EFFECT OF TESTOSTERONE PROPIONATE ON LEUCOPOIESIS IN EXPERIMENTAL CONDITION

Delian P. Delev, Ilia D. Kostadinov, Ivanka I. Kostadinova, Maria Georgieva-Kotetarova
Medical University – Plovdiv, Medical Faculty, Department Pharmacology and Clinical Pharmacology; 15A Vassil Aprilov Ave. 4002, Plovdiv, BULGARIA, Mobile: + 359 88 8 713731 . Correspondence to: delevg@gmail.com

ABSTRACT:

Introduction: The aging of the stronger gender is related with progressive with progressive decrease of the levels of serum testosterone (T). The epidemiological studies show an increase of the morbidity and mortality, associated with low levels of T in men in the process of aging. The benefits of testosterone replacement therapy (TRT) are undisputable. The reduction of the normal levels of T is associated with deprivation of erythropoiesis. There is scarce data about the influence of testosterone propionate on leucopoiesis in acute and chronic treatment, as in humans, thus experimentally.

Aim: To study the dynamics in the values of serum testosterone and the number of the leucocytes during replacement therapy with testosterone propionate in dose 4 and 8 mg/kg body weight. (b.w.) in rat model of androgen deficiency.

Materials and methods: 140 male rats (70 for acute and 70 for chronic trial) were used, distributed in the following groups-control orchiectomized .simulative operated controls treated with 4 and 8 mg/kg b. w. testosterone propionate, orchiectomized animals, aged male controls and trial animals treated with 4 and 8 mg/kg b. w. testosterone propionate.

Results and discussion: Orchiectomy lowered significantly the levels of serum T at the 15 days trial and insignificantly at chronic one. The supplementation with testosterone propionate raised its levels at the higher dose. In the aged rats an increase of the serum levels of T was observed, a result from the application of its propionate salt. Significance was received when the dose 8 mg/kg b. w. was used. Castration significantly increased the number of the leucocytes at the 15 days traced animals, compared with the sham operated ($p = 0, 04$). The application of testosterone does not change significantly this effect in both doses studied. Respectively the groups treated with 4 mg/kg b. w. and 8 mg/kg b. w. testosterone propionate significantly ($p = 0,008$; $p = 0,033$) differ from sham operated controls by the number of leucocytes. At the chronic trial testosterone propionate significantly raises the number of the leucocytes towards controls as in dose 4 mg/kg b. w. ($p = 0,011$; $p = 0,007$), thus in dose 8 mg/kg b. w. ($p < 0,0001$). There is no authoritative difference in the influence over the number of the leucocytes between the two doses.

Conclusions: 1. 8 mg/kg b.w. of testosterone propionate, applied in rat model of androgen deficiency restores the physical T levels. **2.** Leucocyte stimulation was observed in 15 days testosterone propionate treated rats with androgen deficiency.

Key words: *testosterone propionate, leucocytes, leucopoiesis androgen deficiency, rat model.*

Introduction: The process of aging of the stronger gender is related with progressive decrease of the level of the serum testosterone (T)[1]. Epidemiological researches show an increase in morbidity and mortality, associated with low level of T in men with the progress of age. The benefits of testosterone replacement therapy are indisputable. Libido and sexual function are improved, bone density, muscle strength[2], mood and cognitive functions are increased, cardio-vascular risk and the manifestations of metabolic syndrome are diminished[3]. T has favorable effect on vascular reactivity, inflammation, production of cytokines, expression of adhesion molecules, insulin resistance, concentrations of serum lipids and factors of the hemostasis[4] etc.

Androgens influence hemostasis. Before the introduction of the recombinant hemopoetic growth factors, they have been used as basic pharmacological instruments for stimulation of the production of erythrocytes. Indications for treatment have been aplastic anemia[5] and renal

insufficiency[6]. The reduction of the normal serum levels of T is connected with a deprivation of erythropoiesis. The data for the influence of testosterone propionate on leucopoiesis in acute and chronic treatment is scarce.

Aim: To study the dynamics in the values of serum T and the number of the leucocytes during hormone replacement therapy with testosterone propionate in 4 and 8 mg/kg body weight (b. w.) in rat model of androgen deficiency.

Material and method:

140 male Wistar rats were used, weight from 270 to 380 grams. The design of the experiment is approved by the Bulgarian Drug and Food Agency (License №21/19.03.2012) and decision of the Local Ethical Committee at MU Plovdiv, protocol №3/25.07.2012. The animals are distributed in groups (Table 1).

