Visualization by Light and Scanning Electron Microscopy of Reproductive Tract Lesions in Female Mice Treated Transplacentally with Diethylstilbestrol

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ABSTRACT

Pregnant female mice were exposed to diethylstilbestrol or 11β-methoxy-17β-estradiol on Days 9 to 16 of gestation. The female offspring of these animals were then examined for reproductive tract abnormalities. Scanning electron microscopic and histological evaluation of these specimens demonstrated reproductive tract lesions in all treatment groups when compared to matched control mice. These lesions included apparent displacement of the squamocolumnar junction, uterine squamous metaplasia, atypical uterine cell surface specializations, protrusions of uterine cells, vaginal and cervical papillary growths, enlarged uterine cervix, abnormal vaginal and uterine folding patterns, female hypospadias, and the presence of vaginal concretions. Scanning electron microscopic observations proved particularly useful in studying lesions which involved the disruption of the normal structure and shape of the reproductive tract and the displacement of cell types.

INTRODUCTION

Prenatal exposure to synthetic estrogens such as DES2 (10, 13, 14) and ethinylestradiol (22) have been shown to increase reproductive tract abnormalities in female mice. Such abnormalities can not be duplicated by exposure to the natural estrogen 17β-estradiol when given by a similar route of exposure at equivalent estrogenicity (14). The apparent differences in transplacental toxicity between DES and 17β-estradiol have been attributed to their relative binding to AFP and the associated retention of 17β-estradiol by AFP (14, 18). Comparison of DES activity to other estrogens, which have similar affinities for AFP as DES should help determine the role of AFP in the toxicity of synthetic estrogens. 11β-Methoxy-17β-estradiol (moxestrol), like DES, is a potent estrogen and has a low affinity for AFP (18); moxestrol, like 17β-estradiol, has a steroidal rather than a stilbene structure.

Recent preliminary studies showed that surface ultrastructural features can enhance the interpretation of the pathogenesis of DES-associated lesions (10). The correlation of SEM findings to histological characteristics will help determine the ontogeny and extent of such lesions in the female reproductive tract. Previous studies (10, 13–15, 20) have demonstrated that the mouse can provide a useful model of DES toxicity.

The present correlative morphological studies have been focused on the interrelationship of vaginal, cervical, and uterine tissues and their junctional regions. Also, special attention has been paid to lesions which are observed initially with the scanning electron microscope and subsequently examined histologically. The transplacental toxicity of DES was studied in female CD-1 mice. Moxestrol was also given to pregnant CD-1 mice, and the reproductive tracts of the female offspring were studied and compared to those of the DES-exposed female mice.

MATERIALS AND METHODS

CD-1 mice (obtained from Charles River Breeding Laboratories and mates at the National Institute of Environmental Health Sciences) were treated s.c. with DES (100 μg/kg/day) (Sigma Chemical Co.) or moxestrol (100 μg/kg/day) (New England Nuclear) in corn oil on Days 9 to 16 of gestation (plug day is Day 0). Female offspring were sacrificed at 6 weeks (3 DES, 5 controls), 3 months (4 DES, 5 controls), 15 months (5 moxestrol, 2 DES, 3 controls), or 20 months (5 DES, 5 controls) of age.

Immediately after sacrifice (cervical dislocation), the vagina, cervix, and uterus were removed and cut along the dorsal midline of the vagina and cervix and through each uterine horn. The tract was pinned out on moist cardboard, rinsed with 0.1 M phosphate buffer, pH 7.4, and fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.4, for 1 hr. The samples were dehydrated and prepared for SEM observation as described previously (12).

After SEM observation, samples were embedded in paraffin following the technique of Ayres et al. (1). After sectioning at 8 μm, the samples were stained with hematoxylin and eosin for light-microscopic study.

Description and classification of the lesions found in the different treatment groups were made on the basis of SEM observation of the vaginal and uterine lumina and the histological characteristics of the specimens. The SEM findings were used as a guide for light microscopic embedding and sectioning.

Vaginal concretions (see below) were collected, rinsed in deionized water, and air dried. The samples were mounted on a carbon stub using carbon paste and analyzed by energy-dispersive X-ray microanalysis in a scanning electron microscope. No surface coating was added to the samples.

