Celastrol attenuates impairments associated with lipopolysaccharide-induced acute respiratory distress syndrome (ARDS) in rats

Yongjun Wei and Yu Wang

Tianjin First Center Hospital, Tianjin, China

ABSTRACT
Celastrol, a constituent from a traditional Chinese medicinal herb belonging to the family Celastraceae, has been shown to impart anti-inflammatory properties, in part, by inhibiting NF-κB activity and related induction of pro-inflammatory cytokine formation/release. The present study investigated the effects of celastrol in an animal model of acute respiratory distress syndrome (ARDS) induced by intratracheal administration of lipopolysaccharides (LPSs). Celastrol pre-treatment groups received celastrol by intraperitoneal injection on seven consecutive days before LPS treatment. In rats evaluated 24 h after LPS administration, oxygenation indices and lung injury were measured, as were levels of inflammatory cells and cytokines in isolated bronchoalveolar lavage fluid (BALF). Lung tissue expression of proteins involved in NF-κB and ERK/MAPK pathways were measured by Western blot analyses. Celastrol pre-treatments appeared to attenuate LPS-induced lung injury and inflammatory responses in the rats, including decreases in inducible aggregation/infiltration of inflammatory cells and production/release of pro-inflammatory cytokines in the lungs. Celastrol appeared to also inhibit NF-κB activation, but had no effect on ERK/MAPK pathways in the LPS-induced ARDS. The results here thus indicated that celastrol pre-treatment could impart protective effects against LPS-induced ARDS, and that these effects may be occurring through an inhibition of induction of NF-κB signaling pathways.

Introduction
Acute respiratory distress syndrome (ARDS) is a form of severe hypoxic respiratory failure characterized by impairment in gas exchange and widespread inflammation in the lungs, with a high mortality rate (Peter et al. 2008). The inflammatory response plays a critical role in the pathophysiology of ARDS. When inflammatory responses are triggered, alveolar macrophages activation occurs and results in inflammatory cytokines secretion and recruitment of neutrophils and circulating macrophages to the lungs (Han and Mallampalli 2015). This inflammatory burst results in endothelial and epithelial disruption, leading to alveolar edema, impairment of lung mechanics and gas exchange (Crimi and Slutsky 2004). Anti-inflammatory agents, including high-dose corticosteroids, has been applied in treatment of ARDS patients, but failed to show a mortality benefit (Peter et al. 2008; Han and Mallampalli 2015). On the other hand, it was reported that nuclear factor (NF)-κB was activated in alveolar macrophages in patients with ARDS (Schwartz et al. 1996). NF-κB is an transcription factor, which could stimulate the production of multiple early pro-inflammatory cytokines, thus it might be considered as a potential target for therapeutic intervention in ARDS (Cranshaw and Griffiths 2003).

Celastrol is a major component isolated from traditional Chinese medicinal herb, known as Thunder of God Vine, belonging to the family Celastraceae (Allison et al. 2001). Celastrol was shown to display anti-inflammatory properties by inhibiting NF-κB activity and modulating pro-inflammatory cytokines (El-Tanbouly et al. 2017), and might be a putative therapeutic drug in the treatment of various inflammatory diseases such as rheumatoid arthritis and Crohn’s disease (Pinna et al. 2004; Venkatesha et al. 2016). These properties suggested that celastrol might also play a therapeutic role in lung inflammation. In vitro studies showed that celastrol could inhibit LPS induced tumor necrosis factor (TNF)-α and interleukin (IL)-1β production in human lung macrophages (Allison et al. 2001). In a mouse model of allergic asthma, celastrol was shown to reduce the total number of inflammatory cells in the bronchoalveolar lavage fluid (BALF) and mRNA and protein expression levels of inflammatory cytokines such as IL-4, IL-13, TNFα, and interferon (IFN)-γ, by regulating the NF-κB pathway (Kim et al. 2009). To our knowledge, the potential protective effect of celastrol in ARDS remains unclear. Based on the potential role of celastrol in inflammation and NF-κB inhibition, the present study investigated the possible protective effects of celastrol in LPS-induced ARDS in rats.

