Toxicology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Toxicology

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

1
2
Spermatic and testicular damages in rats exposed to ethanol: Influence of lipid peroxidation but not testosterone

3 Q1 Glaucia E.M.L. Siervo [a,d], Henrique R. Vieira [a], Fernanda M. Ogo [a,d], Carla D.B. Fernandez [b],
4 Géssica D. Gonçalves [a], Suzana F.P. Mesquita [a], Janete Ap. Anselmo-Franci [c],
5 Rubens Cecchini [d], Flavia A. Guarnier [d], Glaura S.A. Fernandes [a,*]

6 [a] Department of General Biology, Biological Sciences Center, State University of Londrina – UEL, Londrina, Paraná, Brazil
7 [b] Department of Morphology, Institute of Biosciences, UNESP – Universidade Estadual Paulista, Botucatu, São Paulo, Brazil
8 [c] Department of Morphology, Stomatology and Physiology, Dental School of Ribeirão Preto, University of São Paulo – USP, Ribeirão Preto, São Paulo, Brazil
9 [d] Department of General Pathology, Biological Sciences Center, State University of Londrina – UEL, Londrina, Paraná, Brazil

ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form 18 January 2015
Accepted 26 January 2015
Available online xxx

Keywords:
Testis
Epididymis
Sperm
Ethanol
Vitamin C
Lipid peroxidation

ABSTRACT

Chronic consumption of ethanol causes morphological and physiological changes in the reproductive system of mammals. Vitamin C has an antioxidant role in organisms by neutralizing the ROS (reactive oxygen species) produced by oxidizing agents and this vitamin has an important function in the male reproductive system. The aim of this study was to evaluate whether vitamin C could prevent or attenuate the alterations in the male reproductive system caused by ethanol consumption. To test this hypothesis, male rats were divided into three experimental groups and treated by gavage for 63 days. The ethanol (E) and ethanol + vitamin C (EC) groups received 2 g/kg of ethanol (25% v/v) daily. In addition to ethanol, the EC group received vitamin C at a dose of 100 mg/day, diluted in water. The control group (C) received only the vehicle. On the 64th experimental day, the animals were anesthetized and euthanized, and blood was collected for plasmatic hormonal analysis. The testis, epididymis, vas deferens, and seminal vesicles were removed and weighed. Sperm from the vas deferens was submitted to morphological and motility analysis. The testis and epididymis were used for oxidative stress and histopathological analysis, sperm count, morphometric analysis of the testis, and stereological analysis of the epididymis. The results showed that vitamin C has a protective effect in the testes of adult male rats, entirely normalizing the parameters of sperm count, spermatogenesis kinetics, lipid peroxidation levels, and sperm motility, as well as partially normalizing the histopathological damage in the testis, epididymis, and sperm morphology. Thus, we concluded that lipid peroxidation is a major mechanism by which ethanol affects the testes and sperm, whereas no plasmatic testosterone alterations were found.

© 2015 Published by Elsevier Ireland Ltd.

10
1. Introduction

11
12 Q2
It has long been known that the chronic consumption of ethanol may cause alterations in the male reproductive tract of mammals (Emanuele and Emanuele, 2001; Teixeira et al., 2012; Uygur et al., 2013 Willis et al., 1983). Studies have found evidence of testicular lesions (Mesquita et al., 2011) and decreased testicular weight (Adaramoye and Arisekola, 2013) as the main alterations resulting from abusive and prolonged ethanol intake. Furthermore, it was shown that there is an increased rate of apoptosis (Zhu et al., 2000; Kok and Kim, 2006) and decreased rate of proliferation (Kok and Kim, 2006) in stem cells; reduction in seminiferous tubule diameter (Mesquita et al., 2011); and decreased levels of luteinizing hormone (LH), number of receptors in the testis, as well as LH-releasing hormone (LHRH) (Shi and Emanuele, 1998). Furthermore, Arco et al. (2003) showed that ethanol exposure changes the expression of proteins in the epididymis; alterations of protein expression in the epididymis

13
14
Q3 15
16
17
18
19
20
21
22
23
24
25
26

Abbreviations: PND, post natal day; C, control group; E, ethanol group; EC, ethanol + vitamin C group; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive species; TBA, thiobarbituric acid; GSH, reduced glutathione; DSP, daily sperm production; HTF, human tubal fluid; ADH, alcohol dehydrogenase; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; XO, xanthine oxidase; LH, luteinizing hormone; LHRH, LH-releasing hormone; FSH, follicle-stimulating hormone.

