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DDT Reduced Testosterone and Aromatase Activity Via ER Receptor in Leydig Cell

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DDT의 Aromatase 증가에 의한 Testosterone 감소효과

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요 약

본 연구는 환경호르몬(endocrine disruptors)으로 분류되었으며, 제초제로 널리 사용되었던 Dichlorodiphenyltrichloroethane (DDT)가 설치류의 생식세포 중 Leydig 세포의 testosterone (T) 생성억제 및 그 관련 작용메카니즘을 규명코자 수행되었다. 먼저 흰쥐의 웅성 생식세포주인 R2C 세포에 T의 양 및 aromatase 활성도를 radio immunoassay (RIA)방법을 이용하여 측정하였다. 그 결과 R2C 세포에 황체형성호르몬(LH)를 처리하여 testosterone 생성을 증가시킨 후, DDT를 처리한 군들은 대조군에 비하여 농도 의존적으로 T의 양은 감소하였으며, aromatase 활성도는 증가하였다. 또한 DDT 자체만 처리한 군에서도 대조군에 비하여 testosterone의 생성이 감소하였다. 이러한 aromatase 활성 증가가 estradiol receptor (ER)와의 상호 관련성을 확인하기 위해 ER antagonist인 ICI 182.780를 처리한 후 T의 양 및 aromatase 활성도를 측정한 결과 DDT에 의해 증가된 aromatase 활성도가 ICI 182.780에 의해 다시 감소됨을 확인하였다. 또한 DDT에 의해 감소된 T의 양도 ICI 182.780에 의해 다시 회복되었다. *In vivo* 실험으로 흰쥐에 DDT를 직접 투여한 후 정소 내 성 호르몬들을 측정해 본 결과 T의 양은 유의성 있게 감소하였으며, estradiol (E_2)의 양은 증가하였으며, aromatase 활성도도 감소하였다. 이러한 결과를 종합해 볼 때 DDT는 aromatase를 감소시키고, 이렇게 감소된 aromatase에 의해 testosterone 생성량을 억제하고, 이러한 DDT의 aromatase의 감소는 ER을 경유하는 것으로 추정할 수 있다.

Key words : dichlorodiphenyltrichloroethane, testosterone, aromatase, estrogen receptor, leydig cell

INTRODUCTION

There is increasing evidence that certain environmental contaminants have the potential to disrupt endocrine processes, which may result in reproductive problems, certain cancers and other toxicities related to (sexual) differentiation, growth, and development. A metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), is a widespread environmental pollutant. Earlier studies have shown that exposure to DDT at early developmental stage results

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in altered sexual differentiation in male rats. Affected animals display a number of signs of feminization, including reduced anogenital distance and increased incidence of nipple retention (Kelce *et al.*, 1995; You *et al.*, 1998). Since DDE is able to bind to the androgen receptor and block the actions of testosterone, its effects on reproductive development have been attributed primarily to an androgen receptor antagonism (Kelce *et al.*, 1995; Kelce *et al.*, 1997).

Several classes of relatively persistent pesticides, such as organotin compounds, DDT and several metabolites, and a number of imidazole-like fungicides are suspected or have been shown to interfere with steroidogenesis. Particular attention has been given to the enzyme aromatase (CYP19) which catalyzes the final, rate-limiting step in the conversion of androgens to estrogens. It has been postulated that organotin compounds may cause endocrine-disruptive effects such as "imposex" in molluscs by inhibiting aromatase activity (Fent, 1996). Various imidazole-like fungicides are known to inhibit aromatase activity in human placental (Vinggaard *et al.*, 2000) and rainbow trout ovarian microsomes (De Mones *et al.*, 1993). Recently, DDT, which has antiandrogenic properties (Kelce and Wilson, 1997), has been reported to increase aromatase protein in rat (You *et al.*, 2001). Aromatase catalyzes the conversion of C19 steroids to estrogens, a reaction that involves the removal of the C19 carbon and aromatization of the A ring of the steroid. The expression of aromatase is controlled by regulatory pathways involving gonadotropins, steroid hormones, and growth factors (Roselli and Resko, 1998).

A recent study reported that DDT exposure significantly increased circulating levels of 17β-estradiol (E_2) in male rats (O'Connor *et al.*, 1999). This finding suggests a possibility that the feminization seen in DDT-exposed male rats may also involve an overproduction of estrogen. In the present study, we investigated the effect of DDT on testoesterone production through aromatase and investigated its molecular mechanism in testicular leydig cell, R2C. The involvement of estrogen receptor (ER) in this process was also investigated using the ER antagonist, ICI 182.780.

