Inflammation, Vol. 42, No. 2, April 2019 (© 2019)
DOI: 10.1007/s10753-018-00953-4

ORIGINAL ARTICLE

Umbelliferone Alleviates Lipopolysaccharide-Induced Inflammatory Responses in Acute Lung Injury by Down-Regulating TLR4/MyD88/NF-κB Signaling

Dongqiu Wang,[1] Xia Wang,[1] Wen Tong,[1] Yuhong Cui,[1] Xiuxian Li,[1] and Haiyun Sun[1,2]

Abstract— This study investigated the protective effect and underlying mechanism of action of umbelliferone (Umb) against lipopolysaccharide (LPS)-induced acute lung injury (ALI). An intragastric Umb injection prior to the administration of LPS dramatically decreased the wet/dry lung weight ratio, attenuated inflammatory cell infiltration in lung tissue, and reduced the LPS-induced production of inflammatory cytokines, including monocyte chemotactic protein-1(MCP-1), interleukin (IL)-6, tumor necrosis factor-α (TNF-α), and IL-1β, in broncheoalveolar lavage fluid (BALF). In addition, Umb resulted in significant antioxidative effects as shown by decreased myeloperoxidase (MPO) and malondialdehyde (MDA) activity and increased superoxide dismutase (SOD) activity compared with the LPS group. Finally, the inhibitory effects of Umb on the expression of toll-like receptor 4 (TLR4)/myeloid differentiation protein 88 (MyD88)/nuclear factor-κB (NF-κB) signaling pathway proteins were also measured. Our results clearly indicated that Umb exerted significant protective effects on LPS-induced ALI by inhibiting the activation of the TLR4/MyD88/NF-κB pathway.

KEY WORDS: umbelliferone; acute lung injury; TLR4/MyD88/NF-κB signaling.

INTRODUCTION

Acute lung injury (ALI) is a critical illness that is associated with significant morbidity and mortality [37]. Previous studies have confirmed that ALI is caused by increased neutrophil infiltration into the lungs, resulting in the release of pro-inflammatory mediators such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, and increased oxidative stress [33]. The manifestations of ALI include acute respiratory distress, non-cardiogenic pulmonary edema, and hypoxemia [29, 35, 36]. Unfortunately, although tremendous advances in the pathophysiology and treatment of ALI have been made, the annual incidence and mortality rate of patients with ALI remain extremely high [37]. There remains a lack of effective therapies for this syndrome. Thus, studies investigating the underlying mechanism of and novel treatment methods for ALI are urgently needed.

Lipopolysaccharide (LPS) is a major component of the Gram-negative bacterial cell wall [9, 13]. LPS triggers inflammatory responses, including membrane lipid oxidization and the production of pro-inflammatory cytokines, resulting in damage to the host [14]. Therefore, the intratracheal administration of LPS has been widely used to establish an animal model of ALI [1]. This model, which possesses similar characteristics to human ALI, is essential in exploring the molecular mechanisms underlying ALI and to test the effects of potential treatments [24]. Toll-like receptor 4 (TLR4), a transmembrane receptor, plays a critical role in the initiation and acceleration of the

[1] Iintensive Care Unit, Heze Municipal Hospital, No. 2888, West Caozhou Road, Heze, 274031, China
[2] To whom correspondence should be addressed at Iintensive Care Unit, Heze Municipal Hospital, No. 2888, West Caozhou Road, Heze, 274031, China. E-mail: haiysun66@126.com

inflammatory response induced by LPS [22, 32]. The binding of LPS to TLR4 can activate nuclear factor-κB (NF-κB) signaling and initiate a cascade of responses, including neutrophil infiltration and the accumulation of inflammatory mediators such as TNF-α, IL-1β, and IL-6 [34]. Studies have shown that this process in an ALI-like phenotype following LPS administration is accomplished *via* a myeloid differentiation protein 88 (MyD88)-dependent pathway [4, 8].

Natural products and their derivatives have been recognized as potential treatments for various diseases [26]. Umbelliferone (7-hydroxy coumarin) is an important derivative of coumarin, which is found in many plants [3, 11]. Previous studies had shown that umbelliferone possesses various pharmacological activities, including high antioxidant [12, 16] and anti-tumor effects [16, 18]. However, no study has investigated the effects of umbelliferone on LPS-induced inflammatory responses in ALI.

In the present study, we first examined the effects of umbelliferone on vascular leakage and inflammatory cell infiltration in ALI tissue. Malondialdehyde (MDA) formation and superoxide dismutase (SOD) activity were also tested to confirm the anti-oxidative activity of umbelliferone. Moreover, the effect of umbelliferone on the TLR4/MyD88/NF-κB signaling pathway was investigated to reveal the potential anti-oxidative mechanisms of umbelliferone. Our research demonstrated that umbelliferone exerted significant protective effects against LPS-induced ALI by inhibiting the activation of the TLR4/MyD88/NF-κB signaling pathway, indicating that umbelliferone might be a useful agent for the treatment of ALI.

