JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Licochalcone A Inhibits Lipopolysaccharide-Induced Inflammatory Response in Vitro and in Vivo

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ABSTRACT: Licochalcone A (Lico A), a flavonoid found in licorice root (*Glycyrrhiza glabra*), is known for its antimicrobial activity and its reported ability to inhibit cancer cell proliferation. In the present study, we found that Lico A exerted potent anti-inflammatory effects in in vitro and in vivo models induced by lipopolysaccharide (LPS). The concentrations of TNF-α, interleukin (IL)-6, and IL-1β in the culture supernatants of RAW 264.7 cells were determined at different time points following LPS administration. LPS (0.5 mg/kg) was instilled intranasally (i.n.) in phosphate-buffered saline to induce acute lung injury, and 24 h after LPS was given, bronchoalveolar lavage fluid was obtained to measure pro-inflammatory mediator and total cell counts. The phosphorylation of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) p65 protein was analyzed by Western blotting. Our results showed that Lico A significantly reduced the amount of inflammatory cells, the lung wet-to-dry weight (W/D) ratio, protein leakage, and myeloperoxidase activity and enhances oxidase dimutase activity in mice with LPS-induced acute lung injury (ALI). Enzyme-linked immunosorbent assay results indicated that Lico A can significantly down-regulate TNF-α, IL-6, and IL-1β levels in vitro and in vivo, and treatment with Lico A significantly attenuated alveolar wall thickening, alveolar hemorrhage, interstitial edema, and inflammatory cells infiltration in mice with ALI. In addition, we further demonstrated that Lico A exerts an anti-inflammation effect in an in vivo model of acute lung injury through suppression of NF-κB activation and p38/ERK MAPK signaling in a dose-dependent manner.

KEYWORDS: *licochalcone A, lipopolysaccharide, nuclear factor-κB, mitogen-activated protein kinase*

INTRODUCTION

In the United States, the prevalence of inflammatory diseases has become a major public health concern.[1,2] Macrophages actively participate in inflammatory responses by releasing pro-inflammatory cytokines [TNF-α, interleukin (IL)-1β, and IL-6] and inflammatory factors (NO and PGE2) that recruit additional immune cells to sites of infection or tissue injury.[3] Lipopolysaccharide (LPS)-induced activation of macrophages is mainly mediated through the transmembrane signaling receptor toll-like receptor 4.[4] LPS has been recognized as a principal component causing acute lung injury (ALI).[5] The mechanisms of ALI are gradually becoming clear. Intranasal instillation of LPS has lead to the release of reactive oxygen species, pro-inflammatory cytokines, and chemotactic factors, which cause the aggregation of neutrophilic leukocytes and ultimately lung tissue injury.[6,7]

In fact, when activated, polymorphonuclear neutrophils (PMNs) lead to a rise of neutral protease activity, such as myeloperoxidase (MPO) activities, which are associated with the development of various pulmonary diseases.[8] IL-8 has been proposed as a major chemotactic factor for the recruitment of neutrophils to the sites of inflammation. Neutrophil-mediated tissue injury is a common mechanism underlying the development of organ dysfunction during ALI.[9] Therefore, inhibitors of these inflammatory molecules have been considered as a candidate of an anti-inflammatory drug.[10]

Licochalcone A (Lico A) is a major and biogenetically characteristic chalcone isolated from the root of Xinjiang liquorice, *Glycyrrhiza inflata*,[11] which possesses antitumor activities[12,13] and can modify the Bcl-2 level.[14] It also proves to be a specific inhibitor for Stat3 and would be employed for the treatment of various diseases caused by disorders of the Jak/Stat pathway.[15] Lico A inhibits the proliferation of rVSMCs by suppressing the PDGF-induced activation of the ERK1/2 pathway and Rb phosphorylation, resulting in cell cycle arrest.[16] Moreover, Lico A strongly inhibits the production of inflammation-associated mediators (IL-1β, IL-6, iNOS, and COX-2) by suppressing nuclear factor-κB (NF-κB) and AP-1 activation.[17] This study was therefore aimed to evaluate the anti-inflammatory potential of Lico A in both in vitro and in vivo models. We investigated pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) production at different time points in RAW 264.7 cells treated with Lico A in vitro; we also investigated the influence of Lico A on LPS-induced ALI in vivo. Furthermore, in an attempt to study the possible mechanism of Lico A on LPS-induced ALI in mice, NF-κB activation and MAP kinase phosphorylation have been analyzed by Western blot. Our data showed that Lico A significantly decreases the inflammation in vitro and in vivo and may represent a novel therapeutic strategy for inflammation.

Received: December 16, 2011
Revised: March 3, 2012
Accepted: March 8, 2012
Published: March 8, 2012

MATERIALS AND METHODS

Chemicals. Lico A (purity >98%, Figure 1) used in the experiments was obtained as a lyophilized powder in a 20 mg vial

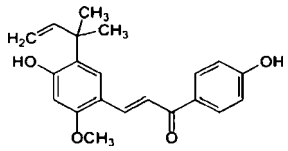

Figure 1. Chemical structure of Lico A.

manufactured by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). LPS (from *Escherichia coli* 0111: B4) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Lico A was resolved in saline with 0.1% DMSO and then sterile filtered before used.

