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Effects of Testosterone Replacement on Lower Urinary Tract Functions in Elderly Male Rats

Shuichi Morizane, Sumiyo Toji, Mayuko Matsumoto and Ikuo Miyagawa

Division of Urology, Department of Surgery, School of Medicine, Tottori University Faculty of Medicine, Yonago 683-8504 Japan

Testosterone has been clinically used to improve hormone deficiency in the aging male; however, investigations how testosterone exerts its effects on lower urinary tract functions are not many. In order to shed light on the efficacy of testosterone on the functions, we replaced teststerone in elderly male Wistar rats aged 19 months. A relevant dose (120 mg) of testosterone was subcutaneously replaced through an implanted silastic tube into 6 rats for 4 weeks (treated group). Another 6 rats received no treatment for 4 weeks (control group). After the end of the 4-week period, we measured plasma testesterone, weight of bladder, prostate and body, bladder muscle content, spontaneous micturition behavior and cystometric parameters, and compared the results between the 2 groups. The daily micturition frequency (18.8 ± 1.5 times/day versus 15.5 ± 1.9 times/day), volume of residual urine at cystometry (0.66 ± 0.10 mL versus 0.24 ± 0.03 mL), bladder capacity (1.03 ± 0.06 mL versus 0.65 ± 0.05 mL), bladder weight (258 ± 9 mg versus 198 ± 19 mg), prostate weight (2.08 ± 0.22 g versus 1.29 ± 0.22 g) and ratio of smooth muscle area/connective tissue area (3.59 ± 0.13 versus 2.59 ± 0.36) were significantly higher in the treated group ($P < 0.05$). In contrast, the average volume of spontaneous micturition was significantly lower in the treated group (0.84 ± 0.07 mL versus 0.98 ± 0.09 mL). Differences in body weight and volume of 24-h urine were not significant between groups. Daily micturition frequency and volume of residual urine at cystometry were increased in the treated group. Testosterone replacement exerted unfavorable effects on the lower urinary functions of elderly rats, including prostatic hypertrophy.

Key words: lower urinary tract function; prostate; rat; testosterone

Male hormone deficiency in the aging male is a popular topic among andrologists, endocrinologists and urologists (Lunenfeld, 2003). Compared with normal ranges for young male adults, total serum testosterone levels are lower in 19% of men in their 60s, 28% of men in their 70s and 49% of men in their 80s (Harman, 2001). Their bladder functions are frequently disrupted mostly by the secondary effects of central neural pathologies (senile dementia, cerebral vascular accident), aging and/or bladder outlet obstruction (Lluel et al., 2003). The bladder activity and various components of bladder outlet are regulated by complex neural mechanisms. During micturition reflexes, the nervous system coordinates the muscles of the detrusor, bladder neck and urethra to promote urine flow (Lluel et al., 2003), modulating the abilities of the lower urinary tract to store and release urine.

Micturition behavior changes with age (Chun et al., 1988), and aging males' alpha adrenergic receptor density is reduced. If chronic testosterone deficiency is prolonged, it might ultimately affect their bladder outlet resistance (Anderson et

al., 1988) and eventually cause functional disorders of the lower urinary tract. Male- and female-hormone receptors are co-localized in the urothelia, bladder smooth muscle cells, proximal urethra striated muscle cells and neurons in the autonomic ganglia of the prostatic plexus, and together exert their effects directly on the lower urinary tract of aged males (Salmi et al., 2001; Keast et al., 1998). Conditions induced by co-localization have recently drawn attention such as partial androgen deficiency in aging males, andropause or male climacteric. Patients are clinically treated with testosterone replacement via injection, orally or percutaneously (Jockenhovel, 2003) in order to alleviate subjective symptoms such as feebleness with loss of concentration, decreased sexual desire, impotence and/or muscle amount degradation (Lunenfeld, 2003).

