Quercetin protects against lipopolysaccharide-induced acute lung injury in rats through suppression of inflammation and oxidative stress

Risheng Huang[1], Tian Zhong[2], Hao Wu[3]

[1]Department of Thoracic Surgery, Wenzhou Central Hospital, Wenzhou, China
[2]Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, China
[3]Department of Clinical Laboratory, Wenzhou Central Hospital, Wenzhou, China

Corresponding author:
Tian Zhong
Sichuan Academy
of Medical Sciences and
Sichuan Provincial
People's Hospital
Chengdu 610031, China
Phone: +862887795180
Fax: +8628-87394243
E-mail: 864591143@qq.com

Abstract

Introduction: Acute lung injury (ALI) is an acute inflammatory disease characterized by excess production of inflammatory factors in lung tissue. Quercetin, a herbal flavonoid, exhibits anti-inflammatory and anti-oxidative properties. This study was performed to assess the effects of quercetin on lipopolysaccharide (LPS)-induced ALI.
Material and methods: Sprague-Dawley rats were randomly divided into 3 groups: the control group (saline alone), the LPS group challenged with LPS (*Escherichia coli* 026:B6; 100 μg/kg), and the quercetin group pretreated with quercetin (50 mg/kg, by gavage) 1 h before LPS challenge. Bronchoalveolar lavage fluid (BALF) samples and lung tissues were collected 6 h after LPS administration. Histopathological and biochemical parameters were measured.
Results: The LPS treatment led to increased alveolar wall thickening and cellular infiltration in the lung, which was markedly prevented by quercetin pretreatment. Moreover, quercetin significantly ($p < 0.05$) attenuated the increase in the BALF protein level and neutrophil count and lung wet/dry weight ratio and myeloperoxidase activity in LPS-challenged rats. The LPS exposure evoked a 4- to 5-fold rise in BALF levels of tumor necrosis factor-α and interleukin-6, which was significantly ($p < 0.05$) counteracted by quercetin pretreatment. Additionally, quercetin significantly ($p < 0.05$) suppressed the malondialdehyde level and increased the activities of superoxide dismutase, catalase, and glutathione peroxidase in the lung of LPS-treated rats.
Conclusions: Quercetin pretreatment effectively ameliorates LPS-induced ALI, largely through suppression of inflammation and oxidative stress, and may thus have therapeutic potential in the prevention of this disease.

Key words: quercetin, acute lung injury, inflammation, oxidative stress.

Introduction

Acute lung injury (ALI) is an acute inflammatory disease, characterized by excess production of inflammatory factors in lung tissue, and followed by non-cardiogenic dyspnea, severe hypoxemia, and pulmonary edema, thus leading to both high morbidity and mortality [1, 2]. A major cause of the development of ALI is sepsis, wherein Gram-negative bacteria are a prominent cause [3]. The intraperitoneal injection of lipopolysaccharide (LPS), a component of the outer cell wall of most Gram-negative

bacteria, mimics human Gram-negative ALI and is one of the most commonly accepted models for ALI [4]. Lipopolysaccharide, binding to its receptor, toll-like receptor 4, provokes the activation of a key pro-inflammatory transcription factor, nuclear factor κB, which induces the expression of various pro-inflammatory cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β, and macrophage inflammatory protein-2 [5]. As a consequence of the strong inflammatory response, alveolar structures change, endothelial and alveolar permeability increase and alveolar fluid clearance decreases, thus critically impairing lung function [3, 6]. Despite significant advances in understanding of the pathophysiology of ALI, currently available treatment options have failed to significantly reduce ALI-related mortality. Therefore, new strategies are still needed to achieve effective treatment of ALI.

