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The protective effect of C-phycocyanin on paraquat-induced acute lung injury in rats

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ABSTRACT

To investigate the potential protective effect of C-phycocyanin (PC) on paraquat (PQ)-induced acute lung injury, rats were divided into control, PQ-treated and PQ + PC-treated groups. Rats in PQ-treated group were orally administered with 50 mg/kg PQ, and rats in PQ + PC-treated group were intraperitoneally injected with 50 mg/kg PC after administration of PQ. At 8, 24, 48 and 72 h after treatments, GSH-Px and SOD activities, MDA levels in plasma and BALF, HYP, NF-κB, IκB-α and TNF-α contents in lung tissues were measured. The pathological changes in lung were observed. After treatment with PC, the levels of MDA and the relative contents of NF-κB and TNF-α were significantly decreased, the activities of GSH-Px and SOD and the relative contents of IκB-α were significantly increased. The degree of rat lung damage was obviously reduced in PQ + PC-treated group. The results suggested that PC treatment significantly attenuated PQ-induced acute lung injury.

1. Introduction

Paraquat (PQ; 1,1′-dimethyl-4,4′-bi-pyridinium chloride) is one of the most widely used herbicide in the world and a highly toxic compound for humans and animals (Suntres, 2002; Parvez and Raisuddin, 2006; Neves et al., 2010). PQ poisoning is more frequently fatal than poisoning caused by other pesticides. Reports showed that PQ poisoning accounted for only 0.34% of pesticides poisoning cases, but PQ poisoning had the highest mortality rate (50–90%), accounting for 13% of all fatal cases (Klein-Schwartz and Smith, 1997; Lock and Wilks, 2001).

Severe PQ poisoning is characterized by multiple-organ failure, involving mainly the lung, kidney, and liver. The lung is the major target organ in PQ poisoning characterized by edema, hemorrhage, interstitial inflammation, and proliferation of bronchial epithelial cells (Berisha et al., 1994; Venkatesan, 2000), and respiratory failure from lung injury is

Abbreviations: PQ, paraquat; PC, C-phycocyanin; GSH-Px, glutathione peroxidase; HYP, hydroxyproline; MDA, maleic dialdehyde; SOD, superoxide dismutase.

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the most common cause of death. The main mechanism of PQ's toxic effects is redox reaction by reactive oxygen species, and lipid peroxidation of cellular membranes is a significant pathway (Yasaka et al., 1986). In addition to redox reaction, inflammatory reaction has been reported as a main mechanism of tissue injury (Orito et al., 2004). Several drugs have been investigated against PQ-induced lung toxicity, but the specific antidote has not been currently founded yet (Bateman, 1987; Suntres, 2002; Dinis-Oliveira et al., 2008).

C-phycocyanin (PC), a biliprotein pigment and an important constituent of the blue-green alga Spirulina platensis, contains phycocyanobilin, an open-chain tetrapyrrole chromophore that is covalently attached to the apoprotein and plays a major role in some of its important biological properties (Lissi et al., 2000). PC has been shown to possess significant antioxidant, radical-scavenging, anti-inflammatory, hepatoprotective, radical scavenging, antiarthritic, neuroprotective and anti-tumor effects (Romay et al., 2003; Li et al., 2005; Cherng et al., 2007; Sathyasaikumar et al., 2007). Therefore, in the present study an attempt was made to investigate whether PC has the protective effect on PQ-induced acute lung injury in a rodent model of rats. The results showed that PC treatment significantly attenuated PQ-induced acute lung injury.

2. Materials and methods

2.1. Materials

Male Wistar rats (weighting 180–200 g, SPF grade) were purchased from the Laboratory Animal Research Center of Shandong University of Traditional Chinese Medicine (Jinan, Shandong, China). Analytical Grade PC (Purity: A620/A280 $\geq$ 4) was provided by Yantai Institute of Coastal Zone Research (Yantai, Shandong, China). PQ was provided by Shandong Kexin Biochemical Co. (Jinan, Shandong, China). All other chemicals were of analytical grade, and procured from local commercial sources.

