Original Article

ACUTE AND CHRONIC TESTOSTERONE PROPIONATE REPLACEMENT THERAPY DOES NOT LEAD TO HEPATOTOXICITY IN OLD MALE WISTAR RATS

Delian P. Delev,
Ilia D. Kostadinov,
Ivanka I. Kostadinova,
Kremena E. Saracheva[1]

Dept. of Pharmacology and Clinical Pharmacology,
[1]Student of Pharmacy,
Pharmacy Faculty,
Medical University – Plovdiv
Bulgaria

Corresponding Author:
Delian P. Delev,
Dept. Pharmacology and Clinical Pharmacology
Medical University Plovdiv
15A, Vassil Aprilov str.
e-mail: delevg@gmail.com

Received: November 10, 2011
Revision received: November 20, 2011
Accepted: December 22, 2011

Summary

The aging of the strong gender is related wth progressive decrease of the level of serum testosterone. Testosterone replacement therapy is beneficial in this population. But it is also correlated with some risks, such as hepatotoxicity and increased blood viscosity. The aim of the current study is to establish the early /15 day/ and the late /15 week/ manifestating changes in alaninaminotransferase (ALT), aspartataminotransferase (AST), lactatedehydrogenase (LDH) and alkaline phosphatase(AP). 24 months aged male wistar rats were divided in six groups: acute and chronic control groups, treated with oleum helianthi and 4 testing groups, treated with 4 and 8 mg testosterone propionate (T) for period of 15 days and 15 weeks. Significant changes in the values of serum transaminases were observed only about AST in the group, treated with 4 mg T for the 15 day period. The values of AP were significantly decreased in the chronic treated groups, compared with the control group. Significant decrease in the levels of the serum LDH was manifested in all testing groups, compared with the controls. The acute and chronic testosterone treatment does not increase the values of the aminotransferases. It is observed age- dependent elevation in the activities of AP and LDH. The values of latter are significantly decreased after acute and chronic testosterone administration.

Key words: liver hepatotoxicity, testosterone, testosterone replacement therapy

Introduction

Serum testosterone levels decline gradually and progressively with aging in men [1]. Epidemiological studies show increased morbidity and mortality, associated with low testosterone levels in aging males. Men with coronary artery disease have significantly lower levels of androgens than normal controls, challenging the preconception that physiologically high levels of androgens in men account for their increased relative risk for coronary artery disease [2]. Testosterone deficiency leads to acceleration of the atherosclerotic changes in the endothelium [3] and takes place in the pathogenesis of the coronary and cardiac diseases [4]. The levels of the serum testosterone are lower in men with diabetes mellitus, compared to non diabetic men [5].

As the duration of life and advance in age increase, the count of the men with partial androgen deficiency will continue to rise up. The benefits of testosterone replacement therapy in elderly men are undeniable.

Several studies indicate that testosterone replacement therapy may produce a wide range of benefits for men with hypogonadism, including improvement in libido, bone density, muscle mass, body composition, mood, and cognition [6], decreases the risk of cardio-vascular diseases [7] ant the manifestations of the metabolic syndrome [8].

Testosterone replacement in the short-term reduces waist circumference, cholesterol and circulating pro-inflammatory cytokines and improves insulin sensitivity and glycemic control in diabetics [9]. In the US alone, approximately 2 billion prescriptions for testosterone were written in 2002. This represents a 30% increase from 2001 and a 170% increase from 1999. There has also been a 500% increase in prescription sales in the past 10 years [10]. Several risks may appear in therapeutic appliance of testosterone preparations as: malignant transformation of the benign prostate hyperplasia, hepatotoxicity and increased blood viscosity.

The 17 alpha-alkylated steroids seem to be implicated in the development of cholestatic jaundice, peliosis hepatis, and liver tumors [11]. There are sporadic reports for hepatocytolysis [12]. On the other hand there is data that the dehydroepiandrostendione stimulates the regeneration of the hepatocytes [13], and testosterone has a protective effect in rats with induced hepatotoxicity [14].

