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The effect of testosterone alone and testosterone + estradiol therapy on bladder functions and smooth muscle/collagen content in surgically menopause induced rats

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ABSTRACT

Objectives: The aim of the study was to investigate the effect of testosterone alone and testosterone + estradiol therapy on bladder functions and smooth muscle/collagen content in surgically menopause induced rat model.
Methods: The study included 34 female Sprague-Dawley rats, and the rats were divided into four groups. After bilateral oophorectomy, during a 60 days period, six rats received IM saline injection for one time, as a control group, and nine rats received testosterone undecanoate 100 mg/kg IM for one time, and nine rats received testosterone undecanoate 100 mg/kg IM for one time + daily 0.50 mg nasal spray of 17β estradiol. Ten rats were taken as sham group. Urodynamic studies were performed in all groups before and after the study. The rats were sacrificed after 60 days, and cystometric findings and smooth muscle/collagen ratio of the bladders were compared between the groups.
Results: Increase in maximal bladder capacity and compliance were significantly higher in the testosterone treatment group and testosterone + estradiol treatment group than in the control group ($p = 0.01$ and $p = 0.002$, respectively for bladder capacity; $p = 0.04$ and $p = 0.005$, respectively for bladder compliance). Smooth muscle/collagen ratio of the bladders was significantly higher in the testosterone and testosterone + estradiol treatment groups than in the control group ($p = 0.04$ and $p = 0.008$, respectively).
Conclusions: This study shows that bladder functions may deteriorate in postmenopausal period. In addition to estrogen replacement therapy, testosterone has a significant role to increase bladder smooth muscle, leading to improvement in bladder functions in postmenopausal women with urogenital system dysfunction.

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1. Introduction

Postmenopausal women have detrimental changes in their urogenital system. It has been estimated that 10–40% of postmenopausal women have symptomatic urogenital atrophy [1]. Urogenital dysfunction in postmenopausal period is related to declining ovarian function. Estrogen deficiency in postmenopausal women can cause urogenital dysfunction and, therefore may lead to disorders of the lower urinary tract. The most significant result of the menopause is estrogen deprivation [2], and therefore, estrogens have been used to treat a spectrum of overactive bladder, urinary incontinence and urinary tract infections in postmenopausal women [3–5].

Numerous studies showed that the gonads appear to be the main sources of testosterone [6,7]. However, the effect of testosterone on smooth muscle of urogenital system in women is not well known. Thus, in addition to estrogen replacement therapy, testosterone might improve deteriorated bladder capacity and compliance in the presence of menopause.

The aim of the study was to investigate the effect of testosterone alone and testosterone + estradiol therapy on bladder functions and smooth muscle/collagen content in surgically menopause induced rat model.

2. Materials and methods

2.1. Animals and study design

The experimental protocol was approved by the Committee on Animal Research at the University of Mersin School of Medicine.

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In this study, 34 female Sprague-Dawley rats, 200–300 g, were used, and the rats were divided into four groups. After bilateral oophorectomy, during a 60 days period, six rats received IM saline injection (for one time), as a control group, and nine rats received testosterone undecanoate (Nebido®, Bayer Schering Pharma, Berlin, Germany) 100 mg/kg IM for one time, and nine rats received testosterone undecanoate 100 mg/kg IM for one time + daily 0.50 mg nasal spray of 17β estradiol (Aerodiol®, Servier, Paris, France). Ten rats were taken as sham group. During a 60 days period, we used daily nasal form of estrogen, because of difficulty in daily vaginal or oral administration of the drugs in rats. We used single injection of long-acting testosterone, again because of difficulty in daily administration of oral testosterone agents in rats. Urodynamic studies were performed in all groups before the treatment as the baseline value and after the treatment at the end of the experiment. The rats were sacrificed after 60 days, and the bladders were removed. Cystometric findings and smooth muscle/collagen ratio of the bladders were compared between the groups.

