Morphologic and morphometric study of the breast parenchyma of rats in persistent estrus treated with tamoxifen and conjugated estrogens

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Summary

Purpose: To evaluate the morphological and morphometric alterations produced by tamoxifen and conjugated estrogens in the mammary epithelium of rats in persistent estrus.

Methods: 33 adult female rats with persistent estrus induced by 1.25 mg testosterone propionate were divided at random into three groups: group I (n=12), receiving only water and used as a control; group II (n=10), treated with 500 μg tamoxifen daily; group III (n=11), treated with 30 μg conjugated estrogens daily. The first abdominal-inguinal pair of breasts was extirpated and processed for morphological and morphometric study. Data were analyzed statistically by the Kruskal-Wallis rank analysis of variance ($p<0.05$).

Results: The morphological study revealed signs of epithelial atrophy and the morphometric study showed a significant reduction in mean number of ducts and alveoli in groups II (10.1 and 1.9, respectively) and III (11.1 and 3.5, respectively) compared to the control group I (25.0 and 6.6, respectively). There was no significant difference between groups II and III.

Conclusions: The present results indicate that, at the doses and during the time of treatment used, both tamoxifen and conjugated estrogens induced atrophy of the mammary epithelium of rats in persistent estrus.

Key words: Normal breast; Tamoxifen; Conjugated estrogens; Rats.

Introduction

Many controversies exist about the effects of conjugated estrogens and tamoxifen on the normal breast. The confirmation of an antiestrogenic action of these drugs could be useful for the reduction of the proliferative activity of the breast of patients submitted to hormonal replacement treatment.

Conjugated estrogens represent a complex combination of sulfate steroids extracted from the urine of pregnant mares [1]. Despite their extensive clinical use for more than 50 years, the literature lacks studies on theirs effects on the normal human breast or on the breast of laboratory animals. Some epidemiological studies have detected no increased risk of breast cancer when conjugated estrogens were used at the dose of 0.625 mg/day for long periods of time [2], whereas others have even shown a reduction of this risk [3]. This leads us to question whether conjugated estrogens may have a different action compared to estradiol or if they may have an action similar to that of tamoxifen.

Tamoxifen can have a partial or total estrogenic effect or an antiestrogenic effect depending on the target tissue and species under study [4, 5]. The drug has proved to be efficient in inhibiting the development of mammary tumors normally induced by carcinogens such as dimethylbenzanthracene in female rats [6]. It also inhibited the effects of estradiol benzoate on mammary duct growth in castrated adult female rats [7]. Thus, we may assume that mammary epithelium under continuous estrogen stimulation, such as that of rats in persistent estrus, could be useful for the study of the effects of tamoxifen and of some similar effects of conjugated estrogens.

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However, little is known about the effects of tamoxifen and conjugated estrogens on normal breast epithelium submitted to constant estrogen stimulation. The objective of the present study was to compare the morphological and morphometric aspects of the mammary epithelium of rats in persistent estrus treated with tamoxifen and conjugated estrogens.

Material and Methods

The study was conducted on 33 virgin female rats (Rattus norvegicus albinus) in persistent estrus obtained by subcutaneous injection of 1.25 mg testosterone propionate on the second day of life [8]. Persistent estrus was confirmed by hormonal colpocytology performed for two weeks starting from the 70th day of life. The animals were then divided at random into three groups: group I, control (n=12), receiving only 1 ml distilled water daily for 30 days; group II (n=10), receiving 500 μg tamoxifen daily diluted in distilled water and administered by gavage for 30 days; group III (n=11), receiving 30 μg conjugated estrogens daily also administered by gavage for 30 days.

On the 31st day the animals were sacrificed and the first abdominal-inguinal breast pair was removed, fixed in Bouin's for 72 h, processed and stained with hematoxylin-eosin for light microscopy. The morphological study characterized the ductal -alveolar pattern and the secretory component. For morphometric analysis concerning counts of alveoli and ducts we used the stereology principles of Weibel and Gomez [9], which recommend counts of only the grid points that intercept the structure under study. A KPL 10X Zeiss ocular micrometer with an integration grid (Integrationplate I) containing 25 points, coupled to a light microscope with 100 X magnification, was used. Ten fields per slide were studied using horizontal scanning, for a total of 250 points. Data were analyzed statistically by Kruskal-Wallis rank analysis of variance ($p<0.05$).

