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 testes 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 DES 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 dysmorphogenic 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 arising from the lining cells of the channels connecting the seminiferous tubules of the testis and the epididymis. The first report of a rete testis tumor in humans was made by Curling in 1853 (5). In a review of the subject, Schoen and Rush in 1959 (6) found only 12 cases described in the literature to which they added one of their own. A more recent summary of the entire clinical literature estimates 21 adenocarcinomas of the rete testis 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 rete testis 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%.
RESULTS

Histology of the Normal Rete Testis. The excretory ducts of the testis in mice 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 albuginea, branches into the efferent ducts. Spermatocytes 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 in the testes of all the males examined at 10 to 18 mo of age. Dilated rete also had inflammation.

Numbers in parentheses, percentages.

Table 1

<table>
<thead>
<tr>
<th>Rete testis lesions in male mice exposed prenatally to diethylstilbestrol</th>
<th>Control</th>
<th>DES-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td>23/96 (24)</td>
<td>130/233 (56)</td>
</tr>
<tr>
<td>Adenocarcinoma-like lesion</td>
<td>0/96 (0)</td>
<td>11/233 (5)</td>
</tr>
</tbody>
</table>

R. R. Newbold, B. C. Bullock, and J. A. McLachlan. Testicular tumors in mice exposed in utero to diethylstilbestrol, submitted for publication.

DISCUSSION

Adenocarcinoma of the rete testis in humans and experimental animals is 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. Shillitoe (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 teratomatous changes (18) and...
since Ewing (19) believed that teratocarcinomas originated in the
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 adeno-
carcinoma 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 associ-
ated 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
malignancies in male offspring of mice given DES during pregnancy
suggests this may be an analogous situation, as naturally occur-
ing 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 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 maledescended testes (30–32), one of which had been
placed in the scrotum by orchiopexy 10 yr previously. Cryptor-
chidism has been implicated as a predisposing factor for testicu-
lar 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 abnor-
mality, rete tumors, also raise the possibility of an association
between cryptorchidism, prenatal DES exposure, and rete testis
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 spermatogenesis occurring in the same
tests.

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 associ-
ation 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 isthmica 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 sur-
veillance 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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LESIONS OF THE RETE TESTIS


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 o flatt 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 testis. 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; mitotic figures are not numerous, but they are abnormal (arrow). H & E, × 60.

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