2.2. Menopause model

The rats were anesthetized with intraperitoneal injection of ketamine (50–100 mg/kg). Under sterile conditions, a midline incision was made to expose the lower abdominal cavity, and the ovaries were removed in total [8–11]. Sham operations were performed in a similar fashion but the abdomen was closed without removing ovarian tissue. The rectus fascia was closed with a running 4–0 plain absorbable catgut suture, and the skin was closed with interrupted 3–0 silk sutures.

2.3. Functional evaluation of the bladder

Urodynamic studies were performed before the treatment and just before the sacrification, as previously described [8,12–14]. The rats received a single 20 mg/kg dose of ciprofloxacin as an antibiotherapy. A 22 G catheter was inserted transurethrally and connected by a polyethylene tube to a pressure transducer using a urodynamic equipment (Life-Tech, Inc., Houston, TX, USA) and a computer program (Urolab Primolus). After the measurement of residual urine volume at the time of catheter insertion, each rat underwent cystometric measurements with infusion of warmed (37 °C) normal saline solution at 0.1 ml/min (Abbott infusion pump). During the study, the baseline pressure (empty bladder), opening pressure (at first leakage) and peak pressure (maximal pressure during voiding) and the maximal bladder capacity were recorded. Bladder compliance (ml/cm H_2O) was calculated according to the following formula: compliance = maximal bladder volume/opening pressure − baseline pressure.

2.4. Histological evaluation

The bladder was removed through a lower midline abdominal incision. After the removal of the bladder, the rats were sacrificed by pentobarbital overdose (200 mg/kg) and bilateral thoracotomy. After both ureters were ligated, the bladder was filled with 10% formalin solution through a catheter transurethrally and kept distended overnight. The specimen was split longitudinally, and the routine tissue processing for light microscopy was performed.

Bladder tissues were embedded in paraffin. Sections (3 μm) were cut by microtome and stained with Masson's trichrome to examine the smooth muscle/collagen ratio [12]. Slides were examined by an Olympus BX50 light microscope and photographed by an Olympus PM10SP photograph system. The slides were analyzed on a PC with Soft Imaging System (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The number of pixels within the bladder wall was counted and set at 100%, and the other structures were erased from the images. The smooth muscle and collagen components of the bladders were identified at 300×, and calculated as mm^2.

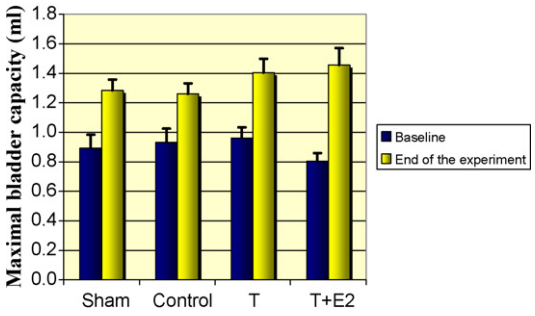

Fig. 1. The mean maximal bladder volume at the beginning and end of the experiment in the sham, control, testosterone (T) and testosterone + estradiol (T + E2) treatment groups. Note that as percentage from the beginning to the end of the experiment, the increase of the mean maximal bladder volume was significantly higher in the testosterone alone and testosterone + estradiol treatment groups than in the control group ($p = 0.01$ and $p = 0.002$, respectively).

2.5. Statistical analysis

Statistical analyses were performed using the "Anova test" to compare the mean body weight among the four groups, "independent *t* test" to compare smooth muscle/collagen ratio and differences as percentage in cystometric findings at the beginning and end of each experiment between the groups. Data are presented as mean ± standard error (S.E.) for cystometric findings, and mean ± standard deviation (S.D.) for smooth muscle/collagen ratio. Probability values of <0.05 were considered statistically significant.

