Effects of Postnatal Administration of Diethylstilbestrol on Puberty and Thyroid Function in Male Rats

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Abstract. To examine the effects of diethylstilbestrol (DES) on male pubertal development and thyroid function, juvenile male Sprague-Dawley rats were given DES daily by oral intubation at doses of 10, 20 and 40 μg/kg/day from postnatal day 33 for 20 days. Prepuce separation was significantly delayed at the dose of 20 μg/kg/day and above in the DES-treated groups. DES treatment induced a significant reduction in the weights of testes, epididymides, the ventral prostate, seminal vesicles plus coagulating glands and fluid, levator ani bulbocavernous muscles, Cowper’s glands and the glans penis. The weights of the liver and adrenals increased in the DES-treated animals. DES caused a dose-dependent reduction in germ cells; in particular the spermatids were mainly affected. The serum levels of testosterone and luteinizing hormone were significantly reduced in the DES-treated groups, but that of estradiol decreased. No differences were observed in the serum thyroxine levels of the control and DES-treated groups. In microscopic observation of the DES-treated animals, degeneration of germ cells and tubular atrophy in the testis were noted, but no microscopic changes in the thyroid. These results indicate that DES affected the pubertal development of juvenile male rats and that its mode of action may be related to alterations in hormone levels.

Key words: Diethylstilbestrol, Male rat, Pubertal development

Concerns have been raised about the potential adverse effects of environmental chemicals that mimic the actions of estrogen on the reproduction and development of humans and wildlife species. Exposure of the developing male to exogenous estrogenic compounds can result in reproductive and developmental abnormalities and an increase in human male reproductive disorders, which include testicular cancer, cryptorchidism, hypospadias and low sperm counts [1–4]. Neonatal treatment with estrogens causes alterations in germ cell development and permanent impairment of testicular function [5–9]. As perinatal exposure of male animals and humans to synthetic estrogens can induce similar adverse changes in male reproductive health, it has been hypothesized that inappropriate exposure to estrogens during juvenile periods may interfere with the development of the reproductive system and with the sperm count in the adult [10, 11]. Faced with growing concern that environmental chemicals might impair human and animal fertility, it is important to investigate the possible influences of these substances on mammalian sexual differentiation and genital development [12, 13].

Diethylstilbestrol (DES) is a stilbene, a non-steroid estrogen, that can bind to estrogen receptors (ERs) and is widely used as a model estrogen for the study of estrogenic effects on the male reproductive function [13–16]. DES has been observed to have endocrine and reproductive toxicity in rats and mice. It is active over a wide dose range, showing effects on the mouse ventral prostate following exposure in utero at 0.02 μg/kg, and carcinogenic effects on the mouse uterus following neonatal exposure to 1,000 μg/kg [17, 18].

The rodent reproductive tract is immature at birth, and precise programs of gonadotropic and steroid hormone secretions are essentially required for sexual development and maturation [19]. When estrogenic chemicals were administered to juvenile animals, they do not induce malformations of the reproductive tract, but rather delay pubertal development [20–23]. The rodent thyroid/pubertal assay using juvenile male rats is used to examine the effects of hormone-like acting substances on the neuroendocrine axis during the sensitive period of puberty. This assay has been used for evaluation of so-called endocrine disrupting chemicals, with several modifications [22–27]. In vivo, estrogen controls many physiological processes and is involved in the development of reproductive organs. Recently, the effect of endogenous or exogenous chemicals with estrogenic activity on male reproductive development and function in adulthood has become the subject of intensive research and debate. A variety of chemicals have been reported to have estrogenic effects by binding estrogen receptors (ERs) or modulating ER-mediated responses [25, 39]. In the present study, we examined the effects of DES treatment on pubertal development and thyroid function in juvenile male rats via endpoints according to the rodent thyroid/pubertal male assay protocol and discussed the practical applications of the sensitivity of various parameters for detecting the endocrine-related effects of test chemicals. The protocol for this

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test has been modified by reducing the treatment period from 30 to 20 days.

