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Original article

High-dose testosterone enanthate supplementation boosts oxidative stress, but exerts little effect on the antioxidant barrier in sedentary adolescent male rat liver

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ABSTRACT

Background: Anabolic-androgenic steroids abuse is on the rise among adolescent boys and young men, mostly in those seeking a 'shortcut' to an improved body image. This approach is associated with the risk of severe adverse health effects, some of which involve the liver and are linked to hepatic oxidative stress. Testosterone and its esters is a cornerstone of most anabolic-androgenic steroid stacking protocols.
Methods: We assessed and compared several hepatotoxicity and liver oxidative stress indices, as well as the contents of some components of the hepatic antioxidant barrier between sedentary adolescent male rats given a 6-week course of weekly im testosterone enanthate (TE, 8 or 80 mg/kg$_{BW}$/week) or vehicle (sesame oil) injections. Blood and livers for the assessments were harvested seven days after the last injection.
Results: TE supplementation dose-dependently elevated blood testosterone and significantly increased the liver content of thiobarbituric acid-reactive substances. Only the high-dose TE supplementation significantly slowed down body weight gain, reduced the liver weight/body weight ratio, increased liver heat shock protein 70/72 content and elevated blood enzyme markers of liver stress. There was no significant difference in reduced glutathione and α- or γ-tocopherol content between the TE-treated and control rats. Of the antioxidant enzymes studied (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase), only the dismutase activity was significantly while moderately elevated and only by the high-dose TE supplementation.
Conclusion: (Sub)chronic supplementation of sedentary adolescent male rats with high TE doses does not exert a lasting major effect on the liver antioxidant barrier and redox homeostasis.

Introduction

Androgens are essential for male sexual differentiation and development, hence androgen therapy is used for the treatment of juvenile hypogonadism and the related delayed growth and puberty. Therapeutic androgens include the 'prototypic' natural androgen testosterone and its esters, and a variety of synthetic derivatives of testosterone, termed anabolic-androgenic steroids (AAS) [1]. Because of their ability to decrease body fat and increase muscularity, these drugs are nowadays increasingly abused by

Abbreviations: AAS, anabolic-androgenic steroids; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BW, body weight; CAT, catalase; CTRL, controls; GGT, γ-glutamyltransferase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; Hsp70/72, 70/72 kDa heat shock protein; LW, liver weight; MDA, malondialdehyde; R_S, Spearman's rank correlation coefficient; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; Tc, core body temperature; TE, testosterone enanthate; TT, total testosterone.

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male adolescents and young men wanting to improve their body image and self-esteem [2,3], hence the incidence of serious adverse health effects associated with the abuse [3–5].

The major site of metabolism and elimination of foreign substances and excess of endogenous metabolites is the liver, which makes this organ a preferred target for drug toxicity [6]. Therefore, the variety of known adverse effects of AAS abuse includes some directly related to liver damage [5–8]. A growing body of reports indicates that this effect is linked to oxidative stress from genomic and non-genomic actions of AAS [9] involving activation of androgen receptors and the receptor for sex hormone binding globulin, as well as activation of stress-signaling pathways by the end products of lipid peroxidation [10,11].

The oxidative stress from substances of exogenous or endogenous origin is an early event in hepatotoxicity and the main cause of acute and chronic liver diseases [12]. The key source of the respective toxic metabolites are the cytochrome P450 enzymes located in the endoplasmic reticulum and in the mitochondrium [13,14]; the latter organelle is generally considered the main site of reactive oxygen species generation by the electron transport chain and a number of other components [15]. Enzymes contributing to the oxidative stress are also present in peroxisomes that are particularly abundant in the liver [10].

Liver cells are equipped with a number of protective antioxidant systems targeted at limiting harmful effects of redox homeostasis impairment [14,16,17]. These systems include free radical and non-radical scavenging enzymes, and low molecular weight antioxidants. The antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), prevent and limit initiation and propagation of free radical chain reactions. The non-enzymatic antioxidants such as reduced glutathione (GSH) and tocopherols are involved in repairing the oxidative stress-induced damage. Yet another component of the liver protective barrier is the 70/72 kDa heat shock protein (Hsp70/72) [18].