MATERIALS AND METHODS

Chemicals and Reagents

Umbelliferone was purchased from the National Institutes for Food and Drug Control (Beijing, China), while LPS (*Escherichia coli* O111:B4) was obtained from Sigma (St. Louis, MO). All the primary antibodies used in this study, including antibodies for NF-κB (#8242), p-NF-κB (#3033), IκB (#4814), p-IκB (#2859), Myd88 (#4283), TLR4 (#14358), and β-actin (#4970), were from Cell Signaling Technology (Danvers, MA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for MCP-1, IL-6, TNF-α, and IL-1β were obtained from R&D Systems (Minneapolis, MN, USA). Myeloperoxidase (MPO), malondialdehyde (MDA), and superoxide dismutase (SOD) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Establishment of the ALI Mouse Model

Thirty male BALB/c mice (7–8 weeks old, weighting about 18–22 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and group-housed in a temperature-controlled room with a 12:12-h light-dark cycle. All animal treatments in this study were performed humanely and following the institutional and national guidelines for ethical animal research. LPS was used to establish acute lung injury (ALI) mice model. Firstly, mice were randomly divided into five groups: normal control group (control), model group (LPS), and the umbelliferone groups (10, 20, and 40 mg/kg). Secondly, mice in umbelliferone groups were pretreated by umbelliferone (10, 20, and 40 mg/kg) through administration by gavage. After 1 h, LPS (50 μL, 3.2 mg/mL) was administered by intranasal administration to induce ALI, while the mice in the control group received an equal volume of PBS without LPS. Twelve hours after LPS challenge, mice were sacrificed and then bronchoalveolar lavage fluid and lung tissue samples were collected.

The Acquisition of Bronchoalveolar Lavage Fluid and Cell Count

The lungs of mice were carefully lavaged three times with a total volume of 1.5 mL sterile PBS solution. The total cell number was counted using a hemocytometer. The rest of the bronchoalveolar lavage fluid (BALF) was centrifuged at 1000*g* for 10 min at 4 °C and the cell-free supernatant was harvested. Five-hundred-microliter supernatant was used to quantify the total protein concentration by a BCA protein assay kit (Beyotime, Shanghai, China). The remainder BALF supernatant was stored at − 80 °C for cytokine analysis.

Measurement of Inflammatory Cytokines in BALF

The levels of inflammatory markers, including MCP-1, IL-6, TNF-α, and IL-1β, in BALF were examined by mouse ELISA kits according to the manufacturer's recommendations (R&D Systems, Minneapolis, MN, USA). The experiment was repeated at least three times.

Histological Evaluation

Lungs were collected 12 h after the administration of LPS. The left lungs were fixed in Bouin's solution,

embedded by paraffin, and then sliced at 4-μm thickness. Sections were stained with hematoxylin and eosin (H&E), and pictures were captured using a microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Lung W/D Ratio

After the mice were sacrificed, the weights of the right lungs were recorded as wet weight. After that, lungs were stored in an incubator at 60 °C for 48 h to get the dry weights. The lung W/D ratio (wet/dry weight) was used to assess the degree of pulmonary edema.

Measurement of MPO, MDA, and SOD

To analysis the neutrophil accumulation in lung tissues, the MPO activity was determined by a MPO activity kit following the manufacturer's protocol. Besides that, the pulmonary content of MDA levels was tested to examine the level of lipid peroxidation, and the SOD level was detected to measure anti-oxidative enzyme activities in the lung tissue. All of these experiments were done according to the instructions recommended by the manufacturers.

Western Blot Analysis

Total protein was extracted from lung tissues and analyzed by western blot with specific antibodies as described [6]. Briefly, lung tissues were collected and flash frozen in liquid nitrogen immediately. After that, lungs were homogenized and lysed in extraction buffer for 1 h on ice. The lysates were centrifuged at 13,000 r for 20 min, and the supernatant was collected. The protein concentration was determined by BCA assay. Equal amounts of protein were separated by 10% SDS-PAGE and electroblotted onto PVDF membranes. After blocking with 5% non-fat milk for 1.5 h at 37 °C, the membranes were incubated with the primary antibody overnight at 4 °C, followed by peroxidase-conjugated secondary antibody for 1 h at room temperature. Subsequently, immunoreactive protein bands were visualized with Tanon 5200 chemiluminescence imaging system.

Statistical Analysis

Values were represented as means ± SEM, and analyzed by using one-way analysis of variance (ANOVA) and Student's *t* test using GraphPad Prism Software (GraphPad Inc., La Jolla, CA, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Umbelliferone Decreased the Number of Total Cells and Protein Concentration in BALF of LPS-Induced ALI Mice

As vascular leakage is a hallmark of ALI, we assessed the total cell number and protein concentration in BALF. Mice were given an intragastric injection of umbelliferone (10, 20, and 40 mg/kg) at 1 h prior to the administration of LPS. BALF was collected at 12 h following LPS challenge. As is shown in Fig. 1a, b, compared with the control group, the challenge of LPS to mice dramatically increased the number of total cells and the protein concentration, while pretreated with umbelliferone dose-dependently inhibited the LPS-induced changes, especially at the concentration of 20 and 40 mg/kg.

Umbelliferone Pretreatment Reduced the Production of Inflammatory Cytokines in the BALF of LPS-Induced ALI Mice

To study the potential anti-inflammatory effects of umbelliferone, the MCP-1, IL-6, TNF-α, and IL-1β levels in BALF were measured by ELISA. As shown in Fig. 2, LPS challenge significantly increased the levels of MCP-1, IL-6, TNF-α, and IL-1β in BLAF in comparison with that in control group mice. The pretreatment of umbelliferone, especially at the concentration of 40 mg/kg, significantly reduced the inflammatory cytokines levels compared to that in the LPS group. All of these results demonstrated that umbelliferone could reduce the inflammatory cytokine levels in LPS-induced ALI mice.

