Food and Chemical Toxicology 91 (2016) 65–72

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Protective effects of resveratrol against cisplatin-induced testicular and epididymal toxicity in rats

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ARTICLE INFO

Article history:
Received 29 December 2015
Received in revised form
22 February 2016
Accepted 23 February 2016
Available online 27 February 2016

Keywords:
Cisplatin
Resveratrol
Steroidogenesis
Spermatogenesis
Oxidative stress
Male rat

ABSTRACT

This study investigated the probable protective effect of resveratrol against cisplatin-induced testicular and epididymal toxicity in rats. Body weights of the animals showed no significant changes after cisplatin administration. Conversely, the weights of testis, and accessory sex organs reduced significantly. The daily sperm production and epididymal sperm quantity and quality were decreased in cisplatin treated rats. The circulatory levels of testosterone and activity levels of testicular 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were significantly decreased after cisplatin treatment. The activity levels of superoxide dismutase and catalase were decreased with an increase in the levels of lipid peroxidation and H_2O_2 generation in the testis and epididymis of cisplatin treated rats, suggesting the cisplatin-induced oxidative stress. The biochemical findings were supplemented by histological examination of testis. Reduced tubular size, decreased spermatogenesis and deterioration in architecture were observed after cisplatin treatment. Administration of resveratrol alone has no significant effect on testicular and epididymal metabolism. On the other hand, administration of resveratrol ameliorated cisplatin-induced alterations in testicular and epididymal oxidative damage, suppressed steroiodgenesis and spermatogenesis and restored testicular architecture. In conclusion, resveratrol possesses multi-mechanistic protective activity that can be attributed to its steroidogenic and antioxidant actions.

1. Introduction

Cisplatin is widely used in the treatment of various types of malignancies, including testicular cancer (Koster et al., 2013). In spite of its therapeutic importance, its use is, however, often limited by its oto-toxicity (Erdem et al., 2012), hepato-toxicity (Waseem et al., 2015), nephro-toxicity (dos Santos et al., 2012) and reproductive toxicity (Amin et al., 2012; Beytur et al., 2012). After treatment for testicular cancer, patients experience a decrease in the number of spermatozoa produced and in their viability, motility, as well as an increase in morphologically abnormal spermatozoa (Jahnukainen et al., 2011; Rives et al., 2012). The mechanism of reproductive toxicity caused by cisplatin is based on the suppression of steroidogenesis and the generation of reactive oxygen species (ROS) (Ahmed et al., 2011). It is suggested that mild and low levels of ROS may enhance the fertilizing potential by promoting hyperactivation motility and capacitation of sperm. On the other hand, it should be noted that mild ROS levels predisposes the sperm to capacitation/acrosome reaction and that more ROS levels are significantly correlated with impaired embryo development (de Lamirande et al., 1997). However, endogenous antioxidant systems prevent their toxic effects to living organism in normal healthy conditions. Superoxide dismutase (SOD) catalase and glutathione peroxidase are the major endogenous antioxidant enzymes which play role in prevention of oxidative injury. The other endogenous radical scavenging agents include glutathione, ascorbic acid, melatonin, etc.

Thus many antioxidant agents including melatonin (Surendran et al., 2012), lycopene (Salem et al., 2012), amifostine (Prieto Gonzalez and Fuchs, 2009) have been considered in experimental and clinical studies to reduce or prevent cisplatin-induced oxidative toxicity. The present study is focused on resveratrol. Resveratrol (trans-3,5,4′-trihidroxystilbene), one of the promising dietary phytoalexins and potent antioxidant is found in a wide range of foods, especially grapes (Das et al., 2010). The resveratrol exhibits pleiotropic health beneficial effects including anti-oxidant, anti-

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http://dx.doi.org/10.1016/j.fct.2016.02.017
0278-6915/

inflammatory, anti-tumor, anti-viral, anti-aging, anti-diabetic, anti-hyperlipidemic, anti-atherogenic and cardio-protective properties (Benayahoum et al., 2013; Dudka et al., 2012). Recently, *In vivo* and *in vitro* studies have shown that resveratrol protects spermatocytes against lipid peroxidation and increases sperm motility, viability, and mitochondrial membrane potential (Bucak et al., 2015; Collodel et al., 2011; Mojica Villegas et al., 2014). In addition, resveratrol is more effective in reducing oxidative DNA damage than melatonin, vitamin E and α-phenyl-N-tert-butyl nitrone (Alturfan et al., 2012; Cadenas and Barja, 1999; Xiao, 2015). Therefore, the aim of the present study was to investigate possible protective effects of resveratrol against cisplatin-induced reproductive toxicity with special focus on testicular steroidogenesis, spermatogenesis, sperm characteristics, testicular and epididymal oxidative toxicity through oxidant/antioxidant parameters and light microscopic evaluation of testicular architecture in Wistar rats.

