Experimental and Toxicologic Pathology 68 (2016) 435–443

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Resveratrol prevents oxidative damage and loss of sperm motility induced by long-term treatment with valproic acid in Wistar rats

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ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 13 June 2016
Accepted 1 July 2016

Keywords:
Antiepileptic
Antioxidant
Testes
Epididymides
Oxidative stress
Male fertility

ABSTRACT

Valproic acid (VPA) is a drug widely use for the treatment of epilepsy in both children and adults. Evidence suggests that long-term use of VPA may lead to an impairment in the male reproductive function. Oxidative stress is considered to play a major role in VPA associated toxicity. In the present work, we demonstrated that the natural antioxidant compound resveratrol (RSV) can be use to prevent VPA oxidative damage. Wistar rats treated with VPA (400 mg kg^{-1}) by gavage for 28 days showed decrease in sperm motility accompanied by increase in oxidative damage to lipids and proteins. Additionally, VPA administration leaded to depletion of reduced glutathione and decrease in total antioxidant potential in testes and epididymides of Wistar rats. The co-administration of RSV (10 mg kg^{-1}) efficiently prevented VPA pro-oxidant effects. In summary, RSV was shown to protect the reproductive system from the damage induced by VPA. Altogether, our data strongly suggests that RSV administration might be a valuable strategy to minimize reproductive impairment in patients requiring long-term VPA treatment.

1. Introduction

Valproic acid (2-propyl-pentanoic acid, VPA) is an antiepileptic drug used to treat a wide range of epileptic conditions in children and adults (Garcia-Morales et al., 2007; Perucca, 2002). VPA is also utilized for the treatment of other diseases such as migraine (Krymchantowski et al., 2002) and bipolar psychiatric disorders (Nasrallah et al., 2006). Besides these applications, VPA has also been shown to be efficient to treat advanced cancer patients, when combined with other chemotherapeutic regimens (Cinatl et al., 2002; Kortenhorst et al., 2009; Wang et al., 2013). The use of VPA for the treatment of different pathologies revealed that toxic effects are associated with long-term use of this drug as, for example, hepatotoxicity (Chang and Abbott, 2006; Kiang et al., 2010; Tong et al., 2005), neurotoxicity (Chaudhary and Parvez, 2012) and impaired fertility (Bairy et al., 2010; Cohn et al., 1982; Khan et al., 2011; Krogenaes et al., 2008; Nishimura et al., 2000; Rättyä et al., 2001; Røste et al., 2002, 2005; Vijay et al., 2008).

Animal (Bairy et al., 2010; Cohn et al., 1982; Khan et al., 2011; Krogenaes et al., 2008; Nishimura et al., 2000; Røste et al., 2002; Vijay et al., 2008) and clinical (Chen et al., 1992; Rättyä et al., 2001; Røste et al., 2005) studies demonstrated adverse effects of the long term use of VPA on male reproductive function. The exact mechanism of VPA toxicity to the male reproductive system is not well defined, but formation of reactive oxygen species (ROS) and the development of oxidative stress have been proposed to explain some of the adverse effects of VPA, including hepatotoxicity (Chang and Abbott, 2006; Kiang et al., 2010; Tong et al., 2005), neurotoxicity (Chaudhary and Parvez, 2012), and teratogenicity (Tung and Winn, 2011). Furthermore, Khan et al. (2011) demonstrated recently that treatment with VPA is toxic to germ cells. The authors have shown that VPA causes depletion of reduced glutathione (GSH) and increases damage to sperm DNA, which demonstrates thatROS generation might be a key mechanism responsible for the toxicity of VPA in the reproductive system (Khan et al., 2011)

Under physiological conditions, ROS are formed during the metabolism of oxygen, and their concentrations are controlled by

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http://dx.doi.org/10.1016/j.etp.2016.07.001
0940-2993/

antioxidant defenses. Several enzymatic systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione *S*-transferase (GST), protect the cells against ROS formation. These antioxidant defenses also include non-enzymatic systems as, for example, GSH (Halliwell and Gutteridge, 1999). Oxidative stress occurs when there is an imbalance between production and removal of ROS (Evelson et al., 2001).

Increased oxidative stress is reported as one of the major causes of male infertility. Spermatozoa and testes, being rich in polyunsaturated fatty acids, show a great susceptibility to attack by ROS and oxidative damage (Turner and Lysiak, 2008). Whereas the generation of low levels of ROS is an important component of the signal transduction stimulating capacity of spermatozoa (Ford, 2004), excessive ROS levels induce lipid peroxidation of sperm cell membrane, malfunction of capacitation, impaired acrosome reactions, and loss of motility (Aitken et al., 1985, 2014).

Resveratrol (3,4′,5-triiidroxiestilbene, RSV) is a natural polyphenolic compound found primarily in grapes and wine, presenting a considerable number of beneficial effects in a variety of organs and systems, which are mainly due to its antioxidant activity (Frémont, 2000). Protective effects of RSV against oxidative damage *in vivo* and *in vitro* are likely due to regulation of endogenous antioxidant cellular systems, in addition to acting as an antioxidant scavenger of ROS (Spanier et al., 2009). RSV also inhibits formation of ROS suppressing pro-oxidant genes (Baur and Sinclair, 2006; Dolinsky et al., 2009; Spanier et al., 2009) and inducing antioxidant enzymes including SOD, CAT, and GPx (Spanier et al., 2009; Tanno et al., 2010; Ungvari et al., 2007). Belguendouz et al. (1997) also reported that RSV chelates copper and other transition metals, which are able to generate free radicals and cause lipid peroxidation. Moreover, RSV has been described to prevent damage to male fertility triggered by different oxidative stress inducers like ethanol (Kasdallah-Grissa et al., 2006), *tert*-butil hydroperoxide (Collodel et al., 2011), triiodotironine (Ourique et al., 2013), and iron/ascorbate (Mojica-Villegas et al., 2014). Altogether, these data suggests that RSV may be used as an alternative to minimize reproductive impairment caused by continuous use of VPA.