Umbelliferone Treatment Attenuated the Inflammatory Cell Infiltration in Lung Tissues in LPS-Induced ALI Mice

To assess the effects of umbelliferone on lung injuries, the histopathological changes of the lung tissue were observed. As shown in Fig. 3, extensive neutrophil infiltration and the obviously destruction of cellular structure were observed in LPS-challenged group compared with the control group. However, pretreatment with umbelliferone attenuated the histological alterations

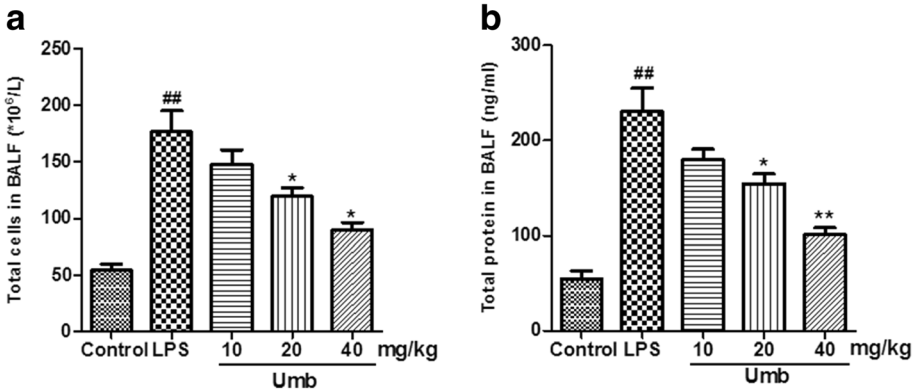

Fig. 1. Effects of umbelliferone on the number of total cells and protein concentration in the BALF of LPS-induced ALI mice. Mice were given an intragastric injection of umbelliferone (10, 20, and 40 mg/kg) a 1 h prior to the administration of LPS. BALF was collected at 12 h following LPS challenge to measure the number of total cells (**a**) and total proteins (**b**). The values presented are the mean ± SEM ($n = 6$ in each group). $^{\#\#}p < 0.01$ *vs* the control group; $^{*}p < 0.05$ *vs* the LPS group.

induced by LPS. Especially, the lung morphology of lung tissue in the 40-mg/kg group was almost approximate to that in control group, indicating that umbelliferone significantly alleviated the lung injuries.

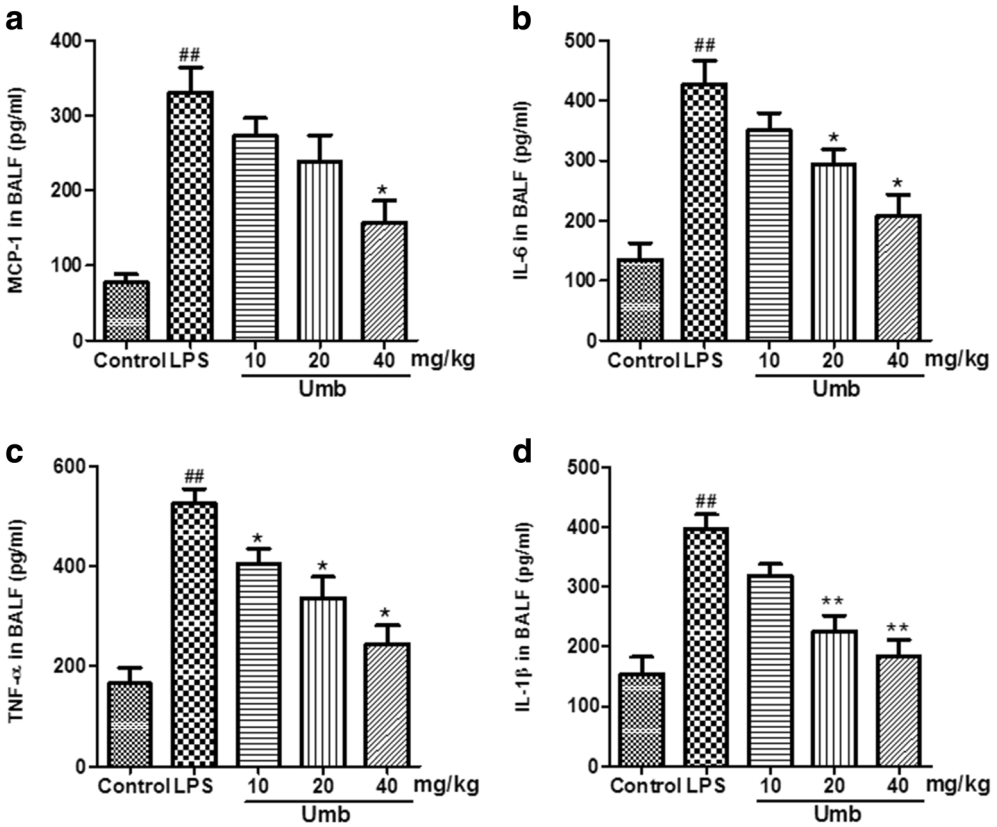

Fig. 2. Effects of umbelliferone on the production of inflammatory cytokines (MCP-1, IL-6, TNF-α, and IL-1β) in the BALF of LPS-induced ALI mice. Umbelliferone (10, 20, and 40 mg/kg) were given by intragastric injection 1 h prior to the administration of LPS. The concentrations of MCP (**a**), IL-6 (**b**), TNF-α (**c**), and IL-1β (**d**) in BALF after LPS i.n. administration were analyzed for the effect of umbelliferone at varied doses. The values presented are the means ± SEM ($n = 6$ in each group). $^{\#\#}p < 0.01$ *vs* the control group; $^{*}p < 0.05$; $^{**}p < 0.01$ *vs* the LPS group.

