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Original article

The protective effect of quercetin on cyclophosphamide-Induced lung toxicity in rats

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ABSTRACT

Cyclophosphamide (CYP) is an anticancer agent widely used in chemotherapy. It has been suggested that CYP causes toxicity in many organs, including the lungs and testes. Many studies have indicated that some antioxidants have possible protective effects against CYP's side effects. This study aimed to investigate the protective effect of quercetin (QUE) on CYP-induced lung toxicity in rats using histologic and biochemical methods.

In the study, 50 male Sprague-Dawley rats weighing 220–250 g were used. There were 4 experimental groups and 1 control group. Group I is the control group, which was given only intragastric (i.g.) solvent (corn oil) for 7 days. Group II was given i.g. corn oil for 7 days as a placebo, and a single dose of intraperitoneal (i.p.) CYP (200 mg/kg) was given on day 7. Groups III and IV, respectively, were given QUE in doses of 50 and 100 mg/kg, dissolved in corn oil, and administered i.g. for 7 days. In addition, a single dose of CYP (200 mg/kg, i.p.) was administered on the 7th day of study. In Group V, a 100 mg/kg dose of QUE was given to rats i.g. for 7 days. On the 8th day of the experiment, all groups of rats' blood and lung tissue samples were collected for analysis of oxidative stress parameters and histopathological examinations.

In the biochemical result (although oxidative parameters were increased in favor of tissue damage) QUE administration revealed attenuated CYP toxicity in the rats 'lungs. In histologic analysis, QUE prevented the CYP-mediated tissue damage and the increase in mast-cell densities in the rats' lung tissues.

The results of the present study have revealed that QUE provides a possible protective effect by inhibiting ROS and mast cell degranulation in induced lung damage.

1. Introduction

Cyclophosphamide (CYP) is an anticancer agent, which is widely used for cancer treatment in clinics. It has also been reported as an immunosuppressant agent. CYP is used to treat chronic and acute leukemia, multiple myeloma, lymphoma, and rheumatoid arthritis [1,2]. It is metabolized by a microsomal cytochrome P450 enzyme in the liver, metabolized phosphoramide mustard, and acrolein. Acrolein is related to CYP's side effects and inhibits the antioxidant system, causing reactive oxygen species production in the cells. Therefore, phosphoramide mustard is related to CYP's therapeutic effects [3]. It was reported that CYP toxicity in lung tissue is interstitial pneumonia and pulmonary fibrosis. These histopathologic patterns are often dose-limiting and even life-threatening. It has been suggested that some types of antioxidants have health benefits that fight CYP toxicity in lung tissue [4,5].

The antioxidants prevent oxidative stress and oxidative-stress-mediated pro-inflammatory responses. QUE is a natural flavonoid in many fruits and vegetables. Several epidemiological and experimental have studies reported that these molecules are effective antioxidants, anti-inflammatories, anti-apaptotic, anti-thrombotics, and anti-ischemics, and are anti-mutagenic, anti-cancer, anti-angiogenic, anti-proliferative, and anti-viral [6–11]. In

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experimental studies performed in several models of cancer toxicity caused by anticancer agents, QEU was reported to prevent this toxicity [12]. Studies have demonstrated that QUE provides antioxidant restoration [13], inhibits inflammatory responses [14], and consequently prevents oxidant-induced inflammatory cell damage by CYP toxicities. Also, previous studies reported that QUE reduced cyclophosphamide-induced cardiotoxicity, urotoxicity, and genotoxicity in mice [15].

CYP-induced lung damage is a common problem in the treatment of many diseases, especially in cancer patients. Because QUE is a powerful antioxidant and has anti-inflammatory features, the aim of the present study of CYP-induced lung injury in rats was to investigate the role of QUE on antioxidant levels, mast cell activation, and lung tissue damage.

