EFFECT OF TESTOSTERONE PROPIONATE ON ERYTHROPOIESIS AFTER EXPERIMENTAL ORCHIECTOMY

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ABSTRACT

INTRODUCTION: Androgen deficiency anemia occurs most frequently in pharmacogenic suppression of androgen synthesis or with advancing age in men. Bilateral orchiectomy is a surgical modality used in the treatment of metastatic prostate carcinoma. It is accompanied by marked decrease in circulating serum levels of androgens.

AIM: The aim of the experimental study was to determine the effect of substitution therapy with testosterone propionate (TP) on some haematological parameters of erythropoiesis in male rats after orchiectomy.

MATERIAL AND METHODS: Eighty Wistar male rats with mean weight of 252.3 g were used in the study. The animals were allocated into 2 control orchidectomized groups, 2 sham-operated groups and 4 experimental orchidectomized groups. Testosterone propionate was administered intramuscularly, once a week at a dose of 4 mg and 8 mg per kilogram of body weight for 15 days and for 15 weeks. Erythrocyte count was performed and hemoglobin and hematocrit levels were measured.

RESULTS: In the chronic experiment there was a significant decrease in red blood cells and hemoglobin, and a tendency of decrease in hematocrit after orchiectomy. The effect of TP on erythropoiesis in orchietomised rats is dose-dependent.

CONCLUSION: TP replacement therapy in doses of 4 mg/kg and 8 mg/kg has a stimulating effect on erythropoiesis only in chronic administration.

Key words: testosterone propionate, erythrocytes, hemoglobin, hematocrit, experimental andropause

INTRODUCTION

Male climacteric is a term used first in 1939 to describe the physiological changes occurring in men after the age of 50.1 They are associated with decreased libido, mood swings, reduced muscle mass and strength, increased visceral fat, reduced bone mineral density etc.2 Similar changes occur in young men with androgen deficiency. With advancing age, the levels of testosterone, produced by the Leydig cells, decline progressively in elderly men3 and rats4.

Androgens affect erythropoiesis.5 They were used as major pharmacological agents to stimulate erythrocyte production before the introduction of recombinant hematopoietic growth factors. In an experimental model of heart failure testosterone improves cardiac function and mobilizes bone marrow stem cells.6 Indications for treatment were aplastic anemia, and renal failure.7 Testosterone plays an important role in circadian rhythm of bone marrow function in adult rats.9 Reduction of normal serum testosterone levels is associated with suppression of erythropoiesis. It has been found in most experimental and case-control studies of testosterone treatment in elderly men that it stimulates erythropoiesis and increases hematocrit 3% - 5% above the normal range. High hematocrit values (55% - 60%) lead to a significant increase in blood viscosity. Erythrocytosis during testosterone therapy has been found in 6% to 25% of adult individu-
Erythrocytosis occurs both in parenteral and enteral administration and it is less frequent when transdermal therapeutic agents are used. Despite abundant clinical data changes in the concentrations of sex hormones that have hemorheological consequences, have not been well studied in a model with orchiectomy. It is known that hemorheological parameters show gender differences that might be altered by experimental bilateral orchiectomy.

**AIM**

The aim of the present experimental study was to investigate the effect of testosterone propionate on the level of erythrocyte count, hemoglobin and hematocrit in conditions of acute and chronic treatment of male orchietomised rats.

### MATERIAL AND METHODS

The study included 80 male Wistar rats with a mean weight of 252.3 g. The design of the experiment was approved by the Bulgarian Food Safety Agency (license No 21 of 19.03.2012) and by the Ethics Committee at the Medical University of Plovdiv (protocol No 3 of 25.07.2012). All animals were divided into groups (Table 1) and treated as shown in Table 2.

**Orchiectomy of male rats**

General anesthesia was performed with midazolam (Dormicum, Roche) (2.5 mg/kg bdw i.p.) and fentanyl (Fentanyl, Richter-Gedeon (0.25 μg/kg bdw i.p.). After the anesthesia the animals fell soundly asleep and woke up after 20 ± 2 minutes.