Generally, testosterone replacement is thought to have little influence on the lower urinary tract, serum prostate-specific antigen and prostate volume (Holmang, 1993; Cooper, 1998; Leder et al., 2004). However, in some animal experiments, testosterone replacement has resulted in prostatic hypertrophy and lower urinary tract passage disorder (Constantinou, 1996), despite favorable results in the bladder functions of female hormone-replaced elderly female rats (Longhearst et al., 1992). To study the effects of testosterone replacement on lower urinary tract functions in elderly male rats, we measured plasma testesterone, bladder, prostate and body weight, bladder muscle content, spontaneous micturition behavior and cystometric parameters using testosterone-replaced and non-treated rats.

Materials and Methods

Animals

Experiments were performed in accordance with the Guidelines of Tottori University Committee for Animal Experimentation at the Division of Laboratory Animal Science, Research Center for Biosience and Technology, Tottori University. The studied animals were 12 male Wistar rats aged 19 months (SLC, Shizuoka, Japan), reared in an air-conditioned room under a 12/12 h light/dark cycle and allowed access to food and water ad libitum. Of the 12, 6 received no treatment for 4 weeks (control group). The remaining 6 had subcutaneous implants for 4 weeks via a silastic tube (inside diameter, 2.5 mm; length, 30 mm) (Kaneda Medix, Osaka, Japan) containing 120 mg of testosterone (Wako, Osaka, Japan) in sesame oil (Kadoya, Tokyo, Japan) (the treated group) according the dose and method reported by Sato et al. (1998).

Micturition behavior

After treatment, micturition behavior was monitored for 24 h in a metabolic cage containing a urine collection funnel. Urine flowed through a duct into a 250-mL plastic beaker placed on an electronic balance (HF200, A.N.D., Tokyo), which was connected to a personal computer (Macintosh iBook G3, Apple Computer, Cupertino, CA) via a multi-port controller (Maclab/400, AD Instruments, Castle Hill, Australia). Volumes of spontaneous micturition were automatically sampled every 150 s for 24 h, and the data including micturition frequency were stored on the compunter's hard disk. Monitoring per each group started at 10:00. The rats were allowed to take water but no food during the observation period.

Cystometry

Cystometry was performed under subcutaneous urethane anesthesia (1.0 g/kg) with a 24-gauge catheter inserted into the apex of the bladder dome to record pressure. On day 31 of the experiment, the bladder was filled with physiological saline by using an infusion pump (5200, TOP, Tokyo) at a constant rate of 0.4 mL/min until micturition was detected. A cystostomy catheter was connected to an external pressure transducer (P2310, Gould, Eastlake, OH) to measure the intravesical pressure, which was recorded on a Macintosh Powerbook G3 via a bridge amplifier

(ML112, AD Instruments) and a multi-port controller Maclab/400. During micturition, filter paper was carefully placed at the meatus to absorb excreted urine without spillage, and the excreted volume was calculated by the difference of the weight. The following parameters were measured: bladder capacity (mL), volume of excreted urine (mL), volume of residual urine (mL) and maximal detrusor pressure (cmH_2O). Maximal detrusor pressure was defined as (instantaneous pressure of the detrusor muscle) minus (resting pressure of the detrusor muscle after contraction). The measurements were performed for 24 h per each rat.

Measurement of plasma testosterone and histological examination of the rat bladder

Blood was drawn by a heart puncture and a volume of 2.5 mL was collected to assess the plasma testosterone level by radioimmunoassay kit (SRL, Tokyo). After blood sampling, the bladder and prostate were surgically removed from the surrounding tissues and weighed. Bladders transected at the urethra level were sectioned into sagittal slices, fixed in 10% formalin and embedded in paraffin, from which 10 μm-thick vertical cross sections were made. The sections were deparaffinized with xylene, rehydrated with a graded series of ethanol and stained with hematoxylin and eosin and the Elastica-van Gieson method which stains smooth muscles yellow and collagen in connective tissues red.

