

Phloretin attenuates LPS-induced acute lung injury in mice via modulation of the NF- κ B and MAPK pathways

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ABSTRACT

Phloretin, which can be isolated from apple trees, has demonstrable *anti*-inflammatory and anti-oxidant effects in macrophages. We previously reported that phloretin could inhibit the inflammatory response and reduce intercellular adhesion molecule 1 (ICAM-1) expression in interleukin (IL)-1 β -activated human lung epithelial cells. In the present study we now evaluate whether phloretin exposure could ameliorate lipopolysaccharide (LPS)-induced acute lung injury in mice. Intra-peritoneal injections of phloretin were administered to mice for 7 consecutive days, prior to the induction of lung injury by intra-tracheal administration of LPS. Our subsequent analyses demonstrated that phloretin could significantly suppress LPS-induced neutrophil infiltration of lung tissue, and reduce the levels of IL-6 and tumor necrosis factor (TNF)- α in serum and bronchoalveolar lavage fluid. We also found that phloretin modulated myeloperoxidase activity and superoxide dismutase activity, with decreased gene expression levels for chemokines, proinflammatory cytokines, and ICAM-1 in inflamed lung tissue. Phloretin also significantly reduced the phosphorylation of nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK), thus limiting the inflammatory response, while promoting expression of heme oxygenase (HO)-1 and nuclear factor erythroid 2-related factor 2, both of which are cytoprotective. Our findings suggest that, mechanistically, phloretin attenuates the inflammatory and oxidative stress pathways that accompany lung injury in mice via blockade of the NF- κ B and MAPK pathways.

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1. Introduction

Acute lung injury (ALI) is a severe pulmonary inflammatory disease, characterized by neutrophil accumulation in the lung, which leads to immersion pulmonary edema, reduced lung capacity, and severe hypoxemia [1]. ALI also causes acute respiratory distress syndrome (ARDS), with fluid accumulating in the alveoli, which leads to difficulty in breathing, a shortness of breath, and rapid breathing; all of which increase the mortality rate [2,3]. Previous studies have found that many factors induce ALI, including the inhalation of toxic gas, bacterial and viral infections, and severe sepsis [1]. In the ALI patient, lung tissue can release proinflammatory cytokines and chemokines, which exacerbate harmful immune responses including potentially injurious oxidative stress [4,5].

Occasionally ALI also occurs as a result of bacterial infection or early sepsis in the respiratory tract [6]. Lipopolysaccharide (LPS) is an important component of the cell wall in gram-negative bacteria, and stimulates innate immunity as part of the early host response to contain pathogenic bacteria [7]. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Haemophilus influenzae* are the most common gram-negative pathogens underlying bacterial pneumonia and ALI in clinical patients [8–11]. The *P. aeruginosa* infected airway has also been employed as an induced lung injury model in mice [9]. However, the intra-tracheal administration of LPS to induce acute lung inflammation is a more commonly used pharmacological research animal model of ALI.

LPS binds to Toll-like receptor 4 and triggers a cascade of inflammatory signaling, resulting in the activation of nuclear factor kappa B (NF- κ B) to induce the production of proinflammatory cytokines and inflammatory mediators [12]. LPS also results in the generation of reactive oxygen species (ROS) that further contribute to cellular injury [13]. Many studies have found that NF- κ B is suppressed by I κ B, with exposure to LPS inducing the activity of inflammatory signals that provoke I κ B phosphorylation and degradation. These events release NF- κ B, such that this

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transcription factor can translocate to the nucleus and promote the transcription of inflammatory mediators [14]. Furthermore, mitogen-activated protein kinase (MAPK) signaling pathways are also stimulated by inflammatory gene expression in the lung tissue of ALI mice [15]. Consequently, reducing both MAPK and NF- κ B activity may be a plausible strategy with which to ameliorate inflammatory responses in ALI.

Phloretin is a natural dihydrochalcone found in apple and pear trees [16].

Recent studies have found that phloretin has anti-inflammatory and anti-obesity properties [16,17]. Previously studies have shown that phloretin could improve the function of glucose transporters (SGLTs and GLUTs) in hepatocytes and renal cells [18,19]. Phloretin also decreased fasting plasma glucose, triglycerides, total cholesterol in diabetic mice [20]. Other study found that phloretin reduced oxidative damage for neuroprotective effects in cerebral ischemia of rats [21]. Phloretin also been demonstrated that has antitumor effect by inducing apoptosis in non-small cell lung carcinoma, breast cells, human leukemia cells, and ovarian cancer cells [22–25]. We also found that phloretin could decrease expression of the intercellular adhesion molecule 1 (ICAM-1), and proinflammatory cytokine expression, by its blockade of the NF- κ B and MAPK pathways in interleukin (IL)-1 β -activated human lung epithelial cells [26]. However, whether phloretin could lessen the effects of acute lung injury were uncertain. In the present study, we investigated whether phloretin could prevent the acute lung injury provoked by intra-tracheal LPS administration in mice, and evaluated the molecular mechanisms of inflammation in this animal model.

