A morphologic and morphometric study of the vesical mucosa and urethra of castrated female rats following estrogen and/or progestogen replacement

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Summary

Purpose: The aim of this study was to analyze the morphologic and morphometric changes in the urethra and vesical mucosa following hormonal replacement

Methods: We analyzed the changes in the urethra and vesical mucosa of 35 castrated adult female rats that had been subjected to estrogen and/or progestogen replacement.

Results: Estrogen replacement, whether or not accompanied with progestogen replacement, provoked metaplasia, hyperplasia and an increased occurrence of stratified epithelia. In the proximal urethra hypoestrogenism caused a higher occurrence of pseudo-stratified and transition epithelia, whereas in the urethral-vesical junction it caused a higher frequency of pseudo-stratified epithelia. The thickness of the epithelium increased following estrogen replacement whereas only a trend towards an increase of the propria lamina thickness was identified. Nuclear volume was only altered in the bladder epithelium.

Conclusion: Estrogen replacement acted both morphometrically and morphologically on the lower urinary tract.

Key words: Urinary tract; Estrogens.

Introduction

The drop in serum levels of estrogen which occurs in menopause causes metabolic and atrophic alterations in many organs with urinary complaints, osteoporosis, cardiovascular diseases and atrophy of the genital organs generally appearing later [1].

Postmenopause urogenital atrophy disorder is characterized by a dry vagina, dyspareunia, dysuria, urinary urgency, pollakiuria and urinary incontinence.

Many researchers have demonstrated the existence of estrogen receptors in the urogenital tract. Iosif *et al.* [2] detected a higher concentration of estrogen receptors in the urethra in comparison to the trigone and bladder while Lindskog *et al.* [3] identified receptors in the vagina and in the urethra, but, in smaller quantities than in the uterus. Wilson *et al.* [4] researched estradiol and progesterone receptors in biopsy fragments of human bladder, trigone and urethra and found a higher concentration of receptors in the distal urethra, in comparison to the trigone and bladder. Batra and Iosif [5] concluded that while the urethra and the bladder are sensitive to estrogen the number of receptors is greater in the uterus and vagina.

Collagen tissue, the main constituent of the support ligaments of the pelvic organs, changes markedly with hypoestrogenism, but improves with hormone replacement [6, 7].

Alterations in the urethral mucosa in postmenopause can cause pollakiuria, urinary urgency and urge-incontinence [8], as well as a decrease in the sealing effect of the mucosa [9] and a drop in the urethral closing pressure [10-13].

It is known that, like vaginal mucosa, urethral mucosa is affected by estrogen. Some researchers have reported alterations following estrogen replacement, such as the transformation of transition epithelia into squamous epithelia in the urethra and vesical trigone [14, 15].

Estrogen administration provokes squamous metaplasia in areas derived from the urogenital sinus [16], as well as a thickening and proliferation of the mucosa in the whole inferior urinary tract [17-19].

Due to the importance of urethral mucosa in urinary incontinence [20, 21], we evaluated the morphologic and morphometric changes in the vesical and urethral mucosa of castrated adult female rats following both separate and combined administration of estrogen and progestogen.

Material and Methods

Thirty-five virgin rats (Rattus Norvegius albinus, Rodentia, Mammalia), weighing 170 to 330 g were used. Female rats were chosen for their small size and the fact that they are easily managed in our animal facilities. The animals were maintained in a temperature controlled environment with unrestricted access to food and water.

At the start of the experiment the animals were submitted to a two-side oophorectomy. After an interval of 28 days, they received daily, subcutaneous injections of either placebo (group

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A), 10 µg/Kg of 17-B estradiol [22] (group B), 10 µg/Kg 17-B estradiol together with 0.2 mg/Kg medroxyprogesterone acetate [23] (group C), or 0.2 mg/Kg medroxyprogesterone acetate (group D). The injections were always given during the same period of the day and continued for 28 days. On the 28th day the rats were sacrificed and the bladder, vagina and part of the cervix, and near the urethra were removed as a block.

These blocks were then macroscopically divided into four distinct regions: distal urethra, proximal urethra, urethral-vesical junction and bladder. Histological sections were colored with hematoxylin and eosin or Masson tricromic.

The type of epithelium (transition, pseudo-stratified or stratified) and areas of hyperplasia or metaplasia were analyzed.

For the morphometric study measurements were taken of the more superficial nuclei of the epithelial tissue, six per lamina on average, and of the thickness of the epithelium and the lamina propria in a minimum of four random regions. Nuclear volume was calculated using SALVATORE and SCHREIBER's formula (1947) of $V = a^2.b/1.91$ (where V = nuclear volume, a = minor nuclear diameter, b = major nuclear diameter and 1.91 = a constant).