Materials and methods
Animal model and drug administration
Wistar rats (male, 180–220 g) were purchased from the Shanghai SLAC Laboratory Animal Co. (Shanghai, China) for use in this experiment. The rats were maintained in a colony room at the Tianjin First Center Hospital under controlled temperature
(23 °C ± 2 °C), and relative humidity (40–60%), and a 12 h light/dark cycle. All rats had *ad libitum* access to standard rodent chow and filtered water. All procedures were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals.

After a 1-week acclimatization, the rats were randomly divided into five groups (N = 8/group): (i) control; (ii) ARDS alone; (iii) ARDS with 0.5 mg celastrol/kg pre-treatment; (iv) ARDS +2 mg celastrol/kg; and, (v) ARDS +5 mg celastrol/kg. The study did not contain a celastrol-only group (although pilot studies were performed using sets of rats treated with dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) and celastrol, as well as ARDS rats treated with DMSO). These doses of celastrol were selected for use here based on preliminary experiments that suggested this dose range could be protective in the ARDS animals, as well as on published papers that indicated that these levels of celastrol could be beneficial in several disease models (Xu et al. 2014; El-Tanbouly et al. 2017).

The ARDS model was induced as was indicated in Hou et al. (2014) and Lang et al. (2017). In brief, rats were anesthetized by intraperitoneal (IP) injection of 2% pentobarbital (40 mg/kg) and then a freshly-prepared solution of lipopolysaccharide (LPS; from *Escherichia coli*, Type O111:B4, Sigma, St. Louis, MO) that had been dissolved in saline was instilled slowly into the trachea (at 2 mg/kg). Control rats were instilled with the same volume (i.e. 100 μl) of saline. The rats were then placed upright and their bodies rotated to ensure equal distribution of LPS (or saline) throughout the lung tissues. For rats in the ARDS + celastrol groups, rats received (at the indicated dose) celastrol on seven consecutive days before the LPS treatment. A stock solution of celastrol (Cat #219465, Sigma, St. Louis, MO) was dissolved in DMSO and then diluted with enough saline such that the dose administered by IP injection each day delivered the desired concentration in the same volume (i.e. 100 μl/rat). For subsequent use in the various protocols outlined below, the rats were then euthanized 24 h after the LPS (or saline) by pentobarbital overdose (IP).

**Arterial blood gas analysis**

At necropsy, blood samples were collected from the abdominal aorta. The oxygenation index in each sample was then measured using an AVL OMNI Blood Gas Analyzer (Basel, Switzerland).

**Lung wet–dry ratio**

To assess lung edema, at necropsy, the right lung middle lobe of each rat was removed and weighed to get the "wet" weight. The tissues were then dried at 80 °C for 72 h and re-weighed to get the "dry" weight. Lung wet/dry weight ratios were then calculated for each rat.

**Histologic examination**

For histological analyses, the right lung upper lobe of each rat was then collected and fixed in 10% formalin for 24 h. The tissues were then dehydrated and embedded in paraffin, sectioned to 4-μm, and stained with hematoxylin–eosin dye (H&E). The tissue specimens were then examined in a blinded manner using a light microscope (Leica Microsystems, Tokyo, Japan) and scored using a semi-quantitative scoring system by two skilled pathologists (Lang et al. 2017). Edema, alveolar and interstitial inflammation, pulmonary hemorrhage, atelectasis and hyaline membrane formation were each scored on a 0–4-point scale, i.e. no injury = 0, injury to < 25% of the field = 1, injury to no more than 50% of the field = 2, injury to 50–75% of the field = 3, and injury throughout the field = 4. Scores were added for the five variables in the same sample, with a maximum possible score of 20.