2. Materials and methods

2.1. Animals

Female BALB/c mice (6–8 weeks, weight 20–25 g) were purchased from the National Laboratory Animal Center in Taiwan. Mice were fed a standard chow diet with water, and were maintained in an animal house equipped with thermostat controlled central air-conditioning. Care and housing protocols for mice and all experimental procedures were approved by the Laboratory Animal Care Committee of Chang Gung University of Science and Technology.

2.2. Phloretin treatment and acute lung injury

Phloretin (from apple wood, $\geq 99\%$ by HPLC) was purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA), and then solubilized for use in DMSO.

Mice were assigned to four groups, each comprising 8 mice. These groups were termed the N group (normal control mice), the LPS group (mice challenged with LPS alone), the PT5 group (mice challenged with 5 mg/kg phloretin plus LPS), and the PT20 group (20 mg/kg phloretin plus LPS). On days 1–7, mice were intra-peritoneally injected with DMSO (N and LPS groups) or phloretin (PT5 and PT20 groups). On day 8 of the experiment, mice were anesthetized with isoflurane (Aesica, Kent, UK), with intra-tracheal administration of 50 μ l LPS (1 μ g/ml) or normal saline. Four hours later, mice were sacrificed and bronchoalveolar lavage fluid (BALF), lung tissue, and serum harvested.

2.3. Histological analyses of lung tissue

Lung tissue was fixed with formalin and paraffin embedded. Six-micron sections were stained with hematoxylin and eosin (H&E). The neutrophil infiltration assay was observed microscopically (Olympus, Tokyo, Japan), as described previously [27].

2.4. Serum collection

Blood was collected from the orbital vascular plexus of mice anesthetized with isoflurane. Samples were then centrifuged at 6000 rpm for 5 min as previously described [28], with serum subsequently stored at -80°C .

2.5. BALF and cell count

All mice were sacrificed, and BALF collected as described previously [28]. Briefly, tracheae were intubated and the lungs flushed with normal saline. Cells were stained with Liu stain solution (Polysciences, Inc., Taipei, Taiwan), and neutrophils counted under the microscope (Olympus, Tokyo, Japan). Additionally, supernatants were assayed for levels of cytokines and chemokines.

2.6. Lung wet-to-dry (W/D) weight ratio

Mice were sacrificed, and the right lung excised and its wet weight (W) obtained. The lung was then dried in an oven at 80°C for 48 h to obtain the dry weight (D). The ratio of W/D was calculated to evaluate lung edema.

2.7. Myeloperoxidase (MPO) activity

MPO activity was measured in the lung tissue using the myeloperoxidase fluorometric activity assay kit (Sigma). Lung tissue was homogenized in cool normal saline, and then processed according to the manufacturer's instructions. Briefly, the MPO could catalyze hypochlorous acid and taurine to form taurine chloroamine, which reacts with 2-nitro-5-thiobenzoic acid to form colorless 5,5'-dithiobis (2-nitrobenzoic acid) [29]. Next, MPO activity was measured using a multi-mode microplate reader (BioTek SynergyHT, Bedfordshire, United Kingdom).

2.8. Superoxide dismutase (SOD) activity

SOD activity was measured in the lung tissue using a SOD determination kit (Sigma).

Lung tissue was homogenized and SOD activity evaluated according to the manufacturer's instructions. SOD could catalyze the dismutation of the superoxide anion into oxygen and hydrogen peroxide. In the assay kit, utilizing water-soluble tetrazolium salt (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) produces a water-soluble formazan dye and reduced superoxide anion [30]. Optical density (OD) was measured at 450 nm using a microplate reader (Multiskan FC, Thermo).

Table 1
Primers used for real-time PCR analyses of mRNA expression.

Gene	Primer forward	Primer reverse
IL-1 β	CACTACAGGCTCCGAGATGA	CGTTGCTTGGTTCTCTTGT
IL-4	TCCGTGCTGAAGAAGAACTC	GTGATGTGGACTTGGACTCATT
IL-6	AGGACCAAGACCATCCAATTCA	GCTTAGGCATAACCGACTAGG
IL-13	GCTCCAGCATTGAAGCAGTG	CGTGGCAGACAGGAGTGTT
TNF- α	GCACCACCATCAAGGACTC	AGGCAACCTGACCACTCTC
MCP-1	TTCCACACCACTCAAGCA	TTAAGGCATCACAGTCCGAGTC
CCL5	CGAAGGAACCGCAAGTGT	AGGACTAGAGCAAGCAATGAC
CCL11	GGCTTCATGTAGTTCCAGAT	CCATTGTGTTCTCAATAATCC
CCL24	AGGCAGTGAGAACCAAGT	GCGTCAATACCTATGTCCAA
iNOS	TTCCACACCACTCAAGCA	TTAAGGCATCACAGTCCGAGTC
COX-2	ACCAGCAGTTCAGTATCAGA	CAGGAGGATGGAGTTGTTGTAG
ICAM-1	AACAGAAATGGTAGACAGCAT	TCCACCGAGTCTCTTAG
β -actin	AAGACCTCTATGCCAACACAGT	AGCCAGAGCAGTAATCTCCTTC