Materials and Methods

Animals
Juvenile Sprague-Dawley male rats acquired from the Laboratory Animal Resources, National Institute of Toxicological Research (Seoul, South Korea), were housed in clear plastic cages on wood chips and given pellet rodent diet (Shinchon, Seoul, South Korea) and tap water ad libitum. Environmental conditions were controlled, i.e., 21–25°C, relative humidity of 50–60%, frequency of ventilation change of >15 air exchanges/h and a 12-h light/dark cycle (light on: 0700–1900 h). Prior to treatment, male rats (weighing 137–147 g) were assigned to each group on a random body weight basis; therefore, at the start of experiments, the mean body weights of the groups were similar. All animals used in this experiments were handled in an accredited South Korea FDA animal facility in accordance with the guidelines for animal experiment of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International Animal Care Policies (Accredited Unit-South Korea Food and Drug Administration: Unit Number-000996).

Study design
Juvenile male rats (33 day of age, 10 rats/group) were treated by oral intubation with DES (Sigma, St. Louis, MO, USA) at 10, 20 or 40 μg/kg/day for 20 days. Corn oil (Sigma) was used as a vehicle at a volume of 5 ml/kg. In the present study, the highest dose level was chosen as the dose to induce general toxic signs, such as a decrease in body weight, in a preliminary pilot study. To evaluate dose-dependent relationships, common division by two from the highest level was selected, and the lowest dose was chosen from the results for the no observed adverse-effect level (NOAEL). The animals were inspected daily between 0900 h and 1000 h for prepuce separation (PPS). PPS was considered complete when the prepuce was completely retracted to expose the glans penis [20, 28].

Necropsy
One day after the last dose, all animals were euthanized under ether anesthesia. The testes, epididymides, ventral prostate, seminal vesicles plus coagulating glands and fluid (SVCGF), Cowper’s glands, glans penis and levator ani plus bulbocavernous muscles (LABC) were carefully excised and weighed. For histological examination, the tissues were placed in 10% neutral buffered formalin fixative. The organs were processed routinely into paraffin sections, stained with hematoxylin and eosin and examined microscopically.

Hormone measurements
Blood was collected from the abdominal aorta approximately 24 h after the last treatment with test compound. Serum was prepared immediately and stored at −75°C until analysis for serum hormone concentrations. The testosterone, estradiol, luteinizing hormone (LH) and thyroxine levels were measured using commercial RIA kits (Coat-A-Count, DPC, Los Angeles, CA, USA) in accordance with the manufacturers’ instructions. For measurement of hormone concentrations, the assay was performed twice by the same method.

Statistics
Means and standard deviations (SDs) were calculated for body weights, organ weights, the onset day of PPS and hormone levels. The means were compared using the Dunnett’s test after one-way analysis of variance using SigmaStat V 2.03 (SPSS, Chicago, IL, USA). Significant differences between values were accepted at P<0.05.