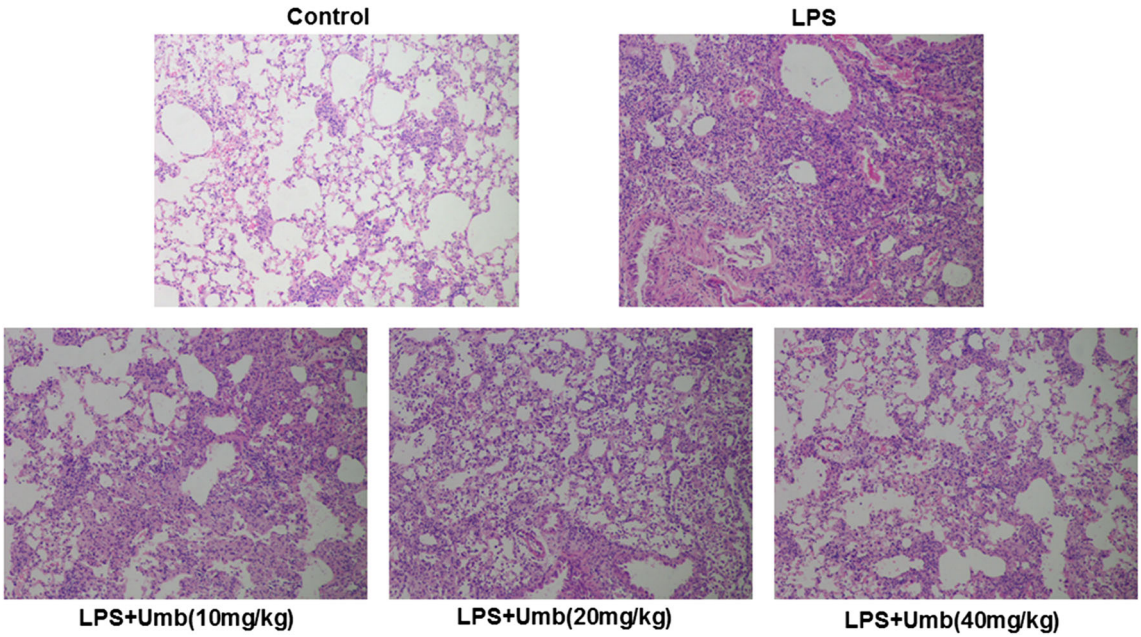

Fig. 3. Effects of umbelliferone on histopathological changes in lung tissues in LPS-induced ALI mice. Umbelliferone (10, 20, and 40 mg/kg) were given by intragastric injection 1 h prior to the administration of LPS. Lungs ($n = 6$) from each experimental group were collected for histological evaluation at 12 h after LPS challenge. Representative histological changes of lung obtained from mice of different groups. Control group. LPS group. Umbelliferone (10, 20, and 40 mg/kg) group.

Umbelliferone Treatment Decreased the Lung W/D Ratio and Suppressed MPO Activity in LPS-Induced ALI Mice

Pulmonary edema and the recruitment of inflammatory cells into the pulmonary compartment are the most important characteristics of LPS-induced ALI. The lung W/D ratio, a reliable index of lung edema, was accessed in this study. As we can see in Fig. 4a, the lung W/D ratio in LPS group was remarkably higher than that in the control group. While the treatment of umbelliferone decreased the LPS induced change. To further demonstrate these effects, the MPO activity in lung tissues, which is an indicator of neutrophil infiltration, was also tested. Results in Fig. 4b showed that the MPO activity was remarkably increased by LPS, and this increase in MPO could be effectively reversed by the treatment of umbelliferone. These results indicated that umbelliferone exerted a protective effect against LPS-induced lung injury.

Umbelliferone Treatment Decreased MDA Formation and Increased SOD Activity in LPS-Induced ALI Mice

The MDA activity and SOD activity were studied to verify the anti-oxidative activities of umbelliferone. In comparison with control group, significant increase in MDA content and decrease in SOD activity in LPS group were shown in Fig. 5a, b. Umbelliferone could effectively decrease the MDA activity and increase the SOD activity in a dose-dependently way. These results indicated that umbelliferone significantly alleviated the LPS-induced oxidative damage in lung tissue.

Umbelliferone Treatment Alleviated the LPS-Induced ALI by Down-Regulating TLR4/MyD88/NF-κB Signaling

To further study the mechanism of umbelliferone on LPS-induced ALI, we tested the protein expression of TLR4/MyD88/NF-κB signaling in LPS-induced ALI mice. As shown in Fig. 6, ALI mice displayed obviously elevation of p-IκBα and p-NF-κB compared with control group. However, the treatment of umbelliferone significantly reversed those, indicating that umbelliferone could inhibit the activation of NF-κB pathway. Besides that, the up-regulated protein expression of MyD88 and TLR4 induced by LPS challenge could also be inhibited by umbelliferone. These data indicated that umbelliferone treatment alleviated the LPS-induced ALI by partly down-regulating TLR4/MyD88/NF-κB signaling.

