Lesions of the Rete Testis in Mice Exposed Prenatally to Diethylstilbestrol

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ABSTRACT

Adenocarcinoma of the rete testis is an exceptionally rare and malignant testicular neoplasm. Although treatment of pregnant women with diethylstilbestrol (DES) results in reproductive tract abnormalities in their male offspring, increased incidence of testicular tumors has not been verified. However, recently three cases of seminoma have been described in men prenatally exposed to DES, suggesting an association of prenatal DES treatment and the subsequent development of testicular tumors. This report describes the treatment of outbred pregnant CD-1 mice with DES (100 μg/kg) on Days 9 through 16 of gestation and its effects on their male offspring. In addition to nonmalignant abnormalities such as retained testes which have been reported in men exposed prenatally to DES, lesions resembling adenocarcinoma of the rete testis were seen in prenatally DES-treated mice at 10 to 18 mo of age (11 of 233; 5%). No comparable lesions were seen in 96 age-matched control male mice. These results suggest an association of prenatal DES exposure and the subsequent development of testicular lesions in the rete testis of mice.

INTRODUCTION

Work in our laboratory has established that the mesonephric duct, as well as the Müllerian duct, is a target for DES2 (1, 2). In fact, in adult female mice (2) and humans3 exposed prenatally to DES, hyperplastic mesonephric remnants are a common feature. The relationship of these dysmorphic mesonephric structures to the pathogenesis of hyperplastic or neoplastic disease in the female is under study. These findings raise the possibility that structures derived from the mesonephric ducts or tubules may also be dysplastic in male offspring. Although the embryology of the rete testis is still controversial, Byskov (3, 4) attributes the rete system to mesonephric tubular origin. Thus, the rete testis, a clearly definable adult structure apparently derived from mesonephric tubules, was evaluated for hyperplastic or neoplastic changes in male CD-1 mice exposed prenatally to DES.

Adenocarcinoma of the rete testis is a rare form of neoplasia occurring in humans (7). In animals, spontaneous adenocarcinoma of the rete testis is also extremely rare; one case was reported in a ram (8), and another in the rodent, Mastomys (9). In a recent report of spontaneous tumors of the mouse testis, Yoshitomi and Morii (7) described one case of retetestis adenocarcinoma in 500 aged mice examined.

Previous studies from this laboratory and others have described some testicular tumors, mainly interstitial cell tumors, in prenatally DES-exposed male mice (1, 10, 11). Likewise, a few recent case reports have appeared, suggesting an association between prenatal DES exposure and the subsequent development of testicular seminoma in men (12, 13). In this paper, we describe a high prevalence of lesions in the rete testis of mice exposed prenatally to DES.

MATERIALS AND METHODS

Animals and Treatment Schedule. Outbred CD-1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA, and were bred to male mice of the same strain in the animal facility at the National Institute of Environmental Health Sciences. The time of vaginal plug detection was considered Day 0 of pregnancy. Pregnant mice were housed separately in a room with controlled temperature (21-22°C) and lighting (14-h light and 10-h dark periods) and were provided with hardwood chip bedding, fresh water, and NIH 31 laboratory mouse chow ad libitum.

Pregnant female mice were untreated or given s.c. injections of DES (Sigma Chemical Co., St. Louis, MO) dissolved in corn oil on Days 9 to 16 of gestation. The daily dose of DES was 100 μg/kg body weight, and the total volume of corn oil administered was 0.01 ml/g maternal body weight. The offspring of these animals are referred to as DES-100 or controls in this study. Purity of the DES was checked by thin-layer chromatography, high-pressure liquid chromatography, and gas chromatography-mass spectrometry and exceeded 99%.

Pregnant mice delivered their young on Day 19 of gestation, and all litter sizes were randomly standardized to 8. At 25 days of age, offspring were weaned, segregated by gender, and housed in groups of 5/cage. At 10 to 18 mo of age, 122 control and 277 DES-100 male offspring were sacrificed by cervical dislocation. The abdominal cavity was quickly opened, and gross structural abnormalities in the reproductive tract including location of testes were observed. Testes and epididymis were removed, fixed in 10% neutral buffered formalin, and embedded in paraffin, and 20 serial 6-μm sections of each testis were made. Sections were stained with hematoxylin and eosin and evaluated. In some cases, paraffin sections were stained by the periodic acid-Schiff reaction. This paper describes only the animals in which the rete testis could be evaluated which are as follows: 10 mo (4 control and 31 DES-100); 11 mo (11 DES-100); 12 mo (9 DES-100); 13 mo (18 controls and 21 DES-100); 14 mo (8 controls and 49 DES-100); 15 mo (23 controls and 52 DES-100); 16 mo (14 controls and 18 DES-100); 17 mo (12 controls and 8 DES-100); and 18 mo (17 controls and 34 DES-100). The results were analyzed statistically using the Fisher exact test, and the limit of significance was set at 5%.