2.2. Animals treatments

After 3 days of acclimatization, the rats were randomly divided into three groups. The rats in control group (n = 24) were treated with the saline solution and sacrificed at 8 h (n = 6, control 8 h group), 24 h (n = 6, control 24 h group), 48 h (n = 6, control 48 h group) and 72 h (n = 6, control 72 h group). The rats in PQ-treated group (n = 24) were orally given aqueous solution of PQ (50 mg/kg) by gastric gavage and sacrificed at 8 h (n = 6, PQ 8 h group), 24 h (n = 6, PQ 24 h group), 48 h (n = 6, PQ 48 h group) and 72 h (n = 6, PQ 72 h group). The rats in PQ + PC-treated group (n = 24) were immediately intraperitoneally injected with 50 mg/kg PC after administration of PQ, then were sacrificed at 8 h (n = 6, PQ + PC 8 h group), 24 h (n = 6, PQ + PC 24 h group), 48 h (n = 6, PQ 48 h group) and 72 h (n = 6, PQ 72 h group). Throughout the study period, each animal was observed carefully for clinical signs of toxicity related to PQ. Handling of animals strictly followed the ethical guidelines set forth by the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

2.3. Sample collection

At 8, 24, 48 and 72 h after treatments, 6 rats were randomly selected in each group and deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg). Blood samples were taken from the jugular vein of the rats, and centrifuged at 4000 rpm for 15 min. The supernatant was collected for the measurement of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities, maleic dialdehyde (MDA) levels.

After blood samples collected, immediately thoracotomy was sterilely performed to obtain bronchoalveolar lavage fluid (BALF). The BALF was collected from the right lung. The bronchus of the left lung was clamped with forceps, and then, the right bronchus was cannulated. Subsequently, 5 ml cold saline 4 °C was instilled and aspirated. This was repeated three times and about 3 ml BALF was obtained and centrifuged at 3000 rpm for 15 min. The supernatant was harvested for the measurement of SOD, GSH-Px and MDA. Right lung was harvested for the contents of hydroxyproline (HYP) and tumor necrosis factor-α (TNF-α) in lung homogenate measurement. The inferior lobe of rat left lung at 72 h was cut into small pieces (1 mm^3) and fixed in 3% glutaraldehyde for ultra-morphological examination, and the remaining inferior lobe of left lung was fixed in 10% neutral buffered formalin solution before immunohistochemical and histological analysis.

2.4. Measurement of biomarkers of oxidative stress

The levels of MDA were determined as an indicator of lipid peroxidation. The activities of GSH-Px and SOD and the levels of MDA both in plasma and BALF of rats were determined using the assay Kits (Nanjing Jiancheng Corp., China), according to the manufacturer's recommendations. The units for MDA levels, GSH-Px and SOD activities were expressed as nmol/ml, U and U/ml, respectively.

2.5. HYP assay of lung tissue

The HYP contents of lung tissues were determined and the data were expressed as ng/g wet lung tissue. The 100 mg frozen lung tissue from control, PQ-treated and PQ + PC-treated rats was thoroughly homogenized in distilled water and determined using an assay Kit (Nanjing Jiancheng Corp., China), according to the manufacturer's recommendations.

2.6. Measurements of NF-κB, IκB-α and TNF-α

Nuclear factor kappa-B (NF-κB), IκB-α (an inhibitor of NF-κB) and TNF-α were measured by immunohistochemistry combined with semiquantitative analysis. In brief, after dewaxing of sections, endogenous peroxidase activity was quenched with 3% H_2O_2, and cross-reactivity was blocked with normal serum. The tissues were incubated overnight at 4 °C with primary antibodies according to introduction. Localization of the primary antibodies was achieved by subsequent use of a biotinylated anti-primary antibody, an avidin–biotin complex conjugated with horseradish peroxidase and 3′,5′-diaminobenzidine (Vectasitain Elite Kit, America). Normal