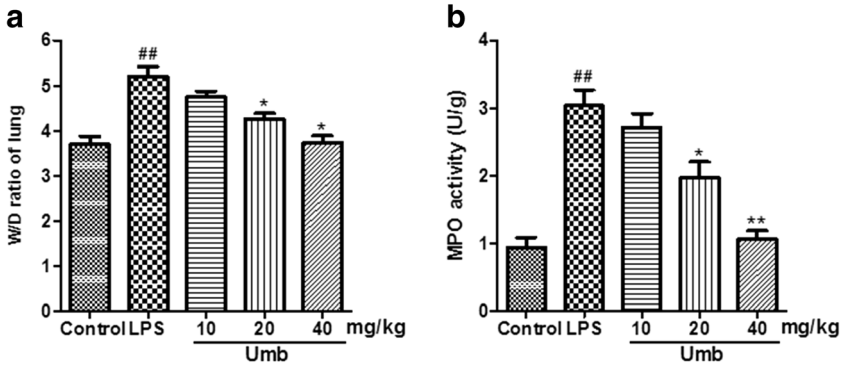

Fig. 4. Effects of umbelliferone on the lung W/D ratio and lung MPO of LPS-induced ALI mice. Mice were given an intragastric injection of umbelliferone (10, 20, and 40 mg/kg) 1 h prior to the administration of LPS. The lung W/D ratio (**a**) and lung MPO (**b**) were determined at 12 h after LPS was given. The values presented are the mean ± SEM ($n = 6$ in each group). $^{\#\#}p < 0.01$ *vs* the control group; $^{*}p < 0.05$; $^{**}p < 0.01$ *vs* the LPS group.

DISCUSSION

ALI is a common clinical disease that results in alveolar-capillary membrane damage, extensive neutrophil infiltration, inflammatory mediator release, and non-cardiogenic pulmonary edema [24]. Numerous studies have confirmed that an increased inflammatory response and excessive oxidative stress play an important role in the pathogenesis of ALI [20].

Umbelliferone, a natural coumarin derivative, is the major effective constituent of the umbelliferae herb family [21, 27]. Previous studies have highlighted multiple pharmacological effects of umbelliferone, including anti-microbial, anti-fungal, anti-inflammatory, and anti-oxidant activities [7, 16, 25, 28, 31]. However, no reports investigating the effect of umbelliferone on ALI exist and the mechanisms underlying the anti-oxidant and anti-inflammatory effects of umbelliferone remain unknown. Therefore, in the present study, we investigated the protective effects of umbelliferone on ALI and explored its underlying mechanism of action.

In the study, LPS was used to establish a mouse model of ALI. Different concentrations of umbelliferone (10, 20, and 40 mg/kg) were used to verify whether its effects are dose-dependent. Our present dates indicated that intranasal LPS administration significantly increased the wet/dry lung weight ratio, which is a marker of pulmonary edema. Umbelliferone attenuated pulmonary edema in a dose-dependent manner, with the largest effect achieved using a concentration of 40 mg/kg. The histological results of our study also demonstrate that LPS resulted in pathological lesions and significant destruction of cellular structures, and the histological alterations were effectively attenuated by high-dose umbelliferone.

These effects were further confirmed by the total cell counts and protein expression in BALF. Indeed,

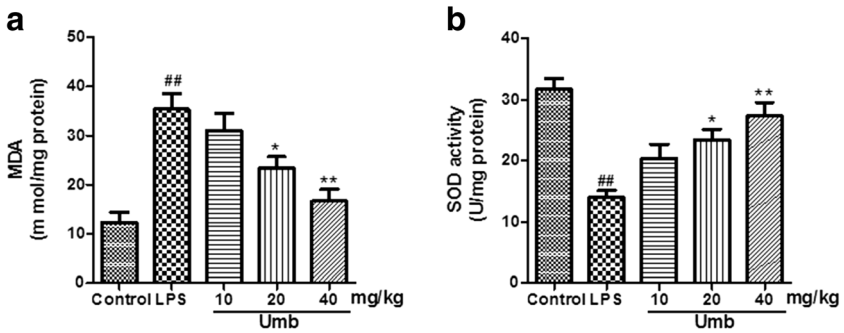

Fig. 5. Effects of umbelliferone on the lung MDA and lung SOD activity of LPS-induced ALI mice. Mice were given an intragastric injection of umbelliferone (10, 20, and 40 mg/kg) 1 h prior to the administration of LPS. The lung MDA (**a**) and lung SOD (**b**) were determined at 12 h after LPS was given. The values presented are the mean ± SEM ($n = 6$ in each group). $^{\#\#}p < 0.01$ *vs* the control group; $^{*}p < 0.05$; $^{**}p < 0.01$ *vs* the LPS group.