3. Results

3.1. Functional study findings

At the beginning of the experiment, no significant differences in the mean body weight were observed among the four groups ($p = 0.816$). Fig. 1 and Fig. 2 show the mean maximal bladder capacity and bladder compliance at the beginning and end of the experiment in the sham, control, testos-

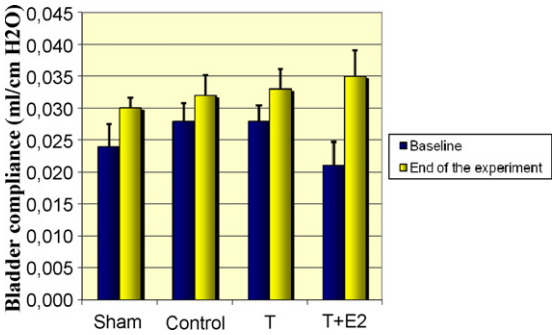

Fig. 2. The mean bladder compliance at the beginning and end of the experiment in the sham, control, testosterone (T) and testosterone + estradiol (T + E2) treatment groups. Note that as percentage from the beginning to the end of the experiment, the increase of the mean bladder compliance was significantly higher in the testosterone alone and testosterone + estradiol treatment groups, compared with the control group ($p = 0.04$ and $p = 0.005$, respectively).

terone alone and testosterone + estradiol treatment groups. The mean maximal bladder volume increased from 0.893 ± 0.15 ml to 1.284 ± 0.22 ml in the sham group, increased from 0.934 ± 0.16 ml to 1.261 ± 0.23 ml in the control group, increased from 0.962 ± 0.21 ml to 1.405 ± 0.12 ml in the testosterone treatment group, and increased from 0.804 ± 0.22 ml to 1.457 ± 0.13 ml in the testosterone + estradiol group (Fig. 1). As percentage from the beginning to the end of the experiment, the mean maximal bladder volume increased 105.6 ± 66.2% in the sham group, 4.9 ± 5.3% in the control group, 53.3 ± 18.8% in the testosterone treatment group and 88.9 ± 15.7% in the testosterone + estradiol treatment group. The increase of the mean maximal bladder volume was significantly higher in the testosterone alone and testosterone + estradiol treatment groups than in the control group ($p = 0.01$ and $p = 0.002$, respectively).

As shown in Fig. 2, the mean bladder compliance increased from 0.024 ± 0.002 ml/cm H_2O to 0.03 ± 0.003 ml/cm H_2O in the sham group, increased from 0.028 ± 0.006 ml/cm H_2O to 0.032 ± 0.005 ml/cm H_2O in the control group, increased from 0.028 ± 0.008 ml/cm H_2O to 0.033 ± 0.006 ml/cm H_2O in the testosterone treatment group, and increased from 0.021 ± 0.007 ml/cm H_2O to 0.035 ± 0.006 ml/cm H_2O in the testosterone + estradiol treatment group. As percentage from the beginning to the end of the experiment, the mean bladder compliance increased 71.76 ± 44.56% in the sham group, 6.6 ± 14.2% in the control group, 37.1 ± 20.9% in the testosterone treatment group and 74.49 ± 15.88% in the testosterone + estradiol treatment group. The increase of the mean bladder compliance was significantly higher in the testosterone alone and testosterone + estradiol treatment groups, compared with the control group ($p = 0.04$ and $p = 0.005$, respectively).

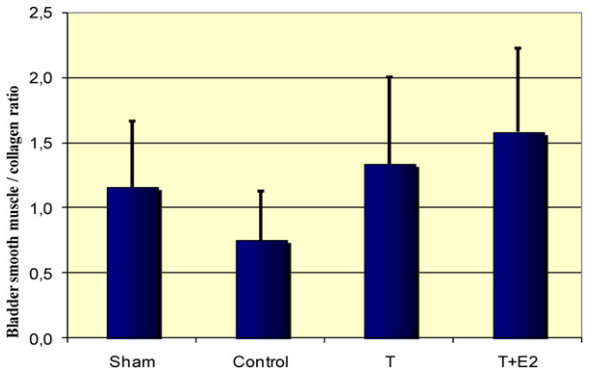

Fig. 3. The mean smooth muscle/collagen ratio in the sham, control, testosterone (T) and testosterone + estradiol (T + E2) treatment groups. Note that smooth muscle/collagen ratio was significantly higher in the testosterone treatment and testosterone + estradiol treatment groups, compared with the control group ($p = 0.04$ and $p = 0.008$, respectively).

