Celastrol alleviates LPS-induced inflammation in BMDMs and acute lung injury in mice via inhibition of p-38 MAPK/MK2 signaling

Zhengxu Chen,1* Xinyi Yang,2* Lu Zhang,3 Man Li,1 Lei Sun2 and Feng Qian2,3

Abstract
Objective: Celastrol is a compound extracted from a medicinal plant Tripterygium wilfordii which has a broad-spectrum anti-inflammatory effect in traditional medicine. However, the effect of celastrol on acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) is still unknown.

Methods: We reported that celastrol alleviated LPS-induced acute lung injury by H&E staining, MPO activity and the expression of cytokines in broncho-alveolar lavage fluid. The effect of celastrol on bone marrow-derived macrophages (BMDMs) after LPS treatment was measured by ELISA and Western blotting.

Results: In vivo, celastrol reduced the LPS-induced lung edema and MPO activity of lung tissue. Furthermore, the production of inflammatory cytokines IL-6, TNF-α, and KC in bronchoalveolar lavage was reduced. In vitro, upon treatment of LPS, celastrol dose-dependently inhibited the expression of iNOS in BMDMs. Meanwhile, the expression of IL-6, TNF-α, and KC in BMDMs were also inhibited by celastrol treatment. Furthermore, we found that celastrol attenuated the phosphorylation of p38 MAPK and MK2, and inhibited the interaction between p38 MAPK and MK2.

Conclusion: Our data indicate that celastrol has an anti-inflammatory effect on LPS-induced inflammatory response in vivo and in vitro, suggesting celastrol is a promising compound for the treatment of ALI and ARDS.

Keywords
acute lung injury, celastrol, MAPK, MK2

Date received: 31 December 2020; accepted: 4 May 2021

Introduction
Acute respiratory distress syndrome (ARDS) is a clinical syndrome caused by non-cardiogenic pulmonary edema and characterized with acute lung injury, with high mortality rate.1 The pathogenesis of ARDS is complex and hasn’t been completely unveiled. Bacterial pneumonia, acute lung injury (ALI) and sepsis are common disorders associated with the development of ARDS.2–4 Sepsis can induce severe inflammatory response and cause multi-organ dysfunction, especially lead to acute lung injury.5 Lipopolysaccharide (LPS), a glycolipid of Gram-negative bacterial outer membranes, is known...
as a common endotoxin that leads to ALI and sepsis. The inflammatory response including nitroxide (NO) production and cytokine generation is the main cause of LPS-induced ALI. Due to the causes of ALI are complicated and the pathogenesis of ALI is still unknown, the clinical treatment is primarily supportive with lung protective ventilation and a conservative fluid management. There is still no effective medicine for ALI in ARDS. Therefore, it is important to find an effective compound for treating ALI and ARDS.

Dysregulated inflammation is a major pathological change of ARDS in clinical, following with diffuse alveolar injury, lung edema and neutrophil infiltration. The lung inflammation in ARDS includes the activation of inflammatory cells and the release of inflammatory mediators. Activated neutrophils migrate into alveolar space and produce pro-inflammatory cytokines to exacerbate pulmonary inflammation. In addition to infiltrated neutrophils, macrophages also play a role in the pathogenesis of ARDS. In lung tissue, alveolar macrophages, and tissue-resident macrophages are important immune cells, which protect against pathogen infection. However, activated macrophages also induce the production of cytokines, reactive oxygen, and nitrogen species, which induce endothelial cell and alveolar epithelial cell injury. The high levels of nitrites/nitrates and NO end-products, as well as elevated iNOS, were detected in the plasma and bronchoalveolar lavage fluid (BALF) of ARDS patients. In addition, inflammatory cytokines have been involved in the development of ARDS. Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 are increased in the plasma and bronchoalveolar lavage fluid (BALF) in ALI, which lead to alveolarcapillary permeability enhancement and neutrophil infiltration in alveolar and epithelial space. The recruitment and activation of neutrophils further aggravate the inflammation and induce alveolar damage. Therefore, inflammatory cells are potential target for the treatment of ARDS and ALI.