Color-assisted quantitative image analysis

The stained smooth muscles were quantified by using color-assisted quantitative image analysis. Sections of stained tissues were observed at a magnification of 400 times by light microscopy (BX50, Olympus, Tokyo), displayed on a color monitor (PVM-20M4V, Sony, Tokyo) and printed with a digital color printer (CP700DSA, Mitsubishi, Tokyo). Prints were digitized with a scanner (GT-8000, Epson, Tokyo) operated on a Macintosh Powerbook G3 (Apple Computer), and quantified by using National Institutes of Health Image 1.55 software (Research Service Branch, National Institutes of Health, Bethesda, MD). We then calculated the components of smooth muscles and connective tissues per full screen. At least 10 fields were examined from each tissue section.

Table 1. Plasma testosterone levels and weight of the rat bladder, prostate and body

	Control group [6]	Treated group [6]
Testosterone (ng/mL)	0.83 ± 0.41	2.61 ± 0.69*
Bladder weight (mg)	198 ± 19	258 ± 9*
Prostate weight (g)	1.29 ± 0.22	2.08 ± 0.22*
Body weight (g)	575 ± 33	598 ± 26

Values are mean ± SD.
[ ], number of animals.
* Significant difference, $P < 0.05$.

Statistical analysis

Experimental values are expressed as mean ± SD. Statistic probabilities were calculated with the non-parametric *t*-test between 2 groups, and $P < 0.05$ was considered significant.

Results

Plasma testosterone levels

The treated group showed significantly ($P < 0.05$) higher plasma testosterone levels than the control group (Table 1).

Bladder, prostate and body weight

The bladder and prostate were significantly ($P < 0.05$) heavier in the treated group than in the control (Table 1). The difference in body weight between groups was not significant (Table 1).