**Western blot analyses**

The remaining right lung tissues were collected, cut into small pieces and homogenized using a Dounce pestle (Wheaton, Millville, NJ). Cytoplasmic and nuclear proteins were then extracted using NE-PER Nuclear and Cytoplasmic Extraction reagents (ThermoScientific, Waltham, MA) according to manufacturer protocols. After determining protein concentrations in each sample with a kit (Bio-Rad Laboratories, Hercules, CA), an aliquot of each (i.e. 30 μg) was subjected to SDS-PAGE separation and the resolved proteins were then electrotransferred to a PVDF membrane (Millipore, Billerica, MA). Additional lanes containing rat IκBα, phosphorylated IκBα (p-IκBα), p65, ERK, p-ERK, JNK, p-JNK, p38, and/or pp38 were run in each gel to provide markers of location for comparison against the rat mixtures (Abcam, Cambridge, MA). For each rat, multiple gels were run (with equal amount of protein samples loaded) and the proteins transferred to dedicated blots that were then probed with an individual primary antibody. Each membrane was then blocked with 5% nonfat milk in Tris-buffered saline containing 0.05% Tween-20 (v/v; TBST) for 1 h at room temperature before being incubated overnight at 4 °C in a solutions of TBST containing specific primary antibodies against rat IκBα, p-IκBα, p65, ERK, p-ERK, JNK, p-JNK, p38, and/or pp38 (Abcam). Antibodies against β-actin and Lamin B were employed as loading controls. The membranes were then washed several times with TBST, and then incubated in TBST containing horseradish peroxidase (HRP)-conjugated secondary antibody against mouse IgG for 1 h at room temperature. All primary antibodies were used at a 1:1000 dilution and the secondary antibody at a 1:200 dilution. Signals were then developed using a SuperSignal West Pico Chemiluminescent Substrate (Pierce, Waukesha, WI). Bands on the films were scanned using a ScanMaker 9800XL densitometer (Microtek, Shanghai, China) and then intensities quantified using Image J software (NIH, Bethesda, MD).

**BALF collection and inflammatory cell analysis**

At necropsy, after tying of the right lung at the bronchus with suture, bronchoalveolar lavages were performed on the left lung of each rat using methods outlined in Nick et al. (2000) and Xie et al. (2012). In brief, the trachea was intubated with a 20-G cannula, and then phosphate-buffered saline (PBS, pH 7.4) was slowly instilled (and withdrawn) via the cannula. The lavage sample was then centrifuged at 1500 × g for 10 min at 4 °C and the supernatant was collected and stored at −20 °C for later analysis. Total protein concentration in the supernatant was measured using the above-noted kit. Each cell pellet was re-suspended in PBS and total cell numbers determined in a hemocytometer (Beckman Coulter, Indianapolis, IN). Cell types were determined by Diff-Quick staining (Sigma, St. Louis, MO) of aliquots of the suspended cells. All slides were counted in a blinded manner by two skilled technicians.
Cytokine assay

Levels of TNFα, IFNγ, IL-6, and IL-10 in the BALF were measured using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) according to manufacturer instructions. The level of sensitivity for each kit was 62.5 pg TNFα/ml, 39.1 pg IFNγ/ml, 62.5 pg IL-6/ml, and 62.5 pg IL-10/ml.

Statistical analysis

All values are expressed as mean ± SD. For discrete variables, Fisher’s exact test was used to determine differences among groups. For continuous variables, when normal distribution and equal variance were shown, data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. When data were not normally distributed or showed unequal variance, a non-parametric test was performed. Significance was set at p < 0.05. All analyses were performed using SPSS software (SPSS Inc., Chicago, IL).

Results

Celastrol pre-treatment attenuated LPS-induced lung injury

Pulmonary function was assessed 24 h after LPS administration. As shown in Figure 1(a), P(A-a)O2 was significantly increased in ARDS alone rats compared with control rats. This value was significantly decreased in rats that received the 2 or 5 mg celastrol/kg pre-treatments. LPS treatment also significantly decreased PaO2/FiO2 ratios, while pre-treatment with either level of celastrol significantly increased this ratio relative to that in the ARDS alone rats (Figure 1b). The results of the lung wet/dry ratio analyses and total protein levels in isolated BALF indicated that the celastrol pre-treatments significantly reduced lung edema caused by the LPS (Figure 1c,d). Note: Data were not shown for the following groups: (i) control treated with DMSO and (ii) ARDS treated with DMSO, because in preliminary experiment it was found that there was no difference between the DMSO treated group and control or ARDS alone groups, respectively. Although a stand-alone celastrol-only group was not evaluated in these studies, the relative safety of the test agent could be inferred from several outcomes in the current study. For example, there was no death or weight loss noted in any celastrol-treated rats during the entire study period. Furthermore, even a dose level of 5 mg celastrol/kg did not induce any significant change in lung function and pathology, including oxygenation index and immune cell profiles in the animals without LPS treatment (Supplementary Figure S1).

