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Effects of testosterone on the orchidectomized rats

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Abstract

The aim of this study was to determine the changes of body weight, organ weight, hematological values and biochemical parameters by testosterone (Testos) on the orchidectomized (Orch) rats. The animals were divided into 4 groups. Intact group (n=10) received no treatment and operation. Sham group (n=10) received only sham operation and no treatment. Orch group received operation and no treatment. Orch+Testos received operation and testosterone. The body weights of each group increased, but that of Orch+Testos group was significantly lower in Orch+Testos group than in all the other groups. There were significant differences ($P<0.05$, $P<0.001$) of body weights between Orch+Testos group and all the other groups. Also, organ weights such as heart, liver, spleen and kidney were measured. The heart weights were significantly lower ($P<0.001$) in the Orch+Testos group than in all the other groups. The liver weights in the Orch+Testos group were significantly differences in comparison with those in the Sham ($P<0.001$) and Orch group ($P<0.05$). On the other hand, there were no significantly differences in the organ weights of spleen and kidney between the Orch+Testos group and the any other groups. The hematological values of white blood cell (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were no significant differences in any other groups. The concentrations of serum total protein and albumin increased significantly ($P<0.05$) in the Orch+Testos group as compared to that in the Orch group. However, there were no significant differences in Ca, IP and Mg in any other groups. We conclude that testosterone was significantly decreased the body weight in the orchidectomized rats. Our findings suggest that testosterone may influence the process of lipid packaging and absorption in the orchidectomized rats.

Key words : Rat, Testosterone, Orchidectomized

INTRODUCTION

The sex hormones testosterone and estrogen play an important but still poorly understood role in age related male physiology and pathology (Carani et al, 1997). Testosterone is the major gonadal sex steroid produced by the testes in mammalian. Testosterone is also produced in smaller amounts by the ovaries in mammalian. Androgens are critical for differentiation of male gonadal structures prior to birth, for sexual maturation during puberty, and for maintenance of male secondary sexual characteristics and genital function, including spermatogenesis, in adulthood (Clark and Khosla, 2009).

Leydig cells are responsible for testosterone production in the mammalian testis. Testosterone production depends upon stimulation of these cells by luteinizing hormone (LH) that is secreted in pulses into the peripheral circulation by the pituitary gland in response to gonadotropin releasing hormone (GnRH) from the hypothalamus (Zirkin and Chen, 2000). The administration of exogenous testosterone to rats similarly is able to suppress endogenous testosterone production by the Leydig cells via its suppression of LH (Ewing et al, 1983). If exogenous testosterone is administered continuously, as it is when its administration is via Silastic

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implants, LH remains suppressed and Leydig cell testosterone production is severely reduced (Ewing et al, 1983; Keeney et al, 1988).

It is well known that blood testosterone concentration decreases during the course of male aging and results in declines in sexual function, muscle function, bone density, and other physiological parameters (Sarkar, 2009; Basaria, 2010; Zuckerman-Levin et al, 2009). To understand the effects of body weight, organ weight, hematological values and biochemical parameters by testosterone on the orchidectomized rat were investigated.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats, aged 63 days, were purchased from Bio-Safety Research, Chonbuk National University and used for the experiment described below when they were 70 days old. The rats weighing 356～452g were placed in stainless steel wire bottomed plastic cages and housed in a room maintained at 23±1℃ and humidity 55% on 12hrs light/dark cycles. All rats were allowed free access to a pelleted commercial diet and drinking water. After 1 week of acclimation, Rats were randomly assigned to 4 groups with 10 rats in each group. Orchidectomy was performed in the Orch and Orch+Testos groups, animals in the Sham group were subjected to sham operation. Intact group was non operation and treatment. Orch+Testos group was given subcutaneously the Durateston (testosterone, 6mg/ml; testosterone phenylpropionate, 12mg/ml; testosterone isocaproate, 12mg/ml; testosterone decanoate, 20mg/ml. Intervet.) 62mg/kg body weight for 3 times per week from 1 week after surgery.

Orchidectomy

After intraperitoneal anesthesia using ketamine (80 mg/kg) and xylazine (10mg/kg), a midline scrotal incision was made. Testis was surgically resected. Specifically, the both spermatic cord were ligated at the level of the upper part of the scrotum and the intact testis was removed. The scrotum of rats, Sham group, was opened and the testis was exposed. The testis was then replaced in the scrotum and the wound was closed.

Sample preparation

Rats were weighted 2 times per week. After 5 weeks of treatment, rats were killed under anesthesia, blood was collected from the vena cava into heparinized or nonheparinized tubes for hematological values (Scil Vet abc^{TM}, ABX Diagnostics, France) and biological parameters (Spotchem EZ^{TM} SP-4430, ARKRAY, Japan). After blood collection to measure the organ weights, heart, liver, spleen and kidney were removed and stored in saline and then weighted.