Results

General observation and organ weights
No clinical signs or abnormalities related to DES treatment were observed in any of the rats during the treatment period. Statistically significant decreases in body weight were observed at 40 μg/kg beginning on day 3 of DES treatment (Fig. 1). A significant delay in PPS onset (Fig. 2) was observed at 20 and 40 μg/kg/day (42.2 ± 3.97 and 43.6 ± 3.37 day, respectively) compared with the control.
(38.4 ± 1.07 day). The mean body weights of the animals on the day of PPS onset in the 20 and 40 μg/kg/day groups were not significantly higher (216.3 ± 35.91 and 208.6 ± 27.01 g, respectively) than that of the control (190.8 ± 10.54 g). The absolute and relative weights of the ventral prostate, SVCGF and Cowper’s glands were significantly reduced in the 20 and 40 μg/kg/day DES-treated groups, and those of the LABC and glans penis were dose-dependently reduced in the DES-treated animals (Table 1 and 2). The absolute weight of the testes was significantly decreased at 20 and 40 μg/kg/day, but the absolute weight of the epididymides was decreased in the 40 μg/kg/day DES-treated group; however, the relative weights of the testis and epididymides were not decreased, indicating a reduction in body weight. The absolute and relative weights of the adrenals were increased in all DES-treated animals in a dose-dependent manner. The absolute liver weight increased at 10 and 20 μg/kg/day, but the relative liver weight increased in all the DES-treated groups. The thyroid weight was significantly increased at 10 μg/kg/day, but was not changed in the 20 and 40 μg/kg/day groups (Table 1 and 2). The weight of the kidney was unaffected by DES treatment. The absolute heart weight was decreased and the relative hypophysis weight was increased in the 40 μg/kg/day DES treated-group. The changes in the heart and hypophysis were considered not reliably attributable to the treatment.

**Histopathological examination**

Spermatogenic cell loss and tubular atrophy accompanied by modifications in seminiferous epithelium composition and morphology were observed in the testes of the DES-treated animals (Fig. 3). Degeneration and deletion of germ cells, especially elongate spermatids, were observed in the seminiferous tubules of the DES-treated animals at the two highest doses, 20 and 40 μg/kg/day, although normal spermatogenesis with meiotic figures was observed in all DES-treated mice including the controls. The observed reduction in testis weight of the DES-treated animals corresponded with a dose-dependent reduction in the number of spermatogenic cells in the seminiferous tubule cross sections. No microscopic changes were observed in the thyroid after DES administration (data not shown).

**Hormone measurements**

Compared with the controls, the serum testosterone and LH levels were significantly decreased in all DES-treated groups, and the serum estradiol level tended to be decreased in the DES-treated groups. No significant differences in thyroxine levels were observed (Table 3).

**Discussion**

In the present study, we evaluated the effects of DES on pubertal development in the juvenile male rat. Delay of PPS onset and

### Table 1. Absolute (A) and relative (R) weights of reproductive organs and accessory sex glands in control and DES treated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Combined tests</th>
<th>Combined epididymides</th>
<th>Ventral prostate</th>
<th>SVCGF</th>
<th>LABC</th>
<th>Cowper's glands</th>
<th>Glans penis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.73 ± 0.16</td>
<td>0.33 ± 0.03</td>
<td>0.28 ± 0.04</td>
<td>0.54 ± 0.09</td>
<td>0.76 ± 0.07</td>
<td>0.056 ± 0.009</td>
<td>0.085 ± 0.006</td>
</tr>
<tr>
<td>R</td>
<td>0.89 ± 0.07</td>
<td>0.107 ± 0.008</td>
<td>0.09 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>0.018 ± 0.003</td>
<td>0.028 ± 0.003</td>
</tr>
<tr>
<td>DES 10 μg/kg</td>
<td>2.64 ± 0.12</td>
<td>0.32 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.52 ± 0.04</td>
<td>0.63 ± 0.03*</td>
<td>0.051 ± 0.007</td>
<td>0.073 ± 0.003*</td>
</tr>
<tr>
<td>R</td>
<td>0.88 ± 0.03</td>
<td>0.105 ± 0.007</td>
<td>0.10 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.21 ± 0.01*</td>
<td>0.017 ± 0.002</td>
<td>0.024 ± 0.002*</td>
</tr>
<tr>
<td>DES 20 μg/kg</td>
<td>2.56 ± 0.13*</td>
<td>0.30 ± 0.03</td>
<td>0.19 ± 0.02*</td>
<td>0.43 ± 0.08*</td>
<td>0.62 ± 0.04*</td>
<td>0.036 ± 0.007*</td>
<td>0.074 ± 0.005*</td>
</tr>
<tr>
<td>R</td>
<td>0.85 ± 0.03</td>
<td>0.102 ± 0.007</td>
<td>0.06 ± 0.01*</td>
<td>0.14 ± 0.02*</td>
<td>0.20 ± 0.01*</td>
<td>0.012 ± 0.002*</td>
<td>0.024 ± 0.003*</td>
</tr>
<tr>
<td>DES 40 μg/kg</td>
<td>2.47 ± 0.13*</td>
<td>0.26 ± 0.01*</td>
<td>0.14 ± 0.03*</td>
<td>0.13 ± 0.02*</td>
<td>0.39 ± 0.03*</td>
<td>0.021 ± 0.005*</td>
<td>0.061 ± 0.004*</td>
</tr>
<tr>
<td>R</td>
<td>0.96 ± 0.05*</td>
<td>0.102 ± 0.006</td>
<td>0.05 ± 0.01*</td>
<td>0.05 ± 0.01*</td>
<td>0.15 ± 0.01*</td>
<td>0.009 ± 0.001*</td>
<td>0.024 ± 0.002*</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=10. SVCGF, seminal vesicles plus coagulating glands and fluid; LABC, levator ani plus bulbocavernosus muscles. *Significantly different from control (P<0.05).