Animals. Male BALB/c mice, weighing approximately 18−20 g, were purchased from the Center of Experimental Animals of Baiqiuen Medical College of Jilin University (Jilin, China). The mice were housed in microisolator cages and received food and water ad libitum. The laboratory temperature was 24 ± 1 °C, and the relative humidity was 40−80%. Mice were housed for 2−3 days to adapt them to the environment before experimentation. All animal experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Reagents. Mouse TNF-α, IL-6, and IL-1β sandwich enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biolegend (San Diego, CA). Mouse IL-8 ELISA kits were purchased from R&D Systems (Minneapolis, MN). Phospho-specific antibodies for ERK1/2, p38, JNK, and IκB as well as antibodies against ERK, p38, JNK, NF-κB p65, β-actin, and IκB proteins were obtained from Cell Signaling Technologies (Beverly, MA). Antirabbit and antimouse horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from PTG (Chicago, IL). Mouse mpo and mouse sod ELISA kits were purchased from Uscn life Co. (Missouri City, TX). All other chemicals were of reagent grade.

In Vitro Study. *Cell Culture.* The RAW 264.7 mouse macrophage cell line was obtained from the China Cell Line Bank (Beijing, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Laboratories Inc.) supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen/Gibco Life Technologies, Carlsbad, CA), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Biological Industries, Kibbutz Beit Haemek, Israel) at 37 °C in a humidified atmosphere of 5% CO_2 in air. The medium was replaced every 3 days. In all experiments, cells were allowed to acclimate for 24 h before any treatments.

MTT Assay for Cell Viability. To measure cell viability, the MTT assay was performed. RAW 264.7 cells were mechanically scraped, seeded in 96-well plates at 4×10^5 cells/mL, and incubated in a 37 °C and 5% CO_2 incubator overnight. After 24 h, the cells were treated with 50 μL of different concentrations of Lico A (0−30 μM) for 2 h and then stimulation with 50 μL of LPS for 18 h. Subsequently, 20 μL of 5 mg/mL MTT in FBS-free medium was added to each well and incubated for an additional 4 h. Cell-free supernatants were then removed and resolved with 150 μL/well DMSO. The optical density was measured at 570 nm on a microplate reader.

Cytokine Assays. To investigate the effect of LicoA on cytokine responses from LPS-treated cells, RAW 264.7 cells (4×10^5) were seeded into 24-well plates and pretreated with 5, 10, or 20 μM Lico A for 1 h prior to treatment with 1 mg/L of LPS for 6, 12, or 24 h. Cell-free supernatants were collected and stored at −20 °C until assayed for cytokine levels. The concentrations of TNF-α, IL-1β, and IL-6 in the cell supernatants were determined using an ELISA kit. Concentrations were determined for six technical replicates form one passage of each sample.

In Vivo Study LPS-Induced ALI Model. After the BALB/c mice were diethyl ether-anaesthetized, 10 μg of LPS was instilled intranasally (i.n.) in 50 μL of PBS to induce lung injury. Control mice were given 50 μL of PBS i.n. without LPS. Lico A (at doses of 20, 40, and 80 mg/kg, respectively) or vehicle (PBS) was intraperitoneal injected 1 h prior to LPS administration. Previous reports have shown that LPS can cause marked pulmonary inflammation as an acute injury after 2−4 h and maximizes at 24−48 h.[18] So, in this study, bronchoalveolar lavage fluid (BALF) and tissue samples were collected 24 h after LPS exposure. The collection of BALF was performed three times through a tracheal cannula with 0.5 mL of autoclaved PBS, instilled up to a total volume of 1.3 mL. The fluid recovered from each sample was centrifuged (4 °C, 3000 rpm, 10 min) to pellet the cells. The cell pellets were resuspended in PBS for total cell counts using a hemacytometer, and cytospins were prepared for differential cell counts by staining with the Wright−Giemsa staining method. At least 200 cells were counted per slide.

Assays for Cytokines and Oxidase Dimutase (SOD). The concentrations of cytokine TNF-α, IL-6, IL-1β, and IL-8 in the supernatants of the BALF were measured by ELISA using commercially available reagents according to the manufacturer's instructions. The SOD activity in the BALF was quantified by using a mouse SOD ELISA kit.

Lung Wet-to-Dry Weight (W/D) Ratio. After mice were euthanized, the lungs were excised. Each lung was blotted dry, weighed, and then placed in an oven at 80 °C for 48 h to obtain the "dry" weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

Protein Analysis. Protein concentrations in the supernatant of the BALF were quantified using the bicinchoninic acid (BCA) method to evaluate vascular permeability in the airways.

MPO Assay. Neutrophil and macrophage parenchymal infiltration, as reflected by MPO activity, was measured as described previously.[19] Mice under diethyl ether anesthesia were killed 24 h after LPS administration. The lung was excised, and 100 mg was homogenized in 50 mM hydroxyethyl piperazine ethanesulfonic acid (HEPES) (pH 8.0) containing 0.5% cetyltrimethyl ammonium bromide (CTAB) and subjected to three freeze−thaw cycles. The homogenate was centrifuged at 13000*g* for 30 min at 4 °C, and the cell-free extracts were stored at −20 °C until further use. The MPO activity was assayed using a mouse MPO ELISA kit. Samples were diluted in phosphate citrate buffer (pH 5.0).

Histopathologic Evaluation. Histopathologic evaluation was performed on mice that were not subjected to BALF. The lung tissue was fixed in formalin, dehydrated, paraffin embedded, and sliced. After hematoxylin and eosin staining, pathological changes in the lung tissues were observed under light microscope.

