Organohalogen Compounds 65, 159-162 (2003) 

# EFFECT OF DDT ON TESTOSTERONE REDUCTION THROUGH AROMATASE IN LEYDIG CELL

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## 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 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 [1,2]. 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 [1,3].

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[4]. Various imidazole-like fungicides are known to inhibit aromatase activity in human placental[4] and rainbow trout ovarian microsomes [6]. Recently, DDT, which has antiandrogenic properties [7], has been reported to increase aromatase protein in rat [8]. 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 [9].

A recent study reported that DDT exposure significantly increased circulating levels of 17b-estradiol (E) in male rats [10]. 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

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.

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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. Aromatase activity of the microsomal samples was determined by a tritiated-water formation assay described by Lephart and Simpson [11].

Testosterone was assayed in duplicate by using a Coat-A-Count radioimmunoassay (RIA) kit. The radioactivity of $^{125}I$ was quantified by a gamma-counter. 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 (Fig. 1, 5A). Furthermore, DDT alone affected testosterone reduction in a dose-dependent manner in R2C cells (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 (Fig. 3, 4) in R2C cell and rat testis. 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[12]. 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[13]. 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. 2). 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[8], and this is confirmed by the present study (Fig. 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 transcription initiation complex to modulate gene transcription. Aromatase regions homologous to the consensus sequence of the estrogen response elements have

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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.

**Acknowledgments**
This work was supported by an endocrine disruptor grant (03132ED410-1) from the Korea Food & Drug Administration, Republic of Korea.

**Fig. 1**

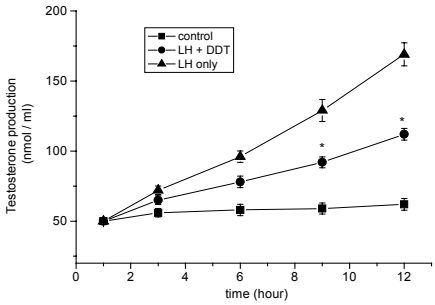


**Fig. 2**

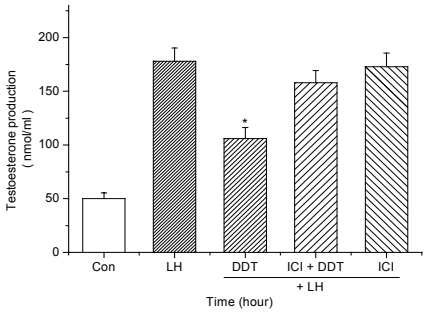


**Fig.1.** Effects of DDT on testosterone production by R2C cells. After being harvested, cells were incubated without or with LH. R2C cells were apparent after 12h of DDT (1 μM) treatment testosterone concentrations were measured in the spent media by RIA. Three experiments were conducted for this determination.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

**Fig. 2.** 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. Testosterone productions were measured in the spent media by RIA. Three experiments were conducted for this determination. *, denotes statistical significance $p < 0.05$)

**Fig. 3**

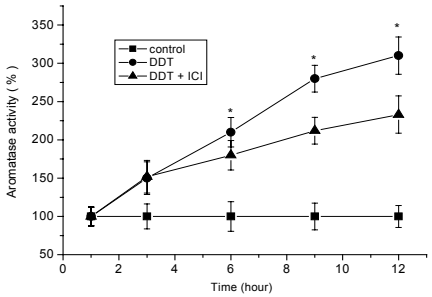


**Fig. 4**

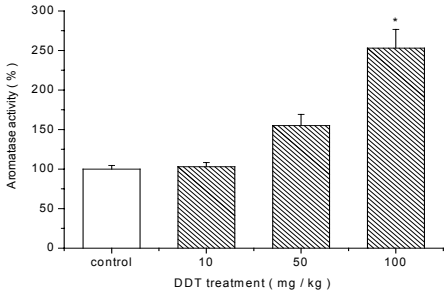

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**Fig. 3.** Effects of DDT on aromatase activity by R2C cells. Cells were treated with DDT (1 μM) for 12h and then aromatase activities were measured in the spent media by RIA. Three experiments were conducted for this determination. *, denotes statistical significance $p < 0.05$)

**Fig. 4.** 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. 5 A** **B**

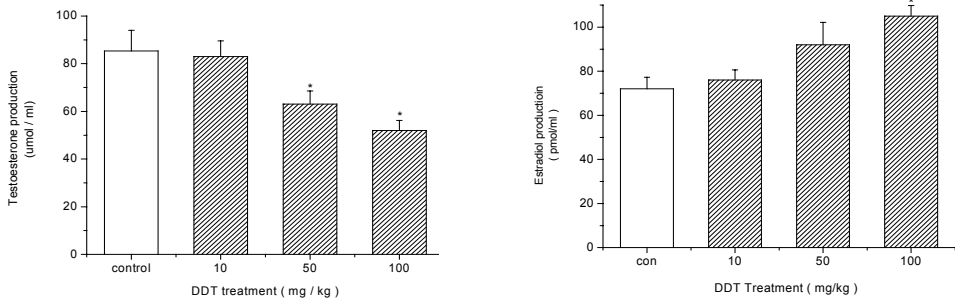


**Fig. 5.** Effects of DDT on testosterone and estradiol in the immature male rats. Rats were dosed with DDT by daily gavage for 3 days. Testosterone and estradiol were measured in the testis by RIA. Three experiments were conducted for this determination. *, denotes statistical significance $p < 0.05$)

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