2. Material and methods

The study involved the use of 50 adult male Sprague Dawley rats, whose weights were 220–250 g. The animals were provided with proper moisture, light, and room temperature, as well as free water and food, until the day of the experiment. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal-care committee of the Local Ethics Committee of Ataturk University for Animal Experiments. The rats were divided into five groups Table 1, consisting of a control and 4 experimental groups, respectively. Group I was a control group, and was given only intragastric (i.g.) solvent (corn oil) for 7 days. Group II was given i.g. corn oil for 7 days as a placebo, and single-dose intraperitoneal (i.p.) CYP (200 mg/kg) was given on the 7th day of the study. Groups III and IV, respectively, were administered i.g. doses of 50 and 100 mg/kg of QUE dissolved in corn oil for 7 days, and a single injection of i.p. CYP (200 mg/kg) was administered on the 7th day. In Group V, a 100 mg/kg dose of QUE was given to rats i. g. for 7 days. On the 8th day of the experiment, the rats in all groups were anesthetized, intracardiac blood samples were taken, and all animals were sacrificed. The blood and lung tissue samples were collected for biochemical analysis of oxidative stress (MDA, SOD, and GSH) and histopathological examination.

3. Biochemical analysis

The lung tissues were homogenized by a tissue homogenizer. The homogenates were centrifuged at 10,000g for 20 min at 4 °C, and the supernatants were obtained; superoxide dismutase (SOD) activity and malondialdehyde (MDA) and glutathione (GSH) levels were determined as previously described [16].

4. Histopathological analysis

The collected lung tissues were fixed in neutral buffered 10% formaldehyde solution for 72 h. After fixation, the tissues were washed in tap water, dehydrated in an ascended series of alcohols, and embedded in paraffin. The paraffin blocks were cut serially at a 5 μm thickness. The lung tissues were then stained with Crossman modified triple staining for histologic analysis. The photomicrographs were taken by conventional light microscope (Nikon eclipse i50, Tokyo).

5. Histochemical chloroacetate esterase (CAE) staining for mast cells (Mc)

To detect mast cells in rat lung tissues, chloroacetate esterase (CAE) staining was performed using naphthol-AS-D chloroacetate and counterstained with hematoxylin. The esterase positive mast cells localizations and densities were evaluated and scored under light microscopy (Nikon Eclipse i50, Japan). The scores were derived semiquantitatively using light microscopy on the preparations from each animal, and were reported as follows: none = –, mild = +, moderate = ++, severe = +++, and very strong = ++++. Histologic examination scale has consisted of A = weak in ≤%25 of tissue; B = mild in ≤%50 of tissue; C = moderate in ≤%75 of tissue; and D = very strong in ≥%75 of tissue. The average degeneration intensity was calculated as (Ax1)+ (Bx2) + (Cx3) + (Dx4))/(A + B + C + D) and reported as follows: + = 0.00–1.00; + + = 1.01–2.00; + + + =2.01–3.00; and + + + + = 3.01–4.00.

6. Statistical analysis

All data were statistically evaluated by one-way ANOVA using SPSS 20.00, followed by Duncan post hoc test. The data were expressed as mean ± SD. $p < 0.05$ was considered statistically significant.

7. Results

7.1. Oxidative parameters

7.1.1. Lung GSH levels

The GSH level of CYP-treated animals was significantly decreased compared to the control group($p < 0.05$). However, the GSH level of 100 mg QUE-treated group at GSH level was significantly increased in the CYP group ($p < 0.05$). The GSH levels and statistics for all groups are seen in Fig. 1A.

7.2. Lung SOD activity

The SOD activity in lung homogenates from CYP-treated rats was significantly decreased when compared with other groups ($p < 0.05$). In addition, 50 mg and 100 mg QUE-treated and CYP-induced groups of SOD activities were higher than in the CYP-induced group ($p < 0.05$). Fig. 1.B shows the SOD activities in the rat lung homogenates.

7.3. Lung MDA levels

In this study, we assessed MDA levels as a marker of oxidative stress. MDA levels of the CYP group were significantly the highest compared to other groups ($p < 0.05$). However, treatment with a 50 mg and 100 mg dose of QUE significantly inhibited the increased MDA level ($p < 0.05$). All levels of MDA and statistics are seen in Fig. 1C.

Table 1
All groups of study and animal protocols.

Groups	Treatment	The number of animals in groups
Control	Control (corn oil i.g.)	10
CYP	Cyclophosphamide (200 mg/kg i.p.)	10
QUE50-CYP	50 mg/kg QUE + Cyclophosphamide (200 mg/kg i.p.)	10
QUE100-CYP	100 mg/kg QUE + Cyclophosphamide (200 mg/kg i.p.)	10
QUE100	100 mg/kg QUE	10