Table 1. *Groups Description*

Група	Легенда	Описание
1.	КМХ	Control group young castrated animals
2.	СОХ	SHAM operated chronic treated young animals
3.	МХ4	Young, chronic treated animals with testosterone 4 mg/kg b.w.
4.	МХ8	Young, chronic treated animals with testosterone 8 mg/kg b.w.
5.	КСХ	Control group chronic old treated animals
6.	СХ4	Old, chronic treated animals with testosterone 4 mg/kg b.w.
7.	СХ8	Old, chronic treated animals with testosterone 8 mg/kg b.w.
8.	КМО	Control group young, castrated, acute treated animals
9.	МСО	SHAM operated, acute treated animals
10.	МО4	Young, acute treated animals with testosterone 4 mg/kg b.w.
11.	МО8	Young, acute treated animals with testosterone 8 mg/kg b.w.
12.	СО4	Old, acute treated animals with testosterone 4 mg/kg b.w.
13.	СО8	Old, acute treated animals with testosterone 8 mg/kg b.w.
14.	КСО	Control group old, acute treated animals

The young animals in this experimental study are 6 months old with average weight 275±5,1grams. The old rats are above 3 years old with average weight 376 ± 6,2 grams. After previously carried out castration or simulative operation and acclimatization of 14 days the rats are injected i. m. (back thigh muscle, gluteus) once a week, as follows (Table 2).

Table 2. *Experimental design*

Group	Abbrev.	N	Treatment	Duration
1.	СОХ	10	0,5 ml Oleum helianti (Sopharma)	15 weeks
2.	КМХ	10	0,5 ml Oleum helianti (Sopharma)	15 weeks
3.	МХ4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
4.	МХ8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
5.	КС	10	0,5 ml Oleum helianti (Sopharma)	15 weeks
6.	СХ4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
7.	СХ8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
8.	КМО	10	0,5 ml Oleum helianti (Sopharma)	15 days
9.	МСО	10	0,5 ml Oleum helianti (Sopharma)	15 days
10.	МО4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
11.	МО8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
12.	СО4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
13.	СО8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
14.	КСО	10	0,5 ml Oleum helianti (Sopharma)	15 days

During the experiment all the animals were bred in standard laboratory conditions. Air temperature 26 ± 1°C, relative humidity 65 ± 5%, free access to food and tap water.

Blood collection was gathered through decapitation under ether narcosis, bellow glass bell filled with vapors of diethyl ether for 60 seconds. The samples received are sent immediately in the Department of Clinical Laboratory at MU Plovdiv. Total testosterone is tested trough ELISA kit of DRG International, USA cat. № EIA – 1559 with analyzer: SIRIO – microplate reader, SEAC, ITALY. The number of leucocytes was observed on automatic hematological counter- Coutler-T 660, USA.

Statistical analyses were carried out with package SPSS 22.0 (Statistical Package for Social Science) for Windows 8.1. For all of the indexes is calculated average value (Mean) and standard error (SEM). In all analyses differences with $p<0.05$ are determined as statistically significant. In normal distribution, the values are juxtaposed through Independent Samples T-test. Tables and figures are built with program package Microsoft Office 2013. Applications MS Word and MS Excel are used.

Results:

Orchiectomy significantly lowered the levels of serum testosterone at the 15 days trial and insignificantly at the chronic one (fig. 1). Supplementation with testosterone propionate raised its levels with significance at the higher dose. We observed an increase in the serum levels of T at the aged male rats too, a result from the application of its propionate salt. Significance was received at the chronic use of dose 8 mg/kg b. w.

Castration significantly raised the number of leucocytes in 15 days traced animals, compared with the sham operated (p = 0, 04). The application of T doesn't change significantly this effect in both doses tested. Respectively the groups treated with mg/kg b. w. and 8 mg/kg b. w. testosterone propionate differ significantly (p = 0,008; p = 0,033) from the simulative operated control by the number of leucocytes (fig. 1).