2. Materials and methods

2.1. Procurement and maintenance of animals

Three month old adult male Wistar rats (body weight 210 ± 10 g) were purchased from an authorized vendor (Sri Raghavendra Enterprises, Bengaluru, India). The animals were kept in groups of four in a cage (18″ × 10″ × 8″) in a temperature (23 ± 2 °C) and humidity (60 ± 5%) controlled room on a 12:12 h light:dark cycle (lights on at 8.00 a.m.). Rats were given a standard rodent chow (obtained from Sai Durga Agencies, Bengaluru, India) and water *ad libitum*. The experiments were carried out in accordance with guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India (CPCSEA, 2003) and experimental protocols were approved by the Institutional Animal Ethical Committee, S.V. University, Tirupati.

2.2. Chemicals

Cisplatin (99% purity) (Cadila pharmaceuticals Ltd., Ahmedabad, India), resveratrol (>99% purity) (TCI Chemicals, Pvt. Ltd., Chennai, India) were purchased from local drug store. Androstenedione (≥98% purity), dihydroepiandrosterone (98% purity), NAD (≥98% purity) and NADPH (97% purity), horse radish peroxidase (>90% purity), were purchased from Sigma Chemical Company (St Louis, MO, USA). Dimethyl sulfoxide (purity ≥99%) (DMSO), malondialdehyde (purity ≥95%) (MDA), and pyrogallol (purity ≥99%) were obtained from Merck (Darmstadt, Germany). All other chemicals used in study were purchased from HIMEDIA (Bangalore, India).

2.3. Experimental design

Animals were randomly divided into four (4) groups with eight animals in each and the details of groups are as under:

Group 1: Control rats given normal saline
Group 2: Rats given resveratrol (20 mg/kg body weight/day) through intraperitoneal injection for 45 days
Group 3: Rats injected with cisplatin (3 mg/kg body weight) on first 3 alternate days
Group 4: Rats given resveratrol (as in group 2) along with cisplatin (as in group 3)

The dose of either cisplatin or resveratrol was based on the published literature (Kishore Reddy et al., 2010 and Turedi et al., 2015 respectively) and the rationale for choosing a 45 day experimental period was to evaluate the effect through a complete testicular spermatogenic cycle (Clermont, 1972). Pilot studies were also performed in our laboratory (data not shown). Since pharmacokinetic data for resveratrol is sparse (Kundu and Surh, 2008), the minimum required dose to exert the antioxidant effect *in vivo* in rats (Yulug et al., 2013) was administered through intraperitoneal route. The human dose of cisplatin was converted to rat dose in relation to body weight/surface area ratio. Rats were treated with 3 mg cisplatin/kg body weight.

2.4. Measurement of general clinical parameters

2.4.1. Determination of body weight and observation of signs of toxicity

The body weight of all animals was recorded in the beginning and on the 46th day of experiment. Rats were observed daily for signs of toxicity such as body position, overall appearance, gait and behavior observations. Food and water intake was recorded once in a week during the treatment period.

2.4.2. Necropsy and determination of tissue somatic indices

The animals were fasted overnight, weighed and killed by cervical dislocation on 46th day. Animals were humanely sacrificed, and the brain, liver, kidney, epididymis (caput, corpus and cauda), seminal vesicles, prostate, vas deferens and penis were removed, cleared of adhering tissues, weighed to nearest milligram by using a Shimadzu electronic balance (Model No: BL- 220H; Kyoto, Japan). Tissue somatic indices (TSI) were calculated by using the following formulas:

$$\text{TSI} = [\text{weight of the tissue (g)}/\text{Body weight of the animal (g)}] \times 100$$

Sizes of testes were also measured using water displacement method. Testes were used for determination of daily sperm production, histological and biochemical studies, and cauda epididymis was used for sperm analysis. Epididymis was also used for biochemical studies.

2.5. Epididymal sperm analysis

The cauda epididymis was removed immediately after autopsy from each rat and spermatozoa were squeezed into petridish containing physiological saline (0.9% NaCl in distilled water). Spermatozoa were counted using an improved Neubauer Chamber, as described by Belsey et al. (1980) and expressed as millions mL^{-1}. Progressive sperm motility was determined within 5 min following their isolation from cauda epididymis at 37 °C by using the method of Belsey et al. (1980), and the data was expressed as percent motility of total sperm counted. The viability based on the ratio of live to dead spermatozoa was determined using 1% trypan blue solution as described earlier by Talbot and Chacon (1981). Sperm membrane integrity was assessed by exposing the sperm to hypo-osmotic solution and observed for tail coiling under the phase contrast microscope (HOS tail coiling). The percent of HOS tail coiled sperm was determined following the method described by Jeyendran et al. (1992). The data expressed as percentage of total sperm.

2.6. Daily sperm production

Testicular daily sperm production was determined by the method of Blazak et al. (1993). In brief, testis was decapsulated and parenchyma was homogenized in 50 ml of ice-cold 0.9% NaCl solution containing 0.01% Triton X-100 using a sterilized mortar and pestle. The homogenate is allowed to settle for 1 min and filtered