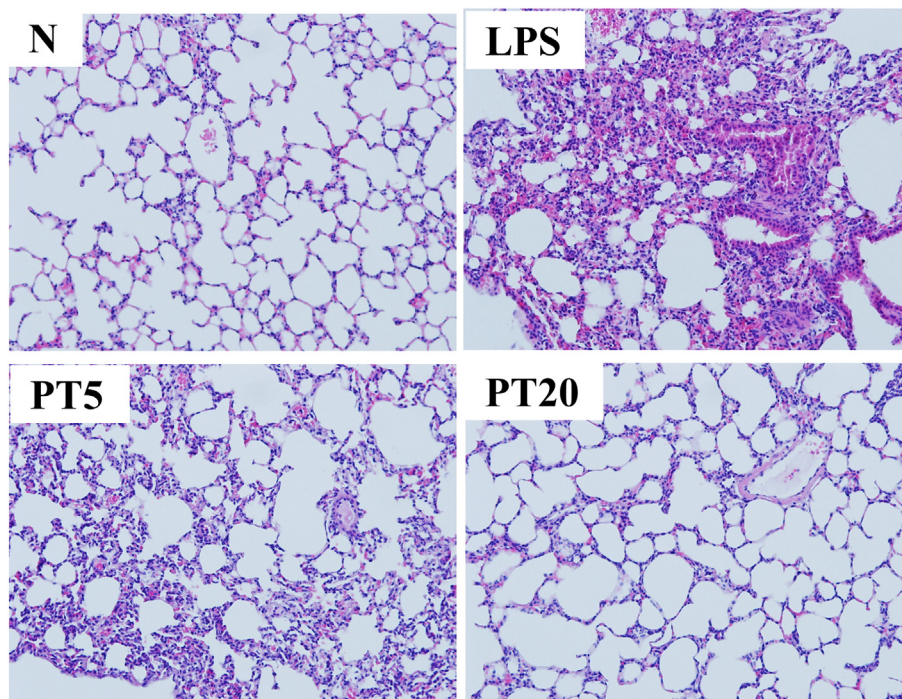


Fig. 1. Phloretin reduces neutrophil infiltration. Representative H&E stains of lung tissue (magnification $\times 200$) for the following groups: N group: normal control mice; LPS group: LPS treatment only; PT5 group: 5 mg/kg phloretin plus LPS; PT20 group: 20 mg/kg phloretin plus LPS.

2.9. ELISA for cytokine production

Specific ELISA kits (R&D Systems, Minneapolis, MN, USA) to quantify IL-6 and tumor necrosis factor (TNF)- α production in serum and BALF were purchased. The OD was spectrophotometrically measured at 450 nm using a microplate reader (Multiskan FC, Thermo).

2.10. Western immunoblot analyses

Lung tissue was homogenized and protein extracted using lysis buffer containing protease and phosphatase inhibitors (Sigma). Nuclear proteins were extracted using the NE-PER nuclear and cytoplasmic extraction reagent kits (Pierce, Rockford, IL, USA). Proteins were resolved

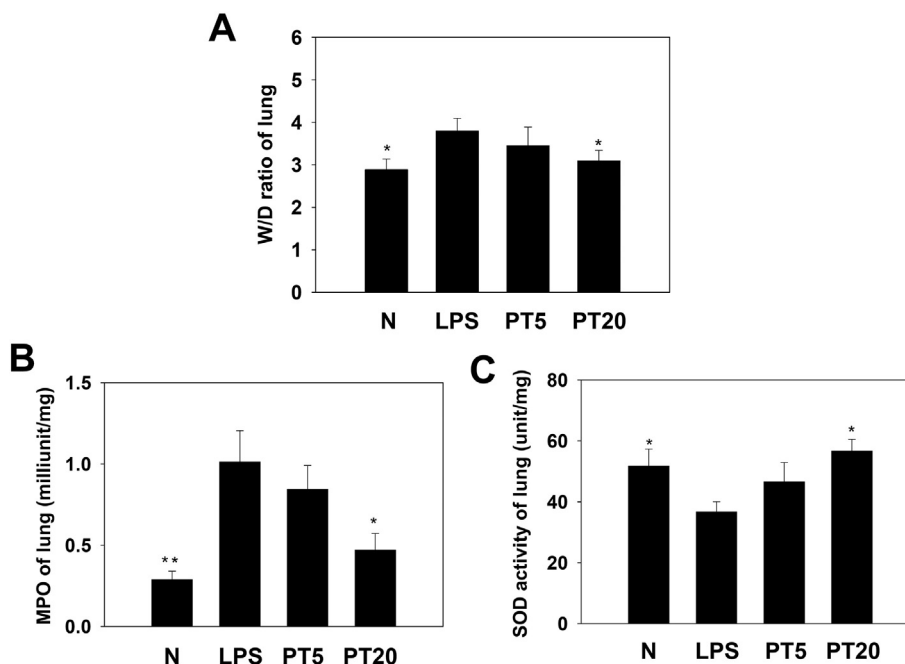


Fig. 2. Phloretin alters the W/D ratio (A), MPO activity (B), and SOD activity (C), in the lung tissue of LPS-induced ALI mice. Data are presented as mean $\pm$ SEM. * $p < 0.05$ compared with LPS control mice, ** $p < 0.01$ compared with LPS control mice.

