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Pathophysiology 22 (2015) 117–123

ISP PATHOPHYSIOLOGY

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Effect of olive leaf extract treatment on doxorubicin-induced cardiac, hepatic and renal toxicity in rats

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Received 12 June 2014; received in revised form 3 April 2015; accepted 30 April 2015

Abstract

Doxorubicin (DOX) is known to increase in oxidative stress in several organs. Olive leaf extract (OLE) has potent antioxidant effects; therefore, we evaluated the ability of OLE to reduce DOX-induced toxicity in the heart, liver, and kidneys of rats. DOX (30 mg/kg; i.p.) was administered to rats, which were sacrificed 4 days after DOX. The rats received OLE (6 and 12 mL/L in drinking water) for 12 days. Serum cardiac troponin I (cTnI) levels, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities, urea and creatinine levels, as well as prooxidant and antioxidant status in organs were measured. DOX was found to increase serum markers that indicate tissue injury, malondialdehyde (MDA), diene conjugate (DC), and protein carbonyl (PC) levels, and to decrease glutathione (GSH) levels in organs. Histopathologic changes were also evaluated. OLE, especially OLE 1000, led to decreases in serum cTnI and urea levels, ALT and AST activities, and amelioration in histopathologic findings. Decreases in MDA, DC, and PC, and increases in GSH levels were observed in organs of DOX-treated rats due to OLE. We conclude that OLE treatment may be effective in decreasing DOX-induced cardiac, hepatic and renal oxidative stress and injury.

Keywords: Doxorubicin; Olive leaf extract; Oxidative stress; Organs; Rat

1. Introduction

Doxorubicin (DOX) is an antineoplastic agent used in the treatment of a variety of human neoplasms. Despite its high antitumor efficiency, the use of DOX in clinical chemotherapy is limited due to its toxic effects, cardiotoxicity that leads to the development of cardiomyopathy and eventually to heart failure is a major limiting factor [1]. Although mechanisms responsible for DOX-induced toxicity on organs are not clearly known, oxidative damage to cellular components is believed to be a major factor in the DOX cardiotoxicity [2–10]. An increase in the formation of reactive oxygen species (ROS) and alteration in prooxidant-antioxidant balance in favor of prooxidation were observed in cardiac muscular tissues of DOX-treated animals [2–7]. Some investigators have also detected increases in hepatic [4,5,8,9] and renal [9,10] oxidative stress parameters associated with tissue damage in DOX-treated animals. Therefore, many investigators have tested the preventive effects of several antioxidants against DOX-induced organ toxicity [2–10].

Olive (*Olea europaea* L.) tree leaves have been widely used for traditional therapies in Mediterranean countries [11]. Several experimental studies have shown that olive leaves have antihypertensive, antiatherogenic, antiinflammatory, hypoglycemic, and hypocholesterolemic actions [11–13]. These effects are attributed to the antioxidant components of olive leaves [11–13]. Oleuropein and its derivatives

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http://dx.doi.org/10.1016/j.pathophys.2015.04.002
0928-4680/

such as hydroxytyrosol and tyrosol are the main phenolic constituents of olive leaves, which are thought to be responsible for their pharmacologic effects. In addition, olive leaves contain caffeic acid, p-coumaric acid, vanilic acid, vanillin, luteolin, diosmetin, rutin, verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside [11–13]. It has been found that olive leaf extract (OLE) or its constituents such as oleuropein have protective effects on oxidative stress-induced cardiac [2,14], hepatic [15–17] and renal [18] damage in experimental animals. However, only one study has investigated the effect of OLE on DOX-induced oxidative stress and organ damage [2].

Therefore, in the present study we aimed to investigate the effect of OLE treatment on organ damage and prooxidant and antioxidant status in the heart, liver and kidneys of DOX-treated rats. We measured serum markers of cardiac, hepatic and renal damage, histopathologic observations, as well as malondialdehyde (MDA), diene conjugate (DC), protein carbonyl (PC), glutathione (GSH) levels, Cu,Zn-superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities in the focus organs.