### Table 2. Absolute (A) and relative (R) organ weights in control and DES treated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Heart</th>
<th>Combined adrenals</th>
<th>Combined kidneys</th>
<th>Thyroid glands</th>
<th>Hypophysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.61 ± 0.07</td>
<td>1.01 ± 0.07</td>
<td>0.046 ± 0.007</td>
<td>2.44 ± 0.10</td>
<td>0.013 ± 0.003</td>
<td>0.011 ± 0.001</td>
</tr>
<tr>
<td>R</td>
<td>3.09 ± 0.18</td>
<td>0.33 ± 0.02</td>
<td>0.015 ± 0.002</td>
<td>0.79 ± 0.05</td>
<td>0.004 ± 0.001</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>DES 10 μg/kg</td>
<td>12.12 ± 0.65*</td>
<td>1.04 ± 0.07</td>
<td>0.069 ± 0.006*</td>
<td>2.42 ± 0.31</td>
<td>0.017 ± 0.003*</td>
<td>0.011 ± 0.002</td>
</tr>
<tr>
<td>R</td>
<td>4.01 ± 0.16*</td>
<td>0.34 ± 0.02</td>
<td>0.023 ± 0.002*</td>
<td>0.80 ± 0.10</td>
<td>0.006 ± 0.001*</td>
<td>0.004 ± 0.001</td>
</tr>
<tr>
<td>DES 20 μg/kg</td>
<td>12.94 ± 0.58*</td>
<td>1.02 ± 0.05</td>
<td>0.071 ± 0.011*</td>
<td>2.31 ± 0.12</td>
<td>0.010 ± 0.003</td>
<td>0.010 ± 0.003</td>
</tr>
<tr>
<td>R</td>
<td>4.26 ± 0.21*</td>
<td>0.34 ± 0.01</td>
<td>0.024 ± 0.003*</td>
<td>0.77 ± 0.04</td>
<td>0.003 ± 0.001</td>
<td>0.004 ± 0.001</td>
</tr>
<tr>
<td>DES 40 μg/kg</td>
<td>9.84 ± 0.73</td>
<td>0.87 ± 0.03*</td>
<td>0.073 ± 0.005*</td>
<td>2.20 ± 0.21</td>
<td>0.013 ± 0.001</td>
<td>0.011 ± 0.001</td>
</tr>
<tr>
<td>R</td>
<td>3.85 ± 0.17*</td>
<td>0.34 ± 0.02</td>
<td>0.029 ± 0.002*</td>
<td>0.86 ± 0.05</td>
<td>0.005 ± 0.001</td>
<td>0.004 ± 0.001*</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=10. *Significantly different from control (P<0.05).
reduction of the weight of the testes and accessory sex organs were observed in the DES-treated animals. A dose-dependent depletion of spermatogenic cells was observed, which was paralleled by a loss in testicular weight. There were decreases in the serum testosterone and LH levels in the DES-treated animals. The present study indicated that DES affected the pubertal development of juvenile male rats and that its mode of action may be via hormone level alterations. Under normal conditions, the control of secretion in the LH-testosterone system involves a number of negative feedback mechanisms. Inhibition of androgen synthesis by DES and other estrogens seems to be mediated via estrogen receptor [29]. By binding to estrogen receptor, DES may inhibit steroidogenic pathways at multiple steps [30–32]. In our experimental system, administration of DES in juvenile male rats may result in derangement of the hypothalamic-hypophyseal-gonadal system, interfering with these feedback systems. These results are in agreement with other findings in DES-exposed animals [33, 34].

