Toxicology and Applied Pharmacology 282 (2015) 285–296

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Amelioration of nandrolone decanoate-induced testicular and sperm toxicity in rats by taurine: Effects on steroidogenesis, redox and inflammatory cascades, and intrinsic apoptotic pathway

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ARTICLE INFO

Article history:
Received 9 September 2014
Revised 15 December 2014
Accepted 16 December 2014
Available online 24 December 2014

Keywords:
Nandrolone decanoate
Taurine
Steroidogenesis
ICAM-1
MMP-9
Cytochrome c

ABSTRACT

The wide abuse of the anabolic steroid nandrolone decanoate by athletes and adolescents for enhancement of sporting performance and physical appearance may be associated with testicular toxicity and infertility. On the other hand, taurine; a free β-amino acid with remarkable antioxidant activity, is used in taurine-enriched beverages to boost the muscular power of athletes. Therefore, the purpose of this study was to investigate the mechanisms of the possible protective effects of taurine on nandrolone decanoate-induced testicular and sperm toxicity in rats. To achieve this aim, male Wistar rats were randomly distributed into four groups and administered either vehicle, nandrolone decanoate (10 mg/kg/week, I.M.), taurine (100 mg/kg/day, p.o.) or combination of taurine and nandrolone decanoate, for 8 successive weeks. Results of the present study showed that taurine reversed nandrolone decanoate-induced perturbations in sperm characteristics, normalized serum testosterone level, and restored the activities of the key steroidogenic enzymes; 3β-HSD, and 17β-HSD. Moreover, taurine prevented nandrolone decanoate-induced testicular toxicity and DNA damage by virtue of its antioxidant, anti-inflammatory, and anti-apoptotic effects. This was evidenced by taurine-induced modulation of testicular LDH-x activity, redox markers (MDA, NO, GSH contents, and SOD activity), inflammatory indices (TNF-α, ICAM-1 levels, and MMP-9 gene expression), intrinsic apoptotic pathway (cytochrome c gene expression and caspase-3 content), and oxidative DNA damage markers (8-OHdG level and comet assay). In conclusion, at the biochemical and histological levels, taurine attenuated nandrolone decanoate-induced poor sperm quality and testicular toxicity in rats.

Introduction

Anabolic androgenic steroids (AAS) are synthetic testosterone analogs that are used to treat growth deficiency, chronic wasting conditions, and post-menopausal osteoporosis (Evans, 2004). However, adolescents and athletes widely abuse anabolic steroids to increase their muscle mass, and improve their physical appearance and sporting performance, with little awareness of their hazardous side effects (Nilsson et al., 2004). Athletes usually take AAS in the form of I.M. injections in cycles lasting 6–12 weeks (Hall and Hall, 2005). The doping doses of anabolic steroids are excessively higher than the therapeutic doses. It has been estimated that AAS abusers take 600–5000 mg of AAS per week that in comparison with the production of testosterone by testes (40–50 mg per week) are 10–100 times more which may cause serious health consequences especially on male fertility (Fronczak et al., 2012).

Nandrolone decanoate or 17β-hydroxy-19-nor-4-androsten-3-one is a commercially available anabolic steroid. Endogenous nandrolone is produced as a byproduct during biochemical transformational reactions and can be detected in picomoles in circulation, while its main metabolite norandrosterone can be detected in urine samples from human subjects in a concentration range of 0.01–0.14 mg/l (Reznik et al., 2001). Similar to testosterone nandrolone decanoate can enhance muscle mass, but without virilization. It is administered in an oily base by intramuscular injection and is mainly indicated in the treatment of osteoporosis in postmenopausal women and in anemia due to chronic renal failure, cytotoxic drug therapy or aplastic anemia (Wood, 2004). Patients with HIV-associated weight loss can be treated by 100 mg of nandrolone decanoate by deep intramuscular injection every 2 weeks for 16 weeks (Gold et al., 1996), whereas chronic obstructive pulmonary disease (COPD) patients suffering from muscle wasting are treated by intramuscular nandrolone decanoate in a dose of 50–200 mg per week for a period of 12 weeks (Velema et al., 2012). Experimentally, nandrolone decanoate administration to male rats induced spermatogenic cells apoptosis (Shokri et al., 2010). Nevertheless, the mechanisms underlying nandrolone decanoate-induced testicular toxicity are not yet fully elucidated.

