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Nephrotoxicity in rabbits after long-term nandrolone decanoate administration

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HIGHLIGHTS

- Nephrotoxic effects of nandrolone decanoate on young rabbits.
- Significant increase in serum urea and creatinine.
- Hypereamia, fibrosis and focal inflammation in kidney tissue of high-dosed rabbits.
- Increased telomerase activity in intramuscularly treated animals.
- Tissue TBARS and GSH levels were significantly altered.

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ABSTRACT

Among the various side effects of supra-physiological dose of anabolic androgenic steroids that are described, renal toxicity remains the least evaluated. The present study provides evidence that long-term administration of nandrolone decanoate could lead to alterations of renal function and structure in the experimental rabbit model. A pronounced increase in serum urea, creatinine, SGOT and SGPT is observed in the treated animals, with intramuscular administration being more detrimental. Histopathological evaluation of kidneys indicated hyperaemia, fibrosis and focal inflammation. Furthermore, the significantly increased telomerase activity found in the kidneys of the intramuscularly treated animals could possibly represent a counteracting survival mechanism. Oxidative stress markers that were influenced the most were TBARS, indicating lipid peroxidation, and GSH. An interesting finding in our study though, was that while intramuscular administration showed the highest biochemical derangement, oxidative stress markers provided mixed results between intramuscularly and subcutaneously treated rabbits. In conclusion, nephrotoxicity of nandrolone decanoate remains a multi-factorial, partly irreversible effect that involves augmented tissue oxidative status.

Abbreviations: AAS, anabolic androgenic steroids; SGOT, serum glutamil oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; ALP, alkaline phospatase; γGT, serum gamma-glutamyltransferase; TBARS, thiobarbituric acid reactive substances; TAC, total antioxidant capacity; GSH, glutathione.

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1. Introduction

Misuse of AASs is becoming a public health problem. AASs represent a group of steroidal hormones related to the male hormone, testosterone. Apart from increasing muscular development and strength, there is emerging evidence that a variety of pathological conditions may arise from their extensive and unsupervised abuse (Darke et al., 2014; Kanayama et al., 2008; Parssinen and Seppala, 2002a,b). Little is known about the potential effect of these drugs on renal function. Scientific knowledge on the subject usually is restricted to case reports or case series and a handful of animal studies. Since the potential effects of AASs on renal function have not been well characterized in humans, hypothesis is mostly driven by the fact that androgen receptors were identified in micro dissected murine glomeruli and cultured mesangial cells and by the knowledge that prognosis in men is worse for various types of chronic kidney disease (Herlitz et al., 2010).

Case reports linking AASs to renal damage include cases of acute kidney injury (Daher et al., 2009), acute renal failure as a complication of rhabdomyolysis (Hageloch et al., 1988), diffuse membrane proliferative glomerulonephritis (Revai et al., 2003), severe cholestasis with kidney failure (Nasr and Ahmad, 2009). Androgens are also known to induce oxidative stress and upregulate components of the renin–angiotensin system (Iliescu et al., 2007; McGuire et al., 2007). In a recent study, dose-related oxidative damage in the kidneys of nandrolone decanoate treated mice was also reported (Riezzo et al., 2014).

Among AASs, nandrolone decanoate possesses a dominant position. Nandrolone (19-nortestosterone, 17b-hydroxy-estr-4-en-3-one) was synthesized in the early 1950s and though it can be regarded as an "old" doping agent, it is still widely used to enhance muscular strength and performance in sports (Bricout and Wright, 2004; Hemmersbach and Grosse, 2010). At the same time, nandrolone therapeutic potential was evaluated, especially in the context of protein deficiency, for a variety of pathological conditions, as in aplastic anaemia, osteoporosis (Geusens, 1995), AIDS (Mulligan et al., 2005), cancer and protein deficiency of the elderly.

The aim of the present study was to investigate the possible detrimental effects of long-term nandrolone decanoate administrationon on renal function of rabbits by monitoring renal specific biochemical parameters, kidney histopathology and oxidative stress markers on serum and tissue level, along with telomerase kidney activity.