Statistical analysis

All values were reported as mean and standard deviation (SD). Significant differences between the values were statistically analyzed using a one-way analysis of variance (ANOVA), followed by a two pairs Student's *t* test. $P<0.05$ or less was considered statistically significant.

RESULTS

Effect of testosterone on body weights

Table 1 shows the effect of body weights on testis re-

Table 1. Effects of testosterone on body weight (BW) of orchidectomized rats

	Intact	Sham	Orch	Orch+Testos
Initial BW (g)	374.8±5.5	402.0±15.2	393.6±26.1	374.4±19.4
After 5 weeks BW (g)	451.2±5.5	471.2±32.6	440.4±36.7	398.6±29.1
BW gain/loss (g)	76.4±5.5	68.4±22.5	46.8±30.9	24.2±14.4^{c,f,l}

Changes in body weight treated with testosterone on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Testos (treated with testosterone on orchidectomized) are shown. ^{c,f,l}indicate the significant differences in values after 5 weeks of Orch+Testos vs Intact, Sham and Orch, respectively. Data are mean±standard deviation. $^{c}P<0.001$ vs. Intact, $^{f}P<0.001$ vs. Sham, $^{l}P<0.05$ vs. Orch

moved rats with testosterone administration. All rats increased body weights by the end of the experiment. Intact (374.8±5.5g) and Orch+Testos group (374.4±19.4g) had similar mean body weights at the start of the study. The body weight of Intact group was increased 76.4±5.5g, whereas it was only increased 24.2±14.4g in the Orch+Testos group. Testosterone prevented the Orch-induced weight gain. The final body weights of Intact (451.2±5.5g), Sham(471.2±32.6g) and Orch group (440.4±36.7g) were significantly higher ($P<0.001$, $P<0.05$) than that of the Orch+Testos group (398.6±29.1g).

Effect of testosterone on organ weights

Organ weights such as heart, liver, spleen and kidney were weighted are shown in Table 2. The hearts of the Orch+Testos group (1.15±0.24g) were significantly lower than those in the Intact (1.60±0.06g), Sham (1.60±0.03g) and Orch group (1.20±0.29g). There were statistically significant differences ($P<0.001$, $P<0.05$) for these organ weights between Orch+Testos and Intact, Sham and Orch group. Also, the livers of the Orch+Testos (14.83±0.44g) were significantly heavier than those in the Orch group (13.68±0.52g) and lower than those in the Sham group (16.00±0.58g) but slightly lower than those in the Intact group (15.25±0.43g). A statistically significant difference in organ weights was observed in the Sham and Orch group when compared with that of the Orch+Testos ($P<0.001$, $P<0.05$), but not between the Orch+Testos and the Intact group. However, the organ weights of spleen and kidney were not significantly different among groups.

Effect of testosterone on hematological values

The hematological values are shown in Table 3. The numbers of WBC were slightly lower in the Intact group (8.1±0.26 10^3/mm^3) than Sham (8.3±0.20 10^3/mm^3), Orch (8.3±0.35 10^3/mm^3) and Orch+Testos group (8.3±0.39 10^3/mm^3). However, there was no statistically significant difference in the numbers of WBC among groups. The numbers of RBC were similar in the Sham (8.1±0.29 10^6/mm^3) and Orch+Testos group (8.1±0.34 10^6/mm^3) and slightly higher than in both the Intact (8.0±0.17 10^6/mm^3) and the Orch group (8.0±0.25 10^6/mm^3). The numbers of RBC were not significantly influenced by the testosterone treatment. Additionally, there was no statistically significant difference for MCV, MCH and MCHC between the groups.

Effect of testosterone on biochemical parameters

The serum concentrations of total protein (T-pro), albu-

Table 2. Effects of testosterone on organ weight of orchidectomized rats

	Intact	Sham	Orch	Orch+Testos
Heart (g)	1.60±0.06	1.60±0.03	1.20±0.29	1.15±0.24^{c,f,l}
Liver (g)	15.25±0.43	16.00±0.58	13.68±0.52	14.83±0.44^{f,l}
Spleen (g)	0.80±0.05	0.80±0.05	0.80±0.09	0.82±0.04
Kidney (g)	3.64±0.35	3.65±0.33	3.27±0.48	3.49±0.35

Changes in organ wet weight treated with testosterone on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Testos (treated with testosterone on orchidectomized) are shown. ^{c,f,l}indicate the significant differences in values after 5 weeks of Orch+Testos vs Intact, Sham and Orch, respectively. Data are mean±standard deviation. $^{c}P<0.001$ vs. Intact, $^{f}P<0.001$ vs. Sham, $^{l}P<0.05$ vs. Orch