on 10% SDS polyacrylamide gels, and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were then incubated with primary antibodies specific for I κ B- α , lamin B1, phosphorylated-p65 (Santa Cruz, CA, USA), heme oxygenase (HO)-1, nuclear factor erythroid 2-related factor 2 (Nrf2), p65, phosphorylated-ERK 1/2, phosphorylated-I κ B- α , phosphorylated-JNK, phosphorylated-p38 (Cell Signaling Technology, Boston, MA USA), ERK1/2, JNK, p38, (Millipore), ICAM-1, and β -actin (Sigma). Membranes were then washed with TBST buffer, and incubated with secondary antibody-HRP conjugates for 1 h. Protein detection was with Luminol/Enhancer Solution (Millipore), using the BioSpectrum 600 system (UVP, Upland, CA, USA).

2.11. RNA isolation and real-time PCR

RNA from lung tissue was extracted using the TRIzol reagent (Life Technologies, Carlsbad, CA, USA). cDNA was synthesized using a cDNA synthesis kit (Life Technologies), with real-time PCR (RT PCR system kit, Bio-Rad Laboratories, Hercules, CA, USA) used to detect and measure gene expression using a spectrofluorometric thermal cycler (iCycler; Bio-Rad). Table 1 details the primers used.

2.12. Statistical analyses

All experimental data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparisons. Values are expressed as mean $\pm$ SEM, with a p value of <0.05 considered to be statistically significant.

3. Results

3.1. Phloretin reduces neutrophil infiltration in LPS-induced lung injury

Mice were sacrificed, and their lung tissue removed, fixed, and H&E stained, in order to determine neutrophil distribution in lung sections. The LPS group showed greater neutrophil infiltration compared to the N group (Fig. 1). However, the histopathological changes induced by neutrophil infiltration were ameliorated by prior exposure to phloretin, as evidenced in the lung biopsy sections (Fig. 1). W/D ratios, used to evaluate lung edema, also revealed that phloretin exposure significantly reduced edema (Fig. 2A).

3.2. The effects of phloretin on SOD and MOP activity in the lung

We found that the LPS group demonstrated a significantly increased MOP activity, and decreased SOD activity, compared to the N group (Fig. 2B–C). Conversely, phloretin significantly repressed LPS-induced MOP activity, and enhanced SOD production.

3.3. Phloretin suppressed the neutrophil count in BALF

Neutrophil infiltration is an important inflammatory marker in acute lung injury [27]. Hence, we evaluated the neutrophil count in BALF, and found that phloretin significantly suppressed neutrophil numbers compared with the LPS group (neutrophil: PT5: $3.5 \times 10^5 \pm 4.4 \times 10^4$ /ml, $p < 0.05$; PT20: $2.5 \times 10^5 \pm 4.3 \times 10^4$ /ml, $p < 0.01$ vs LPS: $5.1 \times 10^5 \pm 7.3 \times 10^4$ /ml) (Fig. 3A).

3.4. The effects of phloretin on cytokine and chemokine levels in BALF and lung tissue

Cytokine levels in BALF were measured by ELISA assays. Phloretin was found to inhibit IL-6 levels compared to the LPS group (IL-6: PT5, 210.9 ± 23.2 pg/ml, $p < 0.05$; PT20, 181.4 ± 29.7 pg/ml, $p < 0.05$ vs LPS: 317.7 ± 15.9 pg/ml) (Table 2). Phloretin also reduced TNF- α levels: (TNF- α : PT5, 354.2 ± 32.6 pg/ml, $p = 0.24$; PT20, 247.7 ± 53.1 pg/ml,

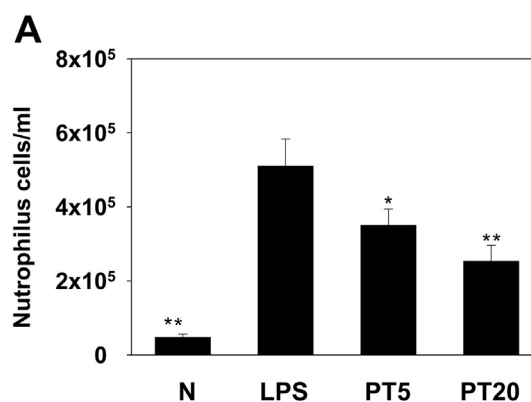


Fig. 3. Phloretin affects neutrophil counts in BALF. Neutrophil cell counts were obtained by microscopic evaluation (A). All data are presented as mean $\pm$ SEM. * $p < 0.05$ compared with the LPS control group. ** $p < 0.01$ compared with the LPS control group.