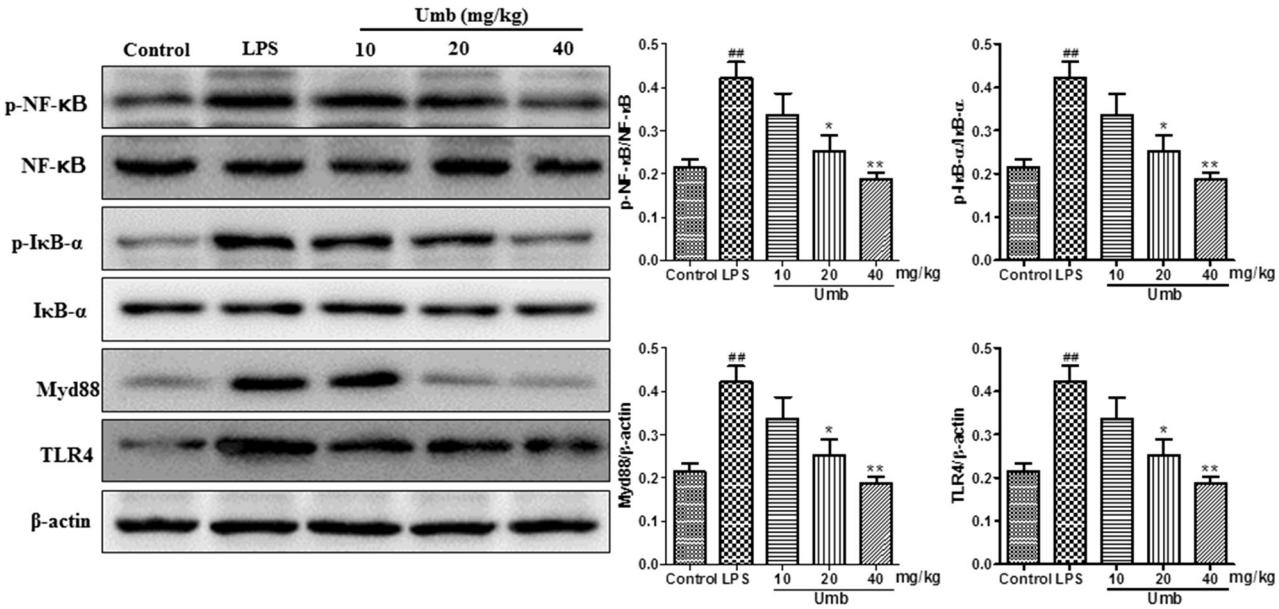

Fig. 6. Effects of umbelliferone on LPS-induced phosphorylation of NF-κB and IκBα in LPS-induced ALI mice with the expressions of MyD88 and TLR4. Mice were given different concentrations of umbelliferone (10, 20, and 40 mg/kg) with an intragastric injection 1 h prior to the administration of LPS. Protein samples were analyzed by western blot with specific antibodies as described. Similar results were obtained in three independent experiments and one of the three representative experiments is shown. The values presented are the mean ± SEM. $^{\#\#}p < 0.01$ *vs* the control group; $^{*}p < 0.05$; $^{**}p < 0.01$ *vs* the LPS group.

umbelliferone significantly reduced the number of total cells and proteins in BALF following an LPS challenge. The recruitment of neutrophils into lung tissues is the main feature of ALI [10]. Myeloperoxidase (MPO) is an enzyme located in the cytoplasmic granules of neutrophils, and its activity reflects the infiltration of neutrophils into lung alveolar or parenchymal spaces [34]. In the present study, umbelliferone significantly decreased the activity of MPO induced by LPS, demonstrating that umbelliferone could suppress neutrophil recruitment to lung tissues. Together, these results demonstrate that umbelliferone inhibited the histopathological changes, pulmonary edema, and lung vascular leakage associated with LPS-induced ALI in mice.

Previous studies have confirmed that inflammatory cytokines are crucial in the pathogenesis of LPS-induced ALI [9]. In this study, the levels of various chemotactic factors (MCP-1) and inflammatory cytokines (IL-1β, TNF-α, and IL-6) were measured using enzyme-linked immunosorbent assays. As expected, all inflammatory mediators in BALF were markedly increased following LPS treatment, and they were significantly reduced with subsequent umbelliferone treatment. These results demonstrate that umbelliferone effectively attenuated pulmonary inflammation in LPS-induced ALI.

Neutrophil accumulation results in the production of reactive oxygen species and granular enzymes, leading to lung tissue damage by inducing an inflammatory cascade [19]. In the present study, LPS significantly increased the MDA content and decreased SOD activity, and these changes were remarkably reversed by umbelliferone treatment. Thus, umbelliferone protected mice against LPS-induced ALI. These findings confirm that the protective effects of umbelliferone on LPS-induced ALI might be attributed to the anti-oxidant effects of the compound.

Moreover, several lines of evidence have demonstrated that TLR4, the major signaling receptor in LPS-induced inflammation, is closely associated with high mortality in ALI, mainly through activation of the NF-κB pathway [8, 17]. NF-κB is a critical regulator of acute inflammation [5]. It is required for the transcription of various cytokines, including IL-6, IL-1β, and TNF-α [2, 15]. Under normal conditions, NF-κB is bound to the inhibitory protein IκB and is sequestered in the cytoplasm. After stimulation by LPS, IκB is immediately phosphorylated and degraded by IκB kinase. IκB degradation releases NF-κB, leading to its translocation to the nucleus and resulting in the transcription of numerous genes involved in inflammation [23, 30]. Therefore, to understand the underlying mechanism of umbelliferone, we studied the expression of proteins in the TLR4/MyD88/NF-κB signaling pathway in mice with LPS-induced ALI. The protein expression of MyD88 and TLR4 was significantly increased by LPS treatment, and

phosphorylated (p)-IκBα and p-NF-κB were also up-regulated. Thus, the recognition of LPS by TLR4 initiated MyD88-dependent signaling. Subsequently, there was degradation of IκB-α, leading to the activation of NF-κB signaling. However, with pretreatment of umbelliferone, the expression of MyD88 and TLR4, as well as p-IκBα and p-NF-κB, was down-regulated. Our results demonstrate that umbelliferone could effectively inhibit NF-κB activation by down-regulating TLR4/MyD88 signaling.

