Effects of tamoxifen administration in rat vaginas: an ultrastructural and light microscopy study

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Summary

Background: Tamoxifen (TAM) inhibits the initiation of carcinogen induced rat mammary tumours and is administrered for extended periods after the initiation of carcinogenesis. It is also a widely used treatment for breast and gynaecological cancer.

Objective: In this study, we aimed to investigate tamoxifen (TAM) administration on vagen development in rats.

Material & Methods: Twenty sexually mature and pregnant Wistar albino rats were chosen as the animal model. They were divided into two groups. Group I: Control group, Group II: Tamoxifen applied (between gestational day 16 and 21 days); 100 μg tamoxifen citrate (TAM) in 0.05 ml saline subcutaneously per day/animal.

After birth, all female rats were sacrificed on the 60th day and were taken vaginal tissue. Transmission electron microscopy and light microscopy have been used to study changes to the vaginal epithelium.

Results: A statistically significant reduction of birth body weight was noted in the experimental group of rats when compared to the control group ($P<0.05$).

We saw increase in the thickness of the epithelium layer and irregularity and disappearance of microscopic papilla, cytoplasmic vacuolation in cells of the surface layer, thin and irregular basal membrane, lateral junction of cells were destroyed in the TAM treated groups.

In conclusion, neonatal tamoxifen administration affects vagina epithelium and lead to decreasing birth body weight and vaginal adenosis.

Key words: Tamoxifen; Vagina; Ultrastructural changes; Rat; Adenosis-like lesion.

Introduction

Tamoxifen is non-steroidal derivative of triphenyl-ethylene and is capable of interfering with the actions of estrogens. Therefore, the antiestrogen tamoxifen has now been widely used for the aim of endocrine therapy of oestrogen receptor positive human breast cancer and gynaecological cancer [1].

Tamoxifen (TAM) inhibits the initiation of carcinogen-induced rat mammary tumors and the administration of TAM for extended periods after the initiation of carcinogenesis prevents the development of tumours for as long as the drug is administered. However me recent observations in mouse models have provided additional insights into the preventive actions of TAM on mammary carcinogenesis. TAM is classified as an oestrogen in short-term tests in the mouse, however, long-term treatment is known is to cause the uterus and vagina to become refractory to exogenous oestrogen administration [2, 3].

Tamoxifen (TAM) has been thought to be antiestrogen because of the competition with oestrogen in binding oestrogen receptors. However, this compound has different pharmacological actions on various organs in different species of animals. TAM acts as a complete oestrogen antagonist to the chicken oviduct [4].

TAM is a nonsteroidal agent, which has demonstrated potent antiestrogenic properties in animal test systems. The antiestrogenic effects may be related to its ability to compete with oestrogen for binding sites in target tissues such as breast. Tamoxifen inhibits the induction of rat mammary carcinoma induced by dimethylbezanthracene (DMBA) and causes the regression of already established DMBA-induced tumours. In this rat model, tamoxifen appears to exert its antitumor effects by binding the oestrogen receptors. It is also recognized that tamoxifen display estrogenic like effects on several sites including the endometrium, bone and lipids [5].

It is apart of the dogma of steroid hormone action that it binds specially to its receptors intracellular and this ligand-receptor complex alters the transcriptional activity of the hormone responsive genes. Antiestrogens inhibit the action of oestrogen on its target organs by binding to the oestrogen receptor; therefore, antiestrogens are commonly used for the therapy of oestrogen receptor-positive human breast cancer and for the induction of ovulation [6].

The purpose of the present study was to investigate ultrastructural changes in the adenosis like effects of neonatal tamoxifen administration on rat vaginal epithelia.

Materials and Methods

Twenty pregnant female Wistar albino rats, 225-250 g in weight, were obtained from the Department of Medical Science Application and Research Centre of Dicle University (DÜSAM). They were housed in invidual cages in temperature-

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controlled environment (22°C) with a 12: 12 h light-dark cycle. All rats were fed with standard pellet food and ad libitum tap water, which were performed according to the Declaration of Helsinki with the permission of the Governmental Animal protection committee. The rats were divided into following groups (n=10):

Group I: Control group. Control rats were injected with equivalent amount (0.05ml.) only saline (between gestational days 16 and 21).

Group II: Tamoxifen applied (between gestational days 16 and 21 days); 100 µg Tamoxifen citrate (TAM) in 0.05 ml saline subcutaneously day/per animal.

Statistical validation (birth body weight) of significant Tamoxifen effects was accomplished using the Two Sample t-Test. All parameters were compared between the tamoxifen treated group and control group [7].