A 10-mm-long skin incision was made on the scrotum apically. The incision was extended 5

### Table 1. Description of the groups in the experimental study

<table>
<thead>
<tr>
<th>Group</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOAC</td>
<td>Sham operated 6-month-old animals in acute treatment</td>
</tr>
<tr>
<td>2</td>
<td>COAC</td>
<td>Control group of 6-month-old orchiectomized rats in acute treatment</td>
</tr>
<tr>
<td>3</td>
<td>O4AC</td>
<td>Orchiectomized 6-month-old testosterone treated rats (4 mg/kg b.w.) in acute treatment</td>
</tr>
<tr>
<td>4</td>
<td>O8AC</td>
<td>Orchiectomized 6-month-old testosterone treated rats (8 mg/kg b.w.) in acute treatment</td>
</tr>
<tr>
<td>5</td>
<td>SOCHR</td>
<td>Sham operated 6-month-old rats in chronic treatment</td>
</tr>
<tr>
<td>6</td>
<td>COCHR</td>
<td>Control group of 6-month-old orchiectomized rats in chronic treatment</td>
</tr>
<tr>
<td>7</td>
<td>O4CHR</td>
<td>Orchiectomized 6-month-old testosterone treated rats (4 mg/kg b.w.) in chronic treatment</td>
</tr>
<tr>
<td>8</td>
<td>O8CHR</td>
<td>Orchiectomized 6-month-old testosterone treated rats (8 mg/kg b.w.) in chronic treatment</td>
</tr>
</tbody>
</table>

### Table 2. Experimental protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>Abbreviation</th>
<th>n</th>
<th>Treatment</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOAC</td>
<td>10</td>
<td>0.5 ml Oleum helianti</td>
<td>15 days</td>
</tr>
<tr>
<td>2</td>
<td>COAC</td>
<td>10</td>
<td>0.5 ml Oleum helianti</td>
<td>15 days</td>
</tr>
<tr>
<td>3</td>
<td>O4AC</td>
<td>10</td>
<td>4 mg/kg b.w. testosterone propionate</td>
<td>15 days</td>
</tr>
<tr>
<td>4</td>
<td>O8CHR</td>
<td>10</td>
<td>8 mg/kg b.w. testosterone propionate</td>
<td>15 days</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>8</td>
<td>O8CHR</td>
<td>10</td>
<td>8 mg/kg b.w. testosterone propionate</td>
<td>15 weeks</td>
</tr>
</tbody>
</table>
mm to the left and right testicular sacs. Cauda epididimis was pulled out with the testis followed by caput epididimis. Vas deferens was ligated along with the spermatic blood vessels. The next step was dissection and removal of the testes. The incisions were then closed using silk threads and treated with topical antibiotic (gentamycin, Sopharma). The castrated animals were placed in individual cages for 48-hour recovery with free access to food and water.

**SHAM ORCHIECTOMY OF MALE RATS**

General anesthesia was performed with midazolam (Dormicum, Roche) (2.5 mg/kg bdw i.p.) and fentanyl (Fentanyl, Richter-Gedeon (0.25 μg/kg bdw i.p.).

A 10-mm-long incision was made on the scrotum apically. The incision was extended another 5 mm to the left and right testicular sacs. Cauda epididimis was pulled out along with the testis followed by caput epididimis. Then cauda epididimis and the testis were returned to their normal anatomic positions leaving the blood vessels intact. Finally the incisions were closed using silk threads and treated with topical antibiotic (Gentamycin, Sopharma). The sham operated animals were placed in individual cages for 48 hours to recover with free access to food and water.

During the experiment, all animals were kept under standard laboratory conditions: air temperature 26 ± 1 °C, relative humidity 65 ± 5%, free access to food and water. TP and oleum helianti were injected once a week in the thigh muscle of the animals’ hind legs. Blood was collected after decapitation under ether anesthesia, under a glass bell filled with vapors of diethyl ether for 60 seconds. The blood samples were sent immediately to the Central Clinical Laboratory of Medical University - Plovdiv.

**HEMATOLOGICAL PARAMETERS**

Hematological parameters (complete blood count) were determined with Coulter counter T-660. Erythrocyte count, hemoglobin and hematocrit levels were followed up. Although hematocrit is a derivative of erythrocyte count, it was monitored for the purpose of completeness of the survey.

**STATISTICAL ANALYSIS**

Statistical analyses were performed with IBM SPSS 20.0 for Windows 7. For each parameter the mean and standard error (SEM) were calculated. The Kolmogorov-Smirnov test was used to determine the distribution. In normal distribution values were compared using the Independent Samples t-test. In non-Gaussian distribution parameters were compared by the nonparametric two independent samples test (Mann-Whitney U test). In all analyses, a difference of p < 0.05 was accepted as statistically significant.

**RESULTS**

The results of the hematology tests are presented in Table 3.