Histological analyses indicated there were significant differences between the control and all LPS-treated rats. Lungs of the control rats were normal, with no inflammatory cell infiltration or interstitial edema (Figure 2a). In contrast, lung tissues obtained from LPS-treated rats showed extensive lung injury (Figure 2b–f). When lung injury scores were calculated (to evaluate edema, alveolar and interstitial inflammation, pulmonary hemorrhage, atelectasis and hyaline membrane formation) (Figure 2f), it was found that LPS treatment resulted in significantly higher scores than with the control rats and that pre-treatment with 2 or 5 mg celastrol/kg significantly attenuated these outcomes (Figure 2d–f).

Celastrol pre-treatment decreased inflammatory cell influx and pro-inflammatory cytokine release in the lungs

Due to LPS, the total numbers of cells, as well as specifically those of macrophages, neutrophils, and lymphocytes were significantly increased (as measured in BALF) compared to corresponding values seen with control rats (Figure 3). In each instance, the pre-treatment with 2 or 5 mg celastrol/kg led to significant decreases relative to values in ARDS alone rats. In concert, levels of pro-inflammatory TNFα, IFNγ, and IL-6 were each decreased.

Figure 1. Celastrol pre-treatment attenuated LPS-induced lung injury. Effects characterized by impact on (a) P(A-a)O2, (b) PaO2/FiO2, (c) lung wet/dry ratio, and (d) BALF total protein levels. N = 8 per group. Data shown are means ± SD. *p < 0.05 and **p < 0.01 vs. control; #p < 0.05 and ##p < 0.01 vs. ARDS alone group.
Figure 2. Changes in lung histopathology. Representative images (H&E staining) of lung tissue in (a) control, (b) ARDS alone, (c) 0.5 (d) 2, and (e) 5 mg celastrol/kg pre-treatment groups. (f) Histological scores. $N = 8$ per group. Data shown are means ± SD. *$p < 0.05$ and **$p < 0.01$ vs. control; ##$p < 0.01$ vs. ARDS alone group.

Figure 3. Immune cell profiles in BALF. BALF was collected 24 h post-LPS (or saline) treatment and immune cell profiles analyzed. (a) Total cells, (b) macrophages, (c) neutrophils, and (d) lymphocytes. $N = 8$ per group. Data shown are means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control; #*$p < 0.05$ and ##$p < 0.01$ vs. ARDS alone group.
significantly increased by LPS administration (Figure 4). Again, 2 or 5 mg celastrol/kg pre-treatment significantly decreased these levels relative to values in ARDS alone rats. The concentration of anti-inflammatory IL-10 was also significantly increased by LPS instillation, and in this case the two celastrol regimens raised these significantly further compared to values in ARDS alone rats. It is worth noting that even when levels of each measured cytokine were evaluated after normalizing to total protein content in the BALF, the same trends/outcomes for the celastrol-treated rats relative to those in the ARDS alone rats were still evident (Supplemental Figure S2). This indicated that celastrol treatment could indeed impact on cytokine production.

Celastrol pre-treatment inhibited LPS-induced NF-κB signal pathway activation

The Western blot analyses revealed that instillation of LPS resulted in activation of the NF-κB signaling pathway in the lungs, as evidenced by decreased IκBα expression and increased p-IκBα expression in cytoplasm, and increases in nuclear levels of p65. Celastrol pre-treatment (5 mg/kg) appeared to significantly attenuate the effects of LPS on these expressions (Figure 5(a)). MAPK signaling pathway was also activated in these tissues. Expression of p-ERK, p-JNK, and pp38 were each significantly increased compared with levels in tissues from control rats. However, celastrol pre-treatment did not result in reductions of the expressions (Figure 5(b)). This indicated celastrol protected against lung injury in ARDS (in rats) through inhibition of NF-κB but not MAPK signaling pathway activation.

