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# Protective effect of quercetin against paraquat-induced lung injury in rats

Hee K. Park [a], Sun J. Kim [a], Do Y. Kwon [a], Jae H. Park [b], Young C. Kim [a,c,*]

[a] College of Pharmacy, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul, Republic of Korea
[b] College of Veterinary Medicine, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul, Republic of Korea
[c] Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul, Republic of Korea

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ABSTRACT

*Aims:* Paraquat (PQ) is known to induce pulmonary injury via a redox cyclic reaction. The present study was aimed to determine the protective effects of quercetin against PQ-induced pulmonary injury in association with its antioxidant activity.

*Main methods:* Male rats were challenged acutely by PQ (50 mg/kg, i.p.) with or without quercetin post-treatment. Pulmonary heme oxygenase-1 (HO-1) expression, malondialdehyde (MDA) level, and the total oxyradical scavenging capacity (TOSC) toward hydroxyl, peroxyl radicals and peroxynitrite were measured 24 h after PQ treatment. Different groups of rats were instilled with PQ (0.5 mg/kg) directly into the right lung. Quercetin was administered to the rats daily for 14 days after PQ instillation. Serum NO, pulmonary glutathione (GSH) and 4-hydroxyproline (4-HP) concentrations were quantified in conjunction with histopathological examination to determine the fibrotic changes in lung.

*Key findings:* Pulmonary MDA level and HO-1 expression were elevated and the TOSC was reduced rapidly by an intraperitoneal dose of PQ. These changes were inhibited by quercetin post-treatment. In rat lungs instilled with PQ 14 days before, NO, MDA and 4-HP were elevated, and GSH was reduced, which were all inhibited significantly by daily quercetin treatment. Histopathological examination also revealed that quercetin ameliorated the increase in fibroblast distribution and collagen deposition in the lungs instilled with PQ.

*Significance:* The present results demonstrate that quercetin administration to rats effectively inhibits the development of PQ-induced pulmonary injury most probably via its antioxidant activity.



## Introduction

Pulmonary fibrosis is a progressive and fatal disease with poor prognosis. The etiology of pulmonary fibrosis is still obscure, but it has been accepted that reactive oxygen species (ROS) is critically implicated in the development of this chronic disease. It is known that under oxidative stress lung cells release inflammatory mediators and cytokines/chemokines, which induces neutrophil recruitment and activation of transcription factors such as nuclear transcription factor κB (NF-κB) and activator protein-1 (AP-1), thereby augmenting the inflammatory response and tissue damage (Brennan et al. 1995; Rahman and MacNee 1998). Accordingly, alveolar and bronchial inflammatory response is considered to be a fundamental process involved in the pathogenesis of many lung diseases including pulmonary fibrosis (Rahman and MacNee 2000). Also generation of ROS appears to play a critical role in pulmonary injury induced by pneumotoxic substances such as paraquat (PQ; 1,1'-dimethyl-4,4'-bipyridinium) and bleomycin (Dinis-Oliveira et al. 2006; Keogh et al. 2005). It is suggested that PQ-induced pulmonary injury involves generation of PQ cation radicals from redox cycling, resulting in oxidation of NADPH to $NADP^+$ in association with production of superoxide anion and other oxygen free radicals (Bus and Gibson 1984).

Quercetin and related flavonoids present in fruits and vegetables have attracted much attention for their beneficial health effects. It has been suggested that daily intake of these substances may reduce the risk of various chronic health disorders such as cardiovascular disease, diabetes, tumor development, stroke and neurodegenerative disease (Hollman and Katan 1999; Skibola and Smith 2000; Boots et al. 2008; Jagtap et al. 2009). The beneficial effects of quercetin have been attributed to multiple mechanisms including antioxidant activity, anti-inflammation, modification of signal transduction pathways, and interactions with receptors and other proteins. However, most investigators accept that the antioxidant activity of quercetin conferred by its phenolic hydroxyl groups is primarily implicated in the therapeutic potential of this flavonoid in different diseases.

Although experimental evidence for the role of a redox imbalance in lung fibrogenesis is substantial, this chronic disease is generally

* Corresponding author. College of Pharmacy, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul, Republic of Korea. Tel.: +82 2 880 7852; fax: +82 2 871 7453.
*E-mail address:* youckim@snu.ac.kr (Y.C. Kim).

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non-responsive to conventional anti-inflammatory and immunomodulatory therapy (Walter et al. 2006). Beneficial health effects of quercetin against various oxidative stress-related diseases have been documented, however, studies examining its potential pneumoprotective effects are scarce. Therefore, it was of interest to determine the protective effects of quercetin against PQ-induced lung injury in association with its antioxidant activity.

## Methods

### *Animals and treatments*

Male Sprague–Dawley rats (250–300 g) were purchased from Samtako (Osan, Korea). Animals were acclimated to temperature (22 ± 2 °C) and humidity (55 ± 5%) controlled rooms with a 12 h light/dark cycle for 1 week before use. The animal experimentation protocol was approved by the Animal Care and Use Committee of College of Pharmacy, Seoul National University. Quercetin (Sigma, St. Louis, MO) was suspended in 0.2% Tween aqueous solution. For acute studies quercetin was administered twice at a dose of 50 mg/kg, ip, 2 and 6 h after PQ (50 mg/kg, ip; Sigma, St. Louis, MO) treatment. PQ was dissolved in normal saline. Rats were sacrificed 24 h following PQ treatment. PQ at this dose was found previously to cause significant changes in the ultra-structure of lung 24 h after exposure (Lee et al. 2008; Mustafa et al. 2002). For induction of chronic pulmonary injury, a single dose of PQ (0.5 mg/kg) was instilled directly into the right lung of a rat (Wyatt et al. 1981). Quercetin (50 mg/kg/day, ip) was administered for 14 consecutive days after PQ instillation (Tieppo et al. 2009).

