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Cardiotoxicity in rabbits after long-term nandrolone decanoate administration

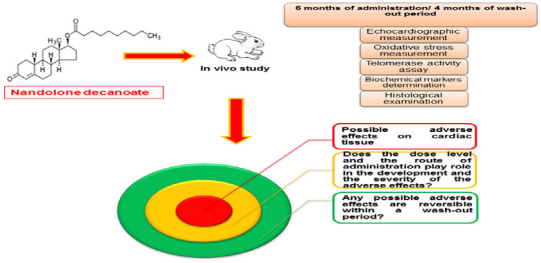
Fotini Vasilaki[a], Christina Tsitsimpikou[b], Konstantinos Tsarouhas[c], Ioannis Germanakis[d], Marias Tzardi[e], Matthaios Kavvalakis[a], Eren Ozcagli[f], Dimitrios Kouretas[g], Aristidis M. Tsatsakis[a,*]

[a] Center of Toxicology Science & Research, Medical School, University of Crete, Heraklion, Crete, Greece
[b] Department of Dangerous Substances, Mixtures and Articles, Directorate of Energy, Industrial & Chemical Products, General Chemical State Laboratory of Greece, Athens, Greece
[c] Department of Cardiology, General Hospital of Karditsa, Terma-Tavropou, Karditsa, Greece
[d] Paediatric Cardiology Unit, Department of Paediatrics, University Hospital Heraklion, Crete, Greece
[e] Department of Pathology, Medical School, University of Crete, Heraklion, Crete, Greece
[f] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Beyazit, Istanbul, Turkey
[g] Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

HIGHLIGHTS

- Cardiovascular effects of nandrolone decanoate on young rabbits.
- Focal fibrosis and inflammatory infiltrations of cardiac tissue in high dose groups.
- Preserved systolic performance, distorted MPI values, diastolic impairment.
- TBARS increased in high dose groups, troponin increased in wash-out period.
- Heart tissue relative telomerase activity increased dose-dependently.

GRAPHICAL ABSTRACT



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ABSTRACT

Abuse of anabolic androgenic steroids is linked to a variety of cardiovascular complications. The aim of our study was to investigate the possible cardiovascular effects of nandrolone decanoate on young rabbits using echocardiography, histology and monitoring of telomerase activity, oxidative stress and biochemical markers. Fourteen rabbits were divided into three administration groups and the control group. Doses of 4 mg/kg and 10 mg/kg of nandrolone decanoate, given intramuscularly and subcutaneously, two days per week for six months were applied. A 4-months wash-out period followed. Focal fibrosis and inflammatory infiltrations of cardiac tissue were observed in the high dose groups. Thiobarbituric acid-reactive species (TBARS) levels were significantly increased in the high dose groups, while catalase activity decreased. Myocardial Performance Index (MPI) is the main echocardiographic index primarily affected by nandrolone administration in rabbits. Despite the preserved systolic performance, histological lesions observed associated with distorted MPI values, point

Abbreviations: AAS, anabolic androgenic steroids; TBARS, thiobarbituric acid reactive species; TAC, total antioxidant capacity; LDH, lactate dehydrogenase; CpK, creatinine kinase; BNP, B-type natriuretic peptide; PW, pulsed wave Doppler; TDI, tissue Doppler imaging; MPI, myocardial performance index; LV, left ventricular.

* Corresponding author at: Center of Toxicology Science & Research, University of Crete, Voutes 71003 Heraklion, Greece. Fax: +30 2810542098.
E-mail address: toxlab@med.uoc.gr (A.M. Tsatsakis).

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Telomerase activity
Biochemical markers

to diastolic impairment of the thickened myocardium due to nandrolone treatment. Oxidative stress accumulates and telomerase activity in cardiac tissue rises. Subcutaneous administration seems to be more deleterious to the cardiovascular system, as oxidative stress, telomerase activity and biochemical markers do not appear to return into normal values in the wash-out period.

1. Introduction

Anabolic androgenic steroids (AAS) are chemical derivatives of the endogenous hormone testosterone and exert two main physiological actions including the promotion of muscle growth and the development of the male reproductive system (Van Amsterdam et al., 2010; Thiblin and Petersson, 2005). Although AAS have valid medical applications, human and animal AAS are frequently misused in order to enhance performance, strength and even for improving the physical appearance and body image (Copeland et al., 2000; Darke et al., 2014; Hakansson et al., 2012; Kao et al., 2012; Larance et al., 2008; Petersson et al., 2010).