Celastrol is a compound derived from the root bark of *Tripterygium wilfordii*, which has anti-inflammatory and anti-oxidant effects. It has been known that celastrol is beneficial to several immune-related diseases, such as colitis and cancer. Celastrol attenuates the DSS-induced colon injury and modulates inflammatory cytokine production and intestinal homeostasis. Additionally, celastrol also has neuroprotective effect on acute spinal cord injury by inhibiting microglial activation and pyroptosis. Although celastrol has protective effects on several diseases, it remains unknown whether celastrol has effects on ALI and ARDS.

In the present study, we found that celastrol alleviated the lung tissue damage and inflammatory response induced by LPS. In addition, celastrol inhibited the expression of inflammatory cytokines and iNOS in LPS-induced BMDMs. Furthermore, celastrol also inhibited the phosphorylation of p38 MAPK and MK2. In conclusion, our study indicates that celastrol has a protective effect on LPS-induced acute lung injury, which is a promising compound for treating ARDS.

**Methods**

**Reagents and animals**

Celastrol (C0869), dimethylsulfoxide (DMSO) (D2650), LPS (L2630), and protease inhibitor cocktail (P8340) were obtained from Sigma-Aldrich (St Louis, MO, USA). The ELISA kits for TNF-α (MТА00B), IL-6 (M6000B), and KC (MKC00B) were purchased from R&D Systems (Minneapolis, MN, USA). Antibodies against iNOS (13120), P-p38 MAPK (4511), p38 MAPK (8690), P-MK2 (3042), MK2 (3041), GAPDH (5174), Myc-Tag (9B11), and Flag-Tag (D6W5B) were purchased from Cell Signaling Technology (Danvers, MA, USA). The Hematoxylin and Eosin staining Kit, cell counting kit-8, Bradford protein assay kit and RIPA lysis buffer (P0013K) were purchased from Beyotime (Shanghai, China). Electrochemiluminescence (32209) and Protein G beads (10003D) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The pcDNA 3.1 plasmids harboring Myc-p38 MAPK or Flag-MK2 were purchased from Genomeditech (Shanghai, China).

C57BL/6 mice (6–10 weeks old, specific pathogen-free) were purchased from SLAC Laboratory Animal Corporation (Shanghai, China). Animals were bred and housed at the Shanghai Jiao Tong University Laboratory Center. The procedures involving mice were approved by the Institutional Animal Care and Use Committee at Shanghai Jiao Tong University.

**LPS-induced acute lung injury model**

Mice were randomly divided into three groups: Vehicle groups, LPS + Vehicle groups and LPS +
celastrol groups. Celastrol was dissolved in vehicle (10% DMSO, 60% cremophor, 20% ethanol, and 10% PBS). ALI was induced in 8-week-old male mice with intratracheal injection of LPS (40 µg in 50 µL PBS, 2 mg/kg) for 6 h. For LPS + Vehicle groups and LPS + celastrol groups, mice were administered with an intraperitoneal injection of vehicle or celastrol (20 mg/kg). Mice were anesthetized with pentobarbital sodium (50 mg/kg) by intraperitoneal injection to collect the lung tissue and BALF for analysis. To saving the consumption of mice, we used five mice per group to perform the experiment and got the significant difference between groups.

**Histological analysis**

The left lobe of lung from each group was fixed by 4% paraformaldehyde, embedded in paraffin, cut into 5 µm sections. The sections fixed in slides were stained with hematoxylin-eosin (H&E) according to the manufacture’s instruction.

**Myeloperoxidase (MPO) estimation**

The largest right lobes of lungs were homogenized in 50 mmol/L potassium phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide (HTAB) and centrifuged to collect the pellet. Then the pellet was resuspended by 0.5% HTAB. The HTAB supernatant was repeatedly frozen and thawed at −80°C three times, frozen for 20 min. After the last thaw, the supernatant was homogenized again, and centrifuged to collect the supernatant. Supernatants were diluted in reaction solution containing 3, 3′, 5, 5′-tetramethylbenzidine (TMB) and H₂O₂ and incubated at 25°C for 5 min, then measured the change of absorbance at 655 nm over 5 min. MPO activity was defined as the change in the absorbance value per minute at 25°C by 1.0 (ΔA min⁻¹), and MPO activity was expressed as unit mass of MPO enzyme activity (ΔA g⁻¹ min⁻¹).