3.2. Histological findings

As shown in Fig. 3, the mean bladder smooth muscle/collagen ratio was 1.16 ± 0.15 in the sham group, 0.76 ± 0.15 in the control group, 1.34 ± 0.21 in the testosterone treatment group and 1.59 ± 0.21 in the testosterone + estradiol treatment group. Compared with the control group, smooth muscle/collagen ratio was significantly higher in the testosterone treatment and testosterone + estradiol treatment groups ($p = 0.04$ and $p = 0.008$, respectively) (Fig. 4).

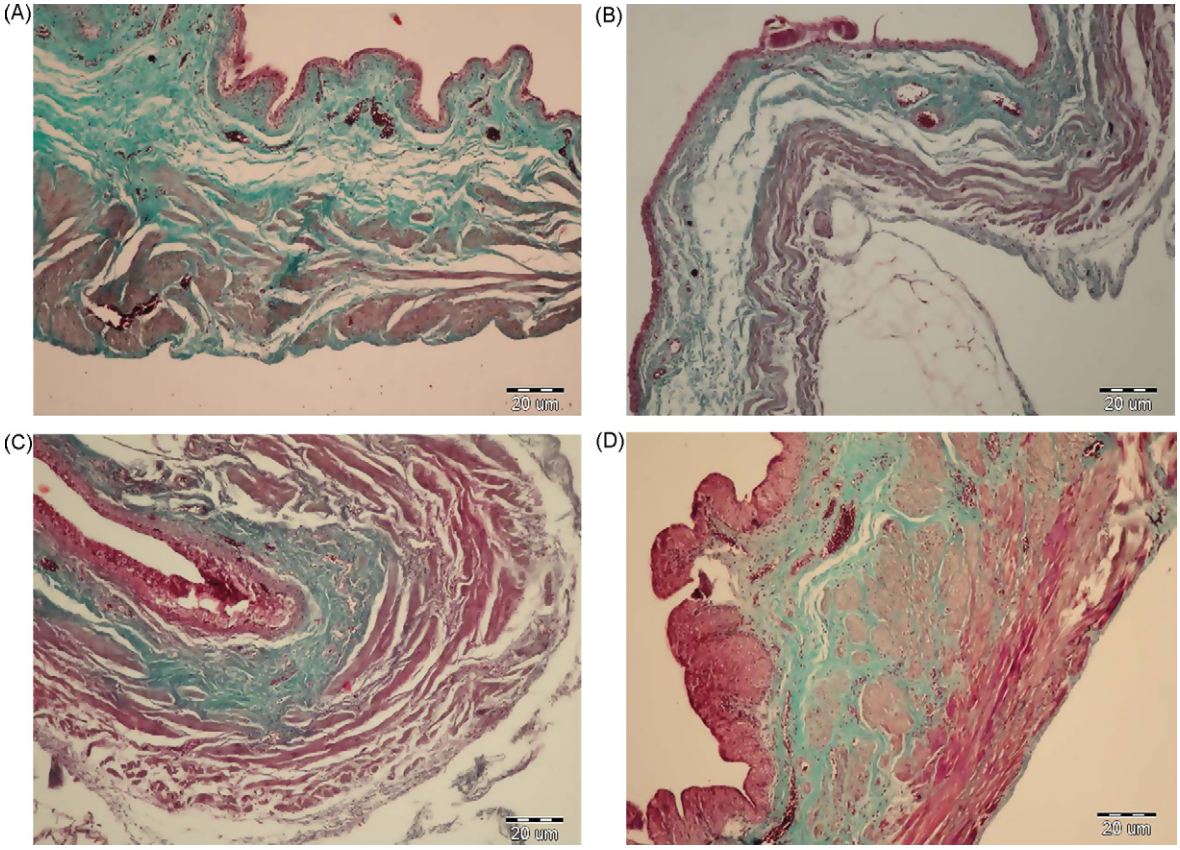

Fig. 4. Masson-trichrome staining of a rat bladder in the sham group (A), control group (B), testosterone treatment group (C) and testosterone + estradiol treatment group (D).

4. Discussion

Ovariectomized rats have been used as a model of menopause induced estrogen deficiency in humans. In addition, Liang et al. demonstrated that ovariectomized rats voided more frequently than sham-operated rats [9]. In their study, estrogen replacement decreased the frequency of voiding but did not return the animals to the pre-ovariectomized voiding pattern. Therefore, we performed bilateral ovariectomy to create a model of menopause, and then we investigated the effect of testosterone alone and testosterone + estradiol therapy on bladder functions and histological changes in surgically menopause induced rat model.

Madeiro et al. studied the association of androgen/estrogen on the bladder and urethra of castrated rats, and after 28 days of medication the animals were sacrificed for the histologic evaluation of the bladders [15]. Dambros et al. investigated the effect of ovariectomy and estradiol replacement therapy for 3 months on the rat bladder histology [2]. Longhurst et al. reported that estrogen treatment for 2 months