Flavonoids are a group of natural substances with a wide range of biological effects, including anti-inflammatory and anti-oxidative effects [7]. For instance, licochalcone A, a flavonoid in licorice root (*Glycyrrhiza glabra*), attenuates alveolar wall thickening, alveolar hemorrhage, and interstitial edema [8]. Quercetin is a flavonoid present in many vegetables, fruits, and beverages and possesses a broad range of pharmacological properties, including anti-inflammatory [9], anti-proliferative [10], and anti-oxidative effects [11]. Recent evidence demonstrates that quercetin can elicit protective effects in different lung injury models [12, 13]. Quercetin administration suppresses paraquat-induced pulmonary injury through its antioxidant activity [12]. Quercetin attenuates cigarette smoke-induced airway inflammation and mucus production in rats [13]. In addition, at the molecular level, quercetin inhibits pulmonary oxidant stress in lung epithelial cells through inducing heme oxygenase-1 (HO-1) and reduces paraquat-mediated oxidative damage via modulating the expression of antioxidant genes [14, 15].

Therefore, we postulated that quercetin could protect against LPS-induced lung injury. In the present study, we tested this hypothesis by using a rat model of LPS-induced ALI.

Material and methods

Animals and experimental groups

All animal experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the Animal Care and Use Committee of Wenzhou Central Hospital (Wenzhou, China). A total of 36 adult male Sprague-Dawley rats weighing 280–300 g were obtained from the Animal Center of Wenzhou Medical College (Wen-

zhou, China). They were housed in a temperature- and humidity-controlled environment with a 12-h light-dark cycle and allowed free access to water and standard laboratory chow. The animals were randomly divided into 3 groups: the control group, the LPS group challenged with LPS alone, and the quercetin group pretreated with quercetin 1 h before LPS challenge.

LPS-induced ALI model

A rat model of ALI was generated as described previously [16]. Briefly, rats were fasted overnight and anesthetized with pentobarbital sodium (50 mg/kg body weight, i.p.). A tracheostomy was performed and LPS (from *Escherichia coli* 026:B6; 100 μg/kg body weight; Sigma, St. Louis, MO, USA) was injected intratracheally through a 24-gauge catheter. Sham-treated animals (control group) received an equal volume of saline alone. In the quercetin group, animals were given a single dose of quercetin (50 mg/kg, by gavage) 1 h before LPS challenge. The quercetin concentration was used according to a previous report [17].

At 6 h after LPS instillation, rats were killed with an overdose of pentobarbital sodium. The left lung was collected from 5 animals of each group for histopathological examination, and the right lung for analysis of lung wet/dry weight ratio. The left lung from the other 7 animals of each group was subjected to bronchoalveolar lavage (BAL), and the right lung was excised for measurement of biochemical parameters.

BAL and differential cell counts

Bronchoalveolar lavage fluid was performed as described previously [18]. The left lung was lavaged 3 times with 2.0 ml of ice-cold phosphate buffered saline (PBS). The BAL fluid (BALF) was immediately centrifuged at 500 × g for 10 min at 4°C, and the cell-free supernatants were stored at –80°C for cytokine and protein analysis. The cell pellets were resuspended in PBS, and the total cell number was determined using a hemocytometer. Cytospin of BAL was prepared and stained with the Wright-Giemsa method. Differential cell counts were evaluated by counting at least 500 cells for the determination of the relative percentage of a specific cell type. Number of neutrophils was calculated as the percentage of neutrophils multiplied by the total number of cells in the BAL.

Protein determination in BALF

Total protein content in BALF was determined by the Bradford method according to the manufacturer's instructions (Tiangen, Beijing, China) using bovine serum albumin as a standard.

Measurement of cytokines in BALF

The concentrations of TNF-α and interleukin-6 (IL-6) in the BALF were measured by standard enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturer's instructions (R&D system, Minneapolis, MN, USA).

Lung wet/dry weight ratio

Lung edema was assessed by measuring the tissue wet/dry weight ratio. The right lung was excised and the wet weight was recorded. The dry weight was determined after the lung was placed in an incubator at 80°C for 24 h, and the wet/dry weight ratios were then calculated.