METHODS AND MATERIALS

Materials

DDT was obtained from the Sigma Chemical Co. (St. Louis, Korea). This compounds was dissolved in ethanol, and the final concentration of ethanol in the cell growth medium was 0.1% (v/v). DMEM and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, Korea). [β-^{3}H] Androstenedione was purchased from Amersham Pharmacia Biotech (Boston, MA).

Cell treatment

R2C cells were obtained from the American Type Culture Collection. Cells in 24-well culture plates containing 1 ml medium per well were exposed to various concentrations of DTT dissolved in dimethyl sulfoxide (DMSO). All treatments were tested in triplicate, For the DMSO at 0.1% had no effect on CYP19 expression or catalytic activity relative to unexposed cells.

Animal

Immature male Sprague-Dawley rats (100~120 g) were purchased from KFDA (Seoul, Korea). The rats were unbiasedly divided into the vehicle control and the treatment groups ($n = 3$ per group) and dosed with DDT (0~100 mg/kg) respectively by daily gavage for 3 days. The rats were killed by decapitation 24 h after the last dose. Testis samples were stored at −20°C until hormone and aromatase assay.

Aromatase assay

The aromatase activity was determined by measuring the [^{3}H]H_2O release upon the conversion of [1β-^{3}H]androstenedione (A) to estrone (E1) (Lephart and Simpson, 1991). Before the experiment, the cells

were cultured in DMEM with 5% DCS (dextran-coated, charcoal-treated FBS) for 48 h. After the cells were treated with DDT, [1β-^{3}H] and rostenedione was added, and then the cells were further incubated for 6 h. The medium (2.0 ml) was extracted with chloroform and then was centrifuged. The aqueous supernatant was mixed with 5% charcoal/0.5% dextran and then was incubated for 30 min. Thereafter, the mixture was centrifuged and the supernatant was added to 5 ml of scintillation fluid and assayed for radioactivity. The amount of radioactivity in [^{3}H]H_2O thus measured was standardized based on the protein concentration which was determined using a micro BCA kit (Pierce Chemical Co., Korea) and expressed as pmol/mg protein/6 h.

Testosterone and Estradiol content assayed by RIA

To ensure that the measured aromatase activity truly reflected the capability of estrogen production, the cells were treated with or without various concentrations of DDT for 24 h, and then the medium was collected and the T and E_2 were assayed in duplicate by using a Coat-A-Count radioimmunoassay kit. The radioactivity of ^{125}I was quantified by a gamma-counter.

Statistics

All experiments were repeated at least three times. Student's t-test was used to assess the statistical significance of differences. A confidence level of <0.05 was considered significant.

RESULTS AND DISCUSSION

Because DDT is known to inhibit LH-induced testosterone production in leydig cell and has been shown to possess estrogenic properties, we decided to investigate the effects of DDT on testosterone production and its effects on aromatase activity in R2C cell. The potent leydig cell activator luteinizing hormone (LH) increased testosterone production compared to the control. DDT exposure significantly decreased testoesterone production in R2C cell and rat testis (Figs. 1, 5A). Furthermore, DDT alone af-

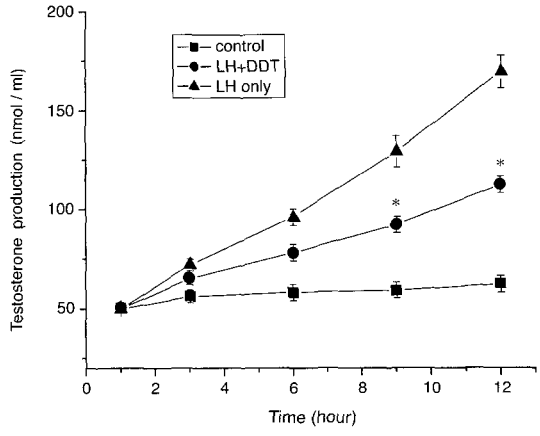

Fig. 1. Effects of DDT on testosterone production by R2C cells. After being harvested, cells were incubated without or with LH. R2C cells were apparent after 12 h of DDT (1 μM) treatment testosterone concentrations were measured in the spent media by RIA. Three experiments were conducted for this determination. *, denotes statistical significance ($p<0.05$)