increased detrusor sensitivity and improved bladder contraction and decreased the elevated voiding frequency of ovariectomized rats to normal, and in addition, interestingly, the voiding frequency of ovariectomized rats after 4 months was lower than that of the sham-operated rats [10]. Therefore, we used a 2 months treatment duration to investigate the effect of testosterone alone and testosterone + estradiol therapy on bladder functions and smooth muscle/collagen content in surgically menopause induced rats.

Fleischmann et al. investigated the behavioral, cystometric and histological changes that occur with long-term estrogen treatment in rat bladders [11]. They found significantly increased collagen to smooth muscle ratio and a trend toward higher voiding pressure, threshold pressure, baseline pressure and mean inter-voiding pressure in the ovariectomy group compared with the estrogen treatment and control groups, although there was no statistical significance. In addition, there was no significant difference in voiding volume and frequency in the three groups. However, they compared voiding studies and cystometric analysis after the treatment at the end of the study. They did not compare the difference before and after the treatment among the groups, because they did not perform baseline cystometric studies. In the present study, from the beginning to the end of the experiment, the increases in the mean maximal bladder capacity and compliance were significantly lower in the control group than in the sham group. In addition, ovariectomized rats showed significantly lower smooth muscle/collagen content than the sham group. Our experimental rat study shows that bladder functions and histology may deteriorate in postmenopausal period.

