Re-establishment of Spermatogenesis by Diethylstilbestrol after 2,5-Hexanedione-induced Irreversible Testicular Atrophy in Rats

Yue QIAN (钱 旭)1, Fuqing ZENG (曾富清)2*
1Department of Dermatology, 2Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Summary: To investigate the effects of diethylstilbestrol (DES) in reestablishing spermatogenesis and the mechanism by which estrogen works on spermatogenesis, rats were exposed to 1% 2,5-HD for 5 week. Then 0.1 mL of DES was given (s.c.) at a rate of 0.3 μg/kg, 30 μg/kg, 3 mg/kg every other day for 2 weeks respectively (DES group) while the other rats received ethyldeate only. Plasma testosterone (T) and LH were measured on the 8th week after the treatment. The rats were killed at the 18th week. The left testis was histopathologically examined. In all the rats in the DES groups, spermatogenesis was re-established and the rats in the 30 μg/kg group showed the best results. Serum T was suppressed markedly in rats of 30 μg/kg and 3 mg/kg groups while T was only mildly inhibited in 0.3 μg/kg group, without significant difference found in serum LH. It is concluded that the nearly complete testicular atrophy could be reversed by DES treatment in rats. Estrogen plays an important part in spermatogenesis, and the role of estrogen in spermatogenesis is more than suppressing the hypothalamo-pituitary-testis axis.

Key words: spermatogenesis; hexanedione; diethylstilbestrol; testicular atrophy

Although it has been known that estrogen was effective in the treatment of male fertility, findings from transgenic mice deficient in estrogen receptors or aromatase suggested an important role of estrogen in male fertility[1]. Evidence has shown that estrogens works at multiple levels on spermatogenesis in males but the exact mechanism has not been fully understood. Previous studies demonstrated that treatment with GnRH-agonist could re-establish spermatogenesis after 2,5-HD-induced testicular injury in rats[2] but the results are still preliminary. In this study, the 2,5-HD-induced testicular atrophy model was used to evaluate the effects of diethylstilbestrol (DES) in the re-establishment of spermatogenesis to explore the effect of estrogen on spermatogenesis.

1 MATERIALS AND METHODS

1.1 Animals

A total of 25 male Sprague-Dawley rats, aged 2 months, were from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Their body weight ranged approximately 175–200 g and monitored individually throughout the study. The testis of both sides were of the same size. The rats were randomly assigned to 5 groups: control group (group A, n=5), testis atrophy group (group B, n=5), 0.3 μg/kg DES group (group C, n=5), 30 μg/kg DES group (group D, n=5), 3 mg/kg DES group (group E, n=5). Four groups (group B, C, D, E) were treated for 5 weeks with 1% 2,5-HD (Emuck, Germany) by feeding them in their drinking water. At the end of the toxicant exposure, the rats were given normal (drug-free) drinking water. Afterwards, groups C, D, E) received a s.c. injection of DES (Sigma, USA) every other day for 2 weeks at a dose of 0.3 μg/kg (group C), 30 μg/kg (group D), 3 mg/kg (group E) while rats in other were given 0.1 mL of ethyldeate only.

1.2 Hormone Measurements

Blood was collected from caudal vein at 8:00 a.m. of the first morning of the 8th week. Plasma LH and T level was measured by RIA.

1.3 Histological Examination

The animals were killed by CO2 asphyxiation at the 18th week. Each testis was trimmed of surrounding adipose tissue and individually weighted. The left testis of each animal was histopathologically examined.

1.4 Statistical Analysis

All data are expressed as the 併±s. ANOVA and student’s t-test were used evaluation of statistical significance. A P<0.05 was considered to be significant.