There are few reports on the effects of AAS on liver antioxidant defenses, mostly concerning adult subjects. Importantly, androgen catabolism changes substantially during adolescence [19], which phenomenon may modify the effects of androgen supplementation during this period. This study aimed at evaluating, in sedentary adolescent rats, the changes in liver oxidant/antioxidant balance and oxidative damage resulting from prolonged treatment with testosterone enanthate (TE). We chose the ester of the native androgen to avoid the hepatotoxicity related to certain modifications of the steroid structure in AAS, consisting in adding more carbon atoms to the sterane core (mainly 17α-alkylation) [1,20]. Notably, testosterone and its esters are a foundation of most, either oral or injectable, anabolic-androgenic steroid stacking plan.

Materials and methods

Animals and experimental protocol

Thirty-seven 4–5-week-old male Wistar rats of initial body weight (BW) 90 ± 10 g (mean ± SD) were used for the study. The rats were housed 4–5 per cage in a temperature- (22–24 °C) and relative humidity- (45–65%)-controlled room, using a 12/12-h light/dark cycle (lights on at 7 a.m.), with free access to standard laboratory rat maintenance chow and tap water. The rats were randomized between three groups: (1) controls (CTRL, $N = 11$), (2) rats receiving low-dose TE (8 mg/kg_{BW}/week) supplementation (TE8 group, $N = 13$), and (3) rats receiving high-dose TE (80 mg/kg_{BW}/week) supplementation (TE80 group, $N = 13$). All the rats received humane care as specified by the Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes. The study protocol was approved by the IVth Local Ethics Committee for Animal Experimentation (Certificate No. 38/2011).

Stock TE (*Testosteronum prolongatum*, Jelfa, Poland; active substance: testosterone 17β-enanthate) diluted with sesame oil (Sigma-Aldrich, St. Louis, MO, USA) as needed to ensure equal volume for both dosages, was injected im once a week for 6 weeks, alternately into the right and left hind limb. CTRL rats were given the same volume of the oil by the same schedule. In all rats, resting core body temperature (Tc) was measured five days after the last injection using a flex-tip digital thermometer inserted 1.5–2 cm deep into the rectum.

Seven days after the last TE injection, the rats were weighed and sacrificed by simple decapitation. Trunk blood was collected, left to clot at room temperature and spun to obtain serum. The trunks were perfused with O_2/CO_2 (95%/5%, v/v) mixture-saturated, glucose- (10 mM)-supplemented Krebs–Henseleit buffer (pH 7.4) to remove remnant blood. Then the livers were quickly dissected, weighed, cut into several pieces and snap-frozen in liquid nitrogen. All the samples were stored at −80 °C pending biochemical analyses.

Assay methods

Blood total testosterone (TT) was determined with a Testosterone RIA DSL-4100 kit (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Blood serum activities of alkaline phosphatase (ALP, EC:3.1.3.1), aspartate aminotransferase (AST, EC:2.6.1.1), alanine aminotransferase (ALT, EC:2.6.1.2) and γ-glutamyltransferase (GGT, EC:2.3.4.2) were measured with a Cobas Integra 400 analyzer (Roche, Rotktreuz, Switzerland). SOD (EC:1.15.1.1) activity was measured using Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). Activities of GPx (EC:1.11.1.9) and GR (EC:1.6.4.2), and GSH levels were assayed using Bioxytech (OXIS International, Portland, OR, USA) kits GPx-340, GR-340 and GSH-400, respectively. CAT (EC:1.11.1.6) activity was assessed according to Aebi [21]. Liver contents of lipid peroxidation products, expressed as malondialdehyde (MDA), were determined by the thiobarbituric acid (TBARS) reaction as described by Ohkawa et al. [22], and that of the non-enzymatic antioxidants α- and γ-tocopherols—according to Sobczak et al. [23]. Hsp70/72 content was determined with an ELISA Kit for Heat Shock Protein 70 (USCN Life Science Inc., Wuhan, China). Total protein content in liver homogenate supernatants was measured using a Protein Assay Kit BCA1 (Sigma-Aldrich).

Statistics

All data are presented as the mean ± SD. BW data were analyzed by a two-way ANOVA with repeated measures on time and TE dose as the main factors, followed by the Tukey test for unequal sample sizes. All other data, except when specified otherwise, were analyzed by a one-way ANOVA with TE dose as the main factor, followed, when appropriate, by the Tukey test. Association between variables was evaluated with the Spearman rank correlation test. In all cases, a $p < 0.05$ was considered significant. All the analyses were performed using Statistica *v.*8 software (StatSoft, Tulsa, OK, USA).

Results

Body and liver weights, serum total testosterone levels and core body temperature

There was no difference in final BW between CTRL and low-dose TE-supplemented rats, whereas the rats given high-dose TE