Urogenital atrophic changes are more prevalent in postmenopausal women and increase with increasing years of estrogen deficiency which is associated with urinary symptoms such as frequency, urgency, nocturia, incontinence, and recurrent infection [3]. Estrogens have been widely used to treat lower urinary tract symptoms in postmenopausal women although its role remains controversial [4]. Urogenital dysfunction in postmenopausal period is related to declining ovarian functions with the association of decreased estrogen and testosterone. Numerous studies including our previous studies showed that administration of estrogens have been resulted in increase in the smooth muscle of urogenital systems including vagina and bladder. However, the effect of testosterone on smooth muscle of urogenital system in women is not well known in the presence of menopause. These urinary symptoms in postmenopausal period might worsen with the decreasing level of testosterone. Thus, we wanted to mainly focus on the effect of testosterone treatment in addition to estrogen replacement therapy. In the present study, we found that bladder functions including bladder capacity and compliance and bladder smooth muscle content decreased in postmenopausal period. Lin et al. concluded that testosterone might be as important as estrogen in the bladder contractile responses [16]. Madeiro et al. studied the association of androgen/estrogen on the bladder and urethra of castrated rats, and after 28 days of medication the animals were sacrificed for the histologic evaluation of the bladders. They reported that the bladders of the rats receiving androgen/estrogen presented a higher number of vessels, epithelial thickness and quantity of muscular fibers than the rats receiving isolated conjugated estrogen [15]. In the present study, increases in maximal bladder capacity and compliance were significantly higher in the testosterone alone and testosterone + estradiol treatment groups than in the control group. In addition, testosterone and testosterone + estradiol treatment groups showed significantly higher bladder smooth muscle/collagen ratio than the control group. Our findings suggest that bladder dysfunction is related to estrogen and androgen deficiency, and the combination therapy may improve bladder functions and histology much better than estrogen therapy alone. To support our findings, Kim et al. reported that testosterone infusion completely restored the vaginal smooth muscle structure and contractility after ovariectomy [17]. Response of the uterus with uterine wet weights to testosterone + estradiol treatment could be provided to get an impression towards the estrogenic response. The main point of our study was to investigate the effect of testosterone treatment, in addition to estrogen therapy, on bladder function and histology, specifically. Unfortunately, we did not focus on the effects of hormone treatment on uterus.

Our findings suggest the hypotheses that estrogen directly affects detrusor function through modifications in muscarinic receptors [18], and inhibits the movement of extracellular calcium ions into muscle cells [19]. Estradiol reduces the amplitude and frequency of spontaneous rhythmic detrusor contractions [20], and may increase the sensory threshold of the bladder in some women [21]. Testosterone has been demonstrated to have an acute, nongenomic action on urinary bladder detrusor neuromuscular transmission, ultimately inhibiting contraction of the muscle in the male and female rats [22]. Lin et al. investigated the effect of letrozole, an aromatase inhibitor, inhibiting estrogen synthesis on bladder contraction with changes in morphology and biochemistry in the female rabbits [16]. They showed that testosterone might be as important as estrogen in the contractile function of the female bladder.

Administration of testosterone to female has been resulted in a variety of side effects including virilization (acne, alopecia, and hirsutism) and a negative impact on the cholesterol–lipoprotein profile [23]. The adverse effect on the lipid profile is less and may even be avoided by the parenteral administration of testosterone [24]. Therefore, postmenopausal women who receive testosterone treatment should be informed for the side effects of the administration of testosterone.

5. Conclusions

This study shows that bladder functions may deteriorate in postmenopausal period. In addition to estrogen replacement therapy, testosterone has a significant role to increase bladder smooth muscle, leading to improvement in bladder functions in postmenopausal women with urogenital system dysfunction.

References

[1] Cardozo L, Robinson D. Special considerations in premenopausal and postmenopausal women with symptoms of overactive bladder. Urology 2002;60(5 (Suppl. 1)):64–71.