RESULTS

Reproductive tract characteristics which were altered by in utero exposure to DES included displacement of the squamocolumnar junction of the cervix, uterine squamous metaplasia, atypical uterine cell surface specializations, protrusions of uterine cells, vaginal and cervical papillary growths, enlarged uterine cervix, abnormal vaginal and uterine folding patterns, female hypospadias, and the presence of vaginal concretions.

The squamocolumnar junction of control mice could be identified within the upper cervical canal, near the bifurcation of the uterine horns. At this junction, the stratified squamous epithelium of the cervix intersected the simple columnar epit-
thelium of the uterine horns. In no case (control or treated) was the junction (or simple columnar cells) ever found below that level. In the DES-treated offspring, the junctional zone was either located in the same area as in controls (this location was observed in 5 of 14 DES-treated mice), or areas of stratified squamous cells were found above the normal demarcation (in DES-treated mice, the incidence of this abnormality was 9 of 14; in controls, 0 of 18). In some cases, the normal simple columnar epithelium was nearly completely replaced by stratified squamous cells throughout the uterine horns (Figs. 1 to 3). Uterine squamous metaplasia was observed in mice exposed to DES in all of the age groups from 6 weeks to 20 months of age.

Even at 6 weeks of age, uterine squamous metaplasia was extensive, and the stratified squamous epithelium extended the full length of the uterine horns in 2 of the 3 mice (Figs. 1 to 3). The extent of squamous metaplasia varied from animal to animal. In one mouse, isolated foci of squamous cells were located among the normal columnar cells (Fig. 4). In another animal, the squamous cells totally replaced the luminal and glandular columnar epithelium. Squamous metaplasia was often accompanied by a cystic endometrium (Figs. 5 and 6). One of the 5 moxestrol uteri was cystic without associated squamous changes. Squamous metaplasia was not observed in moxestrol-treated mice (incidence, 0 of 5).

In addition to alterations in the cell types, the fine structure specializations found on the uterine luminal cell surfaces were also altered. In control mice, the uterine cells were covered only with microvilli. Although the size and number of these microvilli do change with the estrous cycle or hormone treatment (9, 10), one does not normally find a change in the type of cell surface specializations. By contrast, the metaplastic cell surfaces were not covered with microvilli but instead had microridges (Fig. 3); such structures are commonly found on vaginal cell surfaces. The uterine epithelium from the moxestrol animals was always composed of simple cuboidal cells and had flat apical cell surfaces and short stubby microvilli (Fig. 7).

There were intraluminal protrusions of metaplastic squamous cells in the uteri of DES-exposed females. Papillary growths were seen in the vagina of DES-treated mice (Figs. 8 and 9). These were covered with cornified epithelial cells and contained a stromal core. A pitted appearance of the vaginal epithelium was also observed with the scanning electron microscope, but it could only be correlated with irregularities in the vaginal surface by light microscopy.

In addition to vaginal papillary growths, the moxestrol mice had a number of cervical and lower uterine papillary growths of similar morphology (Fig. 10). Cervical protrusions were not observed in DES-exposed mice. However, cervical enlargement was common in treated animals. The cervix of the DES- and moxestrol-treated mice was consistently fibrotic and enlarged.

Instead of the periodic transverse folding characteristic of the control female mouse uterus, the regular pattern of the uterine surface was altered in all of the DES- and moxestrol-exposed mice. In some cases, folds were not present at all (Fig. 7). In others, the folds were disorganized and irregular (Fig. 11).

Vaginal structure was also disturbed in the treated mice. Prenatal exposure to DES led to an increased incidence of female hypospadias (5 of 14 in DES-treated mice; 0 of 18 in control) (Fig. 12) with the associated presence of urethral glands within the remaining part of urogenital sinus (Fig. 13).

Vaginal concretions were also common in the mice treated with DES (7 of 14 DES-exposed mice; 0 of 18 control mice). However, those concretions were not seen in the moxestrol-treated mice or control mice. SEM X-ray microanalysis demonstrated that these calculi contained significant amounts of magnesium and phosphorus. Calcium and potassium were detected in only low levels in these stones (Fig. 14). A correlation between stone formation and hypospadias could not be determined.

