

Effects of the association of androgen/estrogen on the bladder and urethra of castrated rats

A. Madeiro¹, M. Girão², M. Sartori², R. Acquaroli², E. Baracat², G. Rodrigues De Lima²

Department of Gynecology, Piauí State University¹,

Department of Gynecology, Escola Paulista de Medicina - São Paulo Federal University, São Paulo² (Brazil)

Summary

Purpose: To study the effects of methyltestosterone, isolated or associated to estrogens, on the bladder and urethra of castrated adult rats.

Material & Methods: A total of 59 castrated animals were studied. They were divided into the following groups: I - placebo; II - equine conjugated estrogens; III - methyltestosterone and conjugated estrogens; IV - methyltestosterone. After 28 days of medication the animals were sacrificed and bladder and urethra cuts were obtained for the evaluation of the number of vessels, the thickness of the epithelia, and the quantity of collagen and muscular fibers.

Results: The group receiving the androgen/estrogen association presented a higher number of vessels, epithelial thickness and quantity of muscular fibers ($p < 0.05$). A smaller quantity of collagen fibers was observed in the group utilizing isolated conjugated estrogen ($p < 0.05$).

Conclusion: We concluded that the association of androgen/estrogen positively modifies important parameters in the urinary continence mechanism. Therefore, it could constitute an option for hormone replacement in postmenopausal stress urinary incontinence cases.

Key words: Androgens; Estrogens; Urinary incontinence; Collagen.

Introduction

Postmenopausal stress urinary incontinence (SUI) represents one of the main problems afflicting women, thus constituting a very important disease for its psychological and social effects.

Urinary continence is determined by the conjunction of several factors acting synergistically, such as the intraabdominal topography of the vesical neck, the urethral sphincter system, the vascular periurethral cushion, and the musculature of the pelvic and urogenital diaphragms [1]. A urethral pressure higher than the intravesical one is fundamental for urinary continence [2]. Urethral mucosa, vascularization, periurethral musculature and connective tissues are the main determinant factors of intraurethral pressure. Periurethral vascularization is responsible for a third of the urethral pressure; striated muscles are responsible for another third; and the remaining third has been attributed to the periurethral striated muscles and connective tissues [3, 4]. All these factors are highly influenced by estrogens [5, 6].

The use of hormone replacement therapy in SUI is based on the embryologic origin that is common in the genital and urinary tract [7], and also by the presence of steroid receptors in the lower urinary tract [8]. The estrogen influence on the pelvic tissues is long known [6, 9].

Lately, the estrogen/androgen association in the climacterium has earned more attention due to the better knowledge of androgen decrease after surgical or spontaneous menopause, and also, to the evidence of positive

response in women presenting vasomotor symptoms and loss of libido, whenever androgen is added to the HRT [10, 11].

Because of that and also because not much is known about the androgen effects on the female lower urinary tract, we evaluated in this study, the effects of methyltestosterone, isolated or associated to equine conjugated estrogen, on the number of vessels, the epithelium thickness, and the quantity of muscular and collagen fibers in the bladder and the urethra of castrated adult rats.

Material and Methods

A total of 59 virgin adult rats (*rattus norvegicus albinus*) were utilized. These rats were approximately 90 days old and weighed on average 210 grams.

All the animals were confined in plastic cages with covers made out of metal bars and kept at a 22°C room temperature with fluorescent lights and a 12-hour light photo period alternated with a 12-hour dark period.

Bilateral oophorectomy was performed after the acclimatization phase through a dorsal pathway, under inhalation anesthesia with ethyl ether. Hormone administration started four days after the surgical procedure, whenever the hypoestrogenic stage was confirmed by the hormonal colpocytology. The drugs were administered by gavage over 28 uninterrupted days through metal catheters. All drugs and medicines were diluted in 0.5 ml of propylene glycol.

The animals were divided randomly into four groups: I - constituted by 14 animals that received propylene glycol; II - constituted by 15 rats that received a 50 µg/day dose of equine conjugated estrogen; III - 15 rats had 0.075 mg/kg/day doses of methyltestosterone; IV - 15 rats that received a combination of methyltestosterone and equine conjugated estrogens in the same doses above.