Western Blot Analysis. Tissues were harvested and frozen in liquid nitrogen immediately until homogenization. Samples were homogenized in RIPA buffer and lysed for 30 min on ice. Total protein fractionation was performed using a cell lysis buffer for Western blot and IP (Beyotime Institute of Biotechnology, China) according to the manufacturer's protocol. The protein concentration was assayed using the Bio-Rad protein kit, and equal amounts of protein were loaded into wells on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel. Subsequently, proteins were transferred onto polyvinylidene difluoride (PVDF) membranes, blocked overnight with 5% (wt/vol) nonfat dry milk, and probed according to the method described by Towbin et al,[20] with specific antibodies against JNK, ERK1/2, p38, NF-κB p65, β-actin, and IκB proteins, phospho-specific antibodies to JNK, ERK1/2, p38, and IκB proteins in 5% (wt/vol) BSA dissolved in TTBS. With the use of a peroxidase-conjugated secondary antimouse or antirabbit antibody, bound antibodies were detected by ECL plus (GE Healthcare Buckinghamshire, United Kingdom).

Statistical Analysis. All data were presented as the means ± standard errors of the mean (SEMs). Independent groups were compared using Mann−Whitney's U test. Statistical analysis was performed using the Kruskal−Wallis test. A p value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

LPS, a bacterial cell wall component, is known to induce the production of several inflammatory cytokines, tissue edema, and injury.[21] Lung is the most frequently involved organ, and ALI induced by LPS is the major reason of death. One widely used murine model of ALI is produced by the intratracheal administration of LPS. In the present study, we found that Lico A exerted potent anti-inflammatory effects in in vitro and in vivo models induced by LPS. When pretreated with Lico A, the amount of inflammatory cells, W/D ratio, protein leakage, and MPO activity were significantly decreased, and we further demonstrated that Lico A exerta an anti-inflammation effect in in vivo models of ALI through suppression of NF-κB activation and mitogen-activated protein kinase (MAPK) signaling.

Cell Viability and Cytotoxicity. To determine whether Lico A causes toxicity on RAW 264.7 cells, the cell viability was tested at various concentrations of Lico A in the cells by MTT assay (Figure 2). Results showed that Lico A alone at

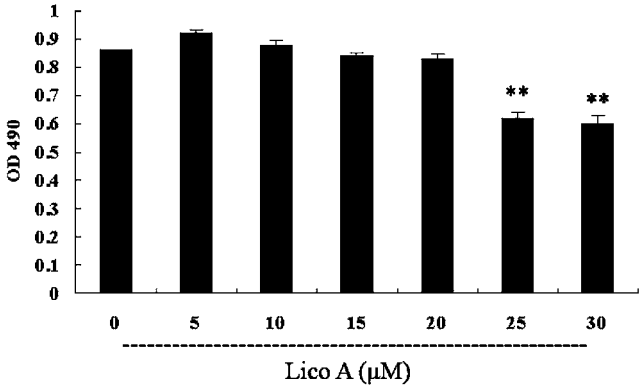

Figure 2. Effect of Lico A on the cell viability of RAW 264.7 cells. The cells were treated with different concentrations of Lico A (0−30 μM) for 24 h. The cell viability was determined by MTT assay. The values presented are the means ± SEMs. $^{**}p < 0.01$ vs untreated control cell cultures.

concentrations of 5, 10, 15, and 20 μM had no effect on the viability of RAW 264.7 cells. At concentrations of 25 and 30 μM, Lico A reduced the cell viability. Therefore, in this study, we used Lico A at concentrations of 20 μM or under, which had no effect on the cell viability for further studies of anti-inflammatory property in RAW 264.7 cells.

Effects of Lico A on Cytokine Production in LPS-Stimulated RAW 264.7 Cells. The production of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β by macrophages in response to inflammatory stimuli and microbial products is well established.[22] Inhibition of inflammatory cytokine and mediator production serves as a key mechanism in the control of inflammation. Our in vitro studies in RAW 264.7 cells demonstrated that, in the absence of Lico A, LPS challenge (1 μg/mL) resulted in an burst of TNF-α (Figure 3A) and a modest peak in IL-6 (Figure 3B) and IL-1β (Figure 3C) concentration, both occurring at 6 h. The concentrations of IL-1β (Figure 3C) in the culture supernatants of RAW 264.7 cells rose steadily over the 12 h period and IL-6 (Figure 3B) concentrations peaked at 12 h and decreased by 24 h. Pretreatment with Lico A prior to LPS induction attenuated TNF-α (Figure 3A) and IL-1β (Figure 3C) levels in a concentration-dependent manner (Figure 3). The concentration of IL-6 was markedly decreased at 24 h in groups