DISCUSSION

Prenatal exposure to DES has been shown to alter normal reproductive tract structure (3, 10, 20) and function (13, 14) in the CD-1 mouse in ways which are strikingly similar to observations reported for women exposed to transplacentally to DES (4, 5, 7, 16). However, complete description of many of these lesions has been greatly enhanced by correlating the SEM and light microscopic findings in these treated mice.

In control mice undergoing a normal estrous cycle (12) or in ovariectomized and hormone-treated mice (9), the uterus is composed of only simple epithelium with microvilli on the apical cell surfaces. However, in DES-treated mice, stratified squamous cells replaced all or part of the normal population of simple columnar cells. To investigate the origin of these atypical cell types, the uterine luminal surface of these animals was carefully studied with the scanning electron microscope. In many specimens, the atypical uterine squamous epithelium was continuous with the cervical squamous epithelium, and the squamocolumnar junction appeared to be displaced cranial to its normal location. However, quite often, isolated foci of squamous cells surrounded by normal-appearing uterine columnar cells were found anterior to those which were in contact with the cervicovaginal cells. This was noted even in very young (6 weeks) mice. Apparently, although the more distal uterine cells may have had a greater propensity for squamous transformation, the direct contact with squamous cells did not seem to be a prerequisite for squamous metaplastic transformation of the uterine epithelium. Also, there was no evidence of overgrowth of squamous cells covering a functional epithelium of columnar cells.

Other investigators (20) have described vaginal adenosis in prenatally treated mice and a caudal movement of the squamocolumnar junction ("cervical metaplasia") by which uterine epithelial glands and cells were found in the mouse cervix and vagina. Although the vaginal fornix was not observed in the current SEM study, in neither this study nor other investigations from this laboratory (14) was the prevalence of vaginal adenosis as extensive as described in other studies. This might be explained by the fact that in other studies mice were treated later in gestation (20) or neonatally (3, 15) which could expose the offspring to the hormones during the period of transformation of the Müllerian vagina (3). Adenosis, in humans (16) and mice (3, 15, 20), has been reported to be the consequence of arrested normal transformation of the Müllerian vagina which normally takes place within 2 weeks after birth in mice, leading to a persistence of columnar glandular epithelium in the vagina. Under the circumstances of this study, the hormone exposure may have been given during a period before the tissue was
sensitive to hormone treatment, and transformation was therefore unaffected.

Although DES caused uterine squamous metaplasia in female mice, moxestrol altered the uterine epithelium in a very different manner. The uteri of prenatally moxestrol-exposed mice were composed of simple epithelial cells which resembled the uterine epithelium of ovariectomized mice (9). Thus, even though DES and moxestrol have similar estrogenic potencies and relatively low AFP binding (18), the actual spectra of lesions observed in female mouse genital tract are not the same for both compounds. The differential toxicities of DES and moxestrol may be attributed to properties of the molecules other than those which are measured in standard estrogen assay systems, but at this time we cannot attribute the difference in toxicity to any particular biochemical property.

The papillary growths in the mouse genital tract observed after prenatal exposure to both DES and moxestrol are especially suited to observation by the scanning electron microscope. Walker (20) described a transverse ridge in a CD-1 mouse which may have been similar to the anomalies observed in the present study. These papillary growths may provide insights to the development of structural cervicovaginal abnormalities in humans such as vaginal ridges and cervical hoods or cockscomb cervices (16).

Perinatal estrogen exposure also altered the development of normal transverse folding of the uterus (11, 12). The moxestrol animals consistently lacked any surface folding. In general, these uteri resembled those of normal 21-day-old immature mice which have not yet developed folds (11). While a number of DES-treated mice also had this appearance, others simply showed an irregular folding pattern (Fig. 1). In both cases, perinatal estrogens inhibited the formation or altered the expression of the normal folding pattern present in adult mice at all stages of the estrous cycle (12). The inability of the mouse uterus to form its normal structure may be similar to the uteri of women exposed prenatally to DES which also have abnormalities in the shape of the lumen (4, 7). Considering both mouse and human findings, DES (and moxestrol) apparently interferes with the developing Müllerian duct system leading to structural abnormalities of the uterine lumen.