The aim of the present study is to determine the early (15th day) and the late (15th week) manifested changes in the values of the hepatocytolytic (ALT, AST), and the holestatic indexes (LDH, AP), that follow testosterone propionate replacement therapy of 24 months old male rats.

Materials and Methods

24 months old male Wistar rats were used, with average weight of 358 grams, divided into the following groups (each group contains 10 animals). All experiments were approved by the local committee for animal experiments welfare of Medical University – Plovdiv. No dead animal trough the experiment was registered.

Cac – control group, for the acute trial (15 days); Cchr – control group, for the chronic trial (15 weeks); 4ac – trial group, treated with 4 mg of testosterone, once a week for 15 days period; 8ac – trial group, treated with 8mg of testosterone, once a week for 15 days period; 4chr – trial group, treated with 4 mg of testosterone, once a week for 15 weeks period and 8chr – trial group, treated with 8 mg of testosterone, once a week for 15 weeks period.

The animals were placed under standard laboratory conditions: 12:12 dark-light cycle, 45% relative air humidity; room temperature 26.5±1^0 C and free access to food and water.

The male rats were treated the following way:

Control group, for the acute trial (Cac) – 0,5 ml Oleum Helianthi (Sopharma) *i.m.* once a week; Control group, for the chronic trial (Cchr) – 0,5 ml Oleum Helianthi (Sopharma) *i.m.* once a week;

Trial group (4ac), treated with 4mg of testosterone propionate *i.m.* once a week for 15 days period;

Trial group (8ac), treated with 8mg of testosterone propionate, once a week *i.m.* for 15 days period;

Trial group (4chr), treated with 8 mg of testosterone propionate *i.m.*, once a week for 15 weeks period;

Trial group (8chr), treated with 8 mg of testosterone propionate *i.m.*, once a week for 15 weeks period.

Our choice of the lower TP dosage used was based on the following facts: a single TP dose of 4 mg/kg BW8 mg/kg body weight is sufficient to restore physiological levels of serum testosterone for about 1 week in castrated sedentary young adult male Wistar rats [15, 16], while it almost doubles circulating blood testosterone levels in their intact counterparts [17].

The blood was collected through decapitation, done under ether narcosis. The tests were sent for analysis by clinic – chemical analyzer: Konelab 60i, Thermo electron Co (USA) in the Central clinic laboratory of the Medical University of Plovdiv. The following three indexes were observed: ALT, AST, AP and LDH.

The received results were interpreted with the method of variation analysis from the software product SPSS 19.0. For each of the indexes were determined mean and standard error of mean (SEM). The comparison of the results for each index between the groups was

done with Independent Samples t-test.

Results

There have been no statistically important differences in the values of ALT found in the treated animals, compared with the control groups (Fig. 1). The values of AST were lowered with statistic significance in trial group, treated with 4 mg of testosterone, once a week for 15 days period (4ac) towards Cac (control group for the acute trial, Fig. 2). Significant decrease in the levels of AP in trial group, treated with 4 mg of testosterone, once a week for 15 weeks period (4chr) and the trial group, treated with 8 mg of testosterone, once a week for 15 weeks period (8chr) towards the control group, for the chronic trial (Cchr) was registered (Fig. 3). Both of the used doses caused significant decrease of the levels of LDH, in the groups of acute and chronic treated animals compared to the control (Fig. 4).

Discussion

Testosterone preparations are applied in therapy of male hypogonadism from decades. In the recent years the indications of their appliance have spread. The benefits of T-supplementation in men with metabolic syndrome, ischemic heart disease and coronary insufficiency are indisputable [6, 18]. However there is also data of the possible risks of the therapy with T-preparations, especially when the serum levels