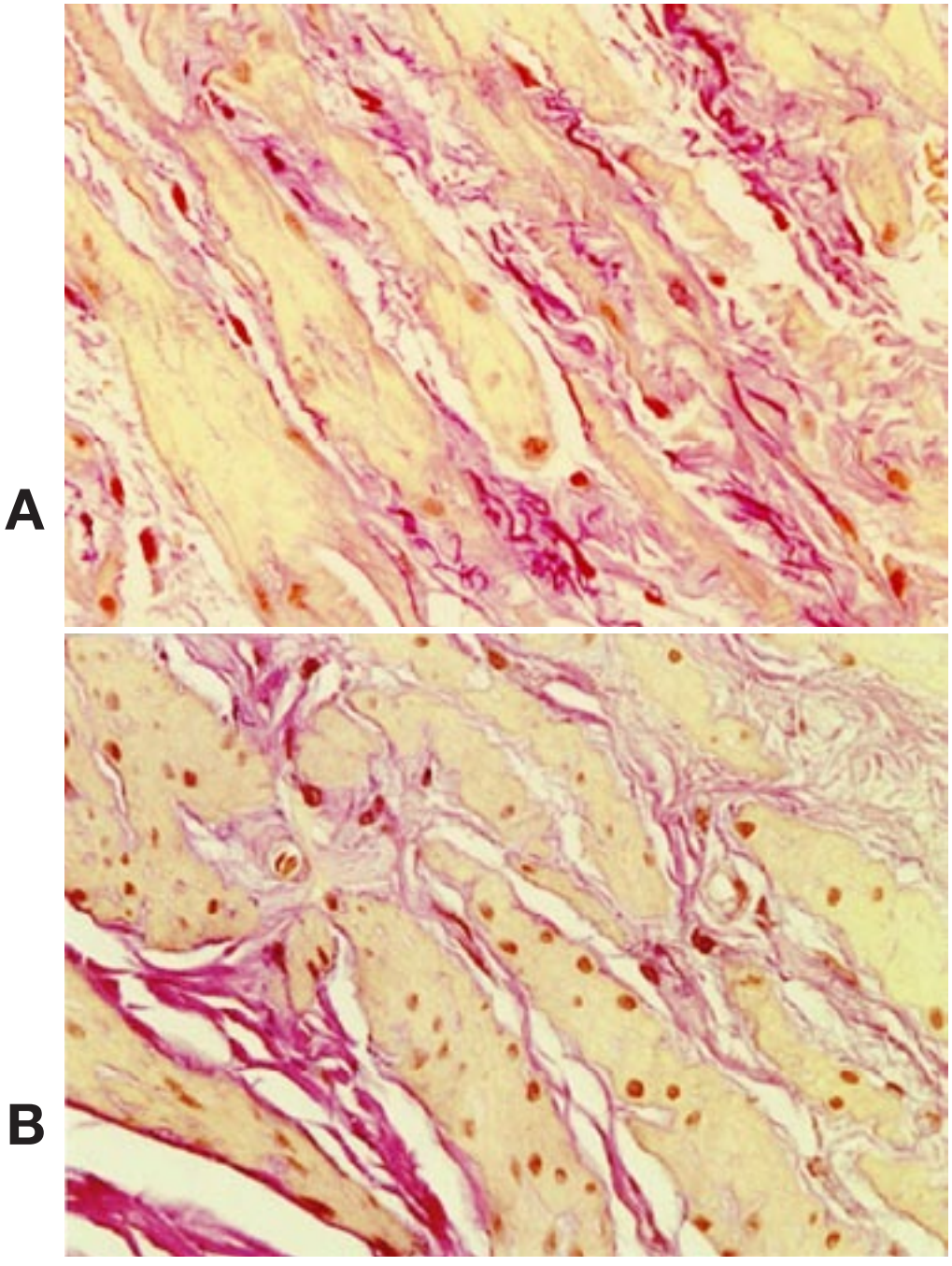

Fig. 1. Light micrographic presentations of sections of the bladder body in elderly male rats (Elastica-van Gieson stain; original magnification, × 400). **A:** an untreated rat (control group). **B:** a testosterone-replaced rat (treated group).

Table 2. Ratio of smooth muscle area/connective tissue area

	Control group [6]	Treated group [6]
Smooth muscle area/connective tissue area	2.59 ± 0.36	3.59 ± 0.13*

Values are mean ± SD of 10 times or more measurements per each rat.
[ ], number of animals.
* Significant difference, $P < 0.05$.

Table 3. Comparison of micturition behavior

Measurement		Control group [6]	Treated group [6]
24-h volume of urine	(mL/day)	15.0 ± 0.8	15.8 ± 1.1
Daily micturition frequency	(time/day)	15.5 ± 1.9	18.8 ± 1.5*
Volume of spontaneous micturition	(mL)	0.98 ± 0.09	0.84 ± 0.07*

Values are mean ± SD.
[ ], number of animals.
* Significant difference, $P < 0.05$.

Table 4. Comparison of urodynamic parameters

		Control group [6]	Treated group [6]
Bladder capacity	(mL)	0.65 ± 0.05	1.03 ± 0.06*
Maximal detrusor pressure	(cmH_2O)	29.8 ± 1.3	34.5 ± 1.3*
Excreted urine†	(mL)	0.42 ± 0.06	0.37 ± 0.07
Residual urine †	(mL)	0.24 ± 0.03	0.66 ± 0.10*

Values are mean ± SD.
* Significant difference, $P < 0.05$.
† During cystometry to measure intravesical pressures.

Histological examination of the rat bladder

The smooth muscle area was significantly ($P < 0.05$) smaller in the control group than in the treated group (Fig. 1 and Table 2)

Micturition behavior

Micturition frequency was significantly ($P < 0.05$) higher in the treated group than in the control group, but the difference in 24-h volume of urine was not significant (Table 3). The average volume of spontaneous micturition was significantly ($P < 0.05$) lower in the treated group than the control group (Table 3).

Urodynamic study

Bladder capacity and volume of residual urine at cystometry were also significantly larger in the treated group. The difference in volume of excreted urine at cystometry was not significant between groups (Table 4).

Discussion

The influence of sex hormones upon the lower urinary tract is of interest. Male and female hormones together regulate the development and functions of lower urinary tract muscles in male rats (Saija et al., 2001). In humans, total plasma testosterone declines by approximately 1% per year on an average from the age of around 40 years (Gray et al., 1991). Due to the concomitant increase of sex hormone binding globulin, free testosterone levels decrease even more steeply from this age. Nonbinding testosterone also decreases with aging (Feldman et al., 2002).