Discussion

Celastrol, known to impart anti-inflammatory properties (Allison et al. 2001), has been widely studied as a potential treatment for inflammatory diseases. In the present study, the effect of celastrol against LPS-induced inflammation in a rat model of ARDS was investigated. In macrophages, LPS activates toll-like receptor 4 (TLR4) to trigger biosynthesis of multiple inflammatory cytokines including TNFα and IL-1β, and stimulates production of co-stimulatory molecules required for immune responses (Raetz and Whitfield 2002). LPS-induced acute lung injury is a commonly used model to replicate several key pathological processes associated with ARDS in humans, including disruption of endothelial barrier, neutrophil infiltration, and accumulation of protein-rich fluid in the airspaces of the lung (Matute-Bello et al. 2008; Dagvadorj et al. 2015). In the present study, a rat model of ARDS was generated by intratracheal administration of LPS. This study found that pre-treatment with two of three doses of celastrol (i.e. 2 or 5 mg/kg) for seven days prior to endotoxin exposure led to reductions in LPS-induced lung injury. These changes also resulted in improved pulmonary function, less lung edema and less pathologic changes than seen in ARDS hosts that did not receive the test agent.

In ARDS patients, markedly elevated levels of inflammatory IL-6 and IL-8 have been detected in isolated BALF (Schutte et al. 1996). There are also significant increases in the numbers of inflammatory cells, i.e. neutrophils, that appeared in the lungs; levels of neutrophils have been correlated with severity of ARDS and patient outcomes (Grommes and Soehnlein 2011). Alveolar macrophages provide a first line of defense against microbes and act as important mediators in inflammatory responses (Cheung et al. 2000). When activated, these cells produce pro-inflammatory cytokines including IL-6 and TNFα as well as chemokines that help to recruit more neutrophils and circulating macrophages to the lung (Han and Mallampalli 2015) that, in turn, lead to even more increases in level of inflammatory mediators (Cranshaw and Griffiths 2003). As celastrol was reported to exert potent anti-inflammatory properties in various experimental models (Allison et al. 2001), we believed it might also be effective against ARDS by impacting on this inflammatory cascade. The current results showed celastrol pre-treatment did in fact lead to...
decreased accumulation of inflammatory cells and in reductions in levels of pro-inflammatory cytokines (and increases in anti-inflammatory cytokine IL-10), in the lungs of ARDS rats.

To further characterize any protective anti-inflammatory role for celastrol against LPS-induced lung injury, the current study also investigated effects of celastrol on NF-κB signaling pathways during LPS-induced ARDS as many inflammatory stimuli, including LPS, activate the NF-κB signaling pathway. In mammals, the NF-κB family is composed of p50, p52, p65, c-Rel, and RelB that could form homodimers/heterodimers that bind to promoters or enhancer regions of genes and so modulate their expression. The most common and best characterized form of NF-κB is the p65/p50 heterodimer (Moynagh 2005). In an activated state, NF-κB proteins are predominantly located in the cytosol, as complexes with members of the inhibitory IκB family such as IκBα. In the activated state, IκBα is phosphorylated – resulting in dissociation of IκBα from NF-κB. The latter then translocates to the nucleus and binds promoters or enhancer regions of genes (Braisier et al. 2007). Among the genes impacted are several that code for important cytokines, including TNFα, IL-1, IL-6, and IL-8, each of which is important in generation of acute inflammatory responses (Blackwell and Christman 1997).

It has been reported that NF-κB was activated in alveolar macrophages of ARDS patients (Schwartz et al. 1996; Moine et al. 2000). As such, specific efficacious inhibitors of NF-κB activation might exert beneficial effects in ARDS. Celastrol has been reported to inhibit NF-κB activation through targeting IκB kinase (Lee et al. 2006). The present results demonstrated that celastrol pre-treatment could reduce LPS-induced phosphorylation of IκBα as well as NF-κB activation and nuclear translocation. It is important to remember that ERK/MAPK signaling pathways are also involved in LPS-induced inflammation (Goncalves-de-Albuquerque et al. 2012) and that this pathway participated in chemoattractant-induced neutrophil chemotaxis during LPS-induced ARDS (Nick et al. 2000; Schuh and Pahl 2009). Combined inhibition of p38 and ERK1/2 induced a suppression of cytokines release in alveolar macrophages (Jarrar et al. 2002).