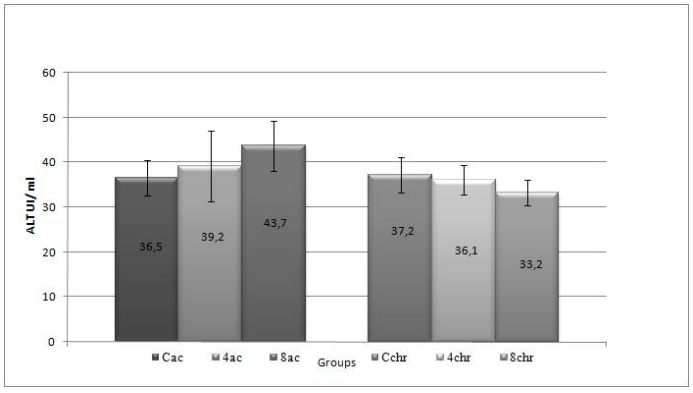

Figure 1. Changes in the values of ALT.
Cac – control group (n=10) for the acute trial (15 days); Cchr - control group (n=10), for the chronic trial (15 weeks); 4ac – trial group (n=10), treated with 4 mg of testosterone, once a week for 15 days period; 8ac–trial group (n=10), treated with 8mg of testosterone, once a week for 15 days period; 4chr – trial group (n= 10), treated with 4 mg of testosterone, once a week for 15 weeks period and 8chr – trial group (n= 10), treated with 8 mg of testosterone, once a week for 15 weeks period. No significant difference between the groups.

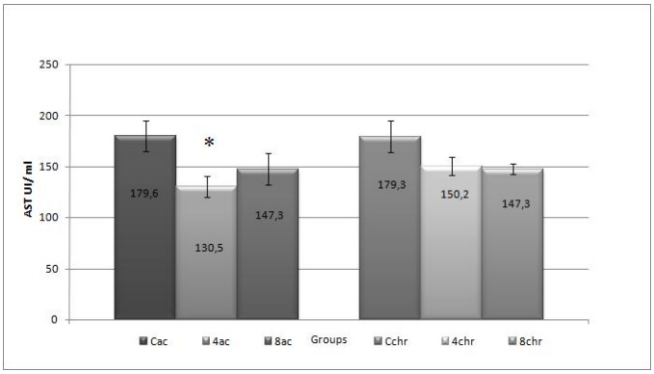

Figure 2. Changes in the values of AST.
Cac – control group (n=10) for the acute trial (15 days); Cchr - control group (n=10), for the chronic trial (15 weeks); 4ac – trial group (n=10), treated with 4 mg of testosterone, once a week for 15 days period; 8ac – trial group (n=10), treated with 8mg of testosterone, once a week for 15 days period; 4chr – trial group (n= 10), treated with 4 mg of testosterone, once a week for 15 weeks period and 8chr – trial group (n= 10), treated with 8 mg of testosterone, once a week for 15 weeks period.
* P = 0.015 compared with Cac.

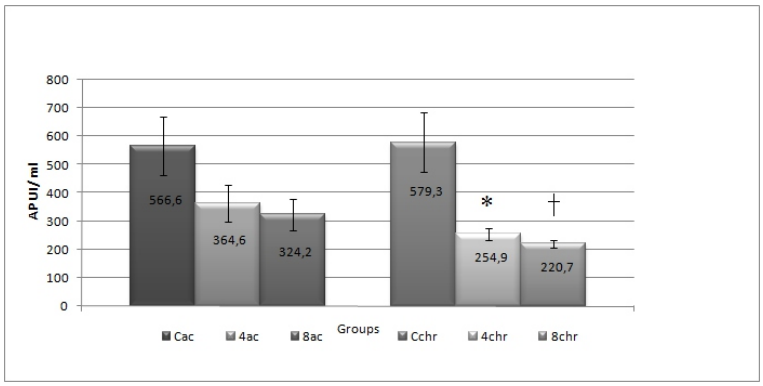

Figure 3. Changes in the values of AP.
Cac – control group (n= 10) for the acute trial (15 days); Cchr - control group (n=10), for the chronic trial (15 weeks); 4ac – trial group (n=10), treated with 4 mg of testosterone, once a week for 15 days period; 8ac – trial group (n=10), treated with 8mg of testosterone, once a week for 15 days period; 4chr – trial group (n=10), treated with 4 mg of testosterone, once a week for 15 weeks period and 8chr – trial group (n=10), treated with 8 mg of testosterone, once a week for 15 weeks period.
* P = 0.008 towards Cchr; † P = 0.003 towards Cchr