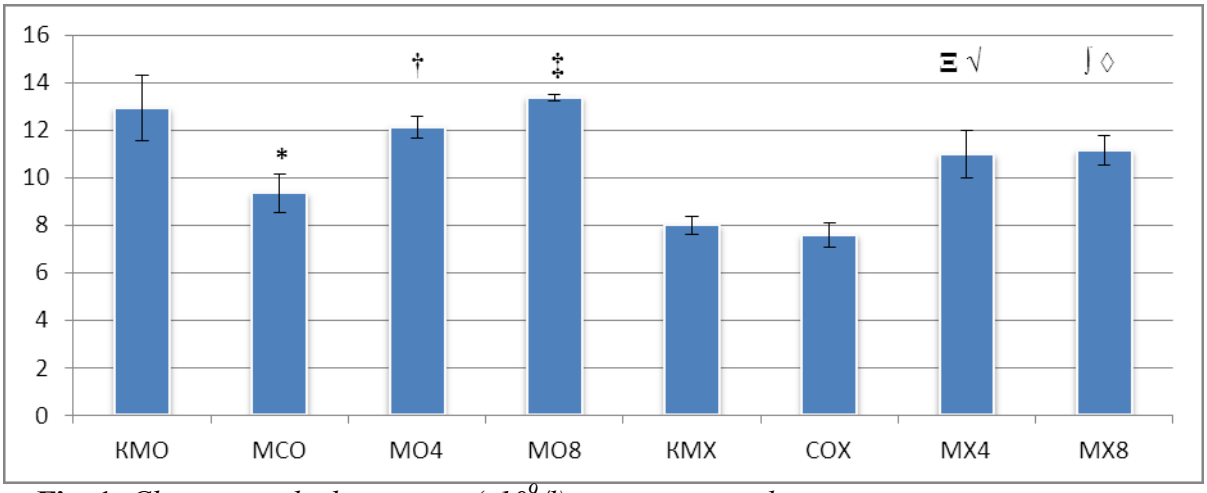

Fig. 1. *Changes in the leucocytes (x10^9/l) – young animals:*

* -P= 0,04 towards КМО; † -P= 0,008 towards МСО; ‡ -P= 0,033 towards МСО; Ξ – P= 0,011 towards КМХ; √ -P= 0,007 towards СОХ; ∫ -P< 0,0001 towards КМХ; ◊ -P< 0,0001 towards СОХ.

There is no statistically significant difference in the traced index between the castrated and the simulative operated controls at the chronic trial. Testosterone propionate significantly raises the number of leucocytes towards control groups as in dose 4 mg/kg b. w. (p = 0,011; p = 0,007), thus in dose 8 mg/kg b. w. (p < 0,0001). There is no authoritative difference in the influence of the number of leucocytes between the two doses (Table 3).

The duration of treatment does not influence significantly the number of leucocytes in both doses tested.

The acute and chronic treatment of aged male rats with testosterone propionate in dose 4 and 8 mg/kg b. w. doesn't change significantly the number of leucocytes. There is no authoritative change in this index in comparison between the two doses and in juxtaposing of acute and chronic treated animals with equal doses.

Table 3. *Leucocyte count ($x10^9$/l) in acute and chronic treated old male rats.*

Group	n	Mean ± SEM	T	p
KCO	10	10,77 ± 0,81	0,74	0,47
CO4	10	11,43 ± 0,37		
KCO	10	10,77 ± 0,81	0,6	0,56
CO8	10	11,41 ± 0,68		
CO4	10	11,43 ± 0,37	0,026	0,98
CO8	10	11,41 ± 0,68		
KCX	10	10,56 ± 0,93	0,65	0,53
CX4	10	11,86 ± 1,59		
KCX	10	10,56 ± 0,93	0,84	0,42
CX8	10	9,57 ± 0,74		
CX4	10	11,86 ± 1,59	1,25	0,24
CX8	10	9,57 ± 0,74		
CX4	10	11,86 ± 1,59	0,2	0,84
CO4	10	11,43 ± 0,37		
CX8	10	9,57 ± 0,74	1,83	0,085
CO8	10	11,41 ± 0,68		

Discussion:

Testosterone participates in the regulation of both erythropoiesis and leucopoiesis. In the literature available exists multiple data for the role of T in the immune regulation, which is realized by the impact over T- and B-lymphocyte population. The thymus is the most probable site of action of the androgens, as its size depends on the androgen status[7]. Orchiectomy is related with the development of thymomegaly, which is seen even in adult animals[8,9]. Significant enlargement of the thymus is established in androgen-resistant conditions[10] too. All this is accompanied by an increase in the number of the circulating T-cells[11]. The expression of androgen receptors is proven as in the thymus, thus in the bone marrow.