* Corresponding author. Tel.: +55 43 33714417.
E-mail address: glaura@uel.br (G.S.A. Fernandes).

http://dx.doi.org/10.1016/j.tox.2015.01.016
0300-483X/© 2015 Published by Elsevier Ireland Ltd.

UNCORRECTED PROOF

Please cite this article in press as: Siervo, G.E.M.L., et al., Spermatic and testicular damages in rats exposed to ethanol: Influence of lipid peroxidation but not testosterone. Toxicology (2015), http://dx.doi.org/10.1016/j.tox.2015.01.016

can lead to diminished sperm quality and decrease in the ability of male gametes, since sperm maturation occurs in the epididymis (Damm and Cooper, 2010).

Ethanol may also decrease testosterone levels (Maneesh et al., 2005; Oliva et al., 2006; Srikanth et al., 1998) and increase oxidative stress (Colantoni et al., 2000; Emanuele and Emanuele, 2001), which results from an imbalance between reactive oxygen species (ROS) generation and antioxidant defenses (glutathione, superoxide dismutase, catalase, and vitamins). At high concentrations, ROS are very toxic to cells, causing DNA damage, lipid peroxidation, and protein degradation (Sun, 1990), in addition to affecting many biological molecules (Damasceno et al., 2002). However, in physiological conditions, ROS are involved in normal biochemical processes, such as control of proliferation and cell signaling (Finkel, 1998).

Ethanol oxidation occurs as a part of alcoholic metabolism. In this process, byproducts such as ROS are formed and these can contribute to cellular damage, playing an important role in reproduction system damage caused by ethanol consumption (Emanuele and Emanuele, 2001). In this context, as a consequence of increasing oxidative stress, increased ethanol consumption leads to lowered levels of ascorbic acid (vitamin C), glutathione, and the activity of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in rat testes (Maneesh et al., 2005; Uygur et al., 2013). Furthermore, it was also reported that increased alcohol consumption promotes decreased activity of steroidogenic enzymes in the testes, which leads to decreased plasma testosterone levels (Maneesh et al., 2005).

Vitamin C is absorbed by the intestine and distributed to different tissues and organs, leading to significant levels of vitamin C in the testes, brain, lungs and adrenal glands (Castro et al., 2008). It is a cofactor for many enzymes and participates in post-transcriptional hydroxylation of collagen, biosynthesis of carnitine, and conversion of the neurotransmitter dopamine to noradrenaline (Duarte and Lunec, 2005; Castro et al., 2008). The antioxidant property of vitamin C in tissues is another important function that protects the cells from ROS (Greco et al., 2005; Lewis et al., 1997; Nazıroğlu, 2003) and is the first line of antioxidant defense against lipid peroxidation. Vitamin C can neutralize free radicals and regenerate other antioxidant molecules, such as glutathione and α-tocoferol (vitamin E) (Duarte and Lunec, 2005; Makker et al., 2009; Sen Gupta et al., 2004).

In the male reproductive system, vitamin C plays a role in testosterone synthesis (Biswas et al., 1996; Fernandes et al., 2011; Sönmez et al., 2005), protection of spermatogenesis, fertility, sperm health, and prevention of sperm agglutination (Agarwal et al., 2005; Eskenazi et al., 2005). In addition, vitamin C has known antioxidant activity in this system (Nazıroğlu, 2003; Shrilatha and Muralidhara, 2007), even when ethanol is consumed (Amanvermez et al., 2005; Rosenblum et al., 1989; Rosenblum et al., 1985), in which lipid peroxidation levels are significantly reduced in the testes after administration of vitamin C. These same authors suggest that lipid peroxidation may be an important mechanism that stimulates testicular pathogenesis and toxic effects caused by ethanol.