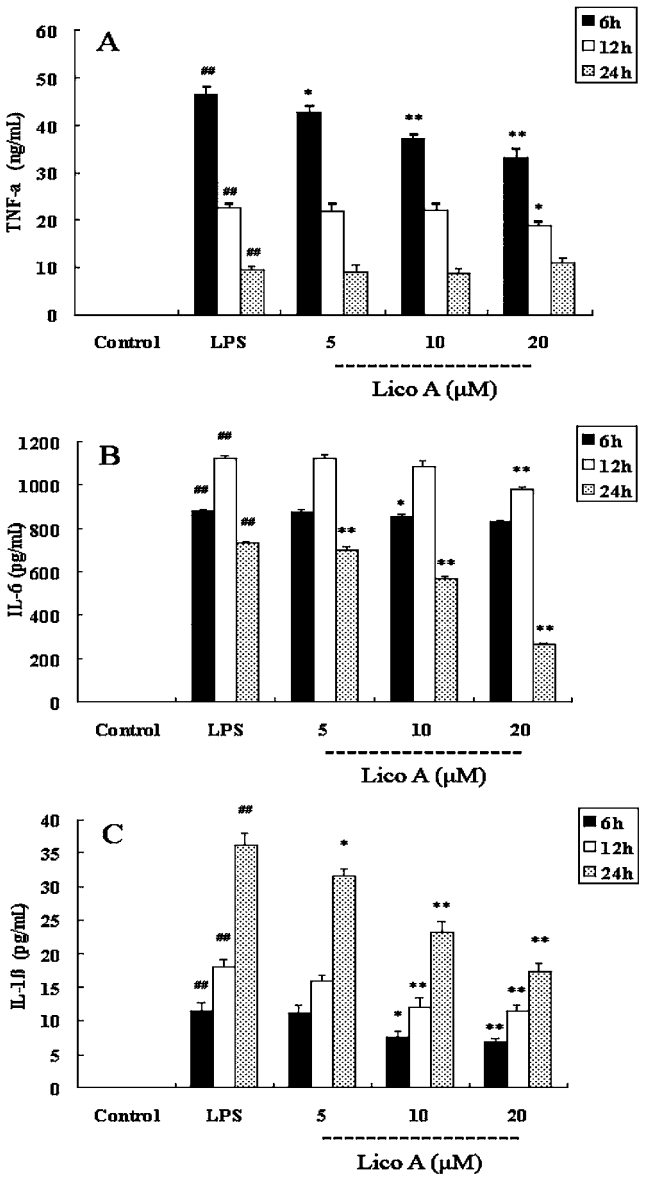

Figure 3. Effect of Lico A (at doses of 5, 10, or 20 μm) on TNF-α (A), IL-6 (B), and IL-1β (C) expression at different time points following LPS (1 μg/mL) administration in RAW 264.7 cells. The values presented are the means ± SEMs. $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

treated with Lico A and showed significant change as compared to the LPS group (Figure 3) ($p < 0.01$). On the basis of this result, we further demonstrated that Lico A had an effect on clinical application using mice with LPS-induced ALI pretreated with Lico A.

Effects of Lico A on Cytokines and Inflammation Cells Production in BALF from Mice with ALI. TNF-α is the earliest and primary endogenous mediator of the process of an inflammatory reaction. It has been reported that the activity of TNF-α is effectively inhibited in the aqueous phase of lung fluids of patients with acute respiratory distress syndrome (ARDS).[23] Giving TNF-α intravenously has caused increased lung inflammation and increased permeability in animals,[24−26] and neutrophil depletion prevented TNF-mediated ALI in guinea pigs.[27] IL-6 is produced by activated macrophages and stimulates acute-phase responses in the liver. Martin has found

dx.doi.org/10.1021/jf2051587 | J. Agric. Food Chem. 2012, 60, 3947−3954

that IL-6 concentrations are very high in the BAL of patients at risk for ARDS and that they remain elevated throughout the course of established ARDS.[23] Furthermore, monocaryon/macrophages can generate TNF-α and IL-6. The data presented here demonstrate that Lico A may significantly inhibit the production of LPS-induced TNF-α, IL-6, and IL-1β in a concentration-dependent manner in vivo (Figure 4). Accordingly, Lico A demonstrated protection to mice ALI induced by LPS through the inhibition of the production of cytokines levels in BALF.

Neutrophils are key cells in the inflammatory response that characterizes ALI,[28] which are the earliest immune cells to be recruited to the site of injury or inflammation. Numerous evidence linking PMNs and lung injury and the critical involvement of cytokines in the recruitment of PMNs into tissues, it was hoped that the study of cytokines in BAL would provide clues about the mechanisms that regulate injury in the lungs.[29−31] MPO, the most abundant granule enzyme in neutrophils,[32] excessive generation has been linked to tissue damage in many diseases, especially those characterized by acute or chronic inflammation, and has also been inhibited after the pretreatment of Lico A. The chemokine CXCL8 (IL-8), also known as neutrophil-activating peptide 1 (NAP1), is a CXC chemokine that is recognized as a potent effector of neutrophil functions.[33] Treatment of the rabbits with a humanized anti-CXCL8 antibody prevented neutrophil infiltration in ALI.[34] Lico A pretreatment efficiently inhibited the release of IL-8 and MPO activity of ALI mice as compared with the LPS group (Figures 4 and 5), which indicates that Lico A exerted antineutrophil influx effects in LPS-induced ALI through inhibited the release of IL-8. Thus, Lico A confers