[2] Dambros M, Rodrigues Palma PC, Mandarim-de-Lacerda CA, Miyaoka R, Rodrigues Netto Jr N. The effect of ovariectomy and estradiol replacement on collagen and elastic fibers in the bladder of rats. Int Urogynecol J 2003;14:108–12.
[3] Robinson D, Cardozo LD. The role of estrogen in female lower urinary tract dysfunction. Urology 2003;62(Suppl. 4A):45–51.
[4] Cardozo L, Lose G, McClish D, Versi E. A systematic review of the effects of estrogens for symptoms suggestive of overactive bladder. Acta Obstet Gynecol Scand 2004;83:892–7.
[5] Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med 1993;329:753–6.
[6] Flores A, Rodriguez JO, Palfox MT, et al. The acute asymmetric effects of hemiovariectomy on testosterone secretion vary along the estrous cycle. The participation of the cholinergic system. Reprod Biol Endocrinol 2006;4:11.
[7] Stahl F, Gotz F, Dorner G. The influence of fetal adrenals on the androgen levels during brain differentiation in human subjects and rats. Exp Clin Endocrinol 1991;98:131–9.
[8] Çayan S, Canpolat B, Çayan F, et al. The effect of chronic inflammatory condition of the bladder and estrogen replacement therapy on bladder functions and histology in surgically menopause and chronic cystitis induced rats. Neurourol Urodyn 2006;25:194–201.
[9] Liang W, Afshar K, Stothers L, Laher I. The influence of ovariectomy and estrogen replacement on voiding patterns and detrusor muscarinic receptor affinity in the rat. Life Sci 2002;71:351–62.
[10] Longhurst PA, Kauer J, Leggett HRE, Levin RM. The influence of ovariectomy and estradiol replacement on urinary bladder function in rats. J Urol 1992;148:915–9.
[11] Fleischmann N, Christ G, Sclafani T, Melman A. The effect of ovariectomy and long term estrogen replacement on bladder structure and function in the rat. J Urol 2002;168:1265–8.
[12] Çayan S, Chermansky C, Schlote N, et al. The bladder acellular matrix graft in a rat chemical cystitis model: functional and histologic evaluation. J Urol 2002;168:798–804.
[13] Çayan S, Coskun B, Bozlu M, et al. Botulinum toxin type A may improve bladder function in a rat chemical cystitis model. Urol Res 2003;30:399–404.
[14] Acar D, Çayan S, Aktaş S, Tek M, Akbay E. The effect of tamoxifene on bladder functions and histology, and the role of estrogen receptor beta in a rat chemical cystitis model. Neurourol Urodyn 2007;26:309–16.
[15] Madeiro A, Girao M, Sartori M, Acquaroli R, Baracat E, Rodrigues De Lima G. Effects of the association of androgen/estrogen on the bladder and urethra of castrated rats. Clin Exp Obstet Gynecol 2002;29:117–20.
[16] Lin WY, Rehfusst A, Whitbeck C, et al. Effect of letrozole on urinary bladder function in the female rabbit. BJU Int 2007;100:1391–5.
[17] Kim NN, Min K, Pessina MA, Munarriz R, Goldstein I, Traish AM. Effects of ovariectomy and steroid hormones on vaginal smooth muscle contractility. Int J Impot Res 2004;16:43–50.
[18] Batra S, Andersson KE. Oestrogen-induced changes in muscarinic receptor density and contractile responses in the female rat urinary bladder. Acta Physiol Scand 1989;137:135–41.
[19] Elliott RA, Castleden CM, Miodrag A, Kirwan P. The direct effects of diethylstilboestrol and nifedipine on the contractile responses of isolated human and rat detrusor muscles. Eur J Clin Pharmacol 1992;43:149–55.
[20] Shenfeld OZ, McCammon KA, Blackmore PF, Ratz PH. Rapid effects of estrogen and progesterone on tone and spontaneous rhythmic contractions of the rabbit bladder. Urol Res 1999;27:386–92.
[21] Fantl JA, Wyman JF, Anderson RL, Matt DW, Bump RC. Postmenopausal urinary incontinence: comparison between non-estrogen-supplemented and estrogen-supplemented women. Obstet Gynecol 1988;71:823–8.
[22] Hall R, Andrews PLR, Hoyle CHV. Effects of testosterone on neuromuscular transmission in rat isolated urinary bladder. Eur J Pharmacol 2002;449:301–9.
[23] Speroff L, Glass RH, Kase NG. Postmenopausal hormone therapy. In: Clinical gynecologic endocrinology and infertility. 6th ed. Lippincott Williams & Wilkins; 1999, p 725–79.
[24] Davis SR, McCloud P, Strauss BJG, Burger H. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. Maturitas 1995;21:227–36.