### *Measurement of malondialdehyde, glutathione, nitric oxide, and 4-hydroxyproline*

Malondialdehyde (MDA) concentration was determined using a thiobarbituric acid (TBA) method (Mengel et al. 1965). The supernatant obtained after initial centrifugation of lung homogenate was used. Lipid peroxidation products reacting with 2-TBA were measured at 540 nm using 1,1,3,3,-tetraethoxypropane as a standard. For measurement of glutathione (GSH) concentration rat lungs were homogenized in a 4-fold volume of cold 1 M perchloric acid. Total GSH concentrations were determined by using an enzymatic recycling method (Griffith 1980). Concentration of nitrite was quantified using Griess reagent (Sigma, St. Louis, MO) with sodium nitrite as a standard (Green et al. 1982). Briefly, an aliquot (70 μl) of serum was added into 96-well plate. Then Griess reagent solution (70 μl) was added into each well and the plate was incubated for 20 min at 37 °C. The absorbance was measured at 540 nm.

Total collagen contents in lung were estimated by quantifying 4-hydroxyproline (4-HP) using the method of Kondo et al. (1997). Lung tissues were hydrolyzed in 9 N HCl in a sealed tube at 110 °C for 24 h. After centrifugation, 3,4-dehydroproline was added to the supernatant as an internal standard. 4-HP was labeled with 9-fluorenylmethyl chloroformate. A HPLC system equipped with a fluorescence detector (FP-920; Jasco Co., Tokyo, Japan) and 5 μm Symmetry C18 reversed phase column (4.6 mm × 250 mm; Kromasil, Bohus, Sweden) was used. The emitted light was monitored at 310 nm with an excitation wavelength of 260 nm.

### *Total Oxyradical Scavenging Capacity (TOSC) assay*

The method developed by Regoli and Winston (1999) was employed. This assay is based on the ethylene-yielding reaction of α-keto-γ-methiolbutyric acid with hydroxyl, peroxyl radicals and peroxynitrite. The supernatant obtained after initial centrifugation of lung homogenate was used. Peroxyl radicals were generated by thermal homolysis of 2,2'-azobis-amidinopropane. Hydroxyl radicals were generated by the iron plus ascorbate-driven Fenton reaction. Peroxynitrite was produced by spontaneous decomposition of 3-morpholinosydnonimine *N*-ethylcarbamide. An aliquot taken from the headspace of a reaction vial was injected into GC equipped with a flame ionization detector and Poropack N column (Supelco, Bellefonte, PA). TOSC value was quantified from the equation $TOSC = 100 - (\int SA / \int CA \times 100)$, where $\int SA$ and $\int CA$ were the integrated ethylene peak areas obtained from the sample and control reactions, respectively. Because TOSC is calculated from the inhibition of ethylene generation relative to control reaction, TOSC is unit-less. The specific TOSC was calculated by dividing the experimental TOSC with mg protein used.

### *Western blotting analysis*

Lung microsomal proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes by electroblotting. The membranes were blocked in 5% non-fat dry milk in 0.05% Tween 20 in PBS. The blots were incubated overnight with antibodies diluted in 5% bovine serum albumin at 4 °C followed by incubation with secondary antibodies conjugated to horseradish peroxidase. Polyclonal antibody against heme oxygenase (HO)-1 (Santa Cruz Biotechnology, Santa Cruz, CA) was used as a probe. Proteins were detected by enhanced chemiluminescence.

### *Histopathological examination*

Lungs were fixed in buffered 20% formaldehyde solution. Tissue samples were embedded in paraffin, sectioned and stained with Haematoxylin-eosin or Masson's trichrome. Development of fibrosis was graded by the degree of infiltration of fibroblast and collagen using a method proposed by Kim et al. (2006). Fibroblast distribution was scored as − (minus) for no expression of fibroblasts, 1 + for partial expression in one layer of the alveolar interstitum, 2 + for partial expression in two layers, 3+ for general expression in two layers, and 4 + for general expression in more than two layers. Collagen infiltration was scored as − (minus) for no expression of collagen, 1 + when the collagenous layer was partly hypertrophied less than two times that of normal control, 2 + for partial hypertrophy more than two times that of normal control, 3 + for general hypertrophy more than two times that of normal control, and 4 + for general hypertrophy more than two times that of control accompanied by infiltration such that it was barely possible to divide the alveolar zone into several parts.

### *Statistical analysis*

All results were expressed as the mean ± SEM. Equality of variances was determined by Bartlett's test prior to comparison of the different groups using Student's *t*-test or one-way ANOVA followed by Newman–Keuls multiple range test. The acceptable level of significance was established at $P < 0.05$ except when otherwise indicated. All statistical analyses were conducted using GraphPad Prism version 4.0 software.

## Results

### *Acute effects of quercetin against PQ-induced oxidative stress*

The early response of rat lung to an acute dose of PQ was firstly determined. Rats were sacrificed 24 h after PQ challenge (50 mg/kg, i.p.). Administration of PQ resulted in a significant increase in pulmonary MDA levels (Fig. 1). Quercetin treatment alone did not affect the MDA level, but blocked its elevation by PQ completely. A dose of PQ decreased the TOSC of lung tissues against hydroxyl radical, peroxyl radical and peroxynitrite significantly (Fig. 2).