**Analysis of protein concentration in BALF**

The BALF was centrifuged at 4°C after collection. The supernatant was collected for analysis of protein concentration using the Bradford protein assay kit according to instruction.

**Cytokines concentrations by ELISA**

The expression of cytokines TNF-α, IL-6, and KC in BALF and BMDMs medium were measured by using ELISA assay kits according to the manufacture’s instruction.

**Bone Marrow-derived Macrophage isolation**

BMDMs were obtained from male C57BL/6 mice. Mice were sacrificed by cervical dislocation and execution. The femurs and tibias were separated from legs. The bones were cut off at both ends, and the cells were pipette into a sterile conical tube after filtered through a 40-µm filter. After collection and red blood cell lysis, the cells were cultured in DMEM medium (100 units/mL of penicillin and 100 µg/mL streptomycin), 10% FBS. BMDMs were derived by MCSF (10 ng/mL). At day 3, the cells were cultured in new medium for 4 days. At day 7, BMDMs were planted into 6-wells plates or 12-wells plates for detection.

**Cell viability assay**

The effect of celastrol on cell viability of BMDMs was detected by cell counting kit-8 (CCK-8) assay. BMDMs were planted in 96-well culture plates with the density of 5000 cells/well and incubated for overnight. Cells were treated with different concentrations of celastrol at 0, 2, 10, and 50 µM for 24 h. Then10 µL of CCK-8 solution was added into each well and incubated for 2 h at 37°C. The absorbance at 450 nm was measured by a microplate reader (ThermoFisher Scientific, USA).

**Western blotting**

BMDMs were planted in 6-well plates and incubated overnight. Cells were treated with celastrol (0, 2, 10, and 50 µM) for 15 min before LPS (100 ng/mL) stimulation for 18 h or 30 min. Cultured cells were collected in ice-cold RIPA buffer containing a cocktail of protease inhibitors. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to a nitrocellulose (NC) membrane. The membranes were incubated in 5% skim milk at room temperature for 1.5 h and incubated by specific antibodies at 4°C overnight (1:1000’ dilution), followed by incubation with
secondary antibodies for 2 h at room temperature. The blots were visualized using an enhanced electrochemiluminescence detection system (solution A: solution B = 1:1). Quantification of Western blots was analyzed with ImageJ software.

**Immunoprecipitation**

HEK293T cells were transfected with the pcDNA 3.1 plasmids of Myc-p38 MAPK and Flag-MK2 for 42 h. The cells were then treated with celastrol (50 µM) for 6 h, washed with cool PBS and lysed on ice with RIPA lysis buffer with protease inhibitor cocktail. Cell lysate was obtained and part of it was used as input for loading control. The remaining lysate was incubated with anti-Myc antibody for 4 h. Protein-G beads were added and shaken overnight at 4°C. The beads were then eluted with PBS and protein was released by adding loading buffer. Immunoprecipitated sample and total cell lysates fractions (input) were analyzed by immunoblotting.

**Statistical analysis**

Data are expressed as means ± SEM. The data were analyzed by one-way ANOVA and Tukey post hoc test was performed to determine the significance of differences between two groups. P < 0.05 was considered statistically significant. Analysis and graphing were performed using Prism software (ver. 5.0; GraphPad, San Diego, CA, USA).

**Results**

**Celastrol alleviates LPS-induced ALI**

Celastrol is an extract compound derived from medicinal herbs Celastraceae family, and has various bioactivities. However, the effect of celastrol on acute lung injury is still unknown. Therefore, we established the mouse model of LPS-induced acute lung injury. Mice were pretreated with celastrol at 20 mg/kg significantly alleviated LPS-induced lung injury, with less infiltration of inflammatory cells (Figure 1(a)).