In summary, we demonstrated the protective effects of umbelliferone on LPS-induced ALI in an animal model, and showed that this process is attributable to a reduced inflammatory response. Indeed, umbelliferone treatment resulted in decreased inflammatory cytokine expression and elevated anti-oxidant enzyme activity *via* the inhibition of TLR4/MyD88-mediated NF-κB signaling. Additional comprehensive studies are warranted to confirm the effects of umbelliferone in ALI. However, our study provides support for the treatment of ALI with umbelliferone.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Atabai, K., and M.A. Matthay. 2002. The pulmonary physician in critical care. 5: Acute lung injury and the acute respiratory distress syndrome: definitions and epidemiology. *Thorax* 57 (5): 452–458. https://doi.org/10.1136/thorax.57.5.452.
2. Baker, R.G., M.S. Hayden, and S. Ghosh. 2011. NF-kappa B, Inflammation, and Metabolic Disease. *Cell Metabolism* 13 (1): 11–22. https://doi.org/10.1016/j.cmet.2010.12.008.
3. Banerji, N., M. Maiti, S. Sem, and P.C. Datta. 1982. Pharmacognosy of Aegle marmelos (L) Correa. seed. A new protein source. *Acta Pharm Hung* 52 (3): 97–101.
4. Barton, G.M., and R. Medzhitov. 2003. Toll-like receptor signaling pathways. *Science* 300 (5625): 1524–1525. https://doi.org/10.1126/science.1085536.
5. Bouchama, A., and J.P. Knochel. 2002. Heat stroke. *The New England Journal of Medicine* 346 (25): 1978–1988. https://doi.org/10.1056/NEJMra011089.
6. Cai, Z., J. Liu, H. Bian, J. Cai, and G. Zhu. 2016. Suppression of P2X7/NF-kappaB pathways by Schisandrin B contributes to attenuation of lipopolysaccharide-induced inflammatory responses in acute lung injury. *Archives of Pharmacal Research* 39 (4): 499–507. https://doi.org/10.1007/s12272-016-0713-0.
7. Chu, L.L., R.P. Pandey, H.N. Lim, H.J. Jung, N.H. Thuan, T.S. Kim, and J.K. Sohng. 2017. Synthesis of umbelliferone derivatives in Escherichia coli and their biological activities. *Journal of Biological Engineering* 11: 15. https://doi.org/10.1186/s13036-017-0056-5.
8. Doyle, S.L., and L.A. O'Neill. 2006. Toll-like receptors: From the discovery of NFkappaB to new insights into transcriptional regulations in innate immunity. *Biochemical Pharmacology* 72 (9): 1102–1113. https://doi.org/10.1016/j.bcp.2006.07.010.
9. Goodman, R.B., J. Pugin, J.S. Lee, and M.A. Matthay. 2003. Cytokine-mediated inflammation in acute lung injury. *Cytokine & Growth Factor Reviews* 14 (6): 523–535. https://doi.org/10.1016/S1359-6101(03)00059-5.
10. Grommes, J., and O. Soehnlein. 2011. Contribution of neutrophils to acute lung injury. *Molecular Medicine* 17 (3–4): 293–307. https://doi.org/10.2119/molmed.2010.00138.
11. Haig, T.J., T.J. Haig, A.N. Seal, J.E. Pratley, M. An, and H.W. Wu. 2009. Lavender as a source of novel plant compounds for the development of a natural herbicide. *Journal of Chemical Ecology* 35 (9): 1129–1136. https://doi.org/10.1007/s10886-009-9689-2.
12. Hoult, J.R., and M. Paya. 1996. Pharmacological and biochemical actions of simple coumarins: Natural products with therapeutic potential. *General Pharmacology* 27 (4): 713–722.
13. Jeyaseelan, S., H.W. Chu, S.K. Young, and G.S. Worthen. 2004. Transcriptional profiling of lipopolysaccharide-induced acute lung injury. *Infection and Immunity* 72 (12): 7247–7256. https://doi.org/10.1128/Iai.72.12.7247-7256.2004.
14. Jiang, W., F. Luo, Q. Lu, J. Liu, P. Li, X. Wang, Y. Fu, K. Hao, T. Yan, and X. Ding. 2016. The protective effect of Trillin LPS-induced acute lung injury by the regulations of inflammation and oxidative state. *Chemico-Biological Interactions* 243: 127–134. https://doi.org/10.1016/j.cbi.2015.09.010.
15. Jobin, C., C.A. Bradham, M.P. Russo, B. Juma, A.S. Narula, D.A. Brenner, and R.B. Sartor. 1999. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. *Journal of Immunology* 163 (6): 3474–3483.
16. Kanimozhi, G., N.R. Prasad, S. Ramachandran, and K.V. Pugalendi. 2011. Umbelliferone modulates gamma-radiation induced reactive oxygen species generation and subsequent oxidative damage in human blood lymphocytes. *European Journal of Pharmacology* 672 (1–3): 20–29. https://doi.org/10.1016/j.ejphar.2011.09.003.
17. Kim, T.W., E.H. Joh, B. Kim, and D.H. Kim. 2012. Ginsenoside Rg5 ameliorates lung inflammation in mice by inhibiting the binding of LPS to toll-like receptor-4 on macrophages. *International Immunopharmacology* 12 (1): 110–116. https://doi.org/10.1016/j.intimp.2011.10.023.
18. Kofinas, C., L. Chinou, A. Loukis, C. Harvala, C. Roussakis, M. Maillard, and K. Hostettmann. 1998. Cytotoxic coumarins from the aerial parts of Tordylium apulum and their effects on a non-small-cell bronchial carcinoma cell line. *Planta Medica* 64 (2): 174–176. https://doi.org/10.1055/s-2006-957398.
19. Kwak, S.H., H.B. Bae, S.J. Kim, M. Li, and C.W. Jeong. 2010. Effect of curcumin on LPS-induced neutrophil activation and acute lung injury. *European Journal Of Anaesthesiology* 27 (1): 187–187. https://doi.org/10.1097/00003643-201006121-00598.
20. Lang, J.D., P.J. McArdle, P.J. O'Reilly, and S. Matalon. 2002. Oxidant-antioxidant balance in acute lung injury. *Chest* 122 (6): 314s–320s. https://doi.org/10.1378/chest.122.6_suppl.314S.
21. Lim, Hun Jai, Ju Hee Lee, Jae Sue Choi, Sang Kook Lee, Yeong Shik Kim, and Hyun Pyo Kim. 2014. Inhibition of airway inflammation by the roots of Angelica decursiva and its constituent, columbianadin. *Journal of Ethnopharmacology* 155 (2): 1353–1361. https://doi.org/10.1016/j.jep.2014.07.033.