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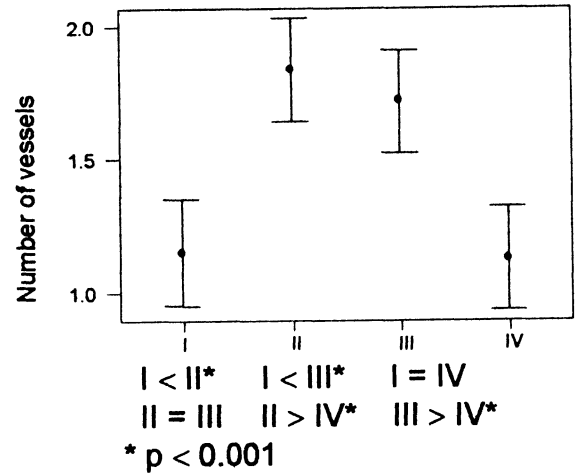
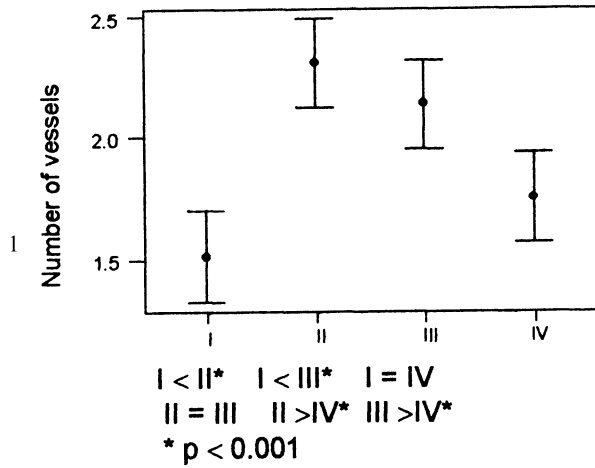


Figure 1. — Confidence intervals ($\gamma=95\%$) for the number of vessels in the bladder.
 Figure 2. — Confidence intervals ($\gamma=95\%$) for the number of vessels in the urethra.

The animals were sacrificed with ethyl ether and, immediately afterwards, they were submitted to lower genital urinary tract removal. The material obtained was divided into two representative fragments of the bladder and the proximal urethra and fixed in formol at 10%. Following that, the pieces were dehydrated with ethyl alcohol, cleared with xylol, and included in liquid paraffin. The histologic sections were obtained in a microtome set to 5 μ and stained with hematoxylin-eosin and picosirius red.

The morphometric analysis was performed through the image representation technique, utilizing a computerized system consisted by a microscope, a color video camera and a computer.

Ten reading fields were selected randomly for the vessel count of the proper slide. The vessels were marked with an asterisk. Epithelia thickness was determined through four linear measurements in regions randomly chosen, always in thinner areas.

A digital image amplified 400x was utilized for the muscular fibers and collagen quantification. A reticulum with 25 points geometrically distributed was attached which allowed the points occurring over the muscular and collagen fibers to be counted. Thus 20 fields were counted with a total of 500 points per animal.

Taking into consideration the nature of the studied variables, variance analysis (ANOVA) and the Tukey multiple comparisons test were utilized, and the significance level was set at 5%.

Results

A larger number of vessels was verified in the urethra as well as in the bladder among the groups receiving estrogen either isolated or associated to androgens (Figures 1 and 2).

The group utilizing the methyltestosterone isolated presented smaller thickness of the epithelium, bladder and urethra, when compared to the group receiving androgen associated to estrogen (Figures 3 and 4).

The quantity of collagen in the bladder and urethra was found to be higher in the group not receiving hormonal medication. Isolated estrogen therapy determined the lesser collagen while the groups receiving androgen were found to be similar to each other but superior to group II (Figures 5 and 6).

The number of muscular fibers in the urethra was larger in the groups utilizing hormones. In the bladder, the combination of conjugated estrogens and methyltestosterone presented the largest number of muscles (Figures 7 and 8).

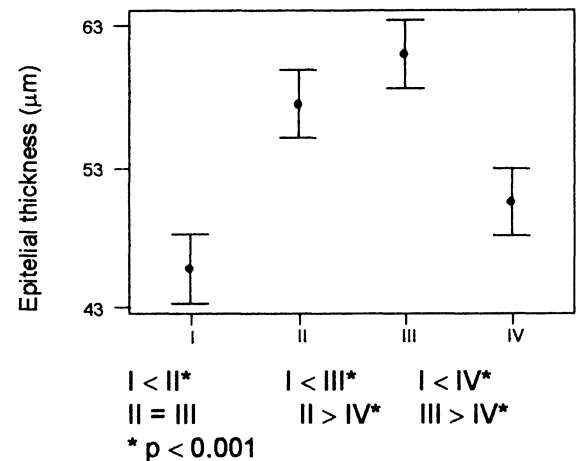
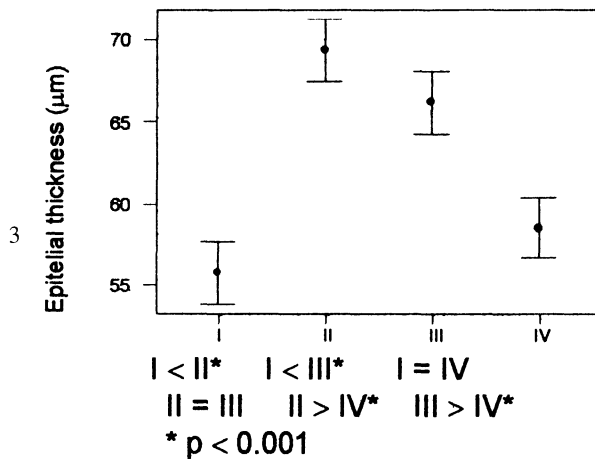