**ERYTHROCYTES**

In the acute castrated rats testosterone propionate in a dose of 4 mg/kg bdw (p = 0.002) reduced the erythrocyte count statistically significantly (Fig. 1). TP in the higher dose (8 mg/kg bdw) had the same effect on erythropoiesis although it could not reach statistical significance. Erythrocyte count was

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Erythrocytes (x10¹²/l)</th>
<th>Hemoglobin (g/l)</th>
<th>Hematocrit (l/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOAC</td>
<td>10</td>
<td>10.14 ± 0.16</td>
<td>155.1 ± 2.98</td>
<td>0.49 ± 0.001</td>
</tr>
<tr>
<td>COAC</td>
<td>10</td>
<td>9.83 ± 0.16</td>
<td>156.4 ± 1.19</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td>O4AC</td>
<td>10</td>
<td>8.51 ± 0.31</td>
<td>141.1 ± 3.94</td>
<td>0.397 ± 0.015</td>
</tr>
<tr>
<td>O8AC</td>
<td>10</td>
<td>9.49 ± 0.17</td>
<td>150.0 ± 2.39</td>
<td>0.45 ± 0.008</td>
</tr>
<tr>
<td>SOCHR</td>
<td>10</td>
<td>6.92 ± 0.17</td>
<td>145.2 ± 2.19</td>
<td>0.37 ± 0.004</td>
</tr>
<tr>
<td>COCHR</td>
<td>10</td>
<td>6.07 ± 0.21</td>
<td>139.4 ± 2.11</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>O4CHR</td>
<td>10</td>
<td>7.62 ± 0.32</td>
<td>151.5 ± 1.87</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>O8CHR</td>
<td>10</td>
<td>7.61 ± 0.06</td>
<td>152.7 ± 3.34</td>
<td>0.404 ± 0.008</td>
</tr>
</tbody>
</table>

Data are presented as $\bar{x} \pm S_x$. 

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*Table 3. Erythrocytes, hemoglobin and hematocrit*
reduced significantly in the sham operated rats by TP in both doses (p < 0.0001, p = 0.011). The higher dose stimulated erythropoiesis significantly (P = 0.013).

In chronically treated animals the erythrocyte count was significantly high (p = 0.006) in sham operated animals in which steroidogenesis is preserved compared to the orchiectomised. The administration of testosterone propionate in both doses significantly increased erythrocyte count compared with the orchiectomised animals (p = 0.001, p < 0.0001, respectively). In sham operated rats the difference reached statistical significance only with the higher dose (p = 0.001) compared to treated animals. There were no significant differences in the examined parameter between the two doses.

**Figure 1.** Changes in the erythrocyte count (×10¹²/l).

**Figure 2.** Changes in hemoglobin (g/l) – young animals.
HEMOGLOBIN

A 15-day treatment with testosterone propionate at doses of 4 and 8 mg/kg bdw decreased hemoglobin statistically significantly (p = 0.002, p = 0.032) in comparison to the orchiectomised controls (Fig. 2). When compared to sham operated animals the lower dose significantly decreased this parameter (p = 0.012), and with the high dose there was no significant change. There were no significant differences in the effect when comparing the two doses.

Both doses increased statistically significantly hemoglobin in chronically treated animals compared with the hemoglobin values in orchiectomised animals (p < 0.0001, p = 0.004). Just like in the acutely treated animals, here there was no significant change in the hemoglobin values between the groups treated with both doses.

The duration of treatment significantly increased hemoglobin values (p = 0.028) only at a dose of 4 mg/kg bodyweight.

Hematocrit

A 15-day supplementation with testosterone propionate in both tested doses decreased statistically significantly hematocrit values compared with the orchiectomised (p < 0.0001, p = 0.044) and the sham operated group (p < 0.0001, p = 0.008). The higher dose significantly increased the hematocrit values (p = 0.006) in acutely treated animals (Fig. 3).

In the chronic experiment hematocrit increased significantly only with the higher dose tested in comparison with the two control groups (p = 0.003, p = 0.004). Logically, testosterone propionate at a dose of 8 mg/kg bodyweight had a statistically significant effect on the examined parameter compared to a dose of 4 mg/kg bodyweight (p = 0.009).

DISCUSSION

The obtained data show that after 15-day acute treatment castrated male rats did not show significant differences in the values of erythrocytes, hemoglobin, and hematocrit compared to sham controls. This effect is probably not due to a stimulating action of the estrogens on erythropoiesis even though the earlier data point at such a possibility.15 There is quite a recent hypothesis that testosterone per se activates erythropoiesis and there is no need to convert it into estrogens to achieve the above effect, unlike some of its biological effects.16 The most likely reason for the lack of statistical difference between the two controls is the short period of monitoring. In the above-mentioned data from clinical trials, the earliest period to monitor these indicators is set at one month. In chronic treatment we can observe a significant decrease in red blood cells and hemoglobin, as well as a tendency of hematocrit decrease.

