

·Complementary Medicine·

The testosterone mimetic properties of icariin

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Abstract

Aim: To evaluate the testosterone mimetic properties of icariin. **Methods:** Forty-eight healthy male Sprague-Dawley rats at the age of 15 months were randomly divided into four groups with 12 rats each: the control group (C), the model group (M), the icariin group (ICA) and the testosterone group (T). The reproductive system was damaged by cyclophosphamide (intraperitoneal injection, 20 mg/kg-day) for 5 consecutive days for groups M, ICA and T, at the sixth day, ICA (gastric gavage, 200 mg/kg-day) for the ICA group and sterandryl (subcutaneous injection, 5 mg/rat-day) for the T group for 7 consecutive days, respectively. The levels of serum testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), serum bone Gla-protein (BGP) and tartrate-resistant acid phosphatase activity in serum (StrACP) were determined. The histological changes of the testis and the penis were observed by microscope with hematoxylin-eosin (HE) staining and terminal deoxynucleotidyl transferase biotin-dUTP-X nick end labeling (TUNEL), respectively. **Results:** (1) Icariin improved the condition of reproductive organs and increased the circulating levels of testosterone. (2) Icariin treatment also improved the steady-state serum BGP and might have promoted bone formation. At the same time, it decreased the serum levels of StrACP and might have reduced the bone resorption. (3) Icariin suppressed the extent of apoptosis of penile cavernosal smooth muscle cells. **Conclusion:** Icariin has testosterone mimetic properties and has therapeutic potential in the management of hypoandrogenism. (*Asian J Androl* 2006 Sep; 8: 601–605)

Keywords: andropause; partial androgen deficiency of the aging male; icariin; hypoandrogenism; testosterone; rats

1 Introduction

The terms, andropause, partial androgen deficiency of the aging male (PADAM) or late-onset hypogonadism (LOH), describe a clinical entity which has been defined as a syndrome associated with advanced age. This syndrome is characterized by a deficiency in serum test-

osterone levels that might result in significant alterations in the quality of life and adversely affect the function of multiple organ systems [1]. Profound and diffuse alterations in the reproductive endocrine function and bone metabolism are associated with hypoandrogenism, and will result in a significant decline of physical function and quality of life. Although reports indicate that testosterone supplement therapy (TST) might be an effective way to manage this condition [1], TST has the risk of therapy, and there might be adverse effects on the prostate and cardiovascular risk factors [2].

Icariin (C₃₃H₄₀O₁₅, molecular weight: 676.67) is a flavonoid isolated from the plant *Herba epimedii* [3, 4], which has been used as a tonic drug in traditional Chi-

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nese medicine (TCM) for centuries [5]. Icariin, the main active component of *Herba epimedii*, can improve erectile function [5, 6] and has properties of antifatigue, immunoregulatory and liver function improving [7–9]. Also, it increases the weights of the pituitary gland, testis and epididymis and the testosterone concentration in immature male rats, and has testosterone mimetic properties [10–12]. It also stimulates the differentiation of osteoblasts and increases the activity of alkaline phosphatase (ALP) and the expression of I-collagen protein in rats and promotes bone formation [13, 14].

The aim of the present study was to evaluate the testosterone mimetic properties of icariin after short-term cyclophosphamide treatment followed by icariin or testosterone treatment in male rats.

2 Materials and methods

2.1 Animals and groups

Forty-eight healthy male Sprague-Dawley rats aged 15 months, weighing 220–300 g, were obtained from the Experimental Animal Centre of Shantou University Medical College. They were housed under standard conditions in a controlled environment with a 12-h photoperiod and had free access to food and water. All rats were divided at random into four groups with 12 rats each: the control group (C), the model group (M), the icariin group (ICA) and the testosterone group (T).

2.2 Model building

It is reported that cyclophosphamide treatment results in a significant inhibition in activities of testicular 3β -hydroxysteroid dehydrogenase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD), a decrease in plasma testosterone level and a decrease in the counts of various germ cells [15]. According to our pre-experiment after injecting cyclophosphamide (20 mg/kg-day) for 5 days [16, 17], compared with the control group, the treatment group rats showed phenomena similar to PADAM, such as lowered bodyweight, reduced activity, kraurotic hair, lowered testosterone level, reduced bone formation, increased bone sorption and accelerated degeneration of reproductive organs, which indicated the success of the model. This model was used as the M group in the present study.