Myeloperoxidase assay

Myeloperoxidase (MPO) activity, a common indicator of neutrophil sequestration, was measured as described previously [19]. In brief, snap-frozen lung tissues were homogenized in PBS solution (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide, sonicated twice for 30 s on ice, and centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was then collected and mixed 1 : 30 (v/v) with assay buffer containing the substrate o-dianisidine hydrochloride (0.2 mg/ml) and 0.2 mM hydrogen peroxide. Standard MPO (Sigma) was used in parallel to determine MPO activity in the sample. Absorbance change was measured at 460 nm for 5 min, and MPO activity was expressed as unit per gram of wet lung tissue per min.

Oxidative stress measurements

Oxidative stress parameters were measured as described previously [20]. A 10% (w/vol) lung homogenate was prepared and centrifuged, and the supernatant was used for assays with commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Malondialdehyde (MDA) levels were measured with the thiobarbituric acid reaction. Superoxide dismutase (SOD) activity was measured at 550 nm by the hydroxylamine method. Catalase (CAT) activity was determined by the breakdown of hydrogen peroxide at 37°C. Glutathione peroxidase (GPx) activity was determined by measuring the oxidation of NADPH at 37°C.

Lung histopathology

Lung tissue was fixed in 10% paraformaldehyde for 24 h, dehydrated by ethanol, embedded in paraffin, and sectioned into 4-µm slices. The deparaffinized sections were stained with hematoxylin and eosin (H + E). Evaluation of lung injury was performed under a light microscope.

Statistical analysis

Data are expressed as means ± standard deviation (SD). Statistical significance was determined by one-way analysis of variance followed by Tukey's multiple comparison test. A p value < 0.05 was considered statistically significant.

Results

Effects of quercetin on LPS-induced histological changes in the lung

As shown in Figure 1 A, sham-operated rats (control group) had normal lung morphology, with a thin alveolar wall and no evident infiltration. In contrast, LPS-treated animals without quercetin pretreatment exhibited extensive lung damage, manifested by profound infiltration of inflammatory cells into lung interstitium and alveolar spaces and alveolar wall thickening (Figure 1 B). Notably, such lung histopathological changes were markedly attenuated by quercetin pretreatment (Figure 1 C).
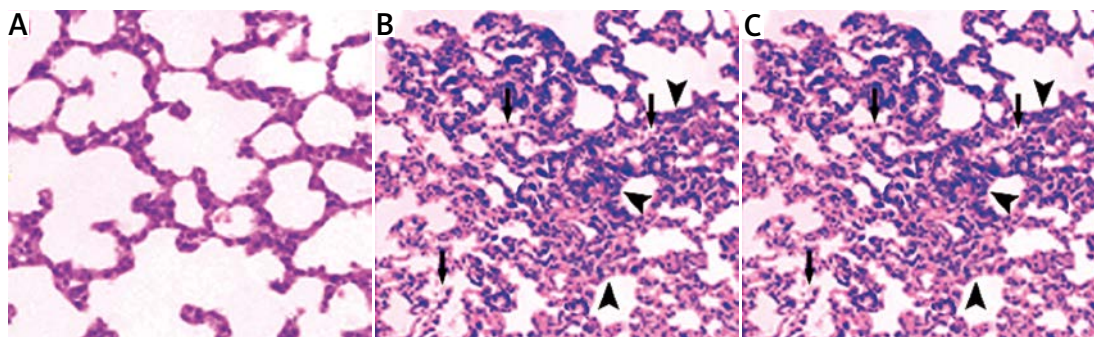