$p < 0.05$ vs LPS: 390.6 ± 32.6 pg/ml) (Table 2). Next, we investigated gene expression using real-time PCR, and found that phloretin significantly decreased levels of IL-1 β , IL-6, TNF- α , MCP-1, and CCL5 in LPS injured lung tissue (Fig. 4). Phloretin also reduced the gene expression of ICAM-1, iNOS, and COX-2 compared to the LPS group. However, phloretin failed to inhibit gene expression of IL-4, IL-13, CCL11, or CCL24.

3.5. The effects of phloretin on serum cytokine levels

Serum analyses showed that phloretin significantly reduced the levels IL-6 and TNF- α compared to the LPS group (Table 3).

3.6. Effects of phloretin on the phosphorylation of NF- κ B and MAPK effectors in injured lung tissue

We found that phloretin significantly inhibited the phosphorylation of I κ B- α and p65 compared with LPS-stimulated, injured lung tissue (Fig. 5). Phloretin also increased the phosphorylation of ERK1/2, p38, and JNK in injured lung tissue (Fig. 6).

3.7. Effects of phloretin on HO-1 and Nrf2 expression in injured lung tissue

When cells suffer oxidative stress, levels of the anti-oxidant HO-1 are increased in order to protect tissue from damage [31]. We found LPS alone induced HO-1 production in lung tissue relative to mice in the control group. However, pre-treatment with phloretin significantly increased HO-1 production, elevating anti-oxidant mediated protection compared to the LPS group. In addition, phloretin also increased Nrf2 expression in nuclear extracts compared to the LPS group (Fig. 7).

4. Discussion

Phloretin is found in apple and pear trees, with its antioxidant properties serving to protect against free radical mediated damage [32]. Previous studies have demonstrated phloretin's pro-apoptotic activity

Table 2

Phloretin decreased the levels of IL-6 and TNF- α in BALF. The data are expressed as mean $\pm$ SEM (n = 8).

Group	IL-6 (pg/ml)	TNF- α (pg/ml)
Normal	13.0 $\pm$ 1.6**	20.1 $\pm$ 3.2**
LPS	317.7 $\pm$ 15.9	354.2 $\pm$ 32.6
PT5	210.9 $\pm$ 23.2*	247.7 $\pm$ 53.1
PT10	181.4 $\pm$ 29.7*	390.6 $\pm$ 32.6*

* $p < 0.05$ compared to LPS group.