MPO activity of lung tissue is an important marker of pulmonary neutrophil infiltration, which indicates the inflammatory injury of lung. As shown in Figure 1(b), the increased MPO activity after LPS induction was remarkably reduced by administration of celastrol. The total protein concentration in BALF was increased 3-fold after LPS induction. Whereas, with celastrol treatment, the protein concentration in BALF was reduced by around 50% (Figure 1(c)). Celastrol treatment also reduced the wet to dry ratio of lung tissue which was induced by LPS stimulation, indicating that celastrol alleviates the LPS-induced lung edema (Figure 1(d)). Furthermore, we detected the phosphorylation of p38 MAPK and MK2 in lung tissue. The expression of P-p38 MAPK and P-MK2 were induced in LPS-induced ALI lung (Figure 1(e)–(g)). Consistent with above results, the phosphorylation of p38 MAPK and MK2 in ALI lung were decreased by celastrol treatment (Figure 1(e)–(g)). These results suggest that celastrol prevents against LPS-induced acute lung injury.

**Celastrol alleviates the inflammatory response**

To further determine the protective effect of celastrol on LPS-induced ALI, we detected the production of pro-inflammatory cytokines in BALF. The expression of TNF-α in BALF was increased approximately 8-fold after LPS treatment for 6 h (Figure 2(a)). And the expression of IL-6 and KC was also dramatically increased after LPS induction (Figure 2(b) and (c)). In response to celastrol treatment at 20 mg/kg, the expression of pro-inflammatory cytokines TNF-α, IL-6, and KC was reduced about 50%. These in vivo data suggest that celastrol alleviates LPS-induced pulmonary inflammation.

**Celastrol inhibits the LPS-induced cytokine production in BMDMs**

To explore the protective effect of celastrol on LPS-induced ALI, we assessed whether celastrol can inhibit macrophage activation. With stimulation of LPS (100 ng/mL) for 3 h, activated BMDMs produced inflammatory cytokines, including TNF-α, IL-6, and KC. Pretreatment with celastrol at 2, 10, and 50 µM for 15 min reduced the production of TNF-α, IL-6, and KC in a dose-dependent manner (Figure 3(a)–(c)). Furthermore, we detected the
effect of celastrol on BMDMs cell viability in different concentration. After the treatment of celastrol for 24 h, the cell viability didn’t decrease significantly, even in 50 µM (Figure 3(d)). These data indicate that celastrol inhibits the production of inflammatory cytokines after LPS stimulation.
Celastrol inhibits the LPS-induced expression of iNOS in BMDMs

As TLR4 ligand, LPS can induce BMDMs to generate inflammatory mediators, such as NO, reactive oxygen species (ROS), and inflammatory cytokines. When macrophages are activated, the generation of NO is related to the expression of iNOS. As shown in Figure 4, the expression of iNOS was 15-fold after LPS stimulation at 100 ng/mL for 18 h. Pretreatment of celastrol at 2, 10, and 50 µM for 15 min reduced the expression of iNOS in LPS-induced BMDMs in a dose-dependent manner. When BMDMs were treated with celastrol at 50 µM, upon treatment with LPS, the expression of iNOS was reduced to 5-fold. These results indicate that celastrol inhibits the LPS-induced expression of iNOS in BMDMs.

Celastrol inhibits the phosphorylation of p38 MAPK and MK2 and the interaction between p38 MAPK and MK2

To determine the anti-inflammatory effect of celastrol on macrophage activation, we detected the activation of p38 MAPK and MK2. BMDMs were treated with celastrol at 0, 2, 10, and 50 µM for 15 min, followed by LPS (100 ng/mL) challenge for 30 min. As shown in Figure 5, the phosphorylation of p38 MAPK was increased approximately 6-fold and the phosphorylation of MK2 was induced 50-fold when BMDMs were treated with 100 ng/mL of LPS. With the pretreatment of celastrol, the phosphorylation of p38 MAPK and MK2 was significantly decreased. To further determine how celastrol inhibited the inflammation induced by LPS, we transfected HEK293T cells with expression vectors of Myc-p38 MAPK and Flag-MK2, and then we immunoprecipitated of lysates with anti-Myc. We found that celastrol inhibited the interaction between p38 MAPK and MK2 (Figure 5(d)). In conclusion, celastrol attenuates LPS-induced activation of p38 MAPK and MK2 in BMDMs, also inhibits the interaction between p38 MAPK and MK2.