2.3 Experimental design

Nothing was administered to the C group. To the M,

T and ICA groups, cyclophosphamide was administered by intraperitoneal injection at a dose of 20 mg/kg-day for 5 consecutive days. At the sixth day, nothing was administered to the M group, and sterandryl was administered by subcutaneous injection at a dose of 5 mg/rat-day for 7 consecutive days in the T group, and icariin (40% purity momoside; Huacui Biotechnology, Xi'an, China) was administered through gastric gavage at a dose of 200 mg/kg-day for 7 consecutive days in the ICA group. Forty-eight rats were sacrificed under 0.3% pentobarital sodium anesthesia, 24 hours after the last icariin and sterandryl treatment. Blood samples (4–5 mL) were obtained from the heart. Serum samples were collected and stored at -70°C for the determination of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), serum bone Gla-protein (BGP) and tartrate-resistant acid phosphatase activity in serum (StrACP). The testes, epididymis and seminal vesicles were dissected and weighed. The left testis and the middle part of the penis were fixed in Bouin's fixative and embedded in paraffin wax. Four-micrometer sections were cut off from the middle portion of the testis and penis, and stained with hematoxylin-eosin (HE) and deoxynucleotidyl transferase biotin-dUDP-X nick end labeling (TUNEL).

2.4 Assessment of effects of ICA on reproduction endocrine function and bone metabolism

Serum testosterone, LH, FSH and BGP levels were determined by radioimmunoassay (RIA). The RIA kits were provided by Beijing North Institute of Biological Products (Beijing, China). Serum StrACP was determined by colorimetry [18]. The kit was provided by the Nanjing Jiancheng Institute of Biological Products (Nanjing, China).

2.5 Cell apoptosis determination

The apoptosis of cavernosal smooth muscle of the penis was determined by TUNEL. The kit was provided by Wuhan Boster Institute of Biological Products (Wuhan, China). All procedures were carried out according to the kit instructions. Cells exhibiting dark brown nuclear staining were considered apoptotic. Negative controls, lacking the labeling enzyme, yielded no reaction product. All counting was carried out on a light microscope using $\times 400$ magnification. Ten visual fields were selected randomly and the average ratio of the number of apoptotic cells to the number of all cells was considered apoptosis rate, that is to say, apoptosis rate = the number of apoptotic cells/

the number of all cells.

2.6 Statistical analysis

The data were expressed as mean ± SD and analyzed by SPSS 10.0 statistical analytical software (SPSS, Chicago, IL, USA). All two-group comparisons were made with unpaired *t*-test. All multiple comparisons were made by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. Differences were considered significant when *P* < 0.05.

3 Results

3.1 Effect of icariin on reproductive organs

As shown in Table 1, the weights of the testis and the seminal vesicles in the C, ICA and T groups were statistically higher than those in the M group. Furthermore, the weight of the seminal vesicles in the T group was statistically higher than that in the ICA and C groups (*P* < 0.05).

3.2 Effect of icariin on reproduction endocrine function

Table 2 shows that in the C, ICA and T groups, the serum testosterone concentration was higher than that in the M group. Furthermore, the concentration of serum testosterone in the ICA group was higher than that in the C group and lower than that in the T group (*P* < 0.05). There were no significant differences in levels of LH and FSH among these four groups.

3.3 Effect of icariin on osseous metabolism

As shown in Table 3, the levels of BGP in the ICA and T groups were statistically higher than those in the M and C groups (*P* < 0.05). Whereas, the levels of StrACP in the C, ICA and T groups were statistically lower than those in the M group (*P* < 0.05). Furthermore, the levels of StrACP in the ICA and T groups were lower than those in the C group (*P* < 0.05).

3.3 Cell apoptosis determination

Table 1. Effect of icariin on the weights of reproductive organs. Data were expressed as mean ± SD (*n* = 12). ^{b,c,h}*P* < 0.05, compared with the M group, C group and ICA group, respectively. C, control group; ICA, icariin group; M, model group; T, testosterone group.

Group	Testis (g)	Epididymis (g)	Seminal vesicles (g)
M	1.1546 ± 0.1214	0.4333 ± 0.0734	0.3608 ± 0.0791
C	1.4296 ± 0.1967 ^b	0.3478 ± 0.1162	0.6064 ± 0.0746 ^b
ICA	1.3714 ± 0.1267 ^b	0.4254 ± 0.0808	0.5117 ± 0.1073 ^b
T	1.4717 ± 0.0579 ^b	0.4776 ± 0.0641	1.0147 ± 0.1581 ^{b,c,h}

Table 2. Effect of icariin on reproduction endocrine function. Data were expressed as mean ± SD (*n* = 12). ^{b,c,h}*P* < 0.05, compared with the M group, C group and ICA group, respectively. C, control group; ICA, icariin group; M, model group; T, testosterone group.

