

IN MALE RATS, CASTRATION LED TO HISTOLOGICAL CHANGES IN THE BLADDER AND BLOOD VESSELS, FURTHER, THE AGE AT WHICH CASTRATION WAS PERFORMED AFFECTED THE DEGREE OF THE HISTOLOGICAL CHANGES.

Hypothesis / aims of study

Although the effects of androgens on the prostate are well established, there are certain symptoms that cannot be explained by changes in prostate size and function alone. Some reports have investigated the relationship between androgens and bladder function, and an evaluation of the effect of androgens on blood vessels has indicated that vascular endothelial cell growth is suppressed due to low testosterone along with enhanced calcification of the blood vessel wall 1). Similarly, a previous report has examined the association between bladder blood flow (BBF) and bladder outlet obstruction; however, there are few studies that examined the association between male hormones and BBF 2). Some reports have compared immature cases with mature cases. For example, rats require testosterone for developing and maintaining the pelvic sympathetic nerve system that controls the bladder 3). From this report, we expect that bladder function is influenced by both the maturity and the time of castration in immature animals. We cannot confirm the clear association between maturity and blood vessels. Therefore, we examined the effects of androgens on BBF, bladder irritability, and histological changes after castration using a castrated Wistar rat model.

Study design, materials and methods

1. Relationship between androgen changes and bladder function: Examination of bladder irritability

Male Wistar rats were classified into the following groups: control unoperated group (control), mature group castrated at the age of 8 weeks (group 8wPC), and immature group castrated at the age of 4 weeks (group 4wPC). Each rat was used at the age of 20 weeks. We assessed androgen and bladder function in all the 3 groups by examining the bladder reaction to irritation. Bladder cystostomy was created under pentobarbital sodium anesthesia. One week later, the rats were placed in metabolic cages, and cystometry was performed without anesthesia or restraint. The bladder was irrigated with normal saline (NS) at room temperature, and 0.25% acetic acid (AA) liquid solution was then injected for 1h. The parameters examined included maximum voiding pressure (cmH₂O) and voiding interval (sec).

2. Differences in BBF caused by androgen changes: Fluorescent microsphere method

Left carotid artery of the rats were cannulated under pentobarbital anesthesia and a constant quantity of fluorescent microspheres were injected intra-arterially, then bladder was excised and weighted. Left femoral artery was simultaneously cannulated to retrieve reference blood. The absorbance of microsphere in blood and bladder tissue were measured by fluorescence microplate reader and the local blood flow rate was calculated. Blood Flow rate is shown as absorbance rate of bladder weight per 1g x reference blood retrieval rate (ml/min) / absorbance rate of entire microsphere within the reference blood (ml/min/g).

3. Examination of androgen-related histological changes in bladder and blood vessels

All rats were sacrificed, and the bladder and iliac artery were removed and histologically examined for differences in smooth muscle and quantity of collagen fiber. We used Mallory-stained specimen for the examination of histological change such as denaturation or fibrosis. Sections of stained tissues were observed under light microscope and the images were captured by a Fujix Digital Camera. The images were analyzed using Photograb-2500 for Macintosh SH-25/MO and Macintosh PowerMac G4, and quantified using Image J 1.46 software. The components of smooth muscles and connective tissues were calculated from at least 10 fields from each tissue section.

Results

1. Relationship between androgen changes and bladder function

No significant difference was noted in the maximum voiding pressure between NS irrigation and AA irrigation among the control, 8wPC, and 4wPC groups (38.7 ± 7.4 to 39.2 ± 1.0 , 40.5 ± 4.3 to 42.6 ± 5.6 , and 36.0 ± 7.2 to 42.0 ± 3.4 [cmH₂O], respectively) (Fig. 1A).

The voiding intervals in each group were shortened ($P < 0.001$) following AA irrigation (482.7 ± 69.2 to 201.3 ± 78.4 , 596.6 ± 79.7 to 270.9 ± 58.7 , and 584.0 ± 60.2 to 283.6 ± 163.2 [sec] for the control, 8wPC, and 4wPC groups, respectively) (Fig. 1B). Further, the voiding intervals for the 8wPC and 4wPC groups were longer than the intervals for the control group; however, the differences were not significant.