Testosterone exerts a powerful influence on male reproductive organs, including spermatogenesis and mounting behavior (Lunenfeld, 2003); however, its effects on lower urinary tract functions have not been fully characterized. In the present study on its effects on elderly male rats, the level of testosterone we replaced was accord-

ing to Sato et al. (1981). They reported average plasma testosterone levels as 2.34 ± 0.21 ng/mL in 12-month-old rats and 0.78 ± 0.17 ng/mL in 24-month-old rats. The corresponding levels in our study were 2.61 ± 0.69 ng/mL in treated rats, almost the same as in their reproductive rats, and 0.83 ± 0.41 ng/mL in control rats, almost the same as in their elderly rats.

The difference in mean body weight was not significant between both groups. The mean bladder weight was significantly greater in the treated group than the control group. Histologically, the amount of muscle tissues significantly increased in the treated group, and the ratio of smooth muscle area/connective tissue area was significantly higher in the treated group than in the control group. The increase in muscle tissues caused an increase in bladder weight. Prostate weight significantly increased in the treated rats as well.

In our observations of rat micturition behavior, differences between the 2 groups were not significant in 24-h volume of urine. However, the treated group showed significantly higher daily micturition frequency and significantly lower average spontaneous micturition. Through cystometry, the bladder capacity, the volume of residual urine and the maximal detrusor pressure were significantly higher in the treated group than in the control group.

The increase in the ratio of smooth muscle area/connective tissue area appeared to raise the detrusor pressure, and bladder capacity then increased with bladder compliance, as we observed. Usually, average micturition volume increases and daily micturition frequency decrease; however, we observed different results in the present experiments, presumably because residual urine increased due to an increase in prostate weight.

As the structures of the prostate of the rat and the human are different, particularly in the way the ventral and dorsolateral prostate loops around the urethra without compression, the rat model could be considered to be non-obstructive. However, in the present study, testosterone replacement enlarged the prostate, which then compressed the urethra. Thus, lower urinary tract obstruction caused an increase in residual urine volume. This disorder of the lower urinary tract passage caused the smooth muscle of the bladder to swell, and increased the ratio of smooth muscle area/connective tissue area, leading to an increase in detrusor pressure. More studies will be necessry to find out if these changes in the bladder are due to the direct effect of testosterone or the secondary effect of increased urethra resistance.

In humans, testosterone replacement does not significantly increase serum levels of prostate-specific antigen or prostate volume (Ebert, 2004), and the lower urinary tract does not appear to be significantly influenced. However, testosterone replacement caused prostatic hypertrophy and influenced lower urinary tract function in the present study with rats. The prostate maintains the ability to respond to androgens throughout life and the proliferative response of prostatic cells to androgens depends on expression of the intracellular androgen receptor. Following prostate growth to its adult size, the rates of cell proliferation and cell death reach equilibrium, preventing further growth.

However, cellular hyperplasia occurs later in the lives of several species, including humans, dogs and some rat strains despite a decrease in testicular androgen production and a concomitant fall in the peripheral levels of androgen reaching the prostate (Banerjee et al., 2001). The imbalance in cell death and cell proliferation that leads to age-dependent prostatic hyperplasia might be related to increased prostatic sensitivity to androgen. Nuclear androgen receptor expression would be changed with cellular aging, and contribute to the evolution of cellular hyperplasia. In fact, nuclear androgen receptor levels are higher in hyperplastic prostate tissues than in normal tissues (Barrack et al., 1983). Age-related increases in the relative estrogen level, as well as other factors, might increase androgen receptor expression in the aging prostate. Further growth or decrease in cell death results, although androgen is decreased in the peripheral circulation with normal dihydrotestosterone levels in the prostate.

In the present testosterone-replaced elderly

male rats, the amount of bladder smooth muscles, the ratio of smooth muscle area/connective tissue area, bladder capacity and detrusor pressure were increased. However, the prostates were swollen and lower urinary tract passage disorder was induced. Average micturition volume was decreased, and daily micturition frequency increased. Testosterone replacement exerted unfavorable effects on the lower urinary functions of elderly rats, including prostatic hypertrophy.

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Corresponding author: Shuichi Morizane