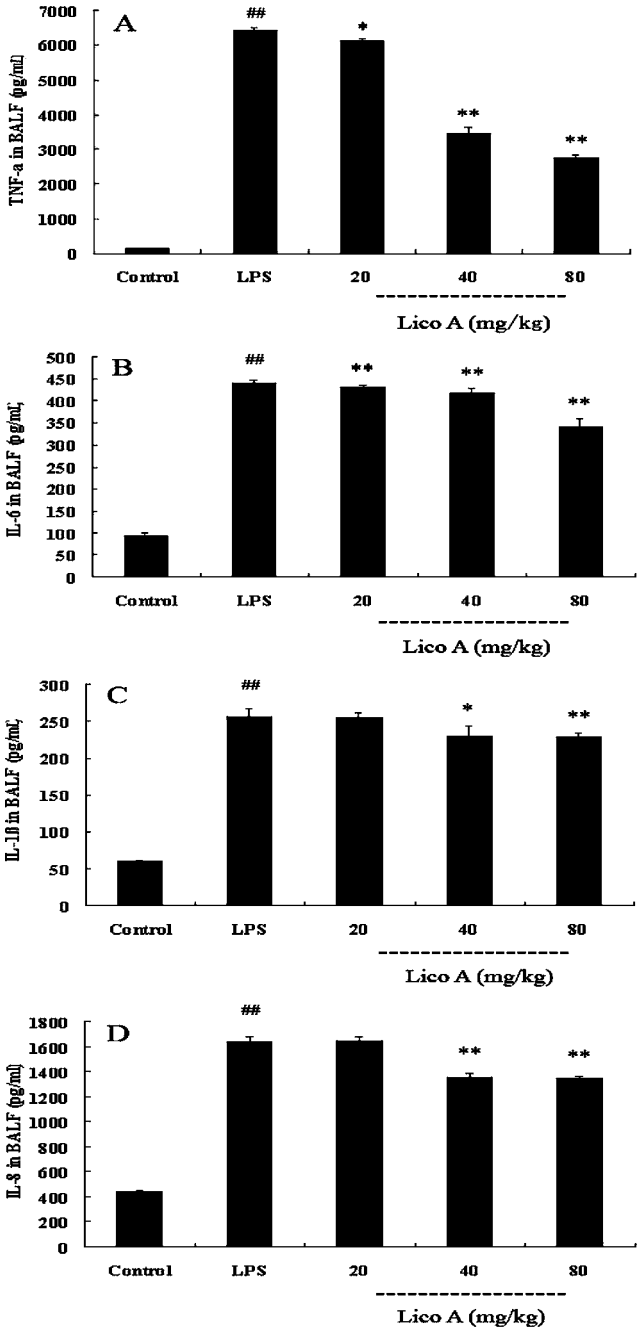

Figure 4. Effect of Lico A on TNF-α, IL-6, IL-1β, and IL-8 expression from mice with ALI. Mice were given Lico A (20, 40, or 80 mg/kg) by intraperitoneal injection 1 h before challenge with LPS. BALF was collected at 24 h following LPS challenge to analyze the inflammatory cytokines TNF-α (A), IL-6 (B), IL-1β (C), and IL-8 (D). The values presented are the means ± SEMs (n = 8 in each group). $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

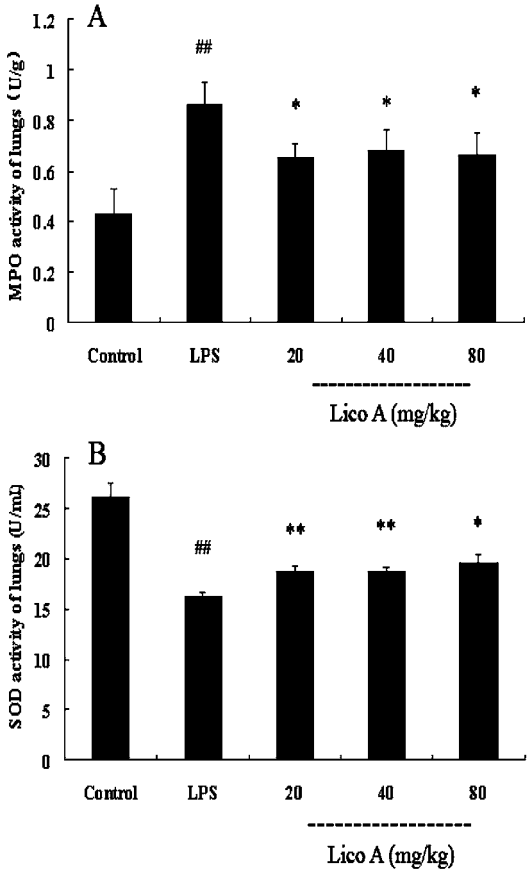

Figure 5. Effects of Lico A on MPO activity in lungs and SOD activity in BALF of LPS-induced mice. BALF was prepared from mice 24 h after LPS challenge, and SOD activity was determined. Lung homogenates were prepared for measuring MPO activity. SOD and MPO activities were determined by SOD- and MPO-specific ELISA kits. The values presented are the means ± SEMs (n = 8 in each group). $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

dx.doi.org/10.1021/jf2051587 | J. Agric. Food Chem. 2012, 60, 3947−3954

protection to mice ALI induced by LPS through the inhibition of the production of inflammatory cell and CXCL8 levels in BALF (Figures 4 and 6).