After birth, all female rats were sacrified on the 60th day and were taken vagina tissue. At sacrifice the rats were injected intraperitoneally with a lethal dose, 0.4 ml Nembutal (sodium pentobarbitone) [8]. Vagina were removed, pinned out on dental wax to their in vivo lenght and placed immediately in a 2.5% phosthate-buffered glutaraldehyde solution at pH 7.4 for 4 hours. Tissue pieces were chosen at random, then split along the long axis into 2 or 3 pieces and placed in fresh fixative for a further 40 min, postfixation was performed in 2% osmium tetroxide, and washed in three changes of phosphate buffer, pH 7.4 dehydrated in graded alcohols.

Sample tissues were embedded Araldyte-Cy 212. Semithin sections were stained with toludine blue. Thin sections were stained with uranyl acetate & lead citrate.

The specimens were examined and photographed using transmission electron microscope.

Results

A. Birth body weight

Mean birth body weights are presented in Figure 1. A statistically significant reduction of birth body weight was noted in rats of experimental group of rats when compared to the control group ($P<0.05$).

B. Histological Changes

Light Microscopicallyy Results

We observed no pathology in the vaginal epithelium of control group. The lumen of vagina was lined by a thick stratified squamous non-keratized epithelium. No columlar cells were present in any of same regions of the control rats. The lamina propria of mucosa is composed of a loose fibro elastic connective tissue containing a rich vascular supply in its deeper regions.

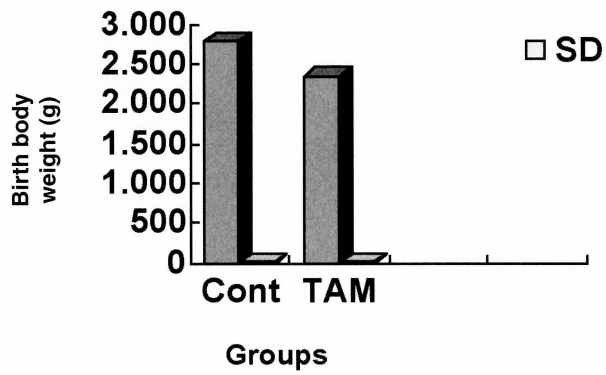


Figure 1. — Birth body weight of control and tamoxifen administration groups.

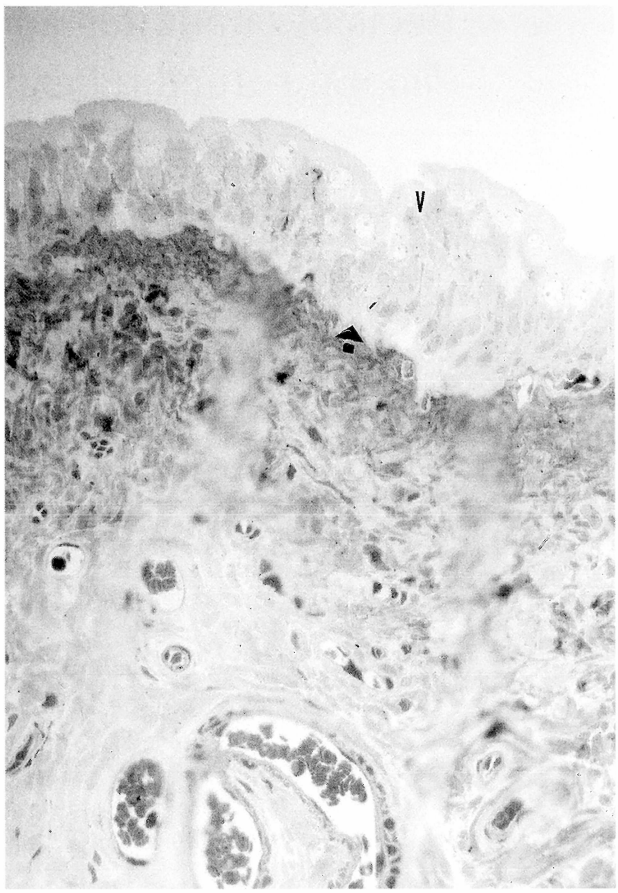


Figure 2. — Vaginal epithelium of a 60-day-old control rat given prenatal injection of tamoxifen. Increased in the thickness of the epithelium layer, irregularity and disappearance of microscopic papilla (arrows), cytoplasmic vacuolation (V) can be seen (Stain: Toludine Blue, original magnification x 200).