2. Methods and materials

2.1. Animals

Fourteen healthy New Zealand male rabbits (3900–5500 g each, in the age of 10–15 months) were used for the purpose of this study. The animals were housed in individual metal cages and kept in a 12-h dark/light cycle, at a temperature between 20 and 23 °C, in the laboratory animal house facilities of the University Hospital of Heraklion, Crete. They were fed with commercial rabbit pellets *ad libitum* and provided with drinking (tap) water. The rabbits were acclimatized under laboratory conditions for 2 weeks, whereupon the treatment period begun.

The animals were divided into four groups. Group 1 and group 2 received intramuscularly a high (HDIM) and a low dose (LDIM) of nandrolone decanoate (10 mg/kg and 4 mg/kg, respectively), two days per week for six months. Group 3 received subcutaneously a high dose (HDSC) of nandrolone decanoate (10 mg/kg) 2 days per week for 6 months. Group 4 served as the control group (C) and its animals were only treated with saline solution. The saline solution was administered intramuscularly. Originally, the appropriate amounts of anabolic were diluted in 2.0 mL of saline solution.

The experimental scheme of exposure was selected in order to simulate the allegedly claimed abuse of steroids by athletes and consisted of two periods: the administration period that lasted six months and the wash-out period, the duration of which was four months. Two animals of the high dosed groups were selected for monitoring in the wash-out period after ceasing administration. The first sacrifice was performed after six months (end of the administration period) and the second at the end of tenth month (end of wash-out period). Serum was collected at baseline, every two months during the administration and wash-out period and at the day of the sacrifice. The animals were sacrificed by intravenous injection of 5 mL pentothal (Thiopental sodium solution, 25 mg/ml), according to the bioethical rules of the University of Crete. During the study period, the animals were weighed and their food consumption was recorded. All rabbits were regularly observed and their condition was closely monitored. No pathological clinical signs were observed at any point.

The present study was approved by the Veterinary Administration Office of Heraklion (Crete, Greece), the Animal Investigation Committee of the University of Crete (Heraklion, Crete, Greece) and conformed to the National and European Union directions for the care and treatment of laboratory animals. All efforts were made to minimize suffering.

2.2. Biochemical markers

Blood samples were individually collected from the vena auricularis of each rabbit in the appropriate glass tubes in order to evaluate the concentration of the following biomarkers: urea, creatinine, SGOT, SGPT, ALP and γGT. Blood serum was separated by centrifugation at 4000 rpm for 15 min and then stored at −18 °C. All biomarkers were spectrometrically measured in Olympus AU2700.

2.3. Histopathological lesions

Kidney tissue block samples, fixed in formalin, embedded in paraffin and sectioned at 3 μm. Then, they were stained with eosin–hematoxylin and subsequently examined under light microscopy. Histopathological examinations were conducted blindly by histopathologists.

2.4. Telomerase activity

Telomerase activity in kidney tissue samples was measured using a commercial telomerase polymerase chain reaction–enzyme linked immune sorbent assay (PCR-ELISA) (Roche Diagnostics Corp., Indianapolis, IN, USA), based on the telomeric repeat amplification protocol.

2.5. Oxidative stress biomarkers

Oxidative stress biomarkers (TBARS concentration, carbonyls, catalase activity, TAC) were measured as previously described (Germanakis et al., 2013; Tsitsimpikou et al., 2013; Zafiropoulos et al., 2014) in the animals' renal tissues. Previously published results on oxidative stress biomarkers in serum from the same animals (Vasilaki et al., 2016) are used for statistical analysis only. Due to sample failure only one tissue sample per HD group in the wash-out period was measured. Therefore the respective results are only discussed qualitatively and not presented.

Briefly, TBARS expressed in nmol/mg protein, were measured in renal tissue homogenate (diluted 1:2) by mixing it with trichloacetic acid (TCA) Tris–HCl, Na_2SO_4 and thiobarbituric acid and