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Testosterone propionate stimulates leuco- and thrombocytopoiesis in rat model of androgen deficiency

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ABSTRACT

There is scarce data about the influence of testosterone propionate on leucopoiesis in acute and chronic treatment, as in humans, thus experimentally. There is clinical and molecular-biological data that the sex hormones, and especially androgens influence the number and the functions of the thrombocytes. The aim of this study is to measure the dynamics in the values of serum testosterone and the number of the leucocytes and platelets during replacement therapy with testosterone propionate in dose 4 and 8 mg/kg body weight. (b.w.) in rat model of androgen deficiency. Conclusions: 1. 8 mg/kg b.w. of testosterone propionate, applied in rat model of androgen deficiency restores the physiological T levels. 2. Leucocyte stimulation was observed in 15 days testosterone propionate treated rats with androgen deficiency. 3. Transient platelet stimulation was observed after testosterone propionate administration in rats with androgen deficiency.

KEYWORDS testosterone propionate, leucopoiesis, thrombocytopoiesis.

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. The data for the influence of testosterone propionate on leucopoiesis in acute and chronic treatment is scarce. There is clinical and experimental data that sex hormones and especially androgens influence the number and function of thrombocytes. There are suggestions that androgens can activate the coagulation factors or thrombocyte activity, thus causing arterial or venous thrombosis [5], [6]. This could be a problem in the androgen replacement therapy.

Aim:
To study the dynamics in the values of serum T and the number of the leucocytes and platelets 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*

group	Legend	Description
1	KMX	Control group young castrated animals
2	COX	SHAM operated chronic treated young animals
3	MX4	Young, chronic treated animals with testosterone 4 mg/kg b.w.
4	MX8	Young, chronic treated animals with testosterone 8 mg/kg b.w.
5	KCX	Control group chronic old treated animals
6	CX4	Old, chronic treated animals with testosterone 4 mg/kg b.w.
7	CX8	Old, chronic treated animals with testosterone 8 mg/kg b.w.
8	KMO	Control group young, castrated, acute treated animals
9	MCO	SHAM operated, acute treated animals
10	MO4	Young, acute treated animals with testosterone 4 mg/kg b.w.
11	MO8	Young, acute treated animals with testosterone 8 mg/kg b.w.
12	CO4	Old, acute treated animals with testosterone 4 mg/kg b.w.
13	CO8	Old, acute treated animals with testosterone 8 mg/kg b.w.
14	KCO	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 (sham) 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 Legend	N	Treatment	Duration
COX	10	0,5 ml Oleum helianthi (Sopharma)	15 weeks
KMX	10	0,5 ml Oleum helianthi (Sopharma)	15 weeks
MX4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
MX8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
KC	10	0,5 ml Oleum helianthi (Sopharma)	15 weeks
CX4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
CX8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 weeks
KMO	10	0,5 ml Oleum helianthi (Sopharma)	15 days
MCO	10	0,5 ml Oleum helianthi (Sopharma)	15 days
MO4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
MO8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 days

CO4	10	4 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
CO8	10	8 mg/ kg b.w Testosterone propionate (Sopharma)	15 days
KCO	10	0,5 ml Oleum helianthi (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 and platelets 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.

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).

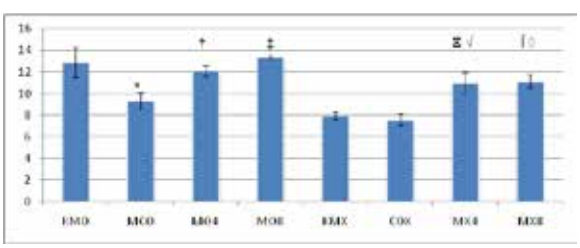

Fig. 1. *Changes in the leucocytes (x10⁹/l) – young animals:*

* -P= 0,04 towards KMO; † -P= 0,008 towards MCO; ‡ -P= 0,033 towards MCO; Ξ – P= 0,011 towards KMX; √ -P= 0,007 towards COX; ∫ -P< 0,0001 towards KMX; ◊ -P< 0,0001 towards COX.

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 (data not shown).

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.

Orchiectomy doesn't change significantly the values of thrombocytes as in acute, thus in chronic study. In line with this fact, there is no statistically authoritative change in the values of this index in both doses of testosterone propionate, applied acute or chronic to the castrated animals. Significant changes in the values of thrombocytes are seen only in the chronic treated old rats in both doses studied (p = 0,012; p = 0,002) (data not shown). The observed change is in direction of rise of their number. It can be affirmed that this process is transitional, as in comparison to the duration of both doses their number significantly lowers (p= 0,043; p = 0,001) (Fig.2).

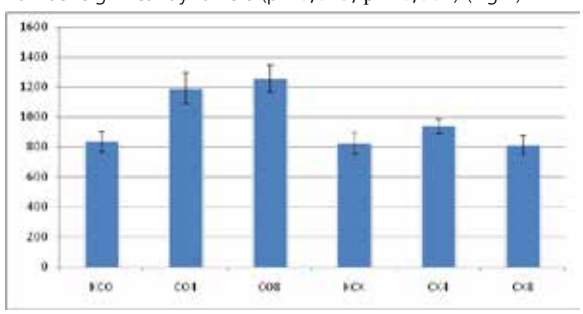

Fig. 2. *Changes in thrombocyte count (x109/l) – aged male rats:* * -P= *0,012 towards* KCO*;* † -P= *0,002 towards* KCO*;* ‡ -P= *0,043 towards* CO4*;* Ξ – P= *0,001 towards* CO8

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].

In the field of hemostasis the major indexes that T influences are fibrinogen, plasminogen activation inhibitor- 1 (PAI-1) and the thrombocyte aggregation. There is data that T lowers fibrinogen and PAI-1 [18]. The experimental data from rats shows that dihydrotestosterone inhibits H_2O_2 – induced thrombocyte aggregation. Besides it is increased in castrated and rats pretreated with the androgen antagonist – flutamid. Because of the action of dihydrotestosterone reduced levels of TxA2 are seen too [4].

In the literature available, we can see experimental and clinical data that T stimulates thrombocytopoiesis. In patients with myelodisplastic syndrome raise the thrombocyte count [19]. In the effect of anabolic preparations and testosterone enantate are marked differences. The latter significantly more expressive raises the number of thrombocytes even in present resistance to the action of the anabolic medicine metenolon acetate [20]. It is affirmed that in women with ovarian cancer the thrombocytosis, which is a bad prognostic mark is androgen mediated [21]. The orchiectomy in mice decreases the thrombocyte number, while T restores the thrombocytopoiesis [22].

In the current study, orchiectomy even insignificantly lowers the thrombocyte number both in the acute and in the chronic trial. The expected increase by the application of testosterone propionate is insignificant too and is seen only in the 15 weeks treatment.

Probably the androgens mediate their effects over the thrombocytopoiesis by receptor means. Megakaryocytes and thrombocytes express iRNA for androgen receptors on the principle of positive feedback with T. Unlike the classical androgen receptors, which are situated in cytoplasm and have to be transported to the nucleus in order to influence the gene expression, the receptors on the thrombocytes are not genome. The latter are localized on the membrane and lead to an increase of the intercellular calcium levels. These receptors lead to more rapid effector response. This fact explains the observed rapid and significant raise of the thrombocyte number only in the group of acute treated aged male rats. This thrombocytosis is transitional and was not established in the group of chronic treated ones probably because of involvement of contra regulatory mechanisms.

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.
3. Transient platelet stimulation was observed after testosterone propionate administration in rats with androgen deficiency.

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