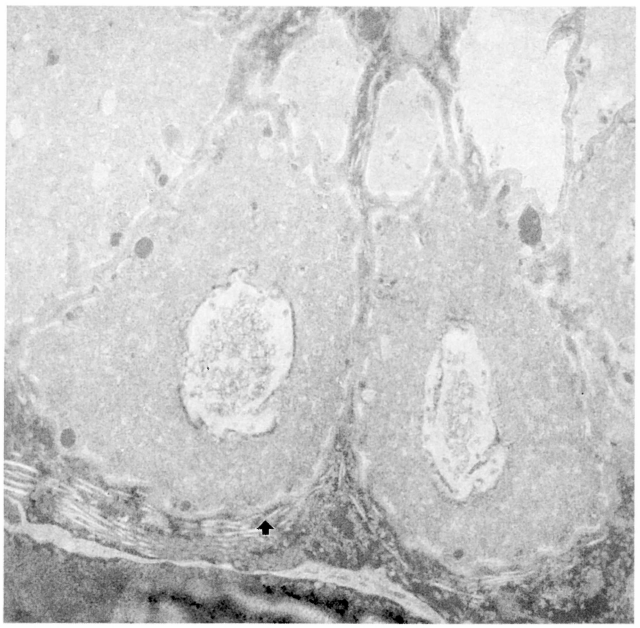
We saw increase in the thickness of the epithelium layer and irregularity and disappearance of microscopic papilla in the TAM treated group. There was cytoplasmic vacuolation present in cells of the surface layer. The columlar epithelium also formed down growth that resembled glandular structure. These lesions may be analogues to the adenosis. The nuclear to cytoplasmic ratio appeared low and nucleoli were prominent. Mitotic figures were found in basal and intermediate layers (Figure 2).

Electron Microscopy Results

We clearly observed regular and normal structure basal membrane in control group.

In the vaginal epithelium of the TAM treated group had a thin and irregular basal membrane, abundant lipid drops (Figure 3), and lateral junction of cells was destroyed (Figure 4).

A nucleus with heterochromatin was very distinct and some tonofibril distinguishable and epithelium cells cytoplasm have a great deal of lipid granule in experimental group (Figure 5).



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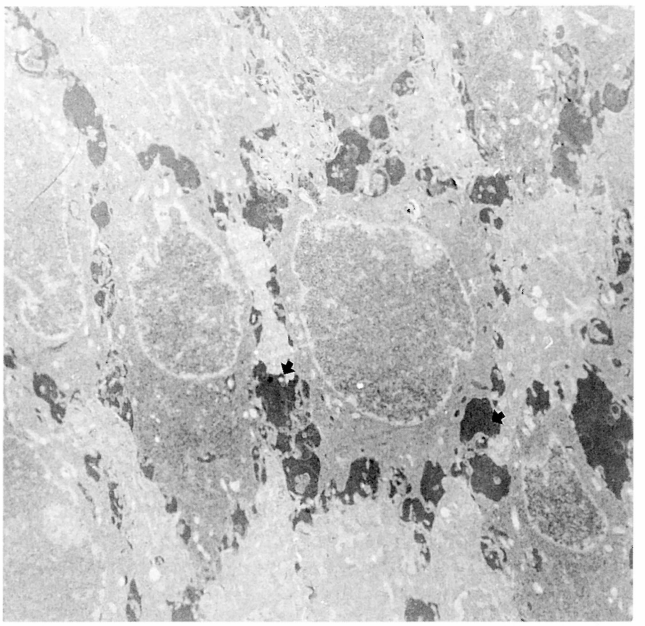

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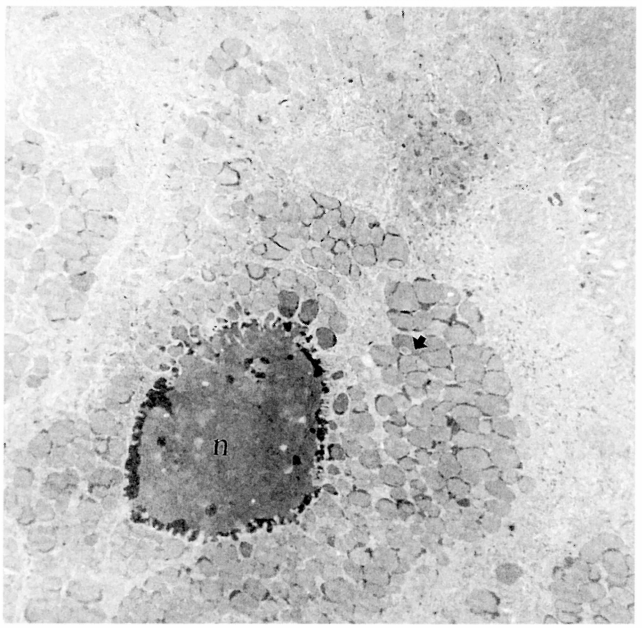