Discussion

In our study, we found that celastrol alleviated LPS-induced acute lung injury. In vitro, upon treatment with LPS, celastrol inhibited the expression of iNOS and inflammatory cytokines in BMDMs. Furthermore, the phosphorylation of p38 MAPK and MK2 was reduced in response to celastrol...
pretreatment. These results suggest that celastrol, a compound extracted from medical herbs, alleviates LPS-induced ALI and inflammatory response.

Celastrol is extracted from the traditional medicinal plant *Tripterygium wilfordii* and has multiple anti-inflammatory activities. Celastrol has multiple molecular targets in different diseases. During the development of hepatic fibrosis, celastrol inhibits the inflammation by activating AMPK-SIRT3 signaling, which attenuates liver fibrosis. Inflammasome plays a critical role in IL-1β production and inflammatory response. Celastrol abolishes the NLRP3 inflammasome activation and reduces inflammasome-mediated inflammatory response. Celastrol reduces lung injury by suppressing the Ednrg/Kng1 signaling pathway and alleviates chronic obstructive pulmonary disease (COPD). According to the published paper and our study, celastrol don’t lead to the damage of lung and also display lower toxic effect on cells or mouse models. In our research, we found that celastrol alleviated LPS-induced lung injury in mice. The elevated production of iNOS and cytokines induced by LPS was decreased after celastrol treatment. This result is consistent with previous study about the anti-inflammation effect. These experimental results indicated that celastrol has an anti-inflammatory effect on inflammatory response and inflammatory disease. Therefore, celastrol is a potential agent for ARDS treatment.

Inflammatory cells play a key role in ARDS and sepsis-induced lung injury. In the initial stage, the dysregulation inflammation induces acute lung injury. The products of microbial or danger associated molecular patterns (DAMP) bind to Toll-like receptors (TLRs) on the lung epithelium and pulmonary macrophages and active these cells. Because tissue resident macrophages are the first line to defense against the pathogenic microorganisms, activated macrophages release various inflammatory cytokines such as IL-6 and TNF-α, which lead to neutrophil and macrophage infiltration in
the lung of sepsis and ARDS. LPS-induced mouse acute lung injury is a classical model to mimic the critical pathological processes in human ARDS, including neutrophil infiltration and protein-rich BALF. In clinical research, it has been reported that the block of IL-6 and TNF-α contribute to inhibit inflammatory response in many inflammatory diseases, such as colitis and arthritis. Celastrol can reduce the production of iNOS and decrease the expression of inflammatory cytokines IL-6, TNF-α, and KC, suggesting celastrol not only alleviates ALI but also relieves other inflammatory diseases.

Mitogen-activated protein kinase (MAPK) is a crucial signaling pathway in inflammation, which leads to inflammatory response and the production of cytokines. The p38 MAPK and its downstream MK2 are important serine/threonine-protein kinases in MAPK, which are associated with inflammatory response, cell migration, and transcriptional regulation. LPS, the agonist of TLR4, can mediate inflammation, which is an interacting process for pathogen and cell. After the activation of TLR4 by LPS, a series of cascades are initiated following the activation of myeloid differentiating factor 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF). Then, the signaling leads to the activation of NF-κB and MAPK. MK2, as a substrate of p38 MAPK, is activated by p38 MAPK, and modulates the stability and translation of cytokine mRNA to induce the synthesis and expression of pro-inflammatory cytokines such as IL-6 and TNF-α. Depletion of MK2 protects against sepsis-induced ALI, and decreases the expression of IL-6 and TNF-α. Therefore, p38 MAPK or MK2 inhibitors can inhibit the expression of IL-6 and TNF-α in monocytes. NF-κB and MAPK are crucial targets of anti-inflammatory agents. Many native compounds have anti-inflammatory effects by inhibiting the activation of p38 MAPK and MK2, such as berberine and Hesperdin. In our study, celastrol inhibited the activation of p38 MAPK and MK2, which was associated with the decreased expression of IL-6, TNF-α, and KC in response to LPS treatment. Our results provide the direct evidence that celastrol have a protective effect on the LPS-induced lung inflammation by inhibiting the activation of p38 MAPK and MK2, which confirms the key role of MK2 and p38 MAPK on inflammatory cytokine production in ARDS and MK2 and p38 MAPK are crucial targets for the treatment of ARDS.