The exact mechanism of testosterone action on erythropoiesis is unclear. Although men and women differ in their erythrocytes count, hemoglobin and hematocrit values (these being higher in men) they have comparable levels of erythropoietin and soluble

![Figure 3. Changes in hematocrit (l/l).](image-url)
transferrin receptor (sTfR). On the other hand, in the androgen-induced erythrocytosis erythropoietin levels are low, rather than high. Use of androgens in the treatment of aplastic anemia and anemia of renal disease is effective, despite the absence of erythropoietin stimulation. In a model of chronic renal failure in rats, testosterone propionate in a dose of 2 mg/kg bodyweight stimulates hematopoiesis and increases the levels of hematocrit and hemoglobin. More recent studies with follow-up of expression of mRNA of serum erythropoietin and its levels in orchietomised rats or those with androgen deficiency (induced by flutamide or cyproterone) demonstrate that expression of EPO and its serum levels are not altered in all three models of androgen deprivation. In an experimental study in rats it was found that the effect of erythropoietin on the proliferation of erythroblasts is totally blocked by flutamide (5-alpha reductase inhibitor), and partially by cyproterone. It is possible that testosterone stimulates erythropoiesis by direct affecting the bone marrow hematopoietic stem cells. This direct effect is mediated through the induction of insulin-like growth factor (IGF-1) of the androgen-receptor mediated mechanism. In the available literature there is evidence that testosterone enhances the absorption of iron, its incorporation into red blood cells and hemoglobin synthesis.

Anemia is partially associated with reduced levels of circulating androgens. Polycythaemia represents one of the undesirable risks of testosterone replacement therapy which requires monitoring.

Literature search we did of mechanism of testosterone action on erythropoiesis explains the observed effect of stimulation in orchietomised rats only at their long-term treatment. The effect was slow, since it is receptor-mediated, and testosterone receptors are genetically active. Therefore, in a 15-day application the monitored parameters of castrated animals (despite the treatment) are more often reduced than increased.

Of the three parameters under observation the effect is dose-dependent only for hematocrit, where the higher dose had a significantly stronger effect. Since this is a leading parameter for the clinical diagnosis of anemia, we can conclude that the effect of testosterone on erythropoiesis in orchietomised rats is dose-dependent.

CONCLUSIONS

In the chronic experiment there was a significant decrease in red blood cells and hemoglobin, and a tendency of decrease in hematocrit after orchietomy. Replacement therapy with testosterone propionate has a stimulating effect on erythropoiesis only in chronic administration.

REFERENCES

ЭФФЕКТ ТЕСТОСТЕРОН - ПРОПИОНАТ НА ЭРИТРОПОЭЗ ПОСЛЕ ОРХИЭКТОМИИ В ЭКСПЕРИМЕНТЕ

Д. Делев, Д. Давчева, И. Костадинов, И. Костадинова

РЕЗЮМЕ

ВВЕДЕНИЕ: Андроген-дефицитная анемия наблюдается чаще всего при фармакологическом подавлении андрогенов или с возрастом у мужчин. Биологическая функция эритропоэза представлена методом, применяемым в терапии метастатической карциномой предстательной железы, сопровождающейся выраженным понижением циркулирующих сывороточных уровней андрогенов.

ЦЕЛЬ: Установить влияние заместительной терапии тестостерон - пропионатом - ТП на некоторые гематологические показатели эритропоза у мужских крыс после орхиэктомии.

МАТЕРИАЛ И МЕТОД: Использовано 80 мужских крыс породы Wistar, средняя масса тела – 252,3 г. Животные разделены следующим образом: 2 контрольные орхиэктомированные, 2 симулированные оперированные и 4 опытные орхиэктомированные группы. ТП применен мышечным раз в неделю в дозе 4 и 8 мг/кг массы тела; период применения – 15 дн. и 15 нд. Пролонгированный: число эритроцитов, уровень гемоглобина и гематокрита.

РЕЗУЛЬТАТЫ: При хроническом применении наблюдается сенситивное снижение эритроцитов и гемоглобина и тенденция к снижению гематокрита после орхиэктомии. Эффект ТП на эритропозу у крыс с орхиэктомией доказаны.

ЗАКЛЮЧЕНИЕ: Заместительная терапия с помощью ТП в дозах 4 и 8 мг/кг оказывает стимулирующий эффект на эритропоз только в случаях продолжительного применения.