Taurine (2-aminoethanesulfonic acid) is the major free β-amino acid that is present in all tissues of most animal species. Taurine can be either synthesized endogenously primarily in the liver from cysteine or methionine, or it can be taken from exogenous dietary sources (Huxtable,

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http://dx.doi.org/10.1016/j.taap.2014.12.007
0041-008X/

1992). Taurine plays important roles in the male reproductive system and sperm cells. Besides its ability to regulate cellular osmosis and stabilize cell membranes, taurine acts as a capacitating agent and as a sperm motility factor (Mrsny et al., 1979). In addition, taurine can inhibit sperm lipid peroxidation by virtue of its antioxidant properties (Manna et al., 2008). Experimentally, taurine showed protective effects against testicular toxicity induced by endosulfan and cadmium (Manna et al., 2008; Aly and Khafagy, 2014). Interestingly, Geiss et al. (1994) reported enhanced performance in athletes supplemented with taurine-enriched beverages. However, up to our knowledge, there are no available studies about the protective effects of taurine on nandrolone decanoate-induced testicular injury. Therefore, this study was carried out to explore the modulatory effects of taurine on testicular and sperm injury induced by nandrolone decanoate in rats and to investigate the possible underlying mechanisms of these protective effects.

Materials and methods

Animals. Adult male Wistar rats weighing 190–200 g were used in this study. They were housed in clean polypropylene plastic cages and kept on a 12 h light/dark cycle and controlled temperature of 22 ± 1 °C. The animals were allowed standard chow pellets and water ad libitum. Rats were left for one week in order to acclimatize, and to minimize the physiological responses to handling and human exposure. All the experiments were carried out in accordance with the international guidelines, and with the approval of the local institutional Research Ethics Committee.

Chemicals. Nandrolone decanoate was obtained as Nandurabolin ampoules from The Nile Company for Pharmaceuticals and Chemical Industries (Egypt). Taurine was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Experimental protocol. Male Wistar albino rats were randomly distributed into four groups of 10 animals each. Rats of the normal control group received single weekly intramuscular injections of peanut oil/benzyl alcohol mixture (90:10 v/v) in addition to daily administration of oral normal saline by gavage as vehicles for nandrolone decanoate and taurine, respectively (Hassan and Kamal, 2013). Rats of the second group were intramuscularly injected in the gluteal muscle once every week by nandrolone decanoate (Nandurabolin ampoules) in the dose of 10 mg/kg. Taurine was dissolved in normal saline and administered to another group of rats in the dose of 100 mg/kg/day by oral gavage. Rats of the combination group received taurine (100 mg/kg/day, oral) and nandrolone decanoate (10 mg/kg/week, I.M.). The doses of nandrolone decanoate and taurine were selected according to preliminary studies, and were matched with literature (Das et al., 2009; Naraghi et al., 2010). The duration of the experiment was selected as 8 weeks to cover the spermatogenic cycle in rats, which was documented as 48–56 days (Kolasa et al., 2004).

Collection of samples. At the end of the experimental period, all rats were sacrificed under light ether anesthesia. Blood was collected and serum was separated by centrifugation. Both testes were isolated immediately, and weighed. All specimens were stored at -70 °C until properly processed as indicated per each assay. Cauda epididymis was used for studying sperm characteristics.

Determination of sperm count. Following the method of Yokoi et al. (2003), cauda epididymis was isolated and immediately minced in physiological normal saline (5 ml), gently rocked on a shaker for 10 min and then incubated for 2 min at 37 °C to allow spermatozoa to leave the epididymal tubules. The supernatant fluid was mixed (1:100) with a solution containing 1 ml formalin (35%), 5 g sodium bicarbonate, and 25 mg eosin in 100 ml distilled water. An aliquot of the diluted sperm suspension (10 μl) was transferred to each counting chamber of a hemocytometer, and was allowed to stand for 5 min then counted under a light microscope at ×200 magnification.

