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Protective effects of silymarin and curcumin on cyclophosphamide-induced cardiotoxicity

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ABSTRACT

Introduction: Cyclophosphamide (CP) is a potent anticancer agent; its clinical use is limited due to its marked cardiotoxicity.
Aim: The present study was aimed at evaluating the cardioprotective effects of silymarin (SLY) and curcumin (CUR), which have strong antioxidant properties, against the toxic effects of high-dose CP on the heart of rats.
Materials and methods: A total of 36 adult Wistar albino female rats were randomly divided into six groups. Group I (control group; nothing was administered), Group II (CP group; 30 mg/kg/day CP was administered intraperitoneally to each animal for seven days), Group III (SLY group; 100 mg/kg/day SLY by gavage for 14 days), Group IV (CUR group; 100 mg/kg/day CUR by gavage for 14 days), Group V (SLY + CP group; 100 mg/kg/day SLY by gavage for 14 days plus 30 mg/kg/day CP intraperitoneally starting from the seventh day) and Group VI (CUR + CP group; 100 mg/kg/day CUR by gavage for 14 days plus 30 mg/kg/day CP intraperitoneally starting from the seventh day). Biochemical, histopathological and immunohistochemical methods were utilised for evaluation of the cardiotoxicity.
Results: The result showed that an increase in heart MDA and DNA fragmentation levels were detected while significant decreases were seen in SOD levels in CP alone group when compared to the other groups. CP caused severe damage in the histopathological status of heart tissue including intersititial oedema, haemorrhage, degeneration and necrosis in muscle fibrils and perinuclear vacuolization. A significant increase in the percentage of TUNEL-positive cells and γH2AX protein expression was detected in the CP-treated group compared to the control and other treated groups. There was significant increase in the percentage of caspase 3-positive cells and decrease in the percentage of Bcl-2 positive cells in the CP group compared to the control group and other treated groups. However, a significant decrease in the percentage of cTnI and cTnT immunoreactivity was also observed in the CP-treated group compared to the control and other treated groups. In the groups in which SLY and CUR were administered concurrently with CP, biochemical parameters, histopathological and immunohistochemical results were found to be significantly lower than in the CP-only group.
Conclusions: These results lead to conclusion that the natural antioxidant SLY and CUR might have protective effects against CP-induced cardiotoxicity and oxidative stress in rats.

1. Introduction

Cyclophosphamide (CP) [*N,N*-bis(2-chloroethyl) tetrahydro-2H-1,3,2-oxphosphorin-2-amine, 2-oxide monohydrate], also known Cytoxan or Endoxan, is a pharmaceutical product used as an antineoplastic agent in the treatment of a wide range of cancers (Dollery, 1999; McCarroll et al., 2008). It also has potent immunosuppressive effects for organ transplantation and autoimmune diseases (Dollery, 1999). Despite its therapeutic importance, a wide range of adverse effects, including cardiotoxicity (Bjelogrlic et al., 2005), nephrotoxicity (McCarroll et al., 2008), hepatotoxicity (Avci et al., 2016; Senthilkumar et al., 2006) and male reproductive

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toxicity (Ceribaşi et al., 2010), have been reported for the drug. The heart toxicity is one of the major side effects of CP and contributes to a high rate of morbidity and mortality (Sayed-Ahmed et al., 2014). The cardiotoxic effects of CP consist of acute, dose dependent cardiac damage morphologically characterized by necrosis, haemorrhage and later development of fibrosis (Goldberg et al., 1986; Senthilkumar et al., 2006). Its cytotoxic effects are the result of chemically reactive metabolites that alkylate DNA and protein, producing cross-link (Hales, 1982; Sladek, 1988). It has been reported that oxidative stress mediated disruption of redox balance of tissues after CP exposure generates biochemical and physiological disturbances (Motawi et al., 2010; Saandeep et al., 2009).

Since the 1980s, medicinal plants and their active ingredients have received increasing attention (Kren and Walterova, 2005). Medicinal plants serve as therapeutic alternatives, safer options, or, in some cases, the only effective treatment, and an increasing number of these plants and their extracts have been shown to produce beneficial therapeutic effects, including antioxidant, anti-inflammatory, anticancer, antimicrobial and immunomodulatory effects (Arafa, 2009; Garcia-Nino and Pedraza-Chaverri, 2014; O'keefe and Harris, 1990). Among the promising medicinal plants, silymarin (SLY) and curcumin (CUR) have been reported to have multiple pharmacological activities, including antioxidant, hepatoprotectant and anti-inflammatory, antibacterial and antineoplastic effects (Arafa, 2009; Kren and Walterova, 2005; Radko and Cybulski, 2007; Sharma et al., 2005). Derived from the milk thistle plant, SLY is a unique flavonoid complex containing silybin, silydianin and silychrisin (Ding et al., 2001). The beneficial properties of these phytochemicals, unique to milk thistle, have been the subject of decades of research (Ding et al., 2001; Flora et al., 1998). CUR, a major active component of turmeric, is extracted from the powdered dry rhizome of *Curcuma longa* Linn (Zingiberaceae) and has been used for centuries in indigenous medicine (Garcia-Nino and Pedraza-Chaverri, 2014; Sharma et al., 2005).