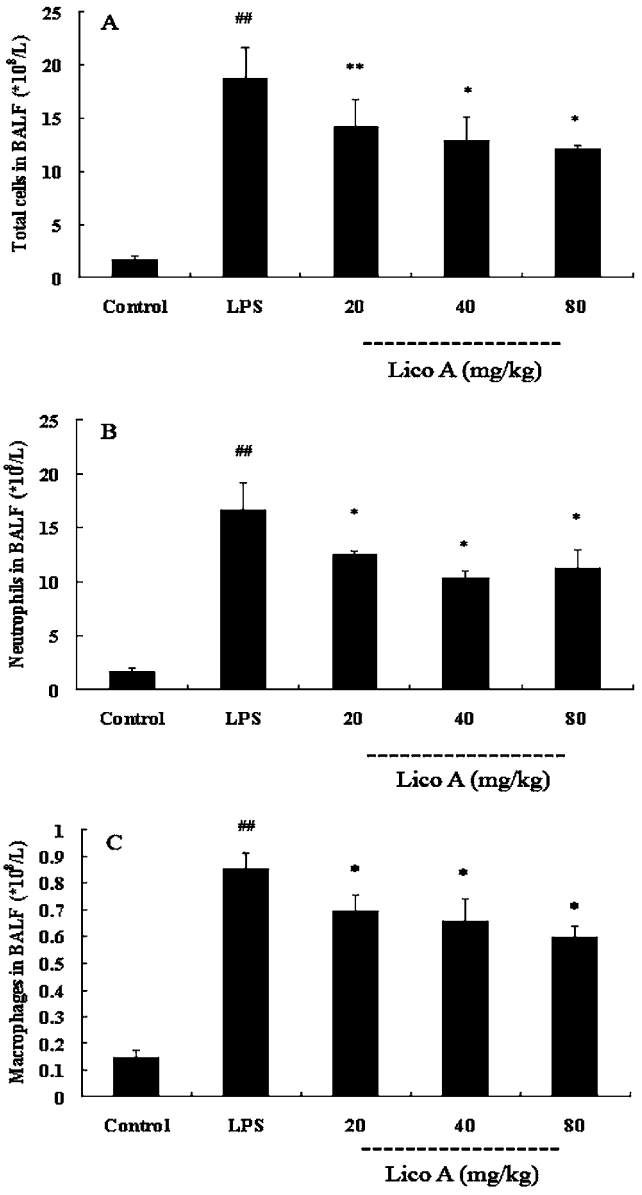

Figure 6. Effects of Lico A on the number of total cells, neutrophils, and macrophages in the BALF of LPS-induced ALI mice. Mice were given Lico A (20, 40, or 80 mg/kg) 1 h prior to an i.n. administration of LPS. BALF was collected at 24 h following LPS challenge to measure the number of total cells (A), neutrophils (B), and macrophages (C). The values presented are the means ± SEMs (n = 8 in each group). $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

Effects of Lico A on Histological Changes in Lung Tissues from Mice with LPS-Induced ALI. To evaluate the histological changes in lung tissue from mice with ALI following treatment with Lico A, lung sections obtained 24 h after administration of LPS were subjected to H&E staining. In the LPS group, the lung showed significant pathologic changes, such as alveolar wall thickening, alveolar hemorrhage, interstitial edema, inflammatory cells infiltration, and even lung tissues destruction (Figure 7). In addition, the control group also showed sight pathologic changes. However, treatment with Lico A (20, 40, or 80 mg/kg) significantly attenuated these changes (Figure 7).

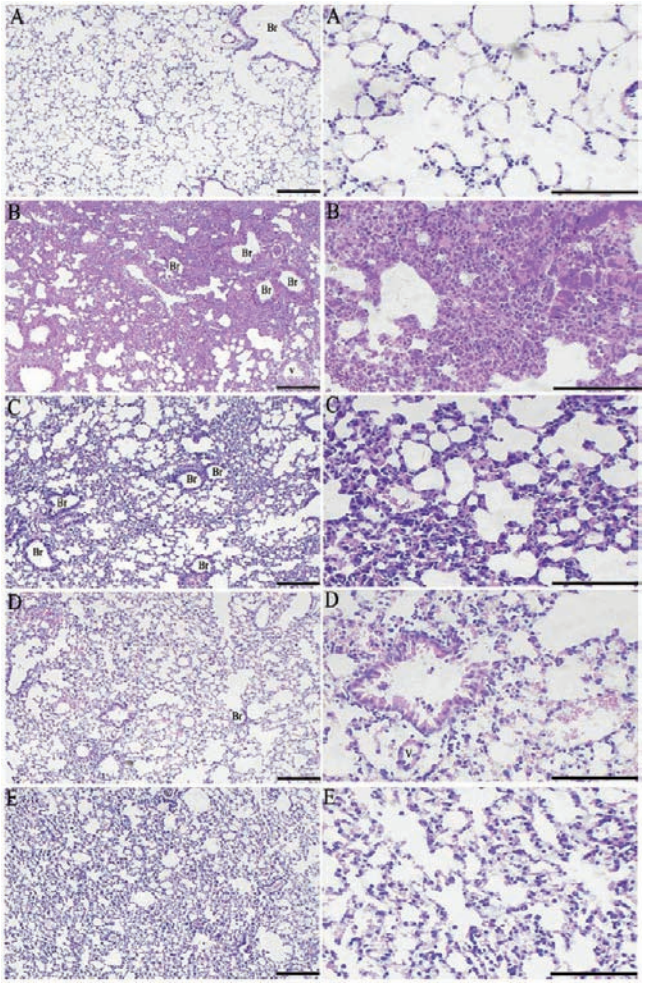

Figure 7. Effect of Lico A on histopathological changes in lung tissues in LPS-induced ALI mice. (A) PBS-challenged mice, (B) LPS-challenged mice, (C) LPS-challenged mice treated with Lico A (20 mg/kg), (D) LPS-challenged mice treated with Lico A (40 mg/kg), and (E) LPS-challenged mice treated with Lico A (80 mg/kg). The left panel is magnified 100×, scale bars = 100 μm. The right panel is magnified 400×, scale bars = 50 μm. Br = bronchi, and V = vessel.

