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Effects of resveratrol on doxorubicin induced testicular damage in rats☆

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ABSTRACT

The purpose of this study was to evaluate the likely protective effect of resveratrol (RES) on doxorubicin (DOX) induced testicular damage. Rats were divided into five groups: control, RES, dimethyl sulfoxide (DMSO), DOX and DOX + RES. At the end of treatment, the rats were sacrificed. Plasma testosterone levels, oxidative status, epididymal sperm parameters and testicular apoptosis were evaluated. MDA levels, GP-x and GSH activities were higher in the DOX group than in the control group. MDA levels were lower in the DOX + RES group than in the DOX group. The DOX group exhibited a significant decrease in plasma testosterone levels, sperm concentration and motility, and a significant increase in abnormal sperm rate and TUNEL (+) cells in the testis. A significant increase was observed in plasma testosterone levels and sperm concentration and motility, and a significant decrease in the abnormal sperm rate and TUNEL (+) cells in the DOX + RES group compared to the DOX group. A marked improvement in severe degenerative alterations in the germinative epithelium was also observed following treatment with RES. In conclusion, RES makes a positive contribution to fertility by exhibiting anti-apoptotic and antiperoxidative effects against DOX-induced testicular damage.

1. Introduction

Anticancer therapy generally compromises physiological hemostasis and causes multi-organ failure during the therapeutic process (Ayla et al., 2011). Doxorubicin (DOX) is an antracycline-group antibiotic that has been used as an antineoplastic agent in the treatment of various hematological malignities, including solid tumors, for many years (Doroshow et al., 1980; Gharanei et al., 2013). Due to its severe side-effects on the testis, as well as toxicity in various organs (Yeh et al., 2009), its clinical use is limited, however (Miranda et al., 2003; Ayla et al., 2011). DOX treatment may damage male fertility by adversely affecting sperm development, production and count (Prahalathan et al., 2005a). Previous studies have shown that it causes apoptosis in the seminiferous epithelium cycle (Sjoblom et al., 1998) and chromosomal damage in germ cells (Au and Hsu, 1980). The exact mechanism responsible for DOX's testicular toxicity is unclear, although recent findings point to oxidative stress and cellular apoptosis as major causes (Prahalathan et al., 2004; Yeh et al., 2009). DOX therapy-related oxidative damage is mediated by an oxyradical complex containing superoxide, hydroxyl radical and a certain amount of iron (Hida et al., 1995). Oxyradicals lead to damage in the mitochondrial and other cytoplasmic organelle membranes through proteins, nucleotides and phospholipids (Muraoka and Miura, 2003).

Resveratrol (RES) (trans-3,4′,5-trihidroksi-stilben) is a natural phytoalexin found in various plants, such as grape, peanut and mulberry (Krasnow and Murphy, 2004; Caruso et al., 2004). RES' biological activities are known to exhibit antioxidant, anti-inflammatory, anticancer, antiatherogenic and cardioprotective properties (Ignatowicz and Baer-Dubowska, 2001; Dudka et al., 2012; Benayahoum et al., 2013). In addition, RES' antioxidant effect may also help protect against DNA damage occurring after a rise in ROS levels and against lipid peroxidation in the cell membrane (Jiang et al., 2008). Studies have shown that it is more effective that vitamins E and C, the best known antioxidants (Belguendouz et al., 1998). In another study, grapeseed extracts containing RES were shown to protect glial cells against oxidative stress (Royshowdhury et al., 2001). The antioxidant activity of RES is associated with its ability to inhibit ribonucleotide reductase and cyclooxygenase transcription in DNA polymerase activity (Frémont, 2000).

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The purpose of this study was to evaluate the likely protective effect of RES on DOX-induced testicular damage using histopathological and biochemical analyses.

2. Materials and methods

2.1. Animals and experimental design

Thirty male Sprague–Dawley rats (5 weeks old, weighing 140–170 g) were used. All animals were kept in standard laboratory conditions. Standard laboratory chow and water were provided ad libitum throughout the experiment. The study was approved by the Institutional Animal Ethical Committee of Karadeniz Technical University, Trabzon, Turkey.

The rats were randomly divided into five groups. The control group (n: 6) was given a single dose of 9% isotonic sodium chloride intraperitoneally (i.p.). The RES group (n: 6) was given 20 mg/kg RES i.p. (R5010-500 mg, Sigma–Aldrich, St. Louis, MO, USA) per day [dissolved in 30% dimethyl sulfoxide (DMSO)] every day throughout the experiment, while the DMSO group (n: 6) was administered 30% DMSO solution i.p. The DOX group (n: 6) was injected with a single dose of 10 mg/kg DOX (Adriblastina 10 mg, DEVA, Turkey) i.p. The DOX + RES group (n:6) was administered a single dose of 10 mg/kg DOX together with 20 mg/kg RES i.p. per day throughout the experiment. Dosages of RES (Oktem et al., 2012) and DOX (Atessahin et al., 2006) were determined on the basis of reports from previous studies.