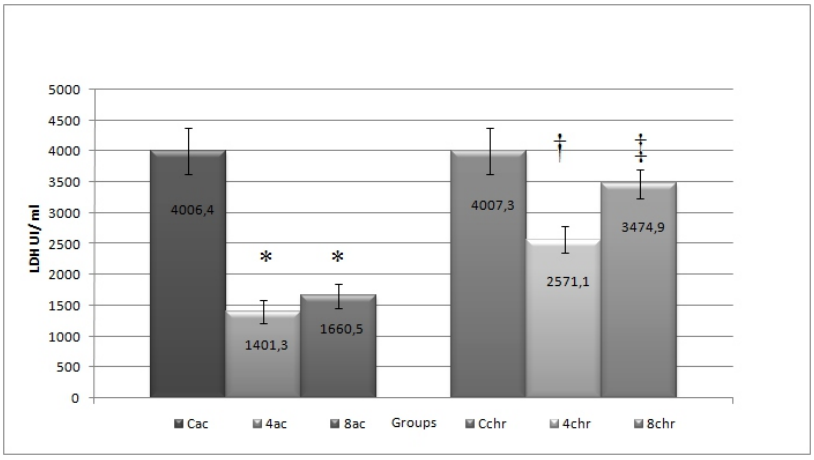

Figure 3. Changes in the values of LDH.
Cac – control group (n= 10) for the acute trial (15 days); Cchr - control group (n=10), for the chronic trial (15 weeks); 4ac – trial group (n=10), treated with 4 mg of testosterone, once a week for 15 days period; 8ac – trial group (n=10), treated with 8mg of testosterone, once a week for 15 days period; 4chr – trial group (n=10), treated with 4 mg of testosterone, once a week for 15 weeks period and 8chr – trial group (n=10), treated with 8 mg of testosterone, once a week for 15 weeks period.
* P < 0.0001 compared to Cac; † P = 0.004 compared to Cchr; ‡ P = 0.008 compared to Cchr.

have reached values above the physiologic, as it is when they are used by healthy people. The androgen anabolic steroids are synthetic hormone derivates of testosterone. The short term and long term therapy with them leads to undesired reactions in various organs, but one of their most serious complications is the toxicity to the liver. Five weeks therapy with nandrolone decanoate increases the values of the transaminases and the AP as a manifestation of hepatolysis and cholestasis. 17-alfa alkilined testosterone derivates (methyltestosterone) find application in the therapy of senile hypogonadism. Then often develops cholestasis with paradoxically low levels of AP [10]. The use of testosterone undecanoate with low serum levels of testosterone and metabolic syndrome does not increase the transaminase activity [19]. On the contrary, it even improves the liver indexes in obesity induced liver steatosis [20]. The received results show, that the propionic salt of testosterone has a similar safety with the undecanoate and does not change the transaminase activity even in continuous treatment. On the contrary the age induced increase in the values of AST [11] are lowered statistically significant in group trial group, treated with 4 mg of testosterone, once a week for 15 days period (4ac). Our results confirm the established by Borhan- Manesh et al. similarity

between the values of AP in chronic treatment with the propionic salt of testosterone and those of 17- alkilined derivates [21]. In the current study we established also age related rise in the values of LDH. The applied T in both doses lowered statistically significantly its serum levels in the acute and chronic treated rats.

Conclusions

The acute and chronic treatment with T doesn't increase the enzyme markers of hepatocytolysis.

An age related rise in the values of the cholestasis enzymes – AP and LDH was registered.

The acute and chronic treatment with T leads to decrease in the values of AP and LDH.

Acknowledgements

This work was supported by a grant from Medical University – Plovdiv.

