouse Leydig TM3 cells exposed to TCDD and reported that the synthesis of proteins and expression of mRNAs were markedly altered by TCDD. Taking those findings together with the present results, we believe that TCDD may have an effect on testis function but that it may not affect testosterone production directly.

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