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Figure 3. — Vaginal epithelium of a 60-day-old control rat given neonatal injection of tamoxifen. Thin-irregular basal membrane (arrows) and abundant lipid drops can be seen (Stain: Uranyl acetate & Lead citrate, x 7,000).
Figure 4. — Vaginal epithelium of a 60-day-old control rat given neonatal injection of tamoxifen. Lateral junction of cells was destroyed (arrows) (Stain: Uranyl acetate & Lead citrate, x 7,000).
Figure 5. — Vaginal epithelium of a 60-day-old control rat given neonatal injections of tamoxifen. Cytoplasm has a great deal of lipid granules (arrows) and nuclei with heterochromatin (n) can be seen (Stain: Uranyl acetate & Lead citrate, x 12,000).

Discussion

It is well known that vaginal tissues in different animal species, including human vaginal tissue, respond to the action of endogenous or exogenous estrogens. Oestrogen receptors (ER) have been reported in vaginal tissues of various species, e.g. rabbit, mouse and human. However no information is available at present on the existence of ER and progesterone receptors (PR) in the vagina during fatal life [1].

The biological effects of tamoxifen (TAM), progesterone (P), or a combination of TAM + P were investigated in the uterus and vagina of newborn guinea pigs after short (2 days) and long (12 days) treatments. Tamoxifen in both tissues (particularly in the vagina) stimulated the progesterone receptor very significantly. Progesterone blocked the number of specific binding sites of progesterone and the stimulatory effect provoked by tamoxifen [3].

Exogenous estrogens have teratogenic and carcinogenic effects on the devoloping female genital tract of both laboratory animals and humans. Steroidal and nonsteroidal estrogens suchas diethylstilbestrol (DES) elitic adverse effects. Certain triphenylethylene compounds, suchas clomiphene, tamoxifen, and nafoxidine arehormonally active and may exhibit antiestrogenic activity.Both clomiphene and tamoxifen are currently administered therapeutically to women [9].

Tamoxifen was administered orally to neonatal rats on days 2-5 after birth and the subsequent effects on the uterus were characterized, morphometrically, over the

following 12 months. Tamoxifen inhibited development of the uterus and glands in the endometrium, indicating a classical estrogen-antagonist action. Between 24 and 35 months after tamoxifen treatment there was a significant increase in the incidence (26%) of uterine adenocarcinomas and a 9% incidence of squamous cell carcinomas of the vagina/cervix in the absence of any oestrogen agonist effect in the uterus [10].

Recent studies have revealed that neonatal TAM treatments result in adesion like-lesions (ADL) and uterine hypoplasia in rats and mice [11, 12, 13].

The effects of tamoxifen (TAM) on uterine carcinogenesis were investigated in female Donryu rats. The uteri in TAM-treated group showed severe atrophy and the incidences of uterine proliferative lesions were limited to a few endometrial hyperplasias in the half TAM group. Most of the vaginas in TAM-treated group showed mucification, while cornification was common in the vaginal epithelium of controls [14].

A statistically significant reduced body weight was noted in rats of the experimental group when compared to those in the control group. Therefore, these results mentioned in previous studies (Figure 1).

We determined in adenosis-like lession of epitheliyal layer TAM-treated rats. This result also was compatible with Taguchi and Nishizuka [11].

The findings in this study of irregularity and disappearance microscopic papilla of vaginal epithelium were not mentioned in previous studies (Figure 2.).

CGS 16949A, an aromatase inhibitor, was administered orally to female rats at doses of 1 and 10 mg/kg/day alone and in combination with tamoxifen (0.5 or 5 mg/kg/day) or 5-fluorouracil (20 mg/kg/day) for 14 days. CGS 16949A and tamoxifen combination: Gross pathological and histopathological changes ascribed to the antiestrogenic action of CGS 16949A, such as increased ovarian weight, decreased uterine weight, cystic follicles and atrophied uterus and vaginal epithelium, were alleviated by combination treatment, and were comparable in severity to those caused by tamoxifen alone [15].

We observed in the vaginal epithelium of the TAM treated group had a thin and irregular basal membrane, abundant lipid drops (Figure 3), and lateral junction of cells was destroyed (Figure 4).

Although the daily dose of tamoxifen in this study was about 10 times that for humans, the administration period was only 5 days. On the other hand, for human therapy, especially for patienents with advanced breast cancer, tamoxifen has been used for many months. Long-term or repeated exposure to tamoxifen may affect some normal estrogen receptor-positive tissues. The therapeutic use of tamoxifen in anovulatory women must be applied especially carefully because of the possible fetal administration.

As a result, maternal tamoxifen treatments caused reduce the development of body and vaginal adenosis in rats postpartum.

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