DIETHYLSTILBESTROL (DES) INDUCES MORPHOLOGICAL ALTERATIONS IN OFFSPRING

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Introduction

The development of the male and female reproductive tracts requires hormonal regulation. Gonadal hormones are responsible for the further differentiation of the reproductive ducts, Wolffian and Müllerian ducts. Moreover, throughout the growth period to adult life, the female reproductive tract is always under the control of hormones, e.g. estrogen and progesterone [1]. Estrogen stimulates cell proliferation, induction of specific proteins and cell differentiation in many organs including the ovary, oviduct and uterus. The actions of estrogen are thought to be mediated by intracellular estrogen receptors (ERs) in target cells [2]. In the middle of the 20th century, diethylstilbestrol (DES), the synthetic estrogen, was frequently prescribed for support of high-risk pregnancies [3]. The negative aspects of this therapy began to emerge in the 1970s. In young women who had been exposed to DES *in utero*, vaginal clear cell adenocarcinoma, a rare type of cancer, was frequently reported to occur very early [4]. Men who were born from DES-controlled pregnancies also have an increased incidence of reproductive tract abnormalities, including epididymal cysts, cryptorchidism, and the presence of Müllerian duct remnants [5, 6]. Thus, DES became the first and one of the most potent members of the expanding list of chemicals known as 'endocrine disruptors' [7]. There is now a great deal of scientific and public interest in the concept that inadvertent exposure to xenoestrogens may negatively affect the reproduction and general health of both humans and animals [8].

To study the postnatal effects of DES on the developing genital tract in an animal model, McLachlan *et al.* [9] injected DES daily into pregnant mice during the phase of growth and differentiation of the fetal reproductive tracts and found that the affected animals had gonadal changes that included intra-abdominal and/or fibrotic testes. In the male hamster, a single injection of DES on the day of birth was shown to be much more potent than that of estradiol-17β in inducing multiple

lesions in adulthood and to induce permanent alterations of androgen responsiveness in the adult male reproductive tract [10]. The majority of male rats treated neonatally with DES exhibited reduction of testis weight with many Sertoli cell-only tubules and very low daily sperm production [11]. Thus, DES was indicated to modulate Sertoli cell development directly. Clinical and experimental animal studies have demonstrated that perinatal DES exposure results in teratogenesis and neoplasia [12,13]. In male mice, 'grandmothers' of which were exposed to DES, fertility appeared to be unaltered, although increased susceptibility to tumors was transmitted to subsequent generations [14]. Reduced fertility was observed in female mice exposed to DES *in utero* but was not transmitted to their offspring [15]. Their susceptibility to tumor formation was apparently transmitted to subsequent generations [15, 16]. These phenomena induced by DES were also thought to be mediated by ER. The mechanisms by which estrogens produce these effects are, however, not clearly understood.

In the present study, we examined morphological abnormalities of female reproductive organs, induced by prenatal single administration of DES to determine the trans-placental effects on female offspring. DES was administered to pregnant mice at 18.5 dpc when these organs are through to have finished their embryonic development, and the effects on the immature offspring were examined.

Materials and Methods

Animals and Treatments

Mature female ICR mice, 12-18 weeks old, were mated with adult male mice of the same strain. They were kept at 23 ± 2°C under a 14 hr light (5:00-19:00) and 10 hr darkness cycle. All animal experiments conformed to the Guidelines for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH #86-23; revised 1985). At noon of the day after mating, mice with a vaginal plug were considered to be at 0.5 days post coitus (dpc). Pregnant mice at 18.5 dpc received subcutaneous injection of DES (200 μg/kg body weight) dissolved in corn oil or corn oil alone (4 ml/kg; vehicle control). Under ether anesthesia, the 16-day-old female offspring from DES- and vehicle-treated pregnant mice were sacrificed, and then ovaries, oviducts and uteri were removed. Oviducts were disentangled by dissecting the mesosalpinx under a surgical microscope, and then the tissues from the ampullae were collected for further processing.

Cell Morphology

The tissues from ovaries, oviduct ampullae and uteri were cut into small pieces and each

sample was fixed in 2.5% glutaraldehyde in 0.1M phosphate buffered saline (PBS; pH 7.4) at 4°C for 2.5 hr, post-fixed in 1% osmium tetroxide in PBS at 4°C for 2 hr, dehydrated through a graded ethanol series, and then embedded in epoxy resin.