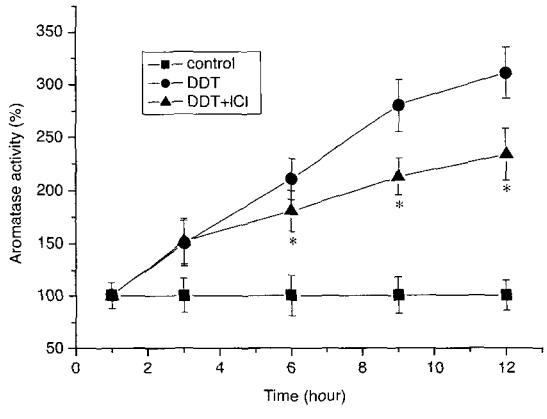

Fig. 2. Effects of DDT on aromatase activity by R2C cells. Cells were treated with DDT (1 μM) for 12 h and then aromatase activities were measured in the spent media by RIA. Three experiments were conducted for this determination. *, denotes statistical significance ($p<0.05$)

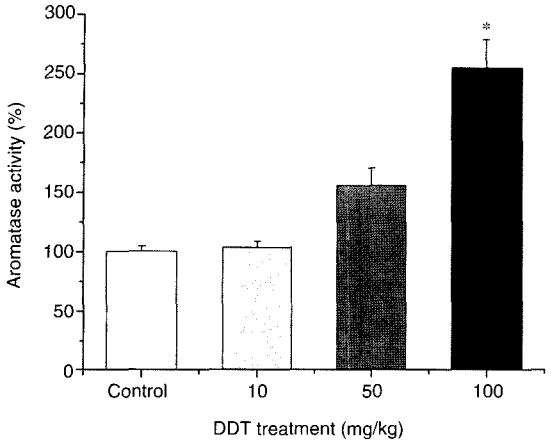

Fig. 3. Effects of DDT on aromatase activity in the immature male rats. Rats were dosed with DDT by daily gavage for 3 days. Aromatase activities were measured in the testis by RIA. Three experiments were conducted for this determination. *, denotes statistical significance ($p < 0.05$)

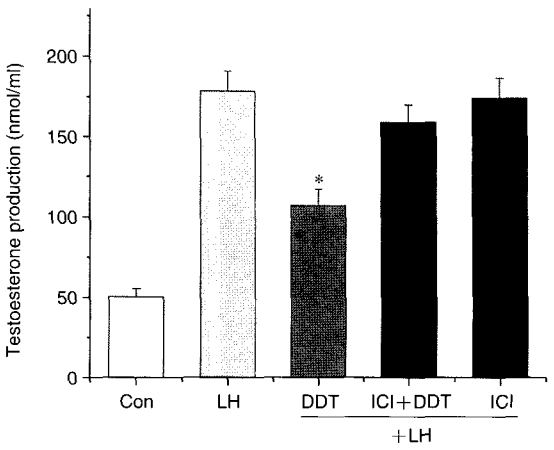

Fig. 4. Antagonism of DDT inhibition of testosterone production by antiestrogen. DDT (1 μM) inhibition of testosterone production was prevented when R2C cells were coincubated with ICI 182,780 (ICI) in media containing 10 ng/ml LH for 12 h. Testosterone productions were measured in the spent media by RIA. Three experiments were conducted for this determination. *, denotes statistical significance ($p < 0.05$)

fected testosterone reduction in a dose-dependent manner in R2C cell (data not shown) slightly. The DDT-mediated suppression of testosterone production was not due to a DDT cytotoxic effect. Cell viability was identical for cultures treated with DDT (data not shown). In addition, DDT was found to increase aromatase activity in R2C cell (Fig. 2) and rat testis (Fig. 3). A recent study reported that HPTE (2, 2-bis(p-hydroxyphenyl)-1, 1, 1-tricholoroethane, DDT metabolite) inhibited production of testosterone in leydig cells, as down-regulation of $P450_{scc}$, the enzyme that catalyzes the first reaction in the testosterone biosynthesis pathway (Benson *et al.*, 2000). However, the mechanism by which DDT causes these effects is not clear. Regarding these results, previous studies have reported that the estrogenic activities of DDT, such as ER binding affinity (Beresford *et al.*, 2000). In order to assess whether the suppressive effects of DDT on LH-inducible testosterone production might be influenced by the ER, ICI 182.780, a pure antiestrogen, was used, and it was found that these inhibitory effects of DDT were antagonized by ICI 182.780, implying that the ER mediates the suppressive effects of DDT (Fig. 4).