Conclusions

Our results unveil that celastrol protects against LPS-induced acute lung injury. Celastrol inhibited the production of IL-6, TNF-α, and KC and reduced the production of iNOS in response to LPS.

Figure 5. Celastrol attenuates the LPS-induced phosphorylation of p38 MAPK and MK2. BMDMs were pre-treated with celastrol for 15 min. And then treated with LPS (100 ng/mL) for 30 min. (a) The protein levels of P-p38 MAPK, p38 MAPK, P-MK2, and MK2 in BMDMs were detected by western blot. (b and c) The quantification of the blots in (a) was shown. (d) Immunoassay of HEK293T cells transfected with expression vector of Myc-p38 MAPK or Flag-MK2, followed by immunoprecipitation of lysates with anti-Myc. All quantitative data shown are mean ± SEM based on triplicate measurement. *p < 0.05, ***p < 0.001.
treatment. Meanwhile, the activation of p38 MAPK-MK2 signaling pathway was inhibited by celastrol treatment. Therefore, celastrol is a potent and effective compound for the treatment of ARDS and other inflammatory diseases.

Author contributions
F.Q. conceived the study. Z.C., X.Y., L.Z., and L.S. designed, performed and interpreted experimental data. X.Y. and F.Q. wrote the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from the National Natural Science Foundation of China (81773741, 81770633, 81973329, 82073858 and 81573438).

Ethics approval
The in vivo experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animal approved by the Biological Research Ethics Committee of Anhui Medical University (LLSC20200649). The date of Ethical Approval is March 1, 2020.