** $p < 0.01$ compared to LPS group.

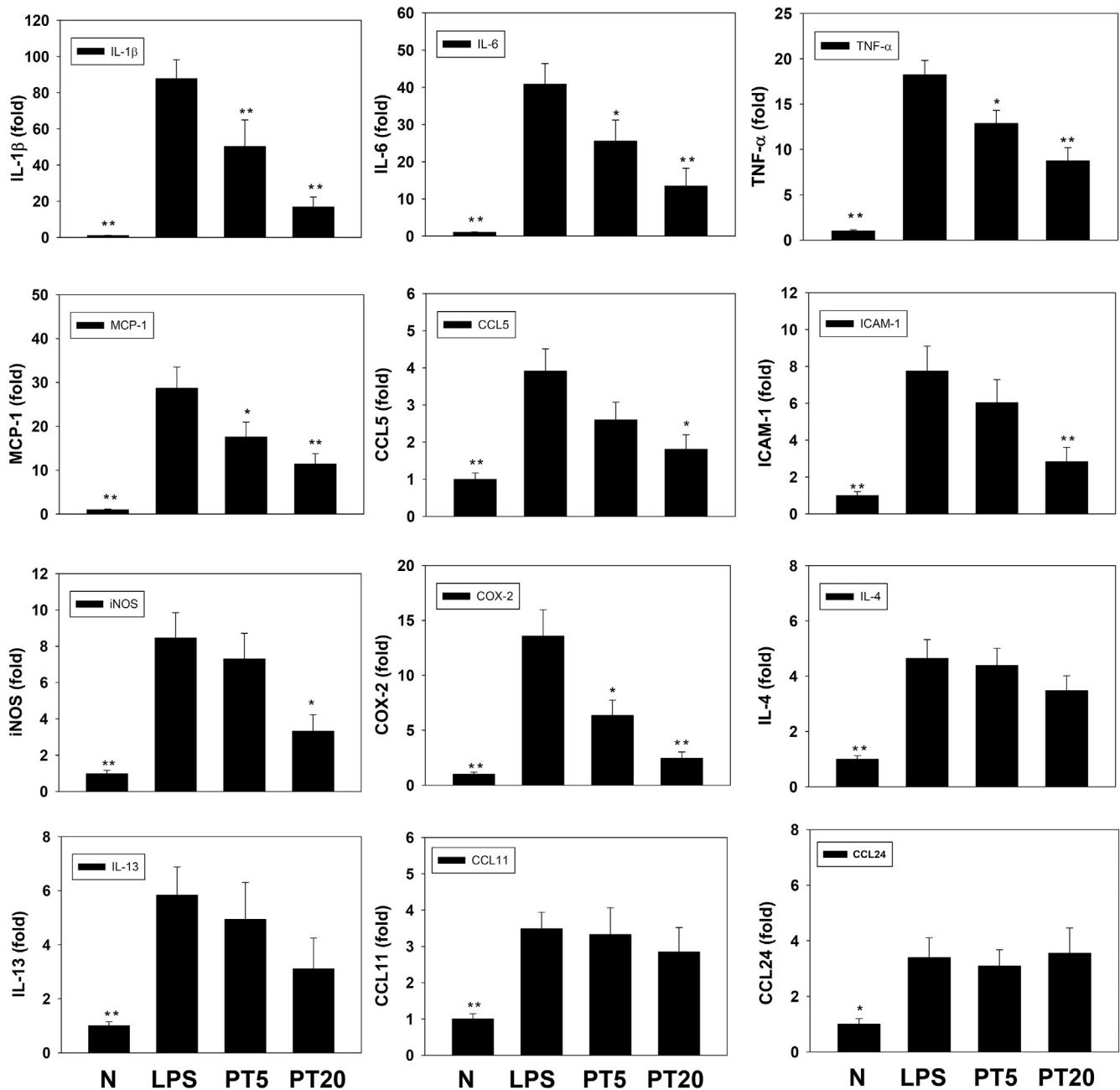


Fig. 4. Phloretin modulated the gene expression levels of cytokines, chemokines, and inflammatory mediators in lung tissue. Fold expression (obtained by real time RT-PCR) is shown relative to β -actin expression (internal control). Data are presented as mean $\pm$ SEM. * p < 0.05 compared with LPS control mice. ** p < 0.01 compared with LPS control mice.

against cells derived from various solid tumors, including glioblastoma, lung, and hepatoma, as well as colon and breast cancer [22,23,33,34]. Phloretin regulates glucose transporters, decreases lipid synthesis in adipocytes, and can suppress the inflammatory effects of LPS-stimulated macrophages, and TNF- α activated HaCaT cells [16,19,35]. Furthermore,

Table 3

Phloretin decreased the levels of IL-6 and TNF- α in serum. The data are expressed as mean $\pm$ SEM (n = 8).

Group	IL-6 (pg/ml)	TNF- α (pg/ml)
Normal	7.2 $\pm$ 1.1**	20.1 $\pm$ 3.2**
LPS	457.9 $\pm$ 42.1	668.0 $\pm$ 35.8
PT5	300.7 $\pm$ 32.7*	526.1 $\pm$ 49.3*
PT10	136.3 $\pm$ 33.6*	247.7 $\pm$ 57.9**

* p < 0.05 compared to LPS group.

** p < 0.01 compared to LPS group.

we have shown previously that phloretin decreases COX-2 and ICAM-1 expression in IL-1 β -induced human lung epithelial cells [26]. However, whether phloretin could attenuate the acute lung injury provoked by LPS administration in mice had not been previously investigated. In the current study, we evaluated whether phloretin could ameliorate neutrophil infiltration, and the inflammatory response in the lungs of ALI mice.

ALI is a severe clinical hypoxemia, with bilateral infiltrates of pulmonary neutrophils that can induce disease progression, favor alveolar edema, decrease tidal volume, and ultimately lead to a loss of lung function [11]. LPS is a major component of the cell walls of gram-negative bacteria [7]. When the host is infected with gram-negative bacteria, those bacteria release LPS to induce inflammation and fever, which then causes tissue edema and/or damage [36]. Without appropriate medical treatment, sepsis, and even death can follow. Additionally, activated neutrophils have the capacity to engulf invading bacteria in order