Figure 1. Quercetin pretreatment ameliorates LPS-induced pathological changes in the lung. The lungs from the control group (**A**), LPS-treated group (**B**), and quercetin group (**C**) with quercetin pretreatment 1 h before LPS treatment were subjected to H + E staining. Representative images of H + E-stained lung sections from each group are shown (magnification: 200×). B – LPS exposure led to infiltration of inflammatory cells into lung interstitium and alveolar spaces (arrow) and alveolar wall thickening (arrowhead). C – Such pathological changes were attenuated by quercetin pretreatment
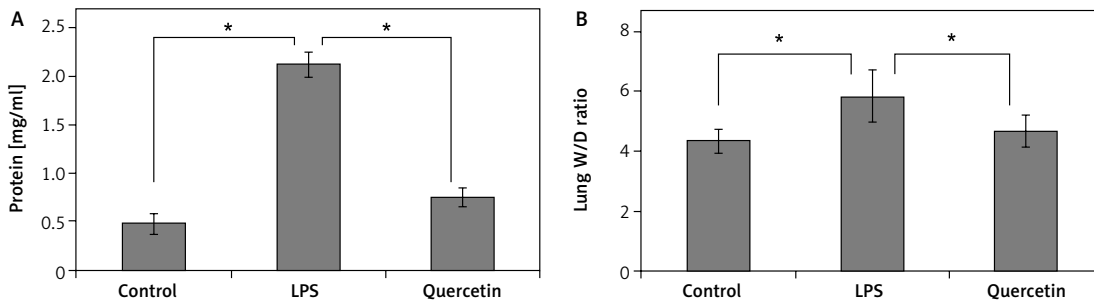
Figure 2. Quercetin inhibits LPS-induced pulmonary hyperpermeability and edema. After LPS challenge, the BALF and the right lung from each group were collected and assessed for the protein concentration (**A**) and wet/dry weight ratio (**B**), respectively

*Data are presented as mean ± SD; *p < 0.05.*

Effects of quercetin on LPS-induced pulmonary hyperpermeability and edema

Lipopolysaccharide exposure resulted in a > 4-fold rise in BALF protein levels and a significant ($p < 0.05$; Figure 2 A) increase in lung wet/dry weight ratios, when compared to sham-operated rats (Figure 2 B). The LPS-induced protein leakage and pulmonary edema were significantly ($p < 0.05$) reduced by quercetin pretreatment (Figure 2).