Effects of Lico A on Oxidative Damage from Mice with ALI. Oxidative damage is a major cause of lung injury during ARDS. One of the major pathological consequences of ALI is ARDS, which is mainly caused by oxidative damage due to inflammatory responses.[35] SOD is the only antioxidant enzyme that can scavenge superoxide, and it has been reported to be markedly decreased in LPS-induced ALI.[36] The data presented here demonstrated that SOD was substantially increased in mice that were preadministered Lico A (Figure 5), as compared to the LPS group. Therefore, in this study, we demonstrated that Lico A may effectively scavenge oxyradicals during the inflammatory response to LPS-induced ALI.

Effects of Lico A on Lung W/D Ratio in Mice with LPS-Induced ALI. Edema is a typical symptom of inflammation not only in systemic inflammation but also in local inflammation. To quantify the magnitude of pulmonary edema, we evaluated the W/D ratio of the lung. Our experiments showed that Lico A may significantly inhibit edema of the lung, as shown by a W/

dx.doi.org/10.1021/jf2051587 | J. Agric. Food Chem. 2012, 60, 3947−3954

D ratio in the Lico A group that was significantly lower than the LPS group (Figure 8). After LPS administration, toll-like

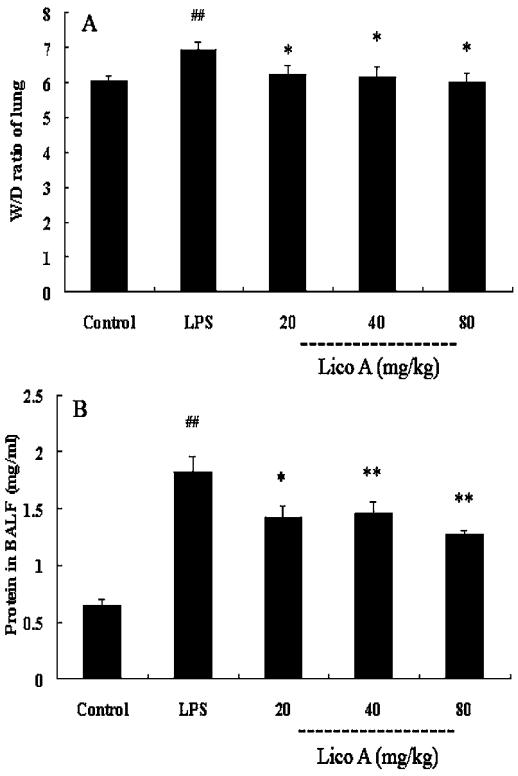

Figure 8. Effects of Lico A on the lung W/D ratio and total protein level in the BALF of LPS-induced ALI mice. Mice were given Lico A (20, 40, or 80 mg/kg) 1 h prior to an i.n. administration of LPS. The lung W/D ratio (A) and total protein concentration in the BALF (B) were determined 24 h after the LPS challenge. The values presented are the means ± SEMs (n = 8 in each group). $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

receptors initiate a series of NF-κB and MAPK cascades and result in expression of cytokines, chemokines, and adhesion molecules; generation of ROS; and secretion of MPO. To further explore the possible underlying mechanism, the effect of Lico A on NF-κB and MAPK pathways was examined.

Effect of Lico A on NF-κB Activation and MAP Kinase Phosphorylation from Mice with LPS-Induced ALI. MAPKs are highly conserved, eukaryotic signal transducing enzymes that respond to environmental stresses, as well as to plasma membrane receptor stimulation, by regulating key molecular targets, up to the transcriptional machinery in the nucleus. This enzyme family includes several subgroups such as JNK, ERK, and p38. Recent studies have shown that p38MAPK is involved in the development of ALI/ARDS with different stimuli.[37−39] It has been shown that p38MAPK is induced by LPS and critical for the LPS-induced cytokine release.[40,41] There is also evidence suggesting that p38MAPK mediated the bronchoconstriction and the neutrophil recruitment provoked by LPS.[42] The ERK pathway participates in chemoattractant-induced neutrophil chemotaxis and the respiratory burst[43,44] and in LPS-induced ALI.[45] Schuh and Pahl reported that the ERK pathway plays an important role in LPS-mediated pulmonary inflammation.[46] Here, we found that phosphorylation of MAPKs substantially increased, concomitant with the increase of cytokines and inflammatory cell infiltration in pulmonary tissue after injection of LPS (Figure 9). We also

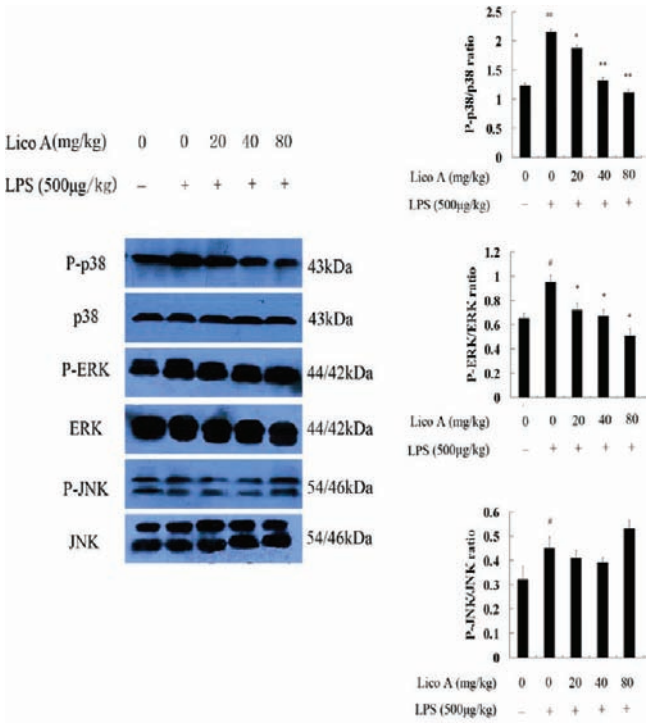

Figure 9. Effects of Lico A on LPS-induced phosphorylation of MAPK molecules in lungs of ALI mice. Mice were pretreated with different concentrations of Lico A for 1 h and stimulated with LPS. The cellular proteins from the lungs were used for the detection of phosphorylated or total forms of three MAPK molecules, ERK, p38MAPK, and JNK. Three independent experiments were performed. The relative quantification of target proteins was calculated by comparison of the bands density levels between samples. The data shown indicate the means ± SEMs. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