2 RESULTS

2.1 General Observations

There were no significant differences in body weight between control group and testis atrophy group, while a small but statistically significant decrease in body weight was observed in 30 μg/kg DES group and 3 mg/kg DES group as compared with control group and testis atrophy group. Testicular atrophy was observed following exposure to 2,5-HD while DES treatment slightly increase of testis weight in 30 μg/kg group and 3 mg/kg group (table 1).
Table 1  General observation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Testis weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>278±9</td>
<td>1.67±0.05</td>
</tr>
<tr>
<td>B</td>
<td>271±8</td>
<td>0.53±0.05</td>
</tr>
<tr>
<td>C</td>
<td>276±9</td>
<td>0.58±0.06</td>
</tr>
<tr>
<td>D</td>
<td>258±10&quot;</td>
<td>0.65±0.05&quot;</td>
</tr>
<tr>
<td>E</td>
<td>253±12&quot;</td>
<td>0.61±0.05&quot;</td>
</tr>
</tbody>
</table>

*P<0.05 as compared with group A; **P<0.05 as compared with group B

2.2 Histopathological Analysis

For each rat, approximately 100 seminiferous tubules were analyzed and categorized as either atrophic, partially recovered, or recovered. By this classification, the rats of group B (testis atrophy group) showed nearly complete testicular atrophy. The recovery of spermatogenesis between the 3 DES groups was significantly different while recovery of spermatogenesis in 30 μg/kg DES group was the best (fig. 1–6).

![Figure 1](image1.png)

Fig. 1 Percentage of seminiferous tubules on cross sections
A: Atrophic; P: Partially recovered; R: Recovered

2.3 Blood Hormone Levels

There were no significant differences in plasma T level between the control group (group A) and testis atrophy group (group B), while significant differences were found in LH level between them. Serum T was suppressed markedly in 30 μg/kg and 3 mg/kg groups and it was slightly inhibited in 0.3 μg/kg group. Serum LH was elevated in all the rats exposed to 2,5-HD (groups B, C, D, E) while there were no significant differences in LH level among all the four 2,5-HD-exposed groups.

![Figure 2](image2.png)

Fig. 2 Control group (HE×40)
Fig. 3 Testis atrophy group (HE×40)
Fig. 4 0.3 μg/kg DES group (HE×40)
Fig. 5 30 μg/kg DES group (HE×40)
Fig. 6 3 mg/kg DES group (HE×40)

Table 2  Blood hormone levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>T (ng/dL)</th>
<th>LH (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>77.6±4.7</td>
<td>3.36±0.12</td>
</tr>
<tr>
<td>B</td>
<td>25.6±2.5*</td>
<td>6.05±0.24*</td>
</tr>
<tr>
<td>C</td>
<td>22.1±2.8*</td>
<td>5.79±0.23*</td>
</tr>
<tr>
<td>D</td>
<td>—</td>
<td>5.85±0.30*</td>
</tr>
<tr>
<td>E</td>
<td>—</td>
<td>5.96±0.25*</td>
</tr>
</tbody>
</table>

*P<0.05 as compared with group A; —: too low to be measured

3 DISCUSSION

2,5-hexanediol (2,5-HD) is a toxicant that causes a long-lasting testicular atrophy and infertility in rats[3,4]. Animal studies have found that when rats were exposed to 1% 2,5-HD in the drinking water for up to 5 weeks, a long lasting testicular atrophy resulted. After 2 weeks of exposure, Sertoli cell microtubules assembly kinetics are altered, which was followed by a reduced fluid secretion of seminiferous tubules of 3 weeks of exposure. When treated by of 2,5-HD for 4 weeks, large basal vacuoles were observed, which was followed by a substantial reduction or loss of germ cells after 5 weeks of toxicant exposure. In this study, the testicular atrophy was considered to be irreversible, because it lasted for more than 70 weeks after toxicant withdrawal. However, some studies showed GnRH-agonist treatment could reverse this irreversible toxicant injury or testicular atrophy following exposure to irradiation or 2,5-HD in rat[5,6].

Although estrogens have been regarded as female steroid hormones, they are now known to have profound effects on both female and male reproductive systems[7]. In males, estrogens are synthesized mainly in the testis, where they are turned to testosterone by P450 aromatase. In male, ERα has been shown to be strongly expressed in the epididymis, efferent ductules and leydig cells. While ERβ has been shown to be expressed by germ cells and leydig cells[8,9]. Therefore, estrogens are considered to be essential to male reproduction.