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2 The abbreviation used is: DES, diethylstilbestrol.

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RESULTS

Histology of the Normal Rete Testis. The excretory ducts of the testis include the rete testis, efferent ducts, epididymis, and ductus deferens. Near the hilus of the testis within the tunica, the seminiferous tubules become straight and are gathered into a network of anastomosing canals, the rete. The rete testis is lined with a simple low cuboidal or flat epithelium resting on a basement membrane (Fig. 1). The network of irregular, broad-lumened canals of the rete opens into a single lacuna which, outside the tunica albugínea, branches into the efferent ducts. Spermatooza and secretions are transported from the seminiferous tubules to the rete and then the efferent ducts which, in turn, carry the secretions toward the head of the epididymis. In this study, the rete testis of 10- to 18-mo-old animals appeared to be normal in 75% (72 of 96) controls and 14% (32 of 233) DES-100 animals.

The abnormalities in control animals were consistent with usual age-related changes, whereas the changes in the DES-100 animals were not.

Histology of the Rete Testis of Prenatally DES-exposed Mice. Prenatal exposure to DES resulted in numerous changes in the testes of all the males examined at 10 to 18 mo of age. Ninety-one % (252 of 277 total number sacrificed) had cryptorchid testes ranging from a location directly under and firmly attached to the kidney, to the region of the inguinal canal. The retained testes were hypoplastic and sometimes fibrotic with multinucleated cells in the seminiferous tubules. In some cases, the testes were necrotic with just scar tissue remaining. Abnormalities including tumors of the corpus testis are described in another report.4

This paper concerns only the animals in which the rete could be evaluated (96 controls and 233 DES-100). In this group, inflammatory changes in the rete testis were observed in one 14-mo control (1%) and 5 DES-100 animals (2%) at 10 mo and 15 mo of age (1 of 96 versus 5 of 233, not significantly different). Dilated rete was seen in one 18-mo control (1%) and 5 DES-100 (2%) at 15 and 18 mo of age (1 of 96 versus 5 of 233, not significantly different). Two of the five DES-100 animals with dilated rete also had inflammation.

The rete testis of a control 10-mo-old male mouse is represented in Fig. 1. Tubules of the rete are lined by cuboidal or flat epithelium. Mild focal hyperplasia of the rete testis was seen in 23 of 96 (24%) control males in this study.

Various degrees of hyperplasia of the rete testis were found among 130 of 233 (56%) DES-100 animals (23 of 96 versus 130 of 233, P < 0.0001) (Table 1). The simplest form of this DES-induced lesion consisted of knob-like proliferation of cuboidal epithelium (Fig. 2) and diffuse hyperplasia of the epithelium (Fig. 3). In other animals, these proliferative overgrowths formed papillary structures consisting of small cuboidal cells with increased hyperchromatism and vacuolated cells over a vascular core. The distribution of rete testis hyperplasia between ages in the DES-100 males was as follows: 21 of 31 (68%), 10 mo; 9 of 11 (82%), 11 mo; 9 of 9 (100%), 12 mo; 12 of 21 (57%), 13 mo; 22 of 49 (45%), 14 mo; 22 of 52 (42%), 15 mo; 11 of 18 (61%), 16 mo; 6 of 8 (75%), 17 mo; and 18 of 34 (53%), 18 mo. Papillary proliferation was not observed in any control animals.

| Table 1 |
| Rete testis lesions in male mice exposed prenatally to diethylstilbestrol | |
| Males were 10- to 18-mo-old offspring of CD-1 mice treated with DES (100 µg/kg s.c.) on Days 9-15 of gestation. Since there was not a statistically significant difference in prevalence of lesions with age, rete testis hyperplasia and lesions resembling adenocarcinoma in each age group have been combined. |
| Control | DES-100 |
| Hyperplasia | 23/96 (24%) | 130/233 (56%) |
| Adenocarcinoma-like lesion | 0/96 (0) | 11/233 (5%) |