To study the effects of agents on the onset of puberty, investigators have searched for reliable and meaningful indices of puberty. While vaginal opening has served as a useful index of female puberty [35], PPS has been widely used as an index of puberty in the male rat [28, 36]. PPS has been shown to be androgen-dependent and, therefore, to be of importance in the study of the hormonal changes necessary for the onset of puberty [28, 37]. Some reports have shown that the age of PPS can be advanced or retarded by hormonal manipulations. Pubertal delays have been detected following exposure to flutamide, an antiandrogen [27]. Peripubertal treatment with p,p’-DDE, methoxychlor, linuron and dibutil phthalate, which show estrogenic activity, delays the onset of androgen-dependent PPS [21, 38–40]. In contrast, carbamazepin, a reproductive toxicant that acts via non-endocrine mechanisms, does not delay PPS even at doses that profoundly reduce testicular sperm production [41]. The timing of puberty is also dependent on the body weight, and the mean body weight of the highest dose group was lower than that of the control according to the daily observations in the present study. However, the mean body weights of the DES-treated groups were higher than that of the control group. Thus, the delay of onset of PPS induced by DES treatment in the present study is considered to be due to the estrogenicity of DES, not its general toxicity.

Throughout puberty and into adulthood, the sex accessory glands and other androgen-dependent tissues continue to be dependent upon testosterone and 5α dihydrotestosterone for maturation and maintenance of function. The important DES-induced change was suppression of androgen action. Significant weight changes were observed in the testes, epididymides, ventral prostate, SVCGF, LABC, Cowper’s glands and glans penis in the DES-treated animals. The most sensitive organs to DES were the LABC and glans penis followed by the ventral prostate, Cowper’s glands, seminal vesicles, testes and epididymides, respectively. In previous studies with DES, decreases in the weights of accessory sex tissues and glands have been shown at various dose levels, and these differences might be due to differences in the experimental conditions [4, 8, 16, 17, 42, 43]. Neonatal administration of the androgen receptor antagonist flutamide delayed PPS and decreased the size of the male reproductive and accessory sex organs [27]. However, in the present study, DES treatment induced similar changes at much lower concentrations (20 μg/kg) than flutamide (5 mg/kg). Estrogen is thought to have greater effects on the male reproductive organs than antiandrogen. Rats treated with gonadotropin-releasing hormone antagonists have suppressed endogenous gonadotropin concentrations, which retards the development of the testes and the male reproductive tract [10, 44]. DES treatment of the male rats in the present study decreased the serum testosterone and LH levels. Neonatal estrogen treatment (days 2–12) cause dose-dependent reductions in plasma testosterone levels in adulthood [5]. The relatively poor spermatogenesis observed in the DES-treated animals seems to be related to a reduction in the testosterone and LH levels. The changes in the serum testosterone and LH levels of the DES-treated groups were higher than that of the control group according to the daily observations in the present study. However, the mean body weights of the DES-treated groups were higher than that of the control group. Thus, the delay of onset of PPS induced by DES treatment in the present study is considered to be due to the estrogenicity of DES, not its general toxicity.