22. Lissauer, M.E., S.B. Johnson, G.V. Bochicchio, C.J. Feild, A.S. Cross, J.D. Hasday, C.C. Whiteford, W.A. Nussbaumer, M. Towns, and T.M. Scalea. 2009. Differential expression of toll-like receptor genes: Sepsis compared with sterile inflammation 1 day before Sepsis diagnosis. *Shock* 31 (3): 238–244. https://doi.org/10.1097/SHK.0b013e3181834991.
23. Mankan, A.K., M.W. Lawless, S.G. Gray, D. Kelleher, and R. McManus. 2009. NF-kappaB regulation: The nuclear response. *Journal of Cellular and Molecular Medicine* 13 (4): 631–643. https://doi.org/10.1111/j.1582-4934.2009.00632.x.
24. Matute-Bello, G., C.W. Frevert, and T.R. Martin. 2008. Animal models of acute lung injury. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 295 (3): L379–L399. https://doi.org/10.1152/ajplung.00010.2008.
25. Muthu, R., N. Selvaraj, and M. Vaiyapuri. 2016. Anti-inflammatory and proapoptotic effects of umbelliferone in colon carcinogenesis. *Human & Experimental Toxicology* 35 (10): 1041–1054. https://doi.org/10.1177/0960327115618245.
26. Newman, David J., and Gordon M. Cragg. 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products* 70 (3): 461–477.
27. Pan, L., X.Z. Li, Z.Q. Yan, H.R. Guo, and B. Qin. 2015. Phytotoxicity of umbelliferone and its analogs: Structure-activity relationships and action mechanisms. *Plant Physiology and Biochemistry* 97: 272–277. https://doi.org/10.1016/j.plaphy.2015.10.020.
28. Pillai, S.P., S.R. Menon, L.A. Mitscher, C.A. Pillai, and D.M. Shankel. 1999. Umbelliferone analogues and their potential to inhibit benzo(a)pyrene- and hydrogen peroxide-induced mutations. *Journal of Natural Products* 62 (10): 1358–1362.
29. Rubenfeld, G.D., E. Caldwell, E. Peabody, J. Weaver, D.P. Martin, M. Neff, E.J. Stern, and L.D. Hudson. 2005. Incidence and outcomes of acute lung injury. *The New England Journal of Medicine* 353 (16): 1685–1693. https://doi.org/10.1056/NEJMoa050333.
30. Sarkar, F.H., Y. Li, Z. Wang, and D. Kong. 2008. NF-kappaB signaling pathway and its therapeutic implications in human diseases. *International Reviews of Immunology* 27 (5): 293–319. https://doi.org/10.1080/08830180802276179.
31. Shokoohinia, Y., S.E. Sajjadi, S. Gholamzadeh, A. Fattahi, and M. Behbahani. 2014. Antiviral and cytotoxic evaluation of coumarins from Prangos ferulacea. *Pharmaceutical Biology* 52 (12): 1543–1549. https://doi.org/10.3109/13880209.2014.907322.
32. Steiner, A.A., S. Chakravarly, A.Y. Ruclaya, M. Herkenham, and A.A. Romanovsky. 2006. Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoietic cells. *Blood* 107 (10): 4000–4002. https://doi.org/10.1182/blood-2005-11-4743.
33. Tasaka, S., F. Amaya, S. Hashimoto, and A. Ishizaka. 2008. Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome. *Antioxidants & Redox Signaling* 10 (4): 739–753. https://doi.org/10.1089/ars.2007.1940.
34. Wang, J., Y.T. Liu, L. Xiao, L.P. Zhu, Q.J. Wang, and T.H. Yan. 2014. Anti-inflammatory effects of Apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway. *Inflammation* 37 (6): 2085–2090. https://doi.org/10.1007/s10753-014-9942-x.
35. Ware, L.B. 2006. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. *Seminars in Respiratory and Critical Care Medicine* 27 (4): 337–349. https://doi.org/10.1055/s-2006-948288.
36. Worthen, G.S., C. Haslett, A.J. Rees, R.S. Gumbay, J.E. Henson, and P.M. Henson. 1987. Neutrophil-Mediated Pulmonary Vascular Injury - Synergistic Effect Of Trace Amounts Of Lipopolysaccharide And Neutrophil Stimuli on Vascular-Permeability And Neutrophil Sequestration In the Lung. *American Review Of Respiratory Disease* 136 (1): 19–28. https://doi.org/10.1164/ajrccm/136.1.19.
37. Zambon, M., and J.L. Vincent. 2008. Mortality rates for patients with acute lung injury/ARDS have decreased over time. *Chest* 133 (5): 1120–1127. https://doi.org/10.1378/chest.07-2134.