Although there are a series of studies addressing the influence of alcohol, there is no available data to evaluate the effects of vitamin C on the morphophysiology of the male reproductive system in ethanol-treated rats. In this context, the damage caused by ethanol in the testis and epididymis of adult male rats can be minimized or prevented after supplementation with an antioxidant such as vitamin C. Thus, the aim of this study was to evaluate whether vitamin C could prevent or attenuate the alterations in the male reproductive system caused by ethanol consumption.

2. Material and methods

2.1. Animals

Adult male Wistar rats with 90 days old (PND 90) and a body weight of around 320 g, were supplied by Biotery of Biological Sciences Centre, State University of Londrina (CCB – UEL) and kept under recommended conditions at the local Laboratory of Toxicology and Metabolic Dysfunction of the Reproduction animal house. The animals were allocated into polypropylene cages (43 × 30 × 15 cm) with laboratory grade pine shavings as bedding, during all the experiment. Temperature and lighting condition was controlled (±23 °C; 12L, 12D photoperiod, lights switched off at 07:00 p.m.). Rat chow and filtered tap water were provided *ad libitum*. Experimental procedures were in accordance with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and were approved by the Ethics Committee on Animal Use of State University of Londrina (CEUA/UEL protocol number 34148/11).

2.2. Experimental design

The animals were randomly assigned to three experimental groups of 15 animals each: control (C), ethanol (E) and ethanol + vitamin C (EC). The last two received ethanol (Nuclear®, Diadema, Brazil 2 g/kg, 25% v/v), and EC group also received vitamin C (100 mg/day/animal). The ethanol and vitamin C doses were according from Srikanth et al. (1998, 1999) and Fernandes et al. (2011), respectively, with minor modifications. Control group received only the vehicle (water). All groups were treated daily, by gavage, during 63 consecutive days. This time corresponds to one spermatogenetic cycle (53.4 days) (Clermont, 1972) further sperm transit (8–10 days) (Robb et al., 1978; França et al., 2005) in rats.

2.3. Preparation of vitamin C

Vitamin C (L-ascorbic acid; Sigma–Aldrich CO, St. Louis, USA) was diluted daily in the corresponding volume of water and stored protected from light.

2.4. Body weight and weight of some reproductive organs

At the end of the treatment, rats were anesthetized with a combination of ketamine (Sedomin® 10% Avellaneda, Argentina) and xylazine (Anasedan®, Paulínia, Brazil), weighed and killed by decapitation. Blood was collected (between 8:00 and 11:30 a.m.) from the ruptured cervical vessels for determination of sexual hormones. Testis, epididymis, vas deferens and seminal vesicle (without the coagulating gland, full and empty) were removed and their weights (absolute and relative to body weights) were determined. Testis and epididymis were used for sperm counts ($n = 10$/group), redox balance status assay ($n = 10$/group) and histopathological analysis ($n = 5$/group).

2.5. Redox balance status assay

2.5.1. Malondialdehyde (MDA) measurement

Lipid peroxidation of testis and epididymis homogenates (25 mg/ml and 5 mg/ml, respectively) of all groups was determined by reaction of thiobarbituric acid reactive substances (TBARS). Malondialdehyde (MDA) is produced as end product of lipid peroxidation chain and reacts with thiobarbituric acid (TBA) to produce a colored product representing the protein adduct TBA–MDA. In acid solution, this adduct absorbs light at 532 nm and is easily extractable in organic solvents such as *n*-butanol. MDA levels

Download English Version:

https://daneshyari.com/en/article/5859101

Download Persian Version:

https://daneshyari.com/article/5859101

Daneshyari.com