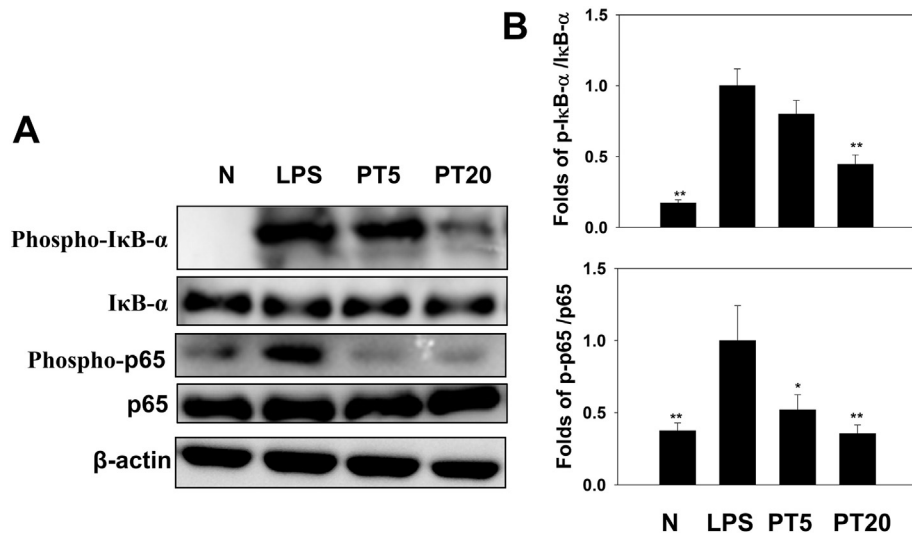


Fig. 5. Phloretin inhibits phosphorylation of the NF-κB pathway, including the phosphorylation of IκB-α and the NF-κB subunit, p65, in lung tissue (A). Three independent experiments were analyzed and compared with the LPS-treated group (B). All data are presented as means ± SEM. * $p < 0.05$ compared with the LPS control group. ** $p < 0.01$ compared with LPS control mice.

to limit tissue damage. However, activated neutrophils also release inflammatory mediators, which cause further immune-mediated, and oxidative injury of the lung [37]. We found that phloretin could effectively reduce neutrophil infiltration in the lung and BALF, leading to a diminished inflammatory response. Pulmonary inflammation also causes edema in ALI. Alveolar rupture and the accumulation of fluid from leaky blood vessels promotes more leukocyte infiltration and leads to

the acute inflammatory response [37,38]. Critically, phloretin decreased the wet-to-dry lung weight ratio, indicating reduced edema and attenuated inflammatory symptoms.

MPO is an abundant enzyme in the granulocytes of neutrophils, and can generate hypohalous acid with which to kill invading bacteria [1, 39]. Hence, MPO activity is a major marker, and indicator of neutrophil infiltration in lung tissue. In this study, our results showed that

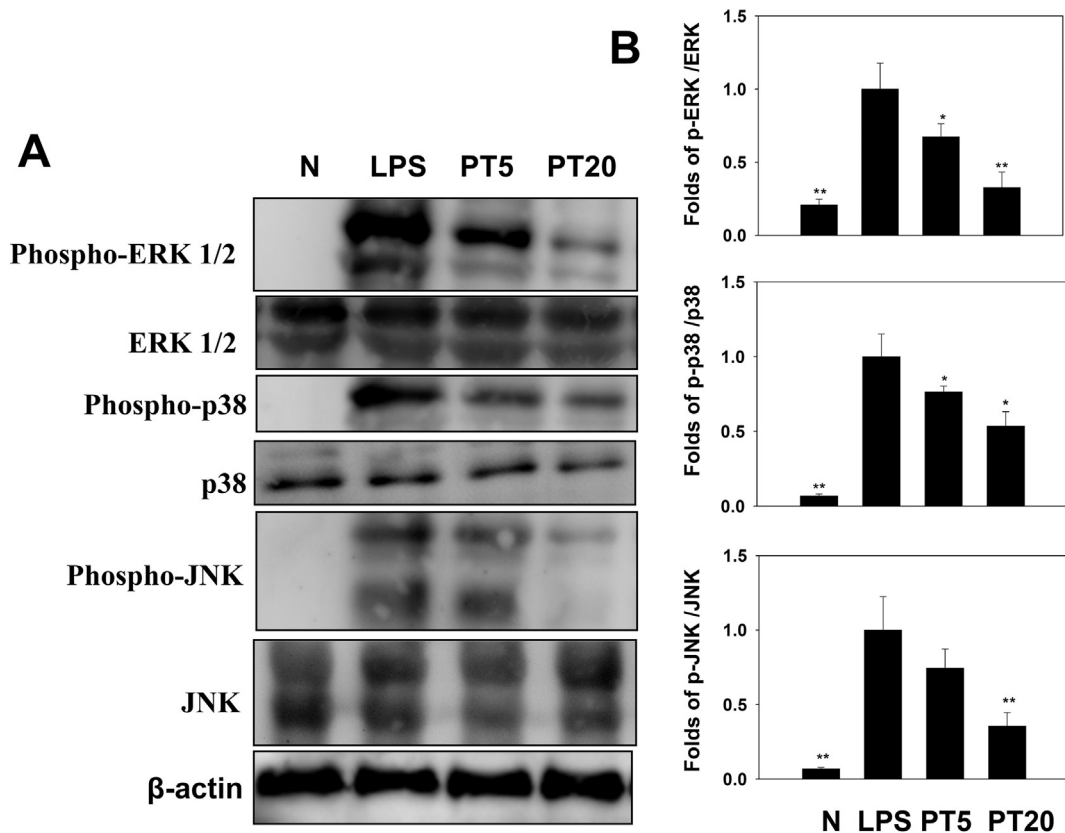


Fig. 6. Phloretin affects LPS-induced phosphorylation of the MAPK pathways. Western blots showed the effects of phloretin on the phosphorylation of ERK, p38, and JNK (A). Three independent experiments were analyzed and compared with the LPS-treated group (B). All data are presented as means ± SEM. * $p < 0.05$ compared with the LPS control group. ** $p < 0.01$ compared with LPS control mice.