2.2. Sample collection

At the end of the 21-day experiment, rats were sacrificed by decapitation under ketamine (Ketalar, Pfizer, Turkey) anesthesia. The abdominal cavity was quickly opened and the testes extracted and separated from the epididymises. The right testicular epididymis was placed in a Petri dish containing 2 mL Tris buffer solution for sperm parameter analysis. The right testis and the left epididymis were fixed in Bouin's solution for histological analysis. Blood and tissue specimens collected for biochemical parameters were stored at −80 °C.

2.3. Biochemical analysis

Plasma malondialdehyde (MDA) levels were determined using the method described by Yagi (1994). Tetramethoxypropane was used as a standard, and MDA levels were calculated as nmol/mL. Tissue MDA levels in testis samples were measured using the method described by Uchiyama and Mihara (1978). Tetramethoxypropane was used as a standard, and MDA levels were calculated as nanomoles per gram wet tissue.

Superoxide dismutase (SOD) and catalase (CAT) activities were determined in the remaining part of the testis tissue. SOD activities were measured using the method described by Sun et al. (1988). Enzyme activity leading to 50% inhibition was taken as one unit, and the results were expressed as U/mg protein. CAT activity was determined using the method described by Aebi (1974), and the results were expressed as k/g protein (k, rate constant). Protein concentrations were determined following Lowrey's method (1951).

Glutathione peroxidase (GP-x) activity was determined using a commercial spectrophotometric kit (Cayman, USA). Glutathione (GSH) levels were analyzed using high-performance liquid chromatography (Afzal et al., 2002) (Agilent 1100 series HPLC and fluorescence detector systems, Waldbronn, Germany; column, Fortis UniverSil C18, 4.6 mm × 250 mm). Results were expressed as μg/mL. Tissue glucose-6-phosphate dehydrogenase (G6PD) and sorbitol dehydrogenase (SDH) activities were measured using the methods described by Deutsch (Aebi, 1986a) and Gerlach (Aebi, 1986b), respectively. The results were expressed as U/g protein. Plasma testosterone levels were measured using a rat testosterone EIA kit (Cayman, USA).

2.4. Histopathological examinations

Testis and epididymis specimens were fixed, embedded in paraffin blocks, sliced into 5-μm sections and stained with hematoxylin–eosin (H&E) for histopathological evaluation. All testicular histology was examined by a histologist under light microscopy (Olympus BX-51; Olympus, Tokyo, Japan). For seminiferous tubule diameter (STD) and germinal epithelium thickness (GET) measurement, 10 different areas were selected at random from each testis section. The Analysis 5 Research program (Olympus Soft Imaging Solutions, Germany) was used under light microscopy at a magnification of 200×, and 20 seminiferous tubules were selected and measured on each testis section. Johnsen's tubular biopsy score (JTBS) was used to evaluate spermatogenesis. Under this system, testis seminiferous tubule sections were scored from 0 to 10 (Johnsen, 1970).

2.5. TUNEL assay

Testicular apoptosis was analyzed using the terminal deoxynucleotidyl transferase (TdT) deoxyuridine triphosphate nick end labeling assay (TUNEL) method. Sections 5 μm in thickness were taken from the paraffin blocks and subjected to standard deparaffinization. TUNEL staining of sections was performed using an in situ cell death detection kit AP kit (Roche, Mannheim, Germany), in accordance with the manufacturer's instructions. The proportion of TUNEL (+) spermatogenetic cells to the number of normal spermatogenetic cells was taken as the testis apoptotic index (TAI) (Karaguzel et al., 2012).

2.6. Epididymal sperm concentration, motility and abnormal sperm rate

The epididymis was broken down in a Petri dish containing 2 mL Tris buffer tampon solution and incubated for 30 min at 37 °C for the sperm to swim in the fluid. Counts, motility and abnormal sperm levels were calculated. A Makler sperm counting chamber (Sefi-Medical Instrument, Haifa, Israel) was used to calculate sperm concentrations. Briefly, 5 μL of semen from the homogenate was dropped into the center of the chamber and the glass lid was closed. Sperm were made to swim at a depth of 10 μm with the assistance of four quartz blades. Sperm count was then calculated under a light microscope at 200× magnification (%10^6).

Sperm motility was calculated by modifying the method described by Sonmez et al. (2005). One drop of sperm fluid diluted in Tris buffer solution was collected by pipette, placed onto a slide and covered with a slide cover. Sperms were evaluated as motile or immotile under a light microscope at 400× magnification. Three different areas were selected for each specimen, and the mean of these was taken as the motility score.

Sperm morphology was calculated by modifying the method described by Trivedi et al. (2011). Sperm containing solution was centrifuged at 1000 rpm for 3 min. Subsequently, 2–3 drops of solution were spread on a slide, dried, fixed with methanol for 3 min and stained with 1% eosin-y. Two hundred sperm from each animal were examined for head and tail anomalies under a light microscope under immersion oil at a magnification of 100×, and were classified as normal, head abnormal or tail abnormal (Xin et al., 2012).