Group	Testosterone (ng/mL)	LH (mIU/mL)	FSH (mIU/mL)
M	0.7878 ± 0.4469	0.1517 ± 0.0407	0.1981 ± 0.0289
C	3.0141 ± 0.4131 ^b	0.1421 ± 0.0114	0.1901 ± 0.1724
ICA	10.9387 ± 2.0392 ^{b,c}	0.1326 ± 0.0125	0.1673 ± 0.0156
T	34.9649 ± 11.4220 ^{b,c,h}	0.1586 ± 0.0613	0.2582 ± 0.2937

Table 3. Effect of icariin on osseous metabolism. Data were expressed as mean ± SD (*n* = 12). ^{b,c}*P* < 0.05, compared with the M group and C group, respectively. C, control group; ICA, icariin group; M, model group; T, testosterone group.

Group	BGP (ng/mL)	StrACP (U/L)
M	1.2210 ± 0.4962	64.8889 ± 12.5748
C	1.8231 ± 0.5004	51.3247 ± 14.1647 ^b
ICA	3.4376 ± 0.5139 ^{b,c}	19.9913 ± 8.6260 ^{b,c}
T	4.5032 ± 1.7013 ^{b,c}	25.7335 ± 7.5724 ^{b,c}

Table 4. Cell apoptosis determination. Data were expressed as mean ± SD (*n* = 12). ^b*P* < 0.05, compared with the M group. C, control group; ICA, icariin group; M, model group; T, testosterone group.

Group	Apoptosis rate
M	0.4828 ± 0.0674
C	0.3968 ± 0.0602
ICA	0.3426 ± 0.0345 ^b
T	0.2265 ± 0.0517 ^b

Table 4 shows that in the ICA and T groups, the apoptosis rate of the cavernosal smooth muscle cells in the penis was lower than that in the M group ($P < 0.05$), and there was no statistical difference between the ICA group and the T group.

4 Discussion

In the aging male, endocrine changes and a decline in endocrine function involve tissue responsiveness, reduced secretory output from peripheral glands and alterations in the central mechanism controlling the temporal organization of hormonal release [19]. Hypogonadism is a clinical condition in which low level of serum testosterone is found in association with specific signs and symptoms, including diminished libido and sense of vitality, erectile dysfunction, reduced muscle mass and bone density, depression and anemia. When hypogonadism occurs in an older man, the condition is often called "late-onset-hypogonadism" or PADAM [20]. By restoring serum testosterone level to the normal range using TST, many of these symptoms can be relieved [21]. Reports indicate that TST might produce a wide range of benefits for men with hypogonadism that include improvement in libido, bone density, muscle mass, body composition, mood, erythropoiesis and cognition [20]. In the present study, after the use of sterandryl in the T group rats, the level of serum testosterone (34.9649 ± 11.4220 ng/mL) increased to the normal range of adult SD rat ($24.24\text{--}39.31$ ng/mL [22]). However, TST has potential undesirable side effects. These concerns are primarily in regards to liver, prostate, lipid profile and cardiovascular system, hematological changes, sleep patterns, social behavior and emotional state [19]. Prostate disease, particularly prostate cancer, and cardiovascular disease are the main safety concerns [23]. Therefore, it is necessary to introduce new treatment drugs.

In the present study, we found that chronic treatment of chemically-induced hypoandrogenic male rats with icariin improved the condition of reproductive organs and increased the circulating level of testosterone. We also found that icariin suppressed the extent of apoptosis of penile cavernosal smooth muscle cells. It seems that icariin possesses testosterone-like activities. However, in the present study, there was no significant difference in serum levels of LH and FSH between ICA group and the others. It seems that icariin mainly affects the testis, but further study is required.

BGP (also called osteocalcin) is the most abundant noncollagenous protein of the bone matrix. It is a small protein (6 000 Da), and is specifically synthesized by the bone-forming cells—the osteoblasts [24]. Studies comparing concentrations of BGP in serum with direct assessment of bone remodeling by iliac crest histomorphometry have shown that this protein is a specific marker of osteoblastic bone formation [25]. Tartrate-resistant acid phosphatase (TRAP) is a functional marker of osteoclasts [26]. Its activity is positively correlated with the activity of osteoclasts. TRAP can be produced by osteoclasts and secreted into the blood. So we can assess the activity of osteoclasts by determining the activity of TRAP in serum [27]. In the present study, we found that icariin treatment also improved the steady-state serum BGP and might have promoted bone formation. At the same time, it decreased the serum levels of StrACP and might have reduced bone resorption. Therefore, icariin treatment is beneficial to bone metabolism.

In summary, icariin shows testosterone mimetic properties, and has therapeutic potential in the management of hypoandrogenism, which is encountered in most of the aging male population. Further experimental and clinical work is required.

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