They are found in the lymphoid and non-lymphoid cells of these organs. In trials with transgenic mice is established, that the androgen receptors localized on the thymus epithelia cells are of more vital importance for the androgen-induced involution of the thymus[12]. The exact mechanism of action is unknown, but it is supposed that T by the means of receptors stimulates the thymocyte apoptosis[13]. In the bone marrow, the androgen receptors are also found in the stromal cells. The castration of C57 BL/6 mice (androgen sensitive cell line) leads to increase of the B-cell subpopulation in the spleen and bone marrow, but subsequent application of T leads to reverse effect only in the bone marrow[14]. Probably the spleen is not a target of the androgens.

Androgen receptors are found in the immature cell elements of the thymus and the bone marrow and disappear before their migration in the peripheral lymphoid organs. This is the main sign these receptors differ from the estrogen ones[15], which are expressed in the peripheral lymphoid tissues and organs[16].

In clinical studies of men with hypogonadothropial hypogonadism and primary hypogonadism is established increased number of the T-lymphocytes in the periphery as in men above 50 this increase has a weaker expression, than in the younger ones. Age probably is a major in the expansion of T-cells in hypogonadial conditions. Replacement therapy with T at this clinical observation returns the lymphocyte number back to the standard[17].

Conclusion:

1. 8 mg/kg b.w. of testosterone propionate, applied in rat model of androgen deficiency restores the physical T levels.

2. Leucocyte stimulation was observed in 15 days testosterone propionate treated rats with androgen deficiency.

[1] Gruenwald DA, Matsumoto AM. Testosterone supplementation therapy for older men: potential benefits and risks. J Am Geriatr Soc. 2003 Jan; 51 (1) : 101- 15; discussion 115.

[2] Sharma V, Perros P. The Management of hypogonadism in aging male patients. Postgrad. Med. 2009 Jan; 121 (1): 113- 21.

[3] Bassil N, Alkaade S, Morley JE. The benefits and risks of testosterone replacement therapy: a review. Ther Clin Risk Manag. 2009 Jun; 5 (3): 427- 48.

[4] Jones TH. Testosterone deficiency: a risk factor for cardiovascular disease? Trends in Endocrinology & Metabolism 2010 Apr 6 [Epub ahead of print] PubMed PMID: 20381374.

[5] Locasciulli A, Porta F, Vossen JM, Bacigalupo A: Treatment of acquired severe aplastic anaemia (SAA) in children: An analysis of the EBMT-SAA working party (abstract). Bone Marrow Transplant (Suppl 2) 4:90, 1989.

[6] Dainiak N: The role of androgens in the treatment of anemia of chronic renal failure. Semin Nephrol 5:147, 1985.

[7] Henderson J. On the relationship of the thymus to the sexual organs: I. The influence of castration on the thymus. J Physiol. 1904; 31:222–229. PubMed: 16992746]

[8] Greenstein B, Fitzpatrick F, Adcock I. et al. Reappearance of the thymus in old rats after orchidectomy: inhibition of regeneration by testosterone. J Endocrinol. 1986; 110:417–422. PubMed: 3760740

[9] Olsen NJ, Watson MB, Henderson GS. Et al. Kovacs WJ. Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. Endocrinology.1991 Nov;129(5):2471-6.

[10] Olsen NJ, Watson MB, Kovacs WJ. Studies of immunological function in mice with defective androgen action. Distinction between alterations in immune function due to hormonal insensitivity and alterations due to other genetic factors. Immunology. 1991 May;73(1):52-7.

[11] Sutherland J, Goldberg G, Hammett M. et al. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol. 2005; 175:2741–53.

[12] Olsen NJ, Kovacs WJ. Effects of androgens on T and B lymphocyte development. Immunol Res. 2001;23(2-3):281-8.

[13] Olsen NJ, Viselli SM, Fan J. et al. Androgens accelerate thymocyte apoptosis. Endocrinology. 1998 Feb;139(2):748-52.

[14] Viselli SM, Reese KR, Fan J. et al. Androgens alter B cell development in normal male mice. Cell Immunol. 1997 Dec 15;182(2):99-104.

[15] Cutolo M, Accardo S, Villaggio B. et al. Arthritis Rheum. 1993 Aug;36(8):1087-97. Presence of estrogen-binding sites on macrophage-like synoviocytes and CD8+, CD29+, CD45RO+ T lymphocytes in normal and rheumatoid synovium.

[16] Cohen JH, Danel L, Cordier G. et al. Sex steroid receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. J Immunol. 1983 Dec;131(6):2767-71.

[17] Nancy J, Kovacs J. Evidence That Androgens Modulate Human Thymic T Cell OutputInvestig Med. 2011 January ; 59(1): 32–35.