Table 3. Effects of testosterone on hematological values of orchidectomized rats

	Intact	Sham	Orch	Orch+Testos
WBC (10^3/mm^3)	8.1±0.26	8.3±0.20	8.3±0.35	8.3±0.39
RBC (10^6/mm^3)	8.0±0.17	8.1±0.29	8.0±0.25	8.1±0.34
MCV (μm^3)	55.2±1.03	55.4±1.77	56.1±1.96	55.7±1.33
MCH (pg)	18.6±0.70	18.7±0.33	19.0±0.65	18.6±0.51
MCHC (g/dl)	34.9±0.50	35.1±0.55	35.2±1.48	34.8±0.90

Changes in hematological values treated with testosterone on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Testos (treated with testosterone on orchidectomized) are shown. Data are mean±standard deviation

Table 4. Effects of testosterone on biochemical parameters of orchidectomized rats

	Intact	Sham	Orch	Orch+Testos
T-pro (g/dl)	7.7±0.49	7.6±0.16	7.5±0.24	7.8±0.28[j]
Alb (g/dl)	5.6±0.31	5.6±0.11	5.5±0.15	5.7±0.28[j]
Ca (mg/dl)	11.2±1.36	11.3±0.72	11.1±0.98	11.3±0.51
IP (mg/dl)	10.1±1.90	10.3±2.13	8.9±1.38	9.0±1.16
Mg (mg/dl)	3.4±0.21	3.4±0.30	3.3±0.29	3.3±0.24

Changes in biochemical parameters treated with testosterone on orchidectomized rats. These data in Intact (non-operated), Sham (Sham-operated), Orch (orchidectomized), Orch+Testos (treated with testosterone on orchidectomized) are shown. jindicates the significant differences in values after 5 weeks of Orch+Testos vs Orch. Data are mean±standard deviation. [j]$P<0.05$ vs. Orch

min (Alb), calcium (Ca), phosphorus (IP) and magnesium (Mg) are shown in Table 4. T-pro and Alb in the Orch+Testos group were 7.8±0.28g/dl and 5.7±0.28 g/dl, respectively, with highest among groups. A statistically significant difference ($P<0.05$) in T-pro and Alb was observed in the Orch+Testos group when compared with that of the Orch group as 7.5±0.24g/dl and 5.5± 0.15g/dl, respectively. However, there was no statistically significant difference in T-pro and Alb between Orch+Testos group and Intact (7.7±0.49g/dl, 5.6± 0.31g/dl) and Sham group (7.6±0.16g/dl, 5.6±0.11 g/dl). In the analysis of Ca, IP and Mg, as shown in Table 4, a statistically significant difference in all of these parameters was not observed in any other groups.

DISCUSSION

In this study, to investigate the effects of testosterone for body weight, organ weight, hematological value and biochemical parameter, Orch+Testos animals were orchidectomized. The results of this study indicate that orchidectomy decreased body weight, and testosterone administration completely prevented the increase in body weight of Orch+Testos group. This finding was in agreement with previous report (del Campo et al, 2008; Evuarherhe et al, 2009). Numerous studies have demonstrated that testosterone is important for skeletal growth during the period of linear growth in males, and is also responsible for maintenance of the skeletal mass in the later stage of life (Guo et al, 1997; Behre et al, 1997). Testosterone deficiency, caused by orchidectomy, has been reported to induce high turnover cancellous osteopenia and cortical osteopenia with decreased periosteal bone formation in rats (Erben et al, 2000; Wakley et al, 1991).

Our findings from this study demonstrated that orchidectomy and testosterone administration significantly decreased rat body weight as compared with intact rats. It is well documented that androgens promote anabolism in the musculoskeletal system while generally repressing adiposity, leading to lean body composition. Circulating androgens decline with age, contributing to frailty, osteoporosis, and obesity (Gentile et al, 2010). Hope et al (1992) demonstrated that decreases in body weight, longitudinal bone growth rate, duodenal calcium transport, and serum Ca and P were exhibited by orchidectomized animals as compared with age matched control animals. Testosterone administration to orchidectomized animals resulted in an increase in body weight, longitudinal bone growth rate, duodenal calcium transport, and serum Ca and P as compared with orchidectomized animals to a level not significantly different from that of age matched control animals. Estradiol administration to orchidectomized animals resulted in an additional decrease in body weight, although no significant effect on duodenal calcium transport, serum Ca, or P was noted as compared with orchidectomized animals. Although our findings demonstrated the loss of sex hormones produced by orchidectomy in male rats changed the serum biochemical parameters, which suggests the occurrence of serum biochemical parameters did not always change in orchidectomized rats (Vandenput et al, 2002; Hope et al, 1992). On the other hand, we have no definitive explanations as to why this contrary result occurs for increase in body weight on the orchidectomized rats by testosterone administration might serve as a source of diet compounds and environments. The changes in testosterone biological charac-

teristics we observed in testis hormone deficiency and testosterone treated animals may have important pathophysiological implications.

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