The association of perinatal hormone exposure to female hypospadias has been made a number of times (2, 6, 8, 14, 16, 19, 21). This appears to be the result of hormonal inhibition of the normal differentiation of the urovascular septum (8). Observation of the medial ventral vaginal wall shows the presence of urethral glands (Fig. 12). Vaginal concretions 3 to 5 mm in diameter were noted in a number of these animals. Similar concretions have been observed in animals exposed neonatally to estrogens (17). However, both the inability to demonstrate hypospadias in certain animals with stone formation and previous indications of paracervical stones (21) indicate that intrauterine urine flow may not be a prerequisite for vaginal calcification formation. Even the chemical similarity of these urinary stones to urinary stones does not prove that the vaginal stones have urinary origin. Tracer studies, such as those proposed by Warner et al. (21), may be necessary to definitively determine the origin of the stones.

REFERENCES


Fig. 1. Abnormal distribution of columnar (C) and squamous (S) cells in the uterine lumen. The squamocolumnar junction, which is usually distinct, has been obscured or displaced, and columnar and squamous cells are distributed throughout the lumen. (DES: 6 weeks of age; bar, 50 μm.)

Fig. 2. Light micrograph from a uterus containing both simple columnar (C) and stratified squamous epithelium (S) cells. The columnar and squamous cells form abrupt junctions with each other, without any indication that one cell type rests upon the other cell type. Paraffin sections were made from the SEM sample illustrated in Fig. 1. (DES: 6 weeks of age; bar, 50 μm.)

Fig. 3. Uterine epithelial cells of DES-exposed mice had either microvilli or microridges on the apical surface, as opposed to only microvilli in control females. These cell surface specialization were important for identifying areas which contained abnormal cell types. (DES: 6 weeks of age; bar, 10 μm.)

Fig. 4. An isolated area of abnormal uterine cells which was surrounded by otherwise normal uterine cells with microvilli (perimeter indicated by arrows). At higher powers of magnification, microridges were identified on the apical surfaces of cells in that area. (DES: 6 weeks of age; bar, 50 μm.)
Fig. 5. Large cyst (arrows) seen as a bulging area within the uterine lumen. The folding pattern of the normal mouse uterus was also absent. (DES; 20 months of age; bar, 1 mm.)

Fig. 6. The bulging area identified by scanning electron microscope in Fig. 5 was subsequently processed for light microscopy and demonstrated to be a uterine cyst. (DES; 20 months of age; bar, 100 µm.)
Fig. 7. The normal transverse uterine folds were completely absent in moxestrol-treated mice. There was no evidence of squamous metaplasia in the moxestrol group. (Moxestrol; 15 months of age; bar, 100 μm.)

Fig. 8. A vaginal polyp, approximately 1 x 2 mm, was identified in this DES-treated mouse. The surface of the polyp was irregular. (DES; 20 months of age; bar, 1 mm.)

Fig. 9. Light micrograph of the polyp identified in Fig. 8 demonstrates a single connective tissue stalk (arrow) near the center of the polyp. (DES; 20 months of age; bar, 250 μm.)

Fig. 10. A cervical papillary growth was observed in the lumen of a moxestrol-exposed mouse. Vaginal papillary growths were also observed in these mice. (Moxestrol; 15 months of age; bar, 500 μm.)

Fig. 11. Abnormal and disorganized folding pattern is present in the uterine lumen. (DES; 6 weeks of age; bar, 500 μm.)
Fig. 12. Female hypospadias was identified in the lower ventral wall of the vagina or the remaining portion of the urogenital sinus. (DES; 3 months of age, bar, 1 mm.)

Fig. 13. The urethral glands (G) area can be demonstrated when the area in Fig. 12 was observed at a higher power of magnification. The glands were present as a result of incomplete closure of the vaginal septum. (DES; 3 months of age; bar, 100 μm.)

Fig. 14. Energy-dispersive X-ray microanalysis indicated that the vaginal concretions were composed of magnesium (Mg) and phosphorus (P). Trace amounts of calcium (Ca) and potassium (K) may be present; aluminum (Al), silicon (Si), iron (Fe), and copper (Cu) have all been identified as background emissions and are not attributable to the sample or its preparation.
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