Determination of sperm viability. For each rat, 20 μl of sperm suspension was carefully mixed with an equal volume of eosin–nigrosin stain, and a thin film was spread on a clean slide. Two hundred sperms were randomly examined for each sample at ×400 magnification and the percentage of viable sperms were calculated. Dead sperms were stained pink and live sperms remained unstained (Wyrobek et al., 1983).

Determination of sperm motility. According to Sönmez et al. (2005), fluid from the cauda epididymis was taken by a pipette and diluted to 2 ml with tris buffer solution. The percentage of motility was estimated microscopically within 2–4 min at ×400 magnification and was expressed as a percentage of motile sperms to the total sperm count.

Determination of sperm abnormalities. Following the method reported by Evans and Maxwell (1987), 300 sperms were microscopically counted per slide at ×400 magnification and the percentages of morphologically abnormal sperms were recorded.

Determination of lactate dehydrogenase-x (LDH-x) activity. Testicular LDH-x enzyme activity was assessed following the method of Cheever et al. (1989). Briefly, a portion of the testis was homogenized in 0.25 M sucrose solution (1:3 w/v) by a glass homogenizer for 1 min, and then centrifuged at 3000 rpm for 30 min. The obtained supernatant was filtered through a 0.45 μm pore-size Acrodisc filter (Sigma-Aldrich, USA). LDH-x enzyme-containing filtrate (20 μl) was incubated in a quartz cuvette with 3 ml of a specific substrate (106 mM trizma, 0.05% sodium azide, 60 mM DL-α-hydroxycaproic acid, and 0.9 mM NAD^+) for 10 min at 30 °C. The change in absorbance was measured at 340 nm by a spectrophotometer. LDH-x enzyme activity was calculated as international units per gram testicular tissue according to the following equation:

$$\text{LDH-x}(\text{U/g testis}) = \frac{\Delta A}{6.3 \times 10^{-3}} \times \frac{3.02}{0.02} \times \frac{3}{1000}$$

Determination of serum testosterone level. Serum testosterone level was measured by ELISA kit purchased from Blue Gene Biotech (Shanghai, PRC). The procedure of the kit was performed as stated in the manufacturer's protocol. The intensity of the finally developed color was measured at 450 nm using a microplate reader. Testosterone concentration in each sample was calculated from a plotted standard curve.

Determination of testicular androgenic enzymes activity; 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD). Briefly, testicular tissue was homogenized using a glass homogenizer for 10 s at 4 °C and pH 7.4 in RIPA buffer (150 mM NaCl, 10 μg/ml PMSF, 1 mM EDTA, 20 mM Tris–HCl, and 1% Triton X-100). The homogenate was centrifuged at 14000 ×*g* for 20 min at 4 °C. The cytosol supernatant was extracted and used as the enzyme source in the assay (Bustamante-Marin et al., 2012). Protein concentrations were determined by a colorimetric kit (Spinreact, Spain). The method of Bergmeyer (1974) was followed to estimate the activities of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD). The reaction mixture in a volume of 2 ml contained 100 μmol sodium pyrophosphate buffer (pH 9.0) and 0.5 μmol cofactor NAD for 3β-hydroxysteroid dehydrogenase and NADPH for 17β-hydroxysteroid dehydrogenase, 0.08 μmol of substrate (dehydroepiandrosterone for 3β-hydroxysteroid dehydrogenase and androstenedione for 17β-hydroxysteroid dehydrogenase), and 100 μl of enzyme source. The reactions were performed in a quartz cuvette of 1.0 cm path length at 23 ± 1 °C. Absorbance was recorded every 20 s for 3 min at 340 nm using a UV–Vis spectrophotometer. Enzyme activity was expressed as nmol of NAD converted to NADH/