Animal welfare
The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

ORCID iD
Feng Qian https://orcid.org/0000-0002-1949-9193

References
1. Wong JJM, Leong JY, Lee JH et al. (2019) Insights into the immuno-pathogenesis of acute respiratory distress syndrome. Annals of Translational Medicine 7(19): 504.
2. Short KR, Kroeze E, Fouchier RAM et al. (2014) Pathogenesis of influenza-induced acute respiratory distress syndrome. The Lancet Infectious Diseases 14(1): 57–69.
3. De Freitas Caires N, Gaudet A, Portier L et al. (2018) Endocan, sepsis, pneumonia, and acute respiratory distress syndrome. Critical Care 22(1): 280.
4. Zhang J, Luo Y, Wang X et al. (2019) Global transcriptional regulation of STAT3- and MYC-mediated sepsis-induced ARDS. Therapeutic Advances in Respiratory Disease 13: 175346619879840.
5. Wang YM, Ji R, Chen WW et al. (2019) Paclitaxel alleviated sepsis-induced acute lung injury by activating MUC1 and suppressing TLR-4/NF-κB pathway. Drug Design, Development and Therapy 13: 3391–3404.
6. Zhang Y, Huang T, Jiang L et al. (2019) MCP-induced protein 1 attenuates sepsis-induced acute lung injury by modulating macrophage polarization via the JNK/c-Myc pathway. International Immunopharmacology 75: 105741.
7. Zheng H, Liang W, He W et al. (2019) Ghrelin attenuates sepsis-induced acute lung injury by inhibiting the NF-κB, iNOS, and Akt signaling in alveolar macrophages. American Journal of Physiology-Lung Cellular and Molecular Physiology 317(3): L381–L391.
8. Wilson JG, Liu KD, Zhuo H et al. (2015) Mesenchymal stem (stromal) cells for treatment of ARDS: A phase 1 clinical trial. The Lancet Respiratory Medicine 3(1): 24–32.
9. Fan E, Brodie D and Slutsky AS (2018) Acute respiratory distress syndrome: Advances in diagnosis and treatment. Journal of the American Medical Association 319(7): 698–710.
10. Soni S, Wilson MR, O’Dea KP et al. (2016) Alveolar macrophage-derived microvesicles mediate acute lung injury. Thorax 71(11): 1020–1029.
11. Peteranderl C, Morales-Nebreda L, Selvakumar B et al. (2016) Macrophage-epithelial paracrine cross-talk inhibits lung edema clearance during influenza infection. The Journal of Clinical Investigation 126(4): 1566–1580.
12. Williams AE, José RJ, Mercer PF et al. (2017) Evidence for chemokine synergy during neutrophil migration in ARDS. Thorax 72(1): 66–73.
13. Aggarwal NR, King LS and D’Alessio FR (2014) Diverse macrophage populations mediate acute lung inflammation and resolution. American Journal of Physiology-Lung Cellular and Molecular Physiology 306(8): L709–L725.
14. Mokra D and Kosutova P (2015) Biomarkers in acute lung injury. Respiratory Physiology & Neurobiology 209: 52–58.
15. Tasaka S, Amaya F, Hashimoto S et al. (2008) Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome. Antioxidants and Redox Signaling 10(4): 739–753.
16. Zhu S, Ware LB, Geiser T et al. (2001) Increased levels of nitrate and surfactant protein A nitration in the pulmonary edema fluid of patients with acute lung injury. American Journal of Respiratory and Critical Care Medicine 163(1): 166–172.
17. Sittipunt C, Steinberg KP, Ruzinski JT et al. (2001) Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome. *American Journal of Respiratory and Critical Care Medicine* 163(2): 503–510.
18. Channappanavar R and Perlman S (2017) Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. *Seminars in Immunopathology* 39(5): 529–539.
19. Cross LJM and Matthay MA (2011) Biomarkers of acute lung injury. *Critical Care Clinics* 27(2): 355–377.
20. Venkatesha SH, Dudics S, Astry B et al. (2016) Biomarkers in acute lung injury: Insights into the pathogenesis of acute lung injury. *Critical Care Clinics* 27(2): 355–377.
21. Shaker ME, Ashamallah SA and Houssen ME (2014) Celastrol ameliorates murine colitis via modulating oxidative stress, inflammatory cytokines and intestinal homeostasis. *Chemico-Biological Interactions* 210: 26–33.
22. Li X, Wang H, Ding J et al. (2019) Celastrol strongly inhibits proliferation, migration and cancer stem cell properties through suppression of Pin1 in ovarian cancer cells. *European Journal of Pharmacology* 842: 146–156.
23. Dai W, Wang X, Teng H et al. (2019) Celastrol inhibits microglial pyroptosis and attenuates inflammatory reaction in acute spinal cord injury rats. *International Immunopharmacology* 66: 215–223.