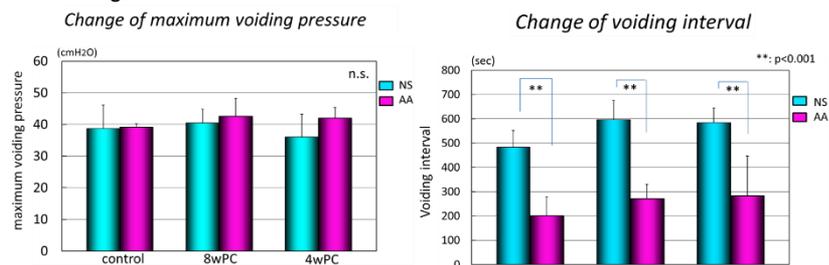


Figure 1A

Figure 1B

2. Differences in BBF caused by androgen changes

The mean BBF rates for the experimental rats were 1.37 ± 0.30 , 1.22 ± 0.46 , and 1.23 ± 0.41 (mL/min/g) for the control, 8wPC, and 4wPC groups, respectively (Fig. 2). Castration led to no changes in the BBF.

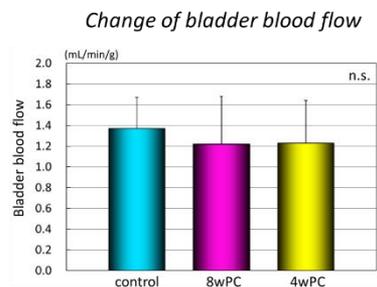


Figure 2

Histological examination of bladder and iliac artery by Mallory staining

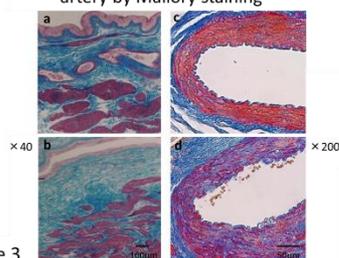


Figure 3

3. Examination of androgen-related histological changes in bladder and blood vessels

Histological examinations of the bladder and iliac artery revealed that the smooth muscles (red) decreased and connective tissues (deep blue) increased in the 4wPC group (Fig. 3). The mean bladder smooth muscle/collagen ratios (m/c ratio) were 3.35 ± 0.72 , 1.75 ± 0.59 , and 1.33 ± 0.35 for control, 8wPC, and 4wPC groups. Compared with the control group, the m/c ratio was lower in the 8wPC and 4wPC groups ($P < 0.01$) (Fig. 4A). The mean m/c ratios at the iliac artery were 2.63 ± 0.67 , 2.24 ± 0.68 , and 0.92 ± 0.46 for control, 8wPC, and 4wPC groups. Compared to the control group, the m/c ratio was lower in 4wPC group ($P < 0.001$) (Fig. 4B).

Smooth muscle/collagen ratio: Bladder

Smooth muscle/collagen ratio: Common iliac artery

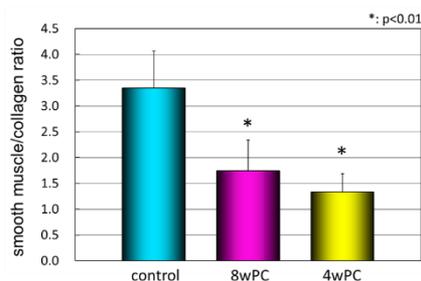


Figure 4A

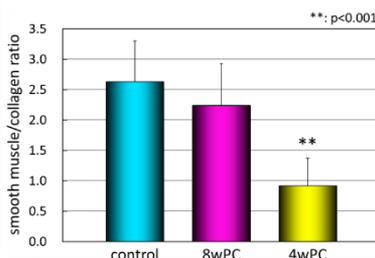


Figure 4B

Interpretation of results

Our findings confirmed that castration led to histological changes not only in the bladder but also in the blood vessels. Further, the age at which castration was performed affected the degree of the histological changes, although bladder irritability was not significantly affected.

Concluding message

This is the first report for considering the age at castration and BBF, bladder function, and histological change by using male Wistar rat. On analysing the relationship between androgen and bladder function, we observed that the histological changes were associated with bladder function but that the contribution of the blood flow was low. We consider that these effects of castration may constitute the key mechanisms underlying LUTS.

References

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Disclosures

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