Abuse of AAS has become a public health issue, as there is mounting evidence suggesting that they affect the myocardial ventricular function through the androgen receptor pathway (Baggish et al., 2010; D'Andrea et al., 2007; Figueredo, 2011; Kasikcioglu et al., 2009; Krieg et al., 2007; Lane et al., 2006; Luijkx et al., 2013), as well as the cardiovascular system in general (Kanayama et al., 2010). Acute myocardial infarction (Fisher et al., 1996; Wysoczanski et al., 2008), cardiomyopathy (Ahlgrim and Guglin, 2009; Mark et al., 2005), severe arrhythmia (Lau et al., 2007; Sullivan et al., 1999) and even cases of sudden death (Fineschi et al., 2001, 2007; Di Paolo et al., 2007; Montisci et al., 2012; Petersson et al., 2006; Thiblin et al., 2000) have been described as the most dramatic cardiovascular manifestations caused by the excessive use of anabolic steroids. However, more clinical and mechanistic studies are needed to evaluate the prevalence of morbidity and mortality in users, as data up to now are scattered and circumstantial and based mainly on case reports.

Moreover, recent studies connect the administration of AAS with changes in oxidative stress, suggesting distinct and different patterns of oxidative stress systemic or local response per substance (Germanakis et al., 2013; Pey et al., 2003). Exercise training did not seem to affect the oxidative status of the individuals (Pey et al., 2003), whereas others report that treatment with stanozolol protected rat skeletal muscle mitochondria against oxidative damage of proteins and changes in membrane fatty acid composition induced by acute exercise (Saborido et al., 2011).

Oxidative stress is known to play a crucial role in the pathogenesis of heart failure. It induces damage or apoptosis of endothelial cells (Aoki et al., 2001; Matthews et al., 2006) and it has also been implicated in the development of atherosclerosis through a variety of mechanisms, especially those leading to endothelial dysfunction (Berliner et al., 1990; D'Agnillo et al., 2000). In addition, cultured vascular smooth muscle cells and endothelial cells exposed to oxidative stress, exhibit shortening of telomeres and accelerated cellular senescence (Matthews et al., 2006). Telomeres are indicators of oxidative stress (Saretzki, 2009). Telomeres and telomerase provide protection against threats to the genome that arise from aninherent difficulty in the asymmetric replication of DNA (Calado and Young, 2009). Recently, telomere and telomerase have been recognized as potential factors involved in the initiation and progression of cardiovascular disease (Samani and van der Harst, 2008; Fuster and Andres, 2006; Edo and Andres, 2005; Wong et al., 2008). There is accumulating evidence that connect telomere length with cardiovascular-related phenotypes, including atherosclerosis and heart failure (Wong et al., 2009). Moreover, alterations in telomerase activity have many clinical implications, such as aging, cancer, and diabetes mellitus (Blackburn, 2005).

Nandrolone (19-nortestosterone, 17b-hydroxy-estr-4-en-3-one) was synthesized in the early 1950s and although it can be regarded as an old doping agent, it is still used to enhance muscular strength and performance in sports and in horse racing. In fact, it is one of the most frequently detected doping agents worldwide (Bricout and Wright, 2004; Hemmersbach and Grosse, 2010; Sauer et al., 1998).

Nandrolone and its esters have been widely used as therapeutic agents mainly in protein deficiency diseases like aplastic anaemia (Gardner, 1985), osteoporosis (Geusens, 1995), AIDS (Mulligan et al., 2005; Storer et al., 2005), cancer (Puccio and Nathanson, 1997) and protein deficiency in the elderly.

The primary aim of our study was to investigate the possible adverse effects of nandrolone decanoate, one of the most commonly used pharmaceutical forms of nandrolone, on cardiac tissue of healthy rabbits. Secondary aim was to evaluate whether the dose level and the administration mode could play any further role and whether any observed adverse effects could be reversible within a wash-out period of 4 months. Thus, echocardiography was applied to the anabolic treated rabbits and histopathological examination of heart tissues was conducted. Furthermore, systemic oxidative stressmarkers and biomarkers related to normal cardiovascular function were measured. To our knowledge, this is the first study that examines all these parameters in order to evaluate the possible cardiotoxic action of nandrolone decanoate.

2. Methods and materials

2.1. Animals

Fourteen healthy New Zealand multicoloured 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 treatment groups. Group 1 and group 2 received 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 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. Two echocardiographic examinations were conducted, both of them the day before the sacrifice sessions. The first sacrifice was performed at