Effects of quercetin on LPS-induced pulmonary inflammation

Lung MPO activity and BAL neutrophil numbers were significantly ($p < 0.05$) higher in animals after LPS exposure than in sham-operated animals (Figure 3 A, B). Quercetin pretreatment significantly ($p < 0.05$) reduced the MPO activity and BAL neutrophil count by 58% and 79%, respectively (Figure 3 A, B).
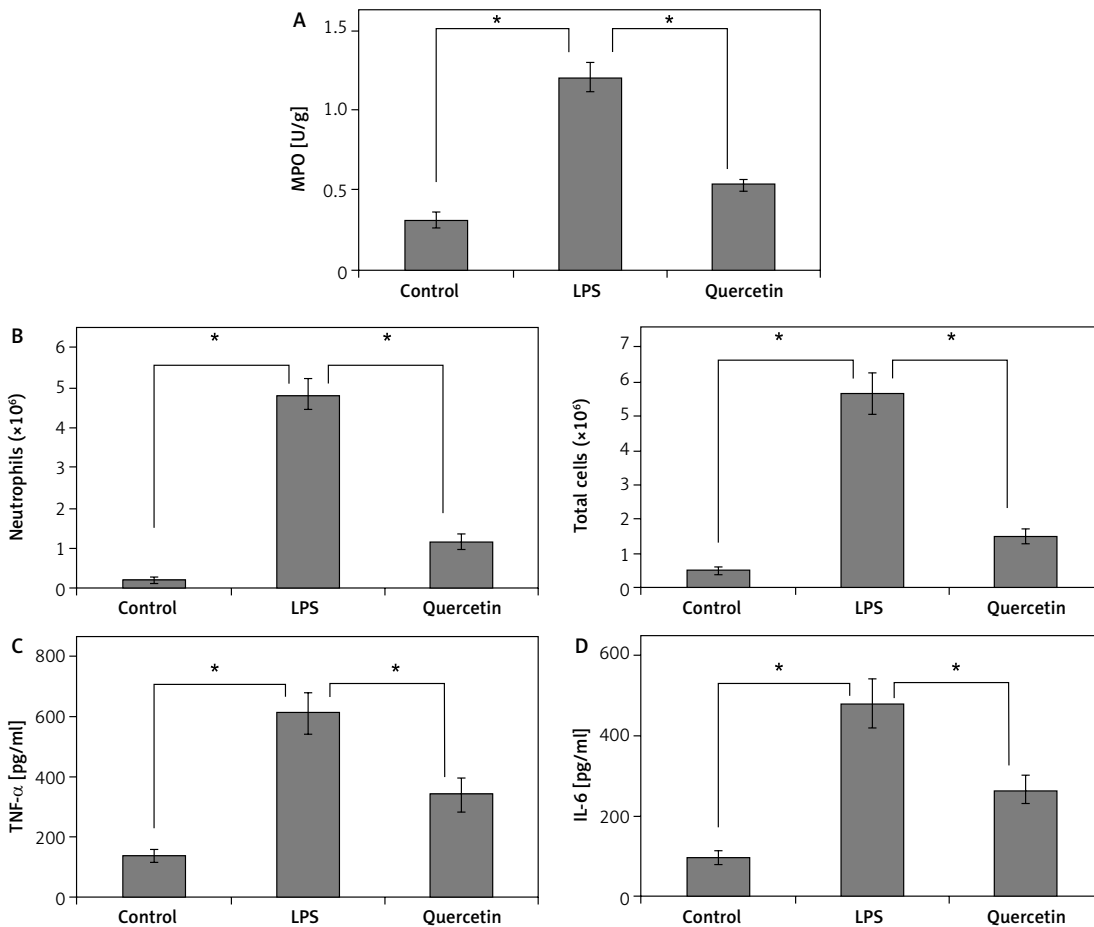

Figure 3. Quercetin pretreatment suppresses LPS-induced neutrophil infiltration and pro-inflammatory cytokine release. The MPO activity in the lung (**A**), the number of neutrophils and total cells (**B**), and the concentrations of TNF-α (**C**) and IL-6 (**D**) in BALF were measured in each group after LPS challenge

*Data are presented as mean ± SD; *p < 0.05.*

As shown in Figures 3 C and D, LPS exposure evoked a 4- to 5-fold rise in the BALF levels of TNF-α and IL-6 in comparison with sham-operated animals. The LPS-induced release of the two cytokines was significantly ($p < 0.05$) counteracted by quercetin pretreatment.

Effects of quercetin on LPS-induced lung oxidative stress

As shown in Table I, the level of MDA, a lipid peroxidation end product, was significantly ($p < 0.05$) increased in LPS-treated animals relative to sham-operated animals. Quercetin pretreatment diminished the MDA level by 43%, when compared to the LPS group. The activities of the 3 antioxidant enzymes (SOD, CAT, and GPx) were significantly ($p < 0.05$) lower in LPS-treated animals than in sham-operated animals (Table I). Notably, such a decline in the activities of these enzymatic antioxidants was significantly ($p < 0.05$) prevented by quercetin pretreatment.

Discussion

In the present study, we found that pretreatment with quercetin attenuated LPS-induced alveolar wall thickening and inflammatory cell infiltration. Quercetin pretreatment diminished the lung wet/dry weight ratio and BALF protein level, indicating a reduction in pulmonary hyperpermeability and edema. LPS-induced TNF-α and IL-6 secretion and neutrophil cell influx in BALF were inhibited by quercetin, accompanied by reduced MPO activity in the lung. Additionally, quercetin pretreatment resulted in a decline in the MDA level and elevation in the activities of SOD, CAT, and GPx. Taken together, our results show that quercetin prevents LPS-induced ALI, at least partially, via suppression of inflammation and oxidative stress.