2. Materials and methods

2.1. Chemicals

DOX (50 mg/25 ml injectable form) was obtained from Fresenius Kabi Oncology Ltd (India). Other chemicals such as thiobarbituric acid (TBA), 2,4-dinitrophenylhydrazine (DNPH), 5,5-dithiobis-(2-nitrobenzoate) (DTNB), cumene hydroperoxide were supplied from Sigma-Aldrich (St Louis, MO, USA).

OLE (liquid) was provided by the Bio-Olive Ltd Company (Ayvalık-Turkey). Olive leaves were collected from *Olea E.* L. trees cultivated under organic farming practices in Ayvalık. Collected leaves were dried at 30 °C for 24 h in an air-oven and then ground to pass a 2-mm screen and stored in a dry and dark place. 100 g of olive leaf powder was extracted with 70% (v/v) aqueous ethanol for 24 hrs at room temperature using a shaking incubator, which was fixed to 180 rpm. The extracts were filtered with Whatman No. 1 filter paper. To remove ethanol, the filtrates were evaporated by using a rotary evaporator under reduced pressure at 38 °C and concentrated under approximately 40 ml in volume. The final quantity of OLE approximately equaled 42 g. Thus, the obtained OLE liquid was dark-green in color, clear, and tasted a slightly sweet. This liquid extract was kept at 4 °C during the period of administration [19].

The total phenol and oleuropein contents of the OLE liquid were analyzed at the İzmir Institute of Science and Technology (İzmir, Turkey) using the method of Folin Ciocalteau [20] and Hewlet-Packard HPLC with a diode array detector [19,21], respectively. Total phenol content was calculated as 196.8 mg gallic acid equivalent/g OLE. Oleuropein content was also detected as 97.0 mg/g OLE.

2.2. Animals and treatments

Male Sprague-Dawley rats that weighed 300–400 g were used in this study. The rats were obtained from the Institute for Experimental Medicine, Istanbul University. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of Istanbul University. The animals were allowed free access to food and water and were kept in wire-bottomed stainless steel cages.

The rats were divided into six groups. (a) Control group: rats were given 0.9% NaCl as vehicle. (b) OLE 500 and 1000 groups: rats were given OLE (6 or 12 ml OLE liquid/L in drinking water) for 12 days. The consumption of OLE was roughly equivalent to 500 and 1000 mg/kg/day in rats. When this was calculated for oleuropein, the dosage was equivalent to 50 mg/kg/day and 100 mg/kg/day. (c) DOX group: a single dose of DOX (30 mg/kg; i.p.) was administered and rats were sacrificed 4 days after DOX injection. (d) OLE-treated DOX groups: rats were given OLE (6 or 12 ml OLE liquid/L in drinking water) for 12 consecutive days, 8 days before and 4 days after the DOX administration.

At the end of treatment period, the animals were fasted overnight and anesthetized with sodium pentobarbital (50 mg/kg; i.p.). The rats were then sacrificed by taking blood through cardiac puncture and blood was collected into dry tubes. The rats' liver, heart and kidney were quickly removed and washed in 0.9% NaCl and kept at −70 °C until they were analyzed. Organs were rapidly excised. Both left and right ventricular walls of the heart, and longitudinal sections of the liver and kidney were fixed in 10% buffered formalin for histopathologic analysis. The remaining parts of the organs were used for biochemical analyses. Homogenates were prepared with ice-cold 0.15 M KCl (10%; w/v) and postmitochondrial fractions were obtained by centrifugation at 10,000 × *g* for 20 min.

2.3. Determinations

Serum cardiac troponin I (cTnI) levels were determined in autoanalyzer (Beckmann Coulter Access immunoanalyzer). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, urea and creatinine levels were measured using a Cobas Integra System (Roche Diagnostics, Mannheim, Germany).

Lipid peroxidation in organs was assessed using two different methods: (a) to evaluate MDA levels, homogenates were added to Buege-Aust reagent (15% trichloroacetic acid: 0.375% TBA: 0.25 N HCl; 1:1:1; w/v) and heated at 100 °C for 15 min, then centrifuged. The absorbance of the supernatants was recorded at 532 nm. The breakdown product of 1,1,3,3-tetraethoxypropane was used as a