Results

Morphological study of the control group revealed lobules exhibiting a high concentration of alveoli lined with cubic cells, dilated and containing eosinophilic secretion. The ducts were present at higher concentrations than alveoli and, like the latter, were dilated and contained eosinophilic material (Figure 1). Groups II (tamoxifen) and III (conjugated estrogens) exhibited a lower concentration of poorly developed ducts and alveoli with scarce or absent secretion (Figures 2 and 3). Analysis of the morphometric data showed a significant reduction in mean number of ducts in groups II and III, 10.1 and 11.1, respectively, compared to the control group, 25.0 (Table 1). A significant reduction in mean number of alveoli was also observed in groups II and III, 1.9 and 3.5, respectively, compared to the control, 6.6 (Table 2). There was no significant difference between groups II and III.

Table 1. — *Mean number of mammary ducts in groups I (control), II (tamoxifen) and III (conjugated estrogens) in 10 fields, observed at 100 X magnification.*

	Groups		
	I	II	III
	23	12	13
	24	12	11
	25	8	12
	33	11	12
	37	8	8
	25	12	8
	27	9	12
	22	11	11
	22	7	12
	23	11	11
	17		12
	22		
Mean	25.0	10.1	11.1

Kruskal-Wallis rank analysis of variance (IxIIxIII). Calculated H = 22.89; critical H (0.05) = 5.99; Multiple comparison test (I>II and III).

Table 2. — *Mean number of mammary alveoli in groups I (control), II (tamoxifen) and III (conjugated estrogens) in 10 fields, at 100 X magnification.*

	Groups		
	I	II	III
	6	1	5
	10	3	2
	3	1	4
	8	3	5
	7	2	2
	10	3	2
	8	2	3
	6	1	2
	4	2	6
	4	1	4
	6		
	7		
Mean	6.6	1.9	3.5

Kruskal-Wallis rank analysis of variance (IxIIxIII). Calculated H = 19.92; critical H (0.05) = 5.99; Multiple comparison test (I>II and III).

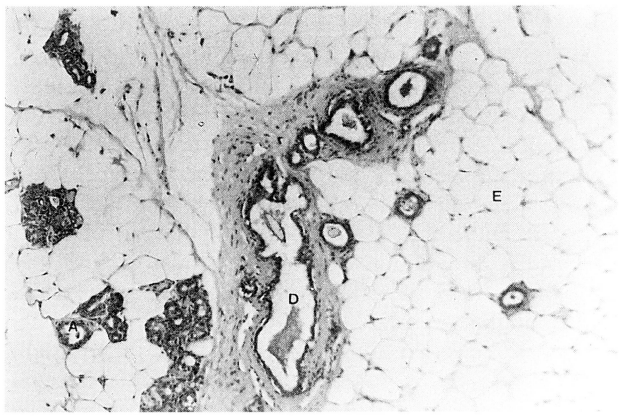

Figure 1. — Photomicrograph of a histological section of the breast from a rat in persistent estrus (control). Note the presence of ducts (D) and alveoli (A) dilated and containing secretion and surrounded by abundant stroma. H-E staining (140X).

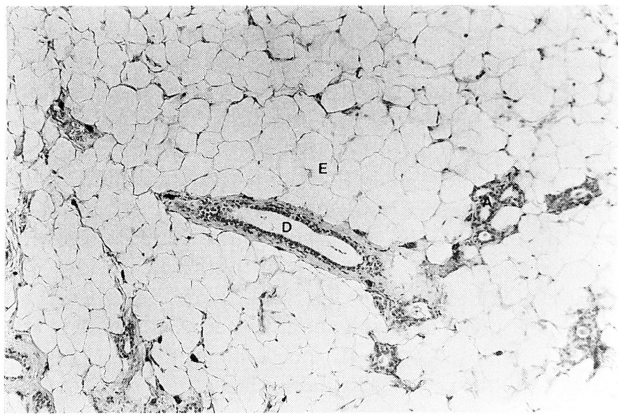

Figure 2. — Photomicrograph of a histological section of the breast from a rat in persistent estrus treated with tamoxifen. Note the presence of poorly developed ducts (D) and alveoli (A) with scarce secretion and surrounded by abundant stroma. H-E staining (140X).