In the present study, we assess the toxic effect of VPA on male reproductive system, where it caused decrease in sperm motility and vigor. These alterations were associated with increase in lipid peroxidation and oxidative damage to proteins in both testes and epididymides of Wistar rats. The co-administration of RSV was efficient to protect against VPA-mediated toxicity in testes and epididymides. Therefore, we demonstrated that RSV has potential to efficiently improve male fertility in VPA treated individuals.

2. Material and methods

2.1. Chemicals

Valproic acid (Valpakine syrup at a concentration of 200 mg mL^{-1}) was obtained from Sanofi Laboratories (São Paulo, SP, Brazil). RSV was purchased from Pharma Nostra (Chengdu Hawk Bio-Engineering, China), and its purity and structure were previously confirmed using chromatography and nuclear magnetic resonance, respectively. All other reagent-grade chemicals were obtained from Sigma (St Louis, Missouri, USA).

2.2. Animals

All animal procedures were approved by the Animal Ethics Committee of the Federal University of Santa Maria (process 076/2013). Adult male Wistar rats (90 days) were obtained from the Central Animal Breeding Facility of the Federal University of Santa Maria, RS, Brazil. The animals were kept in polypropylene cages with controlled temperature (23 ± 2 °C) and a light-dark cycle of 12 h with access to water ad libitum and to approximately 30 g daily per animal of rodent laboratory chow (Supra, São Leopoldo, RS, Brazil). Animals were acclimated to the experimental conditions for a period of two weeks prior to the commencement of the experiment.

2.3. Experimental protocol

Rats (n = 32) were randomly divided in four experimental groups (n = 8 each group) as it follows: (C) control; (RSV) treated with 10 mg kg^{-1} of RSV; (VPA) treated with 400 mg kg^{-1} of VPA; and (VPA + RSV) treated with 400 mg kg^{-1} of VPA and 10 mg kg^{-1} of RSV. VPA was administered daily by gavage at a dose of 400 mg kg^{-1} body weight for 28 days and control groups (C and RSV) received an equal volume of vehicle solution. The dose of 400 mg kg^{-1} (approximately equivalent to the maximum human daily dose on a mg/m^2 basis) of VPA was selected based on the results obtained from different studies carried out for reproductive toxicity in rodents (Khan et al., 2011; Røste et al., 2002; Vijay et al., 2008). Concomitantly with VPA treatment, animals received daily intraperitoneal (i.p.) injections of RSV, freshly prepared in 1% tween 80, at a dose of 10 mg kg^{-1} body weight for 28 days, as used in our previous study (Ourique et al., 2013). Groups C and VPA received i.p. injections of 1% tween 80 in the same conditions.

At the end of the experimental period (four weeks) and 24 h after the last administration, animals were weighed and anesthetised with xylazine and ketamine. Blood was collected through cardiac puncture, and rats were euthanized for removal of their epididymides, testis, prostate, and seminal vesicle which were immediately weighed.

2.4. Assessment of testosterone in serum

Blood was collected in tubes and it was separated using centrifugation (1800*g*, 15 min). Serum was stored at −20 °C for further analysis. Testosterone levels were measured using a competitive electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN). Analyses were performed by the Pasin Laboratory (Santa Maria, RS, Brazil) and expressed as ng dL^{-1}.

2.5. Removal of epididymides and retrieval of spermatozoa

Epididymides were excised and adherent and fat tissues were removed. Cauda epididymidis was collected, transferred to a Petri dishes and immersed in sterile silicone oil, nearby a drop of 200 μL of Fert's medium (114 mM NaCl, 3.22 mM KCl, 0.34 mM NaH_2PO_4, 25 mM $NaHCO_3$, 16 mM $C_3H_5O_3Na$, 0.6% BSA, 0.1% phenol red, 2 mM $CaCl_2{\cdot}2H_2O$, 0.5 mM $MgCl_2{\cdot}6H_2O$, and Milli-Q water). All reagents were adequately preheated. Longitudinal incisions were made in the cauda epididymidis with a fine needle and a scalpel blade to release the spermatozoa. The sperm motility and vigor were evaluated by placing a 4 μL drop of Fert's medium containing the spermatozoa on a slide and then examining the drop using a light microscope (Olympus CX40) at 100×. The percentage of total and progressive motility was estimated from three different fields in each sample, and the mean was used as the final value of motility. Vigor of movement was also estimated, using the following scale: 0, no movement; 1, slight side-to-side movement, no forward progression; 2, rapid side-to-side movement, no forward progression; 3, rapid side-to-side movement, occasional forward progression in spurts; 4, steady, slow forward progression; 5, rapid, steady forward movement (Platz and Seager, 1978).

Epididymal sperm count was determined using Neubauer's hemocytometer. Sperm concentration was expressed as number of sperm per mL of solution containing sperm. To analyse the