24. Ma X, Liu X, Feng J et al. (2019) Fraxin alleviates LPS-induced ARDS by downregulating inflammatory responses and oxidative damages and reducing pulmonary vascular permeability. *Inflammation* 42(5): 1901–1912.
25. Chen SR, Dai Y, Zhao J et al. (2018) A mechanistic overview of triptolide and celastrol, natural products from *Tripterygium wilfordii* Hook F. *Frontiers in Pharmacology* 9: 104.
26. Wang Y, Li C, Gu J et al. (2020) Celastrol exerts anti-inflammatory effect in liver fibrosis via activation of AMPK-SIRT3 signalling. *Journal of Cellular and Molecular Medicine* 24(1): 941–953.
27. Yu X, Zhao Q, Zhang X et al. (2017) Celastrol ameliorates inflammation through inhibition of NLRP3 inflammasome activation. *Oncotarget* 8(40): 67300–67314.
28. Shi K, Chen X, Xie B et al. (2018) Celastrol alleviates chronic obstructive pulmonary disease by inhibiting cellular inflammation induced by cigarette smoke via the Ednrb/Kng1 signaling pathway. *Frontiers in Pharmacology* 9: 1276.
29. Zeng Z, Lin X, Zheng R et al. (2018) Celastrol alleviates airway hyperresponsiveness and inhibits Th17 responses in obese asthmatic mice. *Frontiers in Pharmacology* 9: 49.
30. Zhu B and Wei Y (2020) Antitumor activity of celastrol by inhibition of proliferation, invasion, and migration in cholangiocarcinoma via PTEN/P13K/Akt pathway. *Cancer Medicine* 9(2): 783–796.
31. Xu LM, Zheng YJ, Wang Y et al. (2014) Celastrol inhibits lung infiltration in different animal models by reducing TNF-α and ICAM-1 levels while preserving differentiation in ATRA-induced acute promyelocytic leukemia cells. *PLoS One* 9(8): e105131.
32. Huppert LA, Matthay MA and Ware LB (2019) Pathogenesis of acute respiratory distress syndrome. *Seminars in Respiratory and Critical Care Medicine* 40(1): 31–39.
33. Fan EKY and Fan J (2018) Regulation of alveolar macrophage death in acute lung inflammation. *Respiratory Research* 19(1): 50.
34. Huang X, Xiu H, Zhang S et al. (2018) The role of macrophages in the pathogenesis of ALI/ARDS. *Mediators of Inflammation* 2018: 1264913.
35. Shu M, Tang Y and Liu J (2020) Protective effect of corynoline in sepsis-induced acute lung injury in rats via inhibition of NF-κB. *Natural Product Communications* 15(11): 1934578X20961188.
36. D’Alessio FR (2018) Mouse models of acute lung injury and ARDS. *Methods in Molecular Biology* 1809: 341–350.
37. Fang JY and Richardson BC (2005) The MAPK signalling pathways and colorectal cancer. *The Lancet Oncology* 6(5): 322–327.
38. Menon MB, Groepengießer J, Fischer J et al. (2017) p38(MAPK)/MK2-dependent phosphorylation controls cytotoxic RIPK1 signalling in inflammation and infection. *Nature Cell Biology* 19(10): 1248–1259.
39. Meng L, Li L, Lu S et al. (2018) The protective effect of dexametomidine on LPS-induced acute lung injury through the HMGB1-mediated TLR4/NF-κB and PI3K/Akt/mTOR pathways. *Molecular Immunology* 94: 7–17.
40. Tung C-L, Chen J-C, Ko J-C et al. (2021) Capsaicin acts through reducing P38 MAPK-dependent thymidylate synthase expression to enhance 5-fluorouracil-induced cytotoxicity in human lung cancer cells. *Natural Product Communications* 16(2): 1934578X21993335.
41. Nie Y, Wang Z, Chai G et al. (2019) Dehydrocostus lactone suppresses LPS-induced acute lung injury and macrophage activation through NF-κB signaling pathway mediated by p38 MAPK and Akt. *Molecules* 24(8): 1510.
42. Jiang S, Li D-L, Chen J et al. (2020) Synergistic anticancer effect of gemicitabine combined with
impressic acid or acankoreanogein in Panc-1 cells by inhibiting NF-κB and Stat 3 activation. *Natural Product Communications* 15(12): 1934578X20974239.

43. Wu Y, He H, Ding Y et al. (2018) MK2 mediates macrophage activation and acute lung injury by regulating let-7e miRNA. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 315(3): L371–L381.

44. Fang W, Cai SX, Wang CL et al. (2017) Modulation of mitogen-activated protein kinase attenuates sepsis-induced acute lung injury in acute respiratory distress syndrome rats. *Molecular Medicine Reports* 16(6): 9652–9658.

45. Jeong HW, Hsu KC, Lee JW et al. (2009) Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *American Journal of Physiology-Endocrinology and Metabolism* 296(4): E955–E964.

46. Yeh CC, Kao SJ, Lin CC et al. (2007) The immunomodulation of endotoxin-induced acute lung injury by hesperidin in vivo and in vitro. *Life Sciences* 80(20): 1821–1831.