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![Fig. 3. Induction of abnormalities in the testes of DES-treated rats. A, C: control; B, D: DES-treated rats (40 μg/kg/day). Deletion of the germinal epithelium and tubular atrophy were observed in the group treated with 40 μg/kg/day of DES (B, D) compared with the intact seminiferous tubules of the controls (A, C). Magnification: ×100 for A and B; ×400 for C and D.](image)

### Table 3. Serum hormone levels in control and DES treated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Testosterone (ng/ml)</th>
<th>Estradiol (pg/ml)</th>
<th>Thyroxine (ng/ml)</th>
<th>LH (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.1 ± 0.31*</td>
<td>28.7 ± 8.89</td>
<td>56.6 ± 5.51</td>
<td>23.3 ± 8.520</td>
</tr>
<tr>
<td>DES 10 μg/kg</td>
<td>0.5 ± 0.19*</td>
<td>22.4 ± 3.11</td>
<td>75.6 ± 17.43</td>
<td>7.9 ± 1.033*</td>
</tr>
<tr>
<td>20 μg/kg</td>
<td>0.6 ± 0.69*</td>
<td>22.9 ± 11.57</td>
<td>69.1 ± 13.05</td>
<td>10.7 ± 5.175*</td>
</tr>
<tr>
<td>40 μg/kg</td>
<td>0.3 ± 0.21*</td>
<td>23.5 ± 4.92</td>
<td>54.1 ± 13.48</td>
<td>5.1 ± 0.54*</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=10. *Significantly different from control (P<0.05).
LH levels and the weights of the androgen-dependent tissues observed in the present study may reflect the influence of DES on the negative-feedback mechanism of the hypothalamic-pituitary axis.

Several reports are available on the adverse effects of fetal or neonatal estrogen exposure on spermatogenesis and sperm output in adulthood in rats and hamsters [1, 4, 45, 46]. The findings of the present study that loss of primarily haploid spermatogenic cells differs from those found in other toxic-induced cell death, where the diploid population of spermatocytes are most affected [47, 48] or those affected by the gonadotropin-releasing hormone antagonist [49]. Treatment with gonadotropin antagonist precipitates apoptosis primarily in spermatogonia, spermatocytes, and, to a lesser extent, spermatids [50]. In the present study, meiotic figures of testicular germ cells were observed in the seminiferous tubules of DES-treated animals. Thus, the loss of testicular cells was related to an increase in the apoptotic cell death of spermatogenic cells in the DES-treated animals [17, 18], and both Fas and FasL proteins in the spermatogenic cells are considered to be primary modulators in DES-induced germ cell apoptosis [51]. Estrogen is essential for spermatogenesis by direct interference with spermatogenic cells, which are known to express ERs [52, 53]. DES can also bind to ERs in the pituitary and interfere with the functioning of the pituitary-gonadal axis by controlling the circulating levels of LH and follicle-stimulating hormone through a feedback regulation of steroid hormones, thus leading to the suppression of testosterone levels and increased spermatogenic cell apoptosis [18].

In the thyroid hormone analysis and the microscopic evaluations of the thyroid in the present assay, thyroid function may not be affected by DES. In a previous study, pregnant Sprague-Dawley rats dosed with DES during various gestation periods showed significantly elevated levels of maternal serum thyroxine and a reduction in maternal weight [54]. Cautious interpretation is needed to analyze thyroid-related data because thyroid hormone levels are easily affected by many factors, such as diet, stress, age, and circadian levels. Thus, thyroid histopathology was determined to be the most reliable parameter for detection of compounds that affect thyroid function. Neither changes in thyroxine levels nor induction of follicular cell hypertrophy/hyperplasia were observed after DES treatment in the present study, but the body weights of the DES-treated animals decreased at 40 μg/kg. Therefore, further study may be needed with higher doses of DES.

Acknowledgements

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References


organotin compounds on pubertal male rats. *Toxicology* 2004; 202: 145–158.