Semi-thin sections (0.8 μm) from epoxy-embedded specimens were cut and placed on glass slides, then stained with Giemsa and basic fuchsine and examined histopathologically by light microscopy. Nuclei were stained with Giemsa. The materials in the secretory granules were stained with basic fuchsine [17, 18]. Then, ultra-thin sections (approximately 70 nm in thickness) were cut from the same epoxy-embedded specimens, mounted on copper grids, and double stained with uranyl acetate and lead citrate. They were examined using a transmission electron microscope at 75 kV for observation of changes in ultrastructure.

Results and Discussion

In this study, we observed morphological alterations in the ovary, oviduct and uterus induced by exposure to DES *in utero*.

The walls of the uterus and oviduct are composed of three layers: epithelium, stroma, and muscle. The uterus of the DES-treated 16-day-old offspring appeared contracted compared with those of the vehicle control group. The uterine glands and the uterine epithelium of the DES-treated offspring were poorly developed. Their stroma was thinner than that of controls (Figure). The structure of the muscular layers was similar in both groups. In mice, ER appeared in the stroma cells of the developing reproductive tracts, the Müllerian ducts, from day 13 of pregnancy [19]. In the uterine epithelium, however, ER was detected after birth [20, 21]. These observations indicate that administration of DES at 18.5 dpc down-regulates ER expression in the stroma and that stroma cells became atrophied. Also, this DES exposure would reduce or prevent expression of ER in epithelial cells.

The oviduct epithelium of the DES-treated offspring showed hyperplasia. Although the epithelium of the vehicle-treated offspring was simple columnar epithelium, that of the DES-treated offspring was stratified or multilayered. Some DES-treated epithelial cells were heavily stained with basic fuchsine. Ultrastructural examination revealed that these cells contained secretory granules. In epithelial cells of the control group, basic fuchsine staining was not observed and no such granules were seen by electron microscopy. In mice, oviduct epithelial cells with mucous secretory materials were differentiated at 23 days after birth [22]. These observations indicated that DES treatment at 18.5 dpc promotes secretory activity in the oviduct after birth.

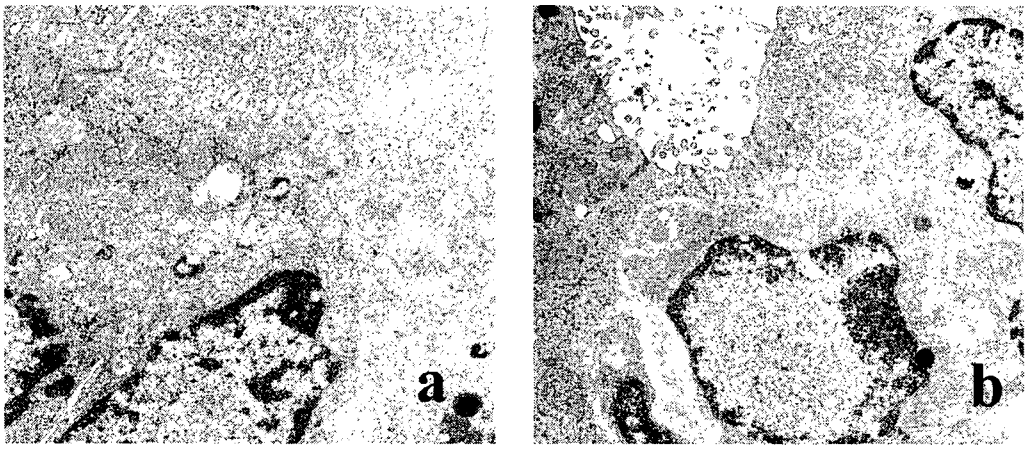

Figure. Electron micrographs of uteri from the vehicle-treated (a) and DES-treated (b) offspring. The uteri of the DES-treated offspring appeared contracted compared with those of vehicle controls. The lamina propria mucosae of the DES-treated offspring were thinner than those of controls.

No marked structural differences were observed, in the ovaries, between the DES-treated offspring and controls. Ovaries from both groups had primary and secondly follicles.

In conclusion, DES treatment at 18.5 dpc induced atrophic alterations of the uterus and hypertrophic alterations of the oviduct. The present results indicated that DES has trans-placental effects on the reproductive tracts of 16-day-old female offspring. Further studies are needed to determine how these alterations affect subsequent development as the offspring grow.

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