Figure 3. — Confidence intervals ($\gamma=95\%$) for epithelial thickness in the bladder.
 Figure 4. — Confidence intervals ($\gamma=95\%$) for epithelial thickness in the urethra.

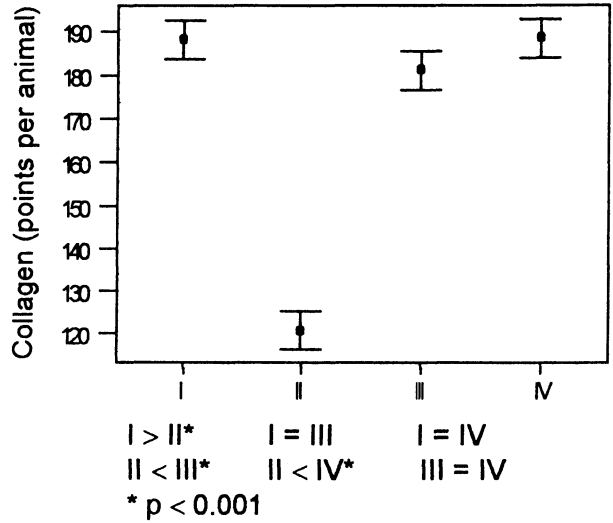
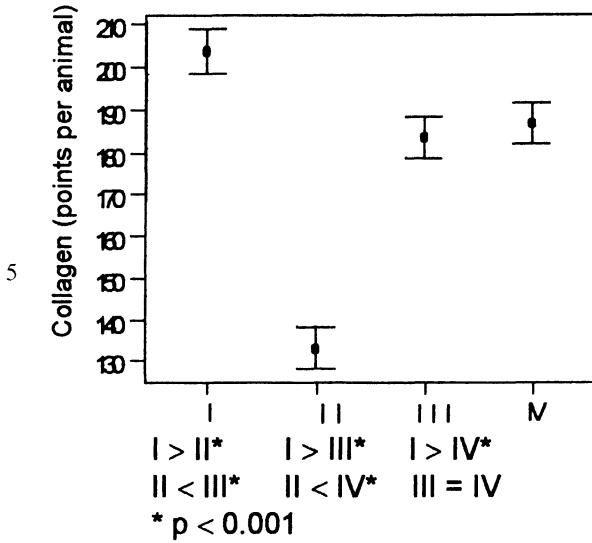


Figure 5. — Confidence intervals ($\gamma=95\%$) for quantity of collagen in the bladder.
 Figure 6. — Confidence intervals ($\gamma=95\%$) for quantity of collagen in the urethra.

Discussion

Despite some sporadic reports, androgen action on the female lower urinary tract still remains unknown.

Methyltestosterone was utilized due to the fact androgen is usually more utilized in climacterium. The standardization of the dosage (0.075 mg/kg/day), was based on reports of other researchers who applied the smallest androgen dosage capable of determining changes in the sexual receptivity of castrated rats without, however, changing the vaginal cytological pattern [12].

As to vascularization, we have observed that methyltestosterone lessened the number of vessels in the bladder as well as in the urethra. On the other hand, the estrogen therapy isolated or associated to androgen led to a significant increase of these vessels, which is concordant with the findings of other authors [13]. Some authors already observed that testosterone causes vasodilation of the

coronary band in animals [14]. In humans, adding androgen to the estrogen therapy did not change vaginal vasodilation which was observed through Doppler velocimetry [15].

We have also observed that in the bladder and urethra, the combination estrogen/androgen contributed to a significant increase of epithelial thickness, a non-verified fact in isolated androgen replacement. These data agree with those reported in the literature [16]. It is known that, beside its plication, the urethral mucosa maintains a sealing effect that helps the urinary continence mechanism [17].