Figure 5. Activation of NF-κB signaling pathway induced by LPS. Expressions of related proteins in (a) NF-κB and (b) MAPK pathways were analyzed by Western blot. β-Actin or Lamin B were used as loading controls. Representative blots are shown. Densitometric measures to permit quantitative analyses/statistical comparisons are shown to the right of each blot set. Data shown in these are means ± SD. *p < 0.01 vs. control; **p < 0.01 vs. ARDS alone rats. Only data from the 5 mg celastrol/kg regimen are shown.
In the present study, it was demonstrated that there was activation of the ERK/MAPK signaling pathways in LPS-induced ARDS. However, celestrol pre-treatment did not exert any inhibitory effect on this activation. Taken together, these data indicated that the protective and anti-inflammatory properties of celestrol in ARDS might be through inhibiting NF-κB signaling pathway.

A key limitation of this study is that no data regarding pharmacokinetics or metabolism of celestrol were derived. Previous studies suggested the half-life of celestrol is around 8 h (Yan et al. 2017), and complexation, enterobacterial recirculation, gastric secretion-enteral reabsorption or others may affect localized levels of celestrol at any given time (Zhang et al. 2012). Although it is certain celestrol could protect against LPS-induced lung injury and inflammation, it is unclear whether the agent might be retained (or accumulate) in the lung. Studies on the pharmacokinetics and metabolism of celestrol in disease models, for example, ARDS, would help to better define detailed mechanisms of the protective effects of celestrol.

Conclusions

Data from this study demonstrated that celestrol inhibit NF-κB activation in a rat model of ARDS. This inhibition could effectively reduce LPS-indcued inflammatory responses and result in a reduction in lung injury. These data suggested that celestrol might have potential as a treatment in ARDS. Further studies are warranted to evaluate the therapeutic potential of celestrol in this severe lung condition.

Disclosure statement

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

References

Allison A, Cabacelas R, Lombardi V, Álvarez X, Vigo C. 2001. Celestrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer’s disease. Prog Neuropsychopharmacol Biol Psychiatry. 25:1341–1357.

Blackwell T, Christman J. 1997. The role of nuclear factor-kB in cytokine gene regulation. Am J Respir Cell Mol Biol. 17:3–9.

Brazier A. 2006. The NF-κB regulatory network. Cardiovasc Toxicol. 6:111–130.

Cheung D, Halsey K, Speert D. 2000. Role of pulmonary alveolar macrophages in defense of the lung against Pseudomonas aeruginosa. Infect Immun. 68:4585–4592.

Cranshaw J, Griffiths M. 2003. Inflammatory processes in the acute respiratory distress syndrome. Curr Anesth Crit Care. 14:66–73.

Crimi E, Slutsky A. 2004. Inflammation and the acute respiratory distress syndrome. Best Pract Res Clin Anaesthesiol. 18:477–487.

Dagvadorj J, Shimada K, Chen S, Jones H, Tumurkhuu G, Zhang W, Crimi E, Slutsky A. 2004. Inflammation and the acute respiratory distress syndrome. Curr Anesth Crit Care. 14:66–73.

El-Tanbouly G, El-Awady M, Megahed N, Salem H, El-Kashef H. 2017. Inhibition of the MAP kinase ERK protects gastric secretion-enteral reabsorption or others may affect local-ized levels of celestrol at any given time (Zhang et al. 2012). Although it is certain celestrol could protect against LPS-induced lung injury and inflammation, it is unclear whether the agent might be retained (or accumulate) in the lung. Studies on the pharmacokinetics and metabolism of celestrol in disease models, for example, ARDS, would help to better define detailed mechanisms of the protective effects of celestrol.

Grommes J, Soehnlein O. 2011. Contribution of neutrophils to acute lung injury. Mol Med. 17:293–307.

Huang S, Mallampalli R. 2015. The acute respiratory distress syndrome: From mechanism to translation. J Immunol. 194:855–860.

Hou S, Ding H, Lv Q, Yin X, Song J, Landen N, Fan H. 2014. Therapeutic effect of intravenous infusion of perfluorocarbon emulsion on LPS-induced acute lung injury in rats. PLoS One. 9:e87826.

Jarrar D, Kuebler J, Rue L, Matalon S, Wang P, Bland K, Chaudry I. 2002. Alveolar macrophage activation after trauma-hemorrhage and sepsis is dependent on NF-κB and MAPK/ERK mechanisms. Am J Physiol. 283:L799.

Kim D, Park J, Jeoung D, Ro J. 2009. Celestrol suppresses allergen-induced airway inflammation in a mouse allergic asthma model. Eur J Pharmacol. 612:98–105.