There are many recent studies on the development of methods for using a variety of antineoplastic chemical agents such as CP at higher doses by preventing their toxic effects (Fraiser et al., 1991; Senthilkumar et al., 2006; Taghiabadi et al., 2012). When these drugs are used frequently at high doses, their treatment effects are no longer seen and deleterious effects occur. The present study was therefore designed to evaluate and compare the protective effects of SLY and CUR in CP-induced cardiotoxicity in rats.

2. Materials and methods

2.1. Chemicals

CP (Endoxan®) was purchased from Eczacıbası-Baxter (Istanbul, Turkey), SLY (code; S0292) and CUR (code; C1386) from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Animals and experimental design

The hearts of animals were obtained from an earlier study (Avci et al., 2016). Thirty-six healthy adult female Wistar albino rats (250 ± 10 g) were used for the study. The rats were obtained from Adnan Menderes University Experimental Research Centre (Aydin, Turkey) and were housed under standard laboratory conditions (24 ± 3 °C, 40–60% humidity, 2-h light-dark cycle). A commercial pellet diet and fresh drinking water were available *ad libitum*. All animals received humane care according to criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, and the experimental protocol was approved by the University of Adnan Menderes Institutional Animal Ethics Committee (No: B.30.2.ADU.0.00.00.00/050.04/2011/043).

Rats were randomly divided into six experimental groups containing six rats each, as follows: Group I (control group; nothing was administered), Group II (CP group; 30 mg/kg/day CP administered intraperitoneally to each animal for seven days), Group III (SLY group; 100 mg/kg/day SLY by gavage for 14 days), Group IV (CUR group; 100 mg/kg/day CUR by gavage for 14 days), Group V (SLY + CP group; 100 mg/kg/day SLY by gavage for 14 days plus 30 mg/kg/day CP intraperitoneally starting from the seventh day) and Group VI (CUR + CP group; 100 mg/kg/day CUR by gavage for 14 days plus 30 mg/kg/day CP intraperitoneally starting from the seventh day). All administrations were applied by gavage as an emulsion in 0.5 mL corn oil.

2.3. Biochemistry

The rats were killed under slight ether anaesthesia at the end of 14 days. For the determination of malondialdehit (MDA) levels and superoxide dismutase (SOD) activity in the heart, the dissected tissues were immediately rinsed in ice-cold phosphate-buffered saline. Tissues were homogenised (2000 rpm/min for 1 min, 1/10 w/v) using a Teflon-glass stirrer (IKA Overhead Stirrer; IKA-Werke GmbH & Co. KG, Staufen, Germany) in a 10% 150 mM phosphate buffer (pH 7.4) in an ice bath. The homogenate was centrifuged (Hettich Zentrifugen, Mikro 200 R, Tuttlingen, Germany) at 6000*g* for 10 min at 4 °C. The supernatants were frozen at −80 °C (Glacier Ultralow Temperature Freezer, Japan) until analysed and then used for determination of MDA levels and SOD activity. The lipid peroxidation levels were determined according to the concentration of thiobarbituric acid reactive substances, and the amount of MDA produced was used as an index of lipid peroxidation. Absorbance was measured with a spectrophotometer at 532 nm. The MDA concentration was calculated by the absorbance complex (absorbance coefficient $\varepsilon = 1.56 \times 10^5$/M/cm) and expressed as nmol/mg of tissue protein (Placer et al., 1966). SOD activity was determined according to the method of Sun et al. (1988), and the absorbance was measured with a spectrophotometer at 560 nm. This method is based on the inhibition of nitro blue tetrazolium reduction using the xanthine-xanthine oxidase system as a superoxide generator. SOD activity was then measured by the degree of inhibition of this reaction. The results are shown as U/mg of tissue protein. The protein levels in the tissues were determined by the method described by Lowry et al. (1951).

For the measurement of DNA fragmentation levels of the heart samples were removed and protected against light, and then stored at −20 °C until analyses. The extent of apoptosis was evaluated by the measurement of DNA fragmentation. This was assessed by quantification of cytosolic oligonucleosome-bound DNA by using the Cell Death Detection ELISA Plus kit (Roche, Mannheim, Germany). The heart of the rats were treated with a homogeniser (Stuart SHM1, UK). The 0.2 g homogenate was made with the lysis buffer and then centrifuged at 20,000*g* for 10 min at 4 °C. The supernatant fraction was used as the antigen source for the immunoassay. This assay is based on the quantitative sandwich ELISA principle using mouse monoclonal antibodies directed against histones (coating antibody) and DNA (peroxidase-labelled antibody), respectively. The amount of peroxidase retained in the immunocomplex is determined photometrically with ABTS [2,29-azino-di-(3-thylbenzthiazoline sulfonate)] as a substrate (Thermo Multiskan FC Microplate Photometer, USA). This allows the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates.

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