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A more severe lesion was also observed in these mice after in utero exposure to DES. This lesion, resembling adenocarcinoma, was not found in any controls but was found in 5% of the prenatally exposed DES-100 mice (Table 1; 0 of 96 versus 11 of 233, P < 0.05). Although distant metastases could not be documented, the lesions often infiltrated into the seminiferous tubules or into the ductuli efferentes; the histological pattern was suggestive of either papillary adenocarcinoma (Fig. 4) or tubulo-papillary adenocarcinoma (Fig. 5a). The lesion in Fig. 5a occupied approximately 10% of the section surface of the testis, and there was an area of local invasion (Fig. 5b). There was increased cellularity of the epithelium, and there were papillary or floridly folded regions in the epithelium. The epithelial cells were mainly cuboidal and appeared to be enlarged. There was considerable pleomorphism of nuclei (Fig. 5c); mitotic figures were not numerous, but they were abnormal (Fig. 5d).

The incidence of this lesion of the rete testis which resembled adenocarcinoma in the DES-100 males was: 1 of 31 (3%), 10 mo; 1 of 21 (8%), 13 mo; 4 of 49 (8%), 14 mo; 1 of 52 (2%), 15 mo; 1 of 18 (6%), 16 mo; and 3 of 34 (9%), 18 mo. The median age of this lesion was 14 mo. Data on rete testis hyperplasia and the more severe lesions have been combined among ages in Table 1.

DISCUSSION

Adenocarcinoma of the rete testis in humans and experimental animals is so rare that it is difficult to estimate how many authentic cases exist. Moreover, investigators disagree on diagnostic criteria, site of origin, and the designation that should be applied to the tumors. Criteria were first formulated in 1945 by Feek and Hunter (14) and later by others (6, 15-17) and have been accepted by most investigators as the basis for identifying this neoplasm. Shillito (16) emphasized that these criteria were not absolute, but they formed significant guidelines in making the diagnosis. In summary, the diagnostic criteria of tumor of the rete testis were: (a) involvement centering on the mediastinum testis rather than the testis proper; (b) lack of direct extension through the parietal tunica; (c) transition from normal epithelial structures to neoplastic structures in the rete testis; (d) no evidence of teratoma; and (e) lack of any other primary tumor.

In the affected mice described in this paper, lesions were seen in the area of the mediastinum testis. There was maximum involvement of the rete but minimal involvement of the corpus. Cells also appeared to be transformed from the normal epithelial structures of the rete testis to abnormal areas. Although certain strains of mice frequently show tumoromatous changes (18) and
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since Ewing (19) believed that teratocarcinomas originated in the rete testis, we examined all of the tumors for evidence of teratocarcinoma, but no neural elements, muscle, cartilage, bone, or other components of teratomas were found. Thus, the tumors described in this paper conform closely to the criteria established for rete adenocarcinoma.

The demonstration of an extremely rare lesion described in this paper is unique in its prevalence alone, since it appears in 5% of the prenatal DES animals. Recently, Yoshitomi and Morii (7) have reported the spontaneous occurrence of a rete adenocarcinoma in a 23-mo-old mouse from a colony of 500 mice (0.2% incidence). Therefore, our data suggest prenatal exposure to DES results in at least a 20-fold increase in prevalence of adenocarcinoma-like lesions. In fact, if these prenatal DES males were allowed to age past 18 mo, they might have a higher incidence of abnormalities of the rete.

The embryology of the rete testis is still controversial. In addition to a mesonephric tubular origin (3, 4), the coelomic epithelium has also been suggested as a precursor for the gonadal rete system (20, 21). This is important because coelomic epithelium also gives rise to the uterus which is also a target for DES dysmorphogenesis and carcinogenesis. Results such as lesions of the rete testis described in this paper may help determine the embryological origin of this poorly understood tissue compartment.

