EFFECT OF TESTOSTERONE PROPIONATE ON THROMBOCYTOPOIESIS IN EXPERIMENTAL CONDITION

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ABSTRACT:

Introduction: There is clinical and molecular-biological data that the sex hormones, and especially androgens influence the number and the functions of the thrombocytes. There are suggestions that androgens can activate the coagulation factors or the thrombocyte activity, thus causing arterial or venous thrombosis. This could be a problem when androgen replacement therapy is taking place.

Aim: To study the dynamics in the values of serum testosterone and the number of the thrombocytes 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, sham 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.

Orchiectomy does not change significantly the values of thrombocytes as in acute, thus in chronic study. Respectively there is no statistically authoritative change in the values of this index from the both doses testosterone propionate studied, applied acute or chronic to the castrated animals. Significant changes in the values of thrombocytes are seen only at the acute treated old rats in both doses studied ($p=0,012$; $p = 0,002$).The observed change is in direction of increase in their number. It can be claimed that this is transitional, as compared by duration of both doses their number significantly lowers ($p = 0,043$; $p = 0,001$).

Conclusions: 1. 8 mg/kg b.w. of testosterone propionate, applied in rat model of androgen deficiency restores the physiological T levels. **2.** Transient platelet stimulation was observed after testosterone propionate administration in rats with androgen deficiency.

Key words: *testosterone propionate, thrombocytes, thrombocytopoiesis, androgen deficiency, rat model.*

Introduction:

The problem about male “menopause” was introduced for the first time in the 16th century in Chinese texts of internal diseases. Even in ancient times it was considered that the testicles maintain man energetic and full of strength. Many authors prove that the level of T decreases in process of aging of men. Christ-Crain reports about a presence of relative hypogonadism in every 5th man above 60 years. Similar changes are also seen in young men with androgen deficiency Likewise the age-determined anemia is at least partially related with lowered levels of circulating androgens. The data mentioned above proves that androgens regulate erythropoiesis by stimulating the puberty up-regulation in boys and play a role in the maintenance of normal hemoglobin levels in adult men[1]. There is clinical and microbiological 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[2,3]. 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 thrombocytes 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	Abbreviation	Description
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	Abbreviation	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^{o}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 2010. 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. 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.

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 ($p = 0,012$; $p = 0,002$) (table.3). 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.1).

Table 1. *Comparison of the thrombocyte count ($x10^9/l$) in acute and chronic treated aged male rats.*

Групи	Брой	Mean ± SEM	T	P
КСО	10	839 ± 68,89	2,86	0,012*
СО4	10	1193 ± 103,22		
КСО	10	839 ± 68,89	3,76	0,002*
СО8	10	1261,9 ± 89,05		
СО4	10	1193 ± 103,22	0,5	0,62
СО8	10	1261,9 ± 89,05		
КСХ	10	826,1 ± 69,37	1,37	0,2
СХ4	10	943,7 ± 50,59		
КСХ	10	826,1 ± 69,37	0,075	0,94
СХ8	10	818,9 ± 65,6		
СХ4	10	943,7 ± 50,59	1,51	0,15
СХ8	10	818,9 ± 65,6		
СХ4	10	943,7 ± 50,59	2,18	0,043*
СО4	10	1193 ± 103,22		
СХ8	10	818,9 ± 65,6	4,01	0,001*
СО8	10	1261,9 ± 89,05		

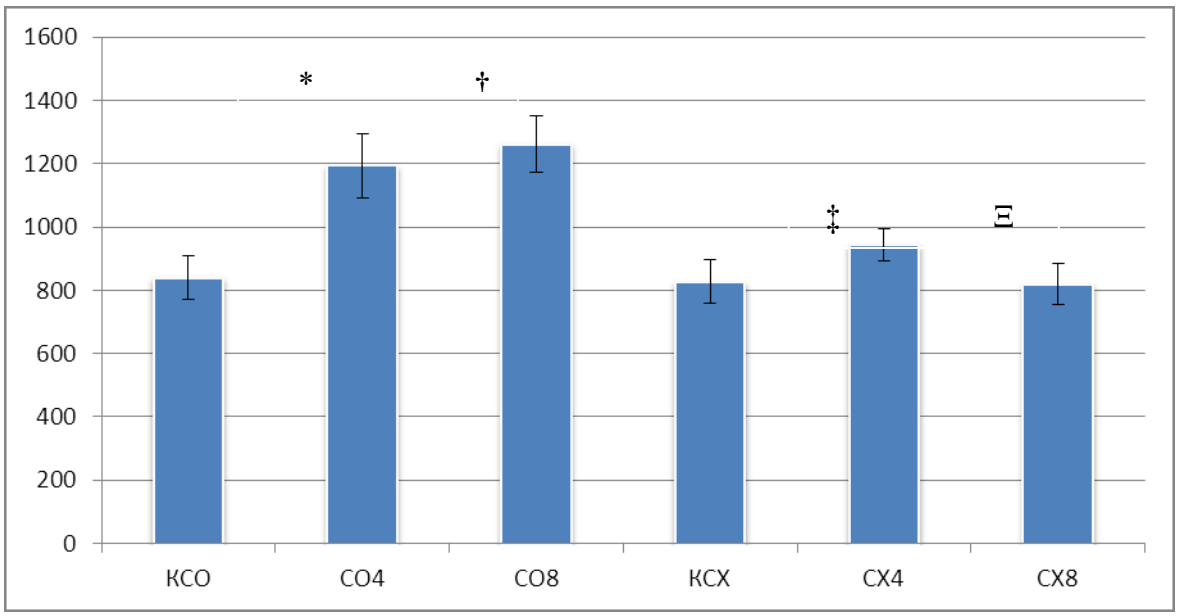
* Significant

Discussion:

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[4]. 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[5]. 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[6]. It is affirmed that in women with ovarian cancer the thrombocytosis, which is a bad prognostic mark is androgen mediated[7]. The orchiectomy in mice decreases the thrombocyte number, while T restores the thrombocytopoiesis[8].



Фиг. 1. *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*

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[9],[10]. 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[11]. 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 physiological T levels.

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

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