noticed that Lico A attenuated the activation of ERK and p38MAPK but not JNK in LPS-stimulated ALI (Figure 9), indicating that the protective effects of Lico A may be due to inhibition of p38MAPK and ERK activation.

NF-κB is a generic name of a series of proteins that have specific binding with the κB site of multiple gene initiators and can promote transcription. Many cytokines and adhesion molecule genes closely related to inflammation and immune reaction have a κB site in their initiating sites. IκB is an inhibitory protein that sequesters NF-κB in the cytoplasm. In response to stimuli, the cytoplasmic NF-κB/IκB complex is activated by phosphorylation on conserved serine residues of IκB. When IκB is ubiquitinated and degraded, NF-κB migrates to the nucleus and turns on transcription. One well-established mechanism of NF-κB suppression is the export of nuclear NF-κB by inhibitor of NF-κB (IκBα), thereby rapidly repressing NF-κB activation.[47] NF-κB was proposed to be the central and critical factor, regulating the production of inflammatory mediators.[48] NF-κB inhibitor could attenuate endotoxin-induced ALI.[49] However, IκB were not degraded in lungs treated with Lico A in the presence of LPS stimulation as compared with the LPS group. In addition, p38MAPK regulates the LPS-induced NF-κB activation pathway in the development of ALI through at least two different mechanisms. Inhibition of

p38MAPK could effectively block the LPS-induced lung injury, which includes neutrophil recruitment into the lung, protein leakage, and apoptosis of BAL cells, via the blocking of NF-κB activation.[50] Here, we found that both NF-κB and p38MAPK were spontaneously activated during LPS attack, and pretreatment of Lico A inhibited the phosphorylation of NF-κB and p38MAPK in a dose-dependent manner (Figures 9 and 10).

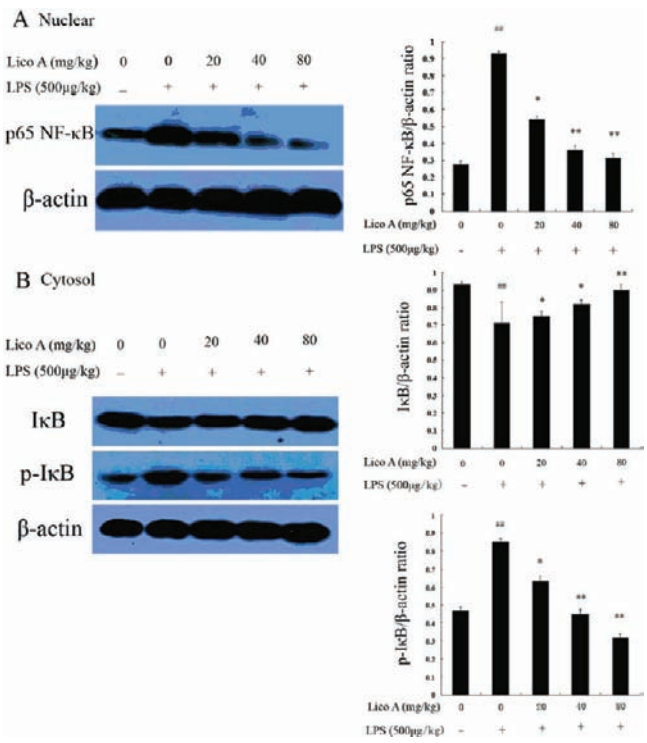

Figure 10. Effects of Lico A on LPS-induced phosphorylation of IκBα and NF-κB activation in lungs of ALI mice. Mice were pretreated with different concentrations of Lico A (20, 40, or 80 mg/kg) for 1 h and then stimulated with LPS (A) for the detection of the NF-κB p65 subunit and cytosolic extracts and (B) for the detection of phosphorylated IκBα by Western blot. Three independent experiments were performed. The relative quantification of target proteins was calculated by comparison of the bands density levels between samples. The data shown indicate the means ± SEMs. $^{\#\#}p < 0.01$ vs control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs LPS group.

These results suggested that the inhibition of NF-κB activation by Lico A may be due to inhibition of p38MAPK and ERK phosphorylation.

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Funding

This work was supported by the National Natural Science Foundation of China (No. 31072168), National High Technology Research and Development Program of China (No. 2011AA10A214), and Natural Science Foundation of Jilin Province of China (No. 20101579).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

Lico A, licochalcone A; LPS, lipopolysaccharide; W/D, lung wet-to-dry weight; SOD, oxidase dimutase; MPO, myeloperoxidase; IL, interleukin; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; ALI, acute lung injury; PMNs, polymorphonuclear neutrophils; BALF, bronchoalveolar lavage fluid; ELISA, sandwich enzyme-linked immunosorbent assay; ARDS, acute respiratory distress syndrome

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