Lang S, Li L, Wang X, Sun J, Xue X, Xiao Y, Zhang M, Ao T, Wang J. 2017. CXCL1/10–PT-10 neutralization can ameliorate lipopolysaccharide-induced acute respiratory distress syndrome in rats. PLoS One. 12:e0169100.

Lee J, Koo T, Yoon H, Jung H, Jin H, Lee K, Hong Y, Lee J. 2006. Inhibition of NF-κB activation through targeting IkB kinase by celestrol, a quinone methide tripterpenoid. Biochem Pharmacol. 72:1311–1321.

Matute-Bello G, Frevert C, Martin T. 2008. Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 295:L379–L399.

Moiné F, McIntyre R, Schwartz M, Kaneko D, Shenkar R, Le Tulzo Y, Moore E, Abraham E. 2000. NF-κB regulatory mechanisms in alveolar macrophages from patients with acute respiratory distress syndrome. Shock. 13:85–91.

Moynagh P. 2005. The NF-κB pathway. J Cell Sci. 118:4589–4592.

Nick J, Young S, Brown K, Avdi N, Arndt P, Suratt B, Janes M, Henson P, Worthen G. 2000. Role of p38 mitogen-activated protein kinase in a murine model of pulmonary inflammation. J Immunol. 164:2151–2159.

Perkins N. 2007. Integrating cell-signalling pathways with NF-κB and IKK function. Nat Rev Mol Cell Biol. 8:49–62.

Peter J, John P, Graham P, Moran J, George I, Bersten A. 2008. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: Meta-analysis. BMJ. 336:1006.

Pinna G, Fiorucci M, Reinmund I, Taquet N, Arondel Y, Muller C. 2004. Celestrol inhibits pro-inflammatory cytokine secretion in Crohn’s disease biopsies. Biochem Biophys Res Commun. 322:778–786.

Raez C, Whitfield C. 2002. Lipopolysaccharide endotoxins. Annu Rev Biochem. 71:635–700.

Schuh K, Pahl A. 2009. Inhibition of the MAP kinase ERK protects from lipopolysaccharide-induced lung injury. Biochem Pharmacol. 77:1827–1834.

Schutte H, Lohmeyer J, Rosseau S, Ziegler S, Siebert C, Kielisch H, Pralle H, Grimminger F, Moor H, Seeger W. 1996. Bronchoalveolar and systemic cytokine profiles in patients with ARDS, severe pneumonia and cardio-genic pulmonary oedema. Eur Respir J. 9:1858–1867.

Schwartz M, Moore E, Moore F, Shenkar R, Moine P, Haelen J, Abraham E. 1996. NF-κB is activated in alveolar macrophages from patients with acute respiratory distress syndrome. Crit Care Med. 24:1285–1292.

Sharif O, Bolsbakov V, Raines S, Newham P, Perkins N. 2007. Transcriptional profiling of the LPS induced NF-κB response in macrophages. BMC Immunol. 8:1.

Venkatesha S, Dudics S, Astry B, Moudgil K. 2016. Control of autoimmune inflammation by celestrol, a natural triterpenoid. Pathog Dis. 74. doi:10.1093/femsdp/ftw059.

Xie X, Yu Y, Huang Y, Zheng L, Li J, Chen H, Han H, Hou L, Gong G, Wang G. 2012. Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis. Shock. 37:548–555.

Xu L, Zheng Y, Wang Y, Yang Y, Cao F, Peng B, Xu X, An H, Zheng A, Zhang D, et al. 2014. Celestrol inhibits lung infiltration in differential syndromic animal models by reducing TNF-α and ICAM-1 levels while preserving differentiation in ATRA-induced acute promyelocytic leukemia cells. PLoS One. 9:e105131.

Yan G, Zhang H, Wang W, Li Y, Mao C, Fang M, Yi X, Zhang J. 2017. Investigation of the influence of glycyrrhizin on the pharmacokinetics of celestrol in rats using LC–MS and its potential mechanism. Xenobiotica. 47:607–613.

Zhang J, Li C, Xu M, Wu T, Chu J, Liu S, Ju W. 2012. Oral bioavailability and gender-related pharmacokinetics of celestrol following administration of pure celestrol and its related tablets in rats. J Ethnopharmacol. 144:195–200.