The Kruskal-Wallis ANOVA was used for statistical analysis and, when significant, was followed by the multiple comparisons test. For the comparison of the four groups with each other, the x^2 partition test was used. For all tests a 5% level of significance was set [24] .

Results

The frequency of hyperplasia was greater in group B (estrogen) in relation to the other groups and was also greater in group C (estrogen with progestogen) in relation to groups A (placebo) and D (progestogen).

Metaplasia was found in seven samples: four in the proximal urethra of animals from group B, two in the urethral-vesical junction (group B) and one in the urethral-vesical junction (group C).

The type of epithelium presented significant alterations. In the proximal urethra, the incidence of transitional epithelium was smaller in group B, while stratified epithelium was more prevalent in group B than in groups A and D. There were no differences among the groups in relation to the presence of pseudo-stratified epithelia in this region. In the distal urethra, stratified epithelium was identified in all the samples, while in the bladder only transition epithelia were found. Although differences in the occurrence of pseudo-stratified epithelium were not observed for any of the regions, among the groups, irrespective of region, a significantly higher frequency was observed in group A.

The thickness of the epithelium in the distal urethra was not significantly different among the four groups. However, in the proximal urethra, the mucosa was significantly thicker in group B in comparison to group A. The epithelium thickness in the urethral-vesical junction was significantly greater in group B than in groups C and D; while in the bladder, the epithelium thickness in group B was greater than in group D.

The thickness of the lamina propria in the bladder was significantly greater in group B than in group D and, although the thickness in the distal urethra, proximal urethra and urethral-vesical junction was not significantly different among the groups, a greater thickness in all these regions in group B was observed.

In the proximal urethra, the nuclear volume in group C was significantly greater than that in group D, while in the bladder the thickness in group D was smaller in comparison to groups A and B. In the urethral-vesical junction there was no difference in nuclear volume among the groups.

Discussion

It is known that the occurrence of hyperplasia in tissues derived from the urogenital sinus is one of the alterations associated with estrogen replacement [10, 12, 13, 18]. In our study hyperplasia was most frequent in the estrogen and estrogen/progestogen groups, thus progesterone apparently did not completely block the proliferation induced by estrogen. Nevertheless, the group which received just estrogen did show a higher occurrence of hyperplasia than the estrogen/progestogen group, although the difference was not significant.

Squamous metaplasia, described as resulting from the effect of estrogen in the urethral mucosa [16], was observed in only seven of our samples; six from the estrogen group and just one from the estrogen/progestogen group.

In the bladder, only transition epithelium was found and neither metaplasia nor hyperplasia were observed which is in agreement with the finding reported by Yosif *et al.* [2].

In the proximal urethra, the results suggest that estrogen increased the prevalence of stratified epithelia and that its absence increased the prevalence of transition and pseudo-stratified epithelia.

Another fact observed in the group which received just estrogen was the occurrence of pyknotic nuclei and thicker epithelium with a greater number of layers. However, when compared to the placebo group, these differences were not statistically significant.

The lamina propria in each of the regions analyzed was thicker in the group which received just estrogen than it was in the other groups. However, this increase was only a tendency and the differences were not significant except in the bladder when the estrogen group had a thickness significantly greater than the group to which just medroxyprogesterone acetate was administrated.

The thickness of the epithelium in the bladder and in the urethral-vesical junction was significantly greater in the estrogen group than in the progestogen group. In the proximal urethra, the thickness was significantly greater in the estrogen group than in the placebo group, and only in the distal urethra were no significant differences in epithelium thickness found among the groups. So, in all the regions studied, the group to which estrogen was administered always presented thicker epithelium in comparison to the other studied groups, demonstrating the trophic action of this hormone in the low urinary tract of female rats.

Conclusions

1) Estrogen replacement, whether or not associated with medroxyprogesterone acetate, caused metaplasia and hyperplasia of the mucosa of the urethra and of the urethral-vesical junction.

2) In the proximal urethra, hormone replacement increased the incidence of stratified epithelia, while in the absence of estrogen, transition and pseudo-stratified epithelia predominated.

3) Hormone replacement induced the presence of stratified epithelium or, in its absence, pseudo-stratified epithelium. In the bladder the effect was smaller in comparison to that in the urethra and in the urethral-vesical junction.

4) Following estrogen replacement in castrated adult virgin female rats, there was an increase in the epithelium thickness of the urethral and vesical mucosa.

5) We did not find significant alterations in the thickness of the lamina propria following hormone replacement except in the bladder, where the group which received estrogen had lamina propria significantly thicker than the group which received progesterone.

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