References

1. Gruenwald DA, Matsumoto AM. Testosterone supplementation therapy for older men: potential benefits and risks. J Am Geriatr Soc. 2003;51(1):101-15; discussion 115.
2. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J. 2000;21:890-4.
3. Akishita M, Hashimoto M, Yumiko Ohike Y, Ogawa S, Iijima K, Eto M, Ouchi Y. Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors, Atherosclerosis. 2010;210(1):232-6.
4. Fukui M, Ose H, Kitagawa Y. Relationship between low serum endogenous androgen concentrations and arterial stiffness in men with type 2 diabetes mellitus. Metabolism. 2007;56:1167-73.
5. Andersson B, Marin P, Lissner L. Testosterone concentrations in women and men with NIDDM. Diabetes Care. 1994;17:405-11.
6. Sharma V, Perros P. The nanagement of hypogonadism in aging male patients. Postgrad Med. 2009;121(1):113-21.
7. Aukrust P, Ueland T, Gullestad L, Yndestad A. Testosterone: a novel therapeutic approach in chronic heart failure? J Am Coll Cardiol. 2009;54: 928-9.
8. Bassil N, Alkaade S, Morley JE. The benefits and risks of testosterone replacement therapy: a review. Ther Clin Risk Manag. 2009;5(3):427-48.
9. Jones TH. Testosterone deficiency: a risk factor for cardiovascular disease? Trends in Endocrinology & Metabolism. 2010 Apr 6 [Epub ahead of print] PubMed PMID: 20381374.
10. Tan RS, Salazar JA. Risks of tertosterone replacement therapy in ageing men. Expert Opin Drug Saf. 2004;3(6):599-606.
11. Westbay D, Ogle SJ, Paradinas FJ, Randell JB, Murray-Lyon IM. Liver damages from long- term methyltestosterone. Lancet. 1977;2(8032):62-3.
12. Stimac D, Milić S, Dintinjana RD, Kovac D, Ristić S. Androgenic /Anabolic steroid-induced toxic hepatitis. J Clin Gastroenterol. 2002;35(4):350-2.
13. Kopplow K, Wayss K, Enzmann H, Mayer D. Dehydroepiandrosterone causes hyperplasia and impairs regeneration in rat liver. Int J Oncol. 2005;27(6):1551-8.
14. Jaya DS, Augstine J, Menon VP. Protective effect of testosterone against alcohol and paracetamol inducedhepatotoxicity in rats. Indian J Exp Biol. 1995;33(3):194-200.
15. Langfort J, Jagsz, S, Dobrzyn P, Brzezinska Z, Klapcinska B, Galbo H, et al. Testosterone affects hormone-sensitive lipase (HSL) activity and lipid metabolism in the left ventricle. Biochem Biophys Res Commun. 2010;399:670-6.
16. Kłapcinska B, Jagsz S, Sadowska- Krepa E, Gorski J, Kempa K, Langfort J. Effects of castration and testosterone replacement on the antioxidant defense system in the rat left ventricle. J Physiol Sci. 2008;58:173-7.
17. Sadowska-Krepa E., Kłapcinska B, Sławomir J, Sobczak A, Stanisław J, Małgorzata C. High-Dose Testosterone Propionate Treatment Reverses the Effects of Endurance Training on Myocardial Antioxidant Defenses in Adolescent Male Rats. Cardiovasc Toxicol. 2011;11:118-27.
18. Nirmal Kumar A, Kalyankar GD Effect of steroid hormones and vitamin B6 on age dependent changes in aminotransferases in rat. Indian J Physiol Pharmacol. 1985;29(4):207-12.
19. Haider A, Gooren LJ, Padungtod P, Saad F. Improvement of the Metabolic Syndrome and of Non- alcoholic Liver Steatosis upon Treatment of Hypogonadal Elderly Men with Parenteral Testosterone Undecanoate. Exp Clin Endocrinol Diabetes 2010;118(3):167-71.
20. Vieira RP, França RF, Damaceno-Rodrigues NR, Dolhnikoff M, Caldini EG, Carvalho CR, Ribeiro W. Dose-dependent hepatic response to sub-chronic administration of nandrolone decanoate. Med Sci Sports Exerc. 2008; 40(5):842-7.
21. Borhan-Manesh F, Farnum JB. Methyl-testosterone- induced cholestasis. The importance of disproportionately low serum alkaline phosphatase level. Arch Intern Med. 1989;149(9):2127-9.