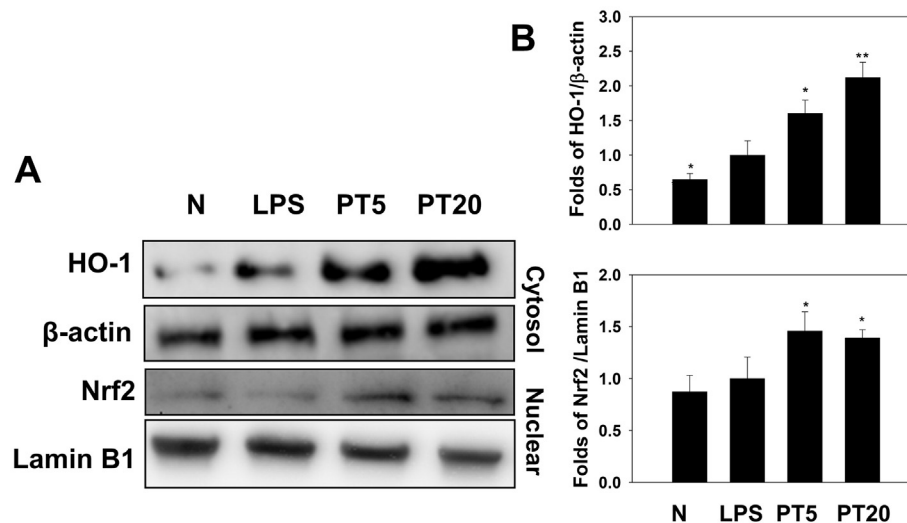


Fig. 7. Effects of phloretin on HO-1 and Nrf2 protein expression in lung tissue. Western blots showed the effects of phloretin on HO-1 and Nrf2 protein expression (A). Three independent experiments were analyzed and compared with the LPS-treated group (B). All data are presented as means $\pm$ SEM. * $p < 0.05$ compared with the LPS control group. ** $p < 0.01$ compared with LPS control mice.

phloretin could suppress neutrophil infiltration and decrease MPO activity, ameliorating the symptoms of lung injury in mice. Phloretin also increased SOD production to protect from oxidative damage. When cells are stimulated by oxidative stress, Nrf2 translocates to the nucleus in order to induce expression of HO-1, which is cytoprotective [40]. Previous studies found that phloretin could reduce lipid peroxidation and scavenge free radicals in injured rat liver [41]. In this study, phloretin significantly increased nuclear Nrf2 expression to induce more HO-1 expression, thereby boosting protection against oxidative stress in the lungs of ALI mice. Hence, we have shown that phloretin can provide some relief from the inflammatory and oxidative damage caused in ALI mice.

Our experimental model is appropriate for the evaluation of early pathological changes in acute lung injury, and assessing the protective effects of phloretin in early ALI mice. Mice exposed to phloretin by intraperitoneal injection for 7 days, then subject to intratracheal administration of LPS to induce lung injury on the 8th day, were sacrificed four hours later. Our experimental model did not induce the death of these mice. In ALI patients, in addition to increased neutrophil infiltration of the lungs, increased levels of proinflammatory cytokines such as IL-6 and TNF- α , cause fever and sepsis [37,38]. Phloretin could significantly suppress both IL-6 and TNF- α in BALF and serum, ameliorating the inflammatory response in ALI mice. Subsequent gene expression profiles for the lung showed that phloretin significantly reduced the levels of proinflammatory cytokines and chemokines, including ICAM-1, COX-2 and iNOS, compared to LPS-induced ALI mice. However, phloretin did not reduce gene expression of the Th2-associated cytokines IL-4, IL-13, CCL11, or CCL24, which promote eosinophilic infiltration.

LPS stimulates pro-inflammatory signals that phosphorylate I κ B, freeing NF- κ B to translocate to the nucleus to promote pro-inflammatory and oxidative- gene expression [36]. Furthermore, MAPK pathway activity also promotes the transcription of proinflammatory genes [15], causing and contributing to lung damage, sepsis and inflammation in ALI mice. Conversely p38 MAPK inhibition significantly attenuates acute lung injury in rats [39,42]. Additionally flavonoids have been identified that reduce LPS-induced acute lung injury by down-regulating the NF- κ B and MAPK pathway [27,39,43]. Wogonin were confirmed that could suppress lung inflammatory response via reduced the phosphorylation of p38 and JNK in acute lung injury of mice [44]. Genipin could also improved acute lung injury by blocked NF- κ B and NLRP3 signaling pathways [45]. In this current study demonstrated that phloretin significantly suppressed MAPK and NF- κ B pathway activity, reduces ICAM-1

expression to diminish neutrophil infiltration, and decreases the secretion of inflammatory mediators.

In conclusion, results from our study show that phloretin has the potential to significantly lessen the inflammatory and oxidative damage by suppressed NF- κ B and MAPK pathways in the lungs of ALI mice. These results provide beneficial experimental evidence for clinical application for ALI in the future.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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