Since 1943 (22), the induction of interstitial cell tumors in adult mice with DES has been well studied; however, no studies of estrogen-treated adult mice (23) have reported abnormalities of the rete. The increased incidence of lesions in the rete testis reported in this paper thus suggests this lesion may be associated with developmental exposure to DES. In addition, early exposure in development may be important, since no reports of this lesion have been made in other rodent models treated neonatally with DES or other estrogenic substances (24–27). An increased prevalence of a rare tumor, vaginal adenocarcinoma, called attention to the adverse effects on female offspring of women given DES while pregnant. The occurrence of rete testis tumors in male offspring of mice given DES during pregnancy suggests this may be an analogous situation, as naturally occurring rete testis lesions are extremely rare among mice. Among men, there does not appear to be a strong age-related factor associated with carcinoma of the rete testis, the reported age range being 20 to 80 yr, and tumors were reported almost equally divided between right and left side with one patient having bilateral tumors (28). Likewise, in the present study, there was no association between ages 10 to 18 mo of the male mouse and prevalence of rete testis lesions. In humans, the tumors usually were present as a testicular mass often associated with a hydrocele (29). It is of special interest that, in the group of human cases that have been reported, there are at least three tumors in maldescended testes (30–32), one of which had been placed in the scrotum by orchiopexy 10 yr previously. Cryptorchidism has been implicated as a predisposing factor for testicular neoplasms (33). The high incidence of retained testes in mice (92%) following prenatal DES exposure in the present study and the occurrence of this specific rare form of testicular abnormality, rete tumors, also raise the possibility of an association between cryptorchidism, prenatal DES exposure, and retic testes cancer. Although cryptorchidism results in decreased or lack of spermatogenesis in male mice, inactivity cannot solely account for higher prevalence of rete abnormalities, since 4 of the 11 mice with the lesion had spermatogenetic occurring in the same testis.

To date, there have been no reports of rete hyperplasia or adenocarcinoma in men that have been attributed to prenatal exposure to DES, but three cases of seminoma have been described in prenatal DES-exposed men, suggesting an association of such treatment and the subsequent development of testicular tumors (12, 13). Since Yoshitomi and Morii (7) report that rete adenocarcinoma can be misdiagnosed as seminoma and since established criteria state seminoma must be ruled out before a diagnosis of rete adenocarcinoma can be made, caution should be taken when examining the testicular lesions associated with prenatal DES exposure.

Although animal studies must be considered thoughtfully if extrapolation to humans is to follow, the prenatal mouse model has provided some interesting comparisons to similarly DES-exposed women such as reduced reproductive capacity (34), uterine structural malformations (35), malformed oviduct (2, 36), and salpingitis isthmic nodosa of the oviduct (2, 37). Moreover, in our earlier report on DES-exposed male mice, we suggested cryptorchid testes and epididymal cysts might be a common finding in exposed humans (10). Therefore, experimental results can be informative and predictive. Hence, continued close surveillance would seem prudent, especially in view of the young age of the exposed men and the high incidence of cryptorchidism and hydrocele in the DES-exposed patients.

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Fig. 1. Normal rete testis, 10-mo-old control CD-1 mouse. Irregular tubules of the rete (R) are located at the mediastinum of the testis. The channels of the rete are lined by cuboidal or flat epithelium. H & E, × 25.
Fig. 2. Focal hyperplasia of the epithelium of rete testis (R) from a 15-mo-old prenatally DES-exposed mouse. There is simple knob-like proliferation of cuboidal cells that have a tendency to pile up. H & E, × 25.
Fig. 3. Diffuse hyperplasia of the epithelium of the rete testis from a 14-mo-old prenatally DES-exposed mouse. The rete joins the efferent ducts at the top of the photomicrograph. H & E, × 25.
Fig. 4. Lesion resembling papillary adenocarcinoma from a 13-mo-old prenatally DES-treated mouse. The rete is largely filled by papillary projections covered by pleomorphic epithelium. H & E, × 25.
Fig. 5. a, lesion of the rete testis from a 16-mo-old prenatally DES-treated mouse. While the papillary pattern is focally apparent, the majority of the tumor has a tubular arrangement of pleomorphic epithelial cells. Rete epithelium in channels at the bottom of the photomicrograph is hyperplastic. This tumor occupies approximately 10% of the section surface of the testes. H & E, × 25. b, higher magnification of the lesion, suggestive of tubulopapillary adenocarcinoma in Fig. 5a; there is an area of local invasion (arrow). H & E, × 60. c, higher magnification of the lesion in Fig. 5a; marked pleomorphism of the tumor with bizarre nuclei (arrow). H & E, × 60. d, higher magnification of the lesion in Fig. 5a; mitotic figures are not numerous, but they are abnormal (arrow). H & E, × 60.
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