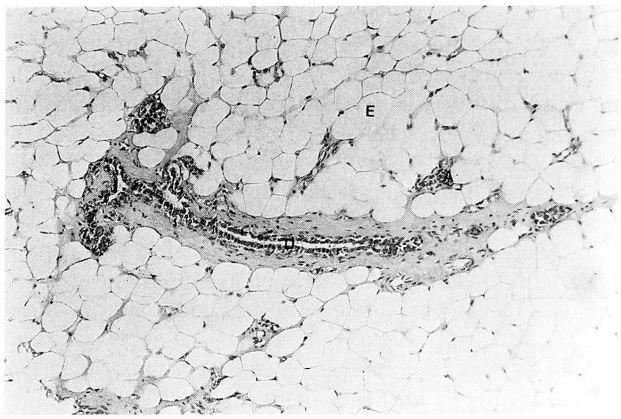

Figure 3. — Photomicrograph of a histological section of the breast from a rat in persistent estrus treated with conjugated estrogens. Observe the presence of barely developed ducts (D) and alveoli (A) with no secretion and surrounded by abundant stroma. H-E staining (140X).

Discussion

Hormonal replacement treatment is a controversial topic because of the possible increased risk of breast cancer, especially in high-risk patients. There is also the possibility of chemoprevention of breast cancer with tamoxifen in high-risk patients, but no consensus has been reached about the relationship between the use of tamoxifen and conjugated estrogens and the risk of breast cancer [10]. In addition, because of ethical reasons it is difficult to study the effects of these drugs on normal human mammary epithelium.

In the present investigation we used rats in persistent estrus in order to study the effects of tamoxifen and conjugated estrogens on the breast of these animals. The drugs were diluted with distilled water and administered orally as commonly done for women. The doses used in the present study may seem high to some authors or low to others, since it is difficult to establish a dose equivalence for the various species, especially in terms of drug absorption and metabolism.

De Gregorio *et al.* [11], in a study on rats concerning routes of administration and dose equivalence, postulated that 1,000 μg tamoxifen per day by the subcutaneous route would result in levels similar to those for women taking 20 mg of the drug per day, i.e., double the dose used in the present study (500 μg) by the oral route. With respect to conjugated estrogens, Orimo *et al.* [12] used 25 and 100 μg a day by the oral route in order to prevent induced osteoporosis in rats and obtained the desired effect at the dose of 100 μg. At a lower dose than this, i.e., 30 μg a day, although about five-fold higher than the equivalent dose commonly used in hormonal replacement, we observed effects on the breast of rats in persistent estrus.

The mammary gland of rats in persistent estrus (control) exhibited a high concentration of ducts and alveoli which were dilated and contained secretions. These findings may have been due to the continuous influx of estrogens and to the higher release of prolactin, and agree with those reported by Jacobson and Norgren [13].

The mammary atrophy produced by tamoxifen may be due to its antiestrogenic action by inhibition in the estrogen receptor molecule of activation function 2 of gene transcription, which is predominant in the breast [14]. Another hypothesis is the down-regulation by tamoxifen of a gene such as CD36, involved in angiogenesis, which has been demonstrated both in human normal and neoplastic mammary tissue [15]. In contrast, the regressive action of the conjugated estrogens is intriguing, since this is proliferated epithelium that receives an increased estrogen stimulus. One of the hypotheses that could be raised to explain the phenomenon could be their complex constitution since conjugated estrogens consist of more than ten sulfate steroids and one of them (equilenin or 17-hydroxyequilenin) can block the effects of estradiol on the mammary epithelium in a manner similar to tamoxifen [3].

Thus, the present study shows that both tamoxifen and conjugated estrogens at the doses tested produced atrophy of the mammary epithelium of rats in persistent estrus. However, we do not know whether these results can be extrapolated to the human species. Additional studies are required to test this possibility.

Conclusion

The most important event observed in the present study was the atrophy produced both by tamoxifen and by conjugated estrogens in the mammary epithelium of rats in persistent estrus, a biological model involving continuous estrogen stimulation. Further studies are needed to better determine the effects of these drugs on normal breasts in view of the possible use of conjugated estrogens alone or in combination with tamoxifen by patients at high risk for breast cancer who require hormonal replacement treatment.

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