Release of various cytokines/chemokines plays a critical role in the pathogenesis of ALI, leading to an increase in the pulmonary permeability of the alveolar-capillary barrier and a consequent impairment in arterial oxygenation [3, 21]. Thus, inhibition of lung inflammation has been a primary target in pharmacologic treatment of ALI patients. Many natural flavonoids such as licochalcone A [8], oroxylin A [22], and astragalin [23] have shown benefits in protecting the lung from LPS-induced ALI via suppression of pro-inflammatory cytokine secretion. Consistently, we found that the natural flavonoid quercetin also ameliorated LPS-induced ALI, which was associated with negative modulation of pro-inflammatory factors including TNF-α and IL-6. Neutrophil recruitment into the lung is a hallmark of ALI. It has been widely accepted that neutrophils are an important source of pro-inflammatory cytokines and contribute to inflammation-induced lung injury [4, 24]. Our data revealed that quercetin pretreatment significantly decreased the number of neutrophils in BALF and the MPO activity in the lung. Tissue MPO activity is a well-established indicator for neutrophil infiltration [25]. Taken together, these results indicate that quercetin exerts anti-inflammatory effects against LPS-induced ALI.

A large body of evidence supports the role of oxidants and oxidative injury in the pathogenesis of ALI [26, 27]. Activated neutrophils produce vast quantities of reactive oxygen species and nitrogen species, which cause cell injury through direct damage to DNA, lipid peroxidation with formation of vasoactive molecules, and oxidation of proteins [28]. Our results also demonstrated that LPS-induced neutrophil recruitment and activation in the lung was accompanied by occurrence of pulmonary oxidative stress, as evidenced by increased MDA levels and reduced antioxidant enzymes. Many efforts have been made to develop antioxidant therapy for the treatment of ALI. Quercetin has shown anti-oxidative activity in various oxidative diseases [29, 30]. Park *et al.* [12] reported that quercetin can effectively inhibit paraquat-induced lung injury in rats through reduction of oxidative stress. Hayashi *et al.* [14] demonstrated that quercetin treatment protects against pulmonary oxidant stress via induction of HO-1 in lung epithelial cells. In a rat model of cigarette smoke-induced lung injury, quercetin pretreatment was found to suppress goblet cell hyperplasia, inflammation, and oxidative stress in the lung [13]. Consistently, our present data demonstrated that pretreatment with quercetin significantly inhibited LPS-induced increase of pulmonary hyperpermeability and lung edema, inflammatory cytokine secretion and neutrophil influx in BALF. Moreover, quercetin administration restored the activities of SOD, CAT, and GPx and reduced the MDA level in the lung.

Table I. Measurement of oxidative stress biomarkers in the lung

Variable	Control group	LPS group	Quercetin group
MDA [nmol/mg protein]	8.21 ±1.54	21.08 ±1.65[*]	12.48 ±1.95[*,#]
SOD [U/mg protein]	260.36 ±17.89	159.75 ±15.05[*]	193.26 ±11.32[*,#]
GPx [U/mg protein]	190.14 ±42.61	136.39 ±61.47[*]	171.28 ±30.86[*,#]
CAT [U/mg protein]	21.26 ±2.54	14.70 ±3.05[*]	18.37 ±2.81[*,#]

MDA – malondialdehyde, SOD – superoxide dismutase, GPx – glutathione peroxidase, CAT – catalase. Value are expressed as mean ± SD; [*]p < 0.05 versus the control group; [#]p < 0.05 versus the LPS group.

These findings collectively demonstrate that quercetin pretreatment has a preventive effect against LPS-induced ALI, which is largely mediated through elevation of antioxidant enzymes and reduction of oxidative stress.

In conclusion, our results indicate that quercetin confers protection against LPS-induced ALI, which involves the reduction of oxidative stress and lung inflammation. These findings warrant further exploration of the clinical applicability of quercetin in the prevention and treatment of ALI.

Conflict of interest

The authors declare no conflict of interest.

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