The action of the sexual steroids, especially estrogens, on the collagen of the urinary tract in postmenopause has controversial results in the literature. The methodology we have utilized for collagen quantification evaluates collagen as a whole. A polarization microscope should be used in order for the type to be specified.

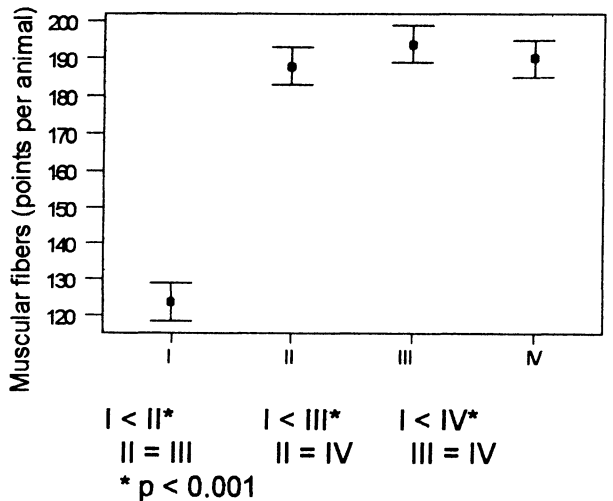
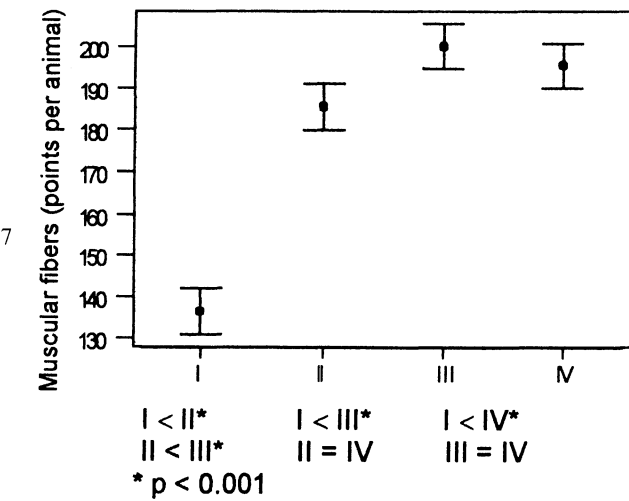


Figure 7. — Confidence intervals ($\gamma=95\%$) for quantity of muscular fibers in the bladder.
 Figure 8. — Confidence intervals ($\gamma=95\%$) for quantity of muscular fibers in the urethra.

We have verified that the use of isolated estrogens promoted an important decrease of collagen among the muscular fascicles of the bladder and in the muscular layer of the urethra, as also demonstrated by other studies [18]. Regarding androgen our findings differ from those obtained by another study that observed a collagen decrease in the bladder and in the ureter of female rabbits treated with testosterone and methandrostenolone [19].

Other researches demonstrated a collagen increase in the skin of postmenopausal women treated with estradiol and testosterone [20]. There was also an increase of collagen production in women utilizing the association of estradiol and testosterone of type III, which though is more flexible [21]. Yet, androgen receptors were demonstrated in the skin fibroblasts, which suggests the susceptibility of these collagen producing cells to these hormones [22].

It is known that an abnormal depot of collagen type III in the bladder between the muscular fibers can alter detrusor contractility thus leading to urinary symptoms [23].

Our results show that the association of methyltestosterone and equine conjugated estrogens acts positively on the number of vessels, on the thickness of the epithelium, and on the number of muscular fibers in the bladder and in the urethra.

The activity of the urethra could improve with an increase in the number of vessels, the epithelium thickness, collagen, and muscular fibers since all of these parameters are involved in the maintenance of intraurethral pressure. Contrary to the bladder, it seems that urethral function is positively correlated to the amount of collagen [24].

Therefore, it seems possible to infer that the association of estrogen/androgen in postmenopause would positively affect the female lower urinary tract. These data, however, need validation in future clinical studies.

Conclusion

Our results show that the utilization of androgens associated with estrogens increased the number of vessels, the thickness of the epithelium, and the number of muscular fibers, but did not alter the amount of collagen. Therefore, the androgen/estrogen association could become one more important alternative in the therapy of postmenopausal stress urinary incontinence.

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Address reprint requests to:
A. MADEIRO, M.D.
R. Cel. Costa Araújo, 3033
Horto Florestal - Teresina - Piauí
Cep 64049-460 (Brazil)