Furthermore, the inducible effects of DDT on aromatase might be influenced by the ER, ICI 182.780 was used, and it was found that these enhancing effects of DDT were antagonized by ICI 182.780, implying that the ER mediates the inducible effects of DDT (Fig. 3). Therefore, we believe that decreased LH-inducible testosterone production by DDT is regulated through aromatase. Several previous studies have shown that DDT treatment leads to reduce LH-inducible testosterone production (You *et al.*, 2001), and this is confirmed by the present study (Figs. 1, 5A). And we showed that DDT exposure significantly increased levels of E in male rats testis (Fig. 5B).

According to the classical hypothesis, the cellular effects of estrogens are mediated by the intracellular ER, which serve as transcription factors. ER belongs to the superfamily of ligand-activated transcription factors, the nuclear receptors. E-ER complexes bind to the genomic estrogen response elements. The estrogen-occupied receptor interacts with additional transcription factors and components of the transcrip-

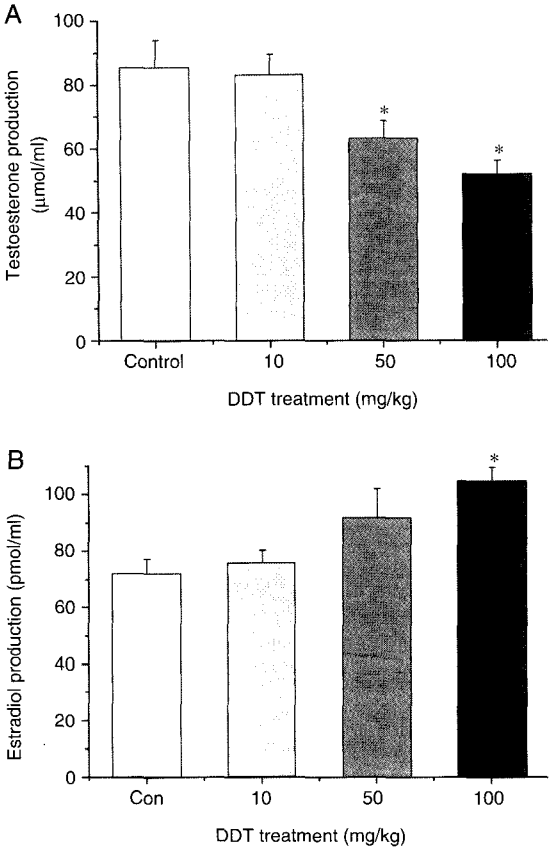

Fig. 5. Effects of DDT on testosterone and estradiol in the immature male rats. Rats were dosed with DDT by daily gavage for 3 days. Testosterone and estradiol were measured in the testis by RIA. Three experiments were conducted for this determination. *, denotes statistical significance ($p < 0.05$)

tion initiation complex to modulate gene transcription.

Aromatase regions homologous to the consensus sequence of the estrogen response elements have been identified in the 5′-flanking regions of the aromatase genes. So, DDT may induce the transcription of the aromatase genes by interacting with these sequences.

Our results indicated that DDT inhibition of LH-inducible testosterone production in R2C and testis is mediated through aromatase. However, the precise mechanisms by which DDT enhance in leydig cell remains unknown. The current study suggests the possibility that DDT might act as an modulrator aromatase gene transcription.

ABSTRACT

Dichlorodiphenyltrichloroethane (DDT), is a widespread environmental pollutant. In this study, we investigated the effect of DDT on testosterone production through aromatase and investigated its molecular mechanism in testicular leydig cell, R2C. We investigated that the effects of DDT on testosterone production and its effects on aromatase activity in R2C cell by radio immunoassay (RIA). As the results, the potent leydig cell activator LH increased testosterone production compared to the control. DDT exposure significantly decreased testosterone production in R2C cell and DDT alone affected T reduction in a dose-dependent manner in R2C cell slightly. In addition, DDT was found to increase aromatase activity in R2C cell in a dose dependent manner. In order to assess whether the suppressive effects of DDT on LH-inducible testosterone production might be influenced by the ER, ICI 182.780, a pure antiestrogen, was used, and it was found that these inhibitory effects of DDT were antagonized by ICI 182.780, implying that the ER mediates the suppressive effects of DDT. Furthermore, the inducible effects of DDT on aromatase might be influenced by the ER, ICI 182.780 was used, and it was found that these enhancing effects of DDT were antagonized by ICI 182.780, implying that the ER mediates the inducible effects of DDT. Our results indicated that DDT inhibition of LH-inducible testosterone production in R2C is mediated through aromatase. However, the precise mechanisms by which DDT enhance in leydig cell remains unknown. The current study suggests the possibility that DDT might act as a modulator aromatase gene transcription.

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