Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

The protective effect of cinnamaldehyde on lipopolysaccharide induced acute lung injury in mice

H. Huang[1], Y. Wang[2*]

[1] Department of Critical Care Medicine, Liaocheng People's Hospital, Dongchang Road, Liaocheng, Shandong, 252000, China
[2] Department of respiration, Liaocheng People's Hospital, Liaocheng, Shandong,252000, China

Correspondence to: wywangyu05@hotmail.com

Received April 11, 2016; **Accepted** August 5, 2017; **Published** August 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.8.13

Abstract: The study wasintended to examinethe protective effect of cinnamaldehyde (CM) on lipopolysaccharide (LPS)-induced acute lung injury (ALI) mice model.The results of the investigation confirmed that, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were significantly decreased by CM. CM also up-regulated level of IL-10. We found that CM significantly attenuated LPS-induced TLR4 expression, NF-κB activation and MPO activity in the lung tissues. It markedly reduced lung wet/dry ratio, and improved typical and severe pathological changes including pulmonary edema in the LPS induced ALI mice. Moreover, CM significantly inhibited neutrophils, macrophages and total cell number in the bronchoalveolar lavage fluid (BALF). In our study, we noted that TLR4–NF-κB signaling pathway was involved in acute lung injury.The results revealed the protective effects of CM in LPS-induced ALI, through suppressionTLR4–NF-κB signaling pathway. Our finding suggests that Cinnamaldehyde is a potential anti-inflammatory agent in treatment the acute lung injury.

Key words: Cinnamaldehyde; Lung injury; Anti-inflammatory agent; TLR4–NF-κB.

Introduction

Acute lung injury (ALI) and it's sever conditions, acute respiratory distress syndrome (ARDS) is rapidly growinggrave clinical disorder, generally resulting from sepsis, trauma, and severe pulmonary infections (1). The pathogenesis of ALI are often characterized by enlarged diffuse lung inflammation, alveolar-capillary obliteration, hypoxia, non-cardiogenic pulmonary edema and diminished lung compliance.First time, ARDS was defined by Ashbaugh and associatesin 1967 (2), followed by the American-European Consensus Conference's (AECC) in 1994 (3). About 190,000 cases of ARDS estimated in the United States as per the latest population data (4).Despite the availability of many therapeutic interventions (like ventilation and nutritional supports), ALI and ARDS are often coupled with low survival rates (35% to 50%) in the critically ill patients (5, 6, 7). The morbidity and mortality associated with this critical disease remain very high. At present, there is no any effective pharmacological treatment for the acute lung injury. Therefore, a safe and effective new drug or treatments are urgently needed for ALI and ARDS.

Lipopolysaccharide (LPS)is considered as a major constituent of the cell wall of Gram-negative bacteriaand considered as a potent biological inducer of macrophages (8), thus, can act as suitable inducing agent of ALI in experimental rats.Moreover, it stimulates the NF-κB activation, which stimulates the over-production of pro-inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and IL-8 (9, 10). Consequently, excessive production of these inflammatory mediators induces the systemic inflammation and which further leads to the development of ALI/ARDS.It has been well-established that NF-κB singling pathway is significantly involved in the inflammatory processes in LPS induced ALI/ARDS. While, Toll-like receptor 4 (TLR4) expressed on the cell surface, is ananotherimportant receptor of LPS that can able to activate the NF-κB signaling pathway in and associated cytokines in ALIinduced by LPS (11, 12).

Cinnamaldehyde, an essential oil obtained from the plant of *Cinnamomum cassia*, comprises cinnamic acid, cinnamon ether, cinncassiol, glycosides and some other constituents (13). From the ancient times, it has been used a source of medicine for numerous of ailments. Together with this, various studies have confirmed showed scientific evidences of cinnamaldehyde to act as antibacterial, anti-inflammatory, anti-oxidant, anti-ulcer, anti-diabetic, and anti-tumor effects accompanied with low cellular toxicity (13, 14).However, the anti-inflammatory and anti-oxidant properties of cinammaldehyde have been recently demonstrated (15, 16), but no investigation has been carried out to elucidate its protective role inacute lung injury. Therefore, the present study was intended to investigate the an anti-inflammatory effect of cinnamaldehyde in LPS-induced acute lung injury mice (ALI) model.

Materials and Methods

Animals

Male BALB/c mice weighing 20-24g of age of7 to 10 weeks wereused for the study. Mice were housed under a 12:12 h light-dark (LD) cycle under regular tempe-

rature (23 ± 2°C) in standard polypropylene laboratory cages. The animals were provided standard laboratory, food and water ad labitumprior to the experiment.All animal experiments were approved by Institutional animal ethical committee.

Reagents

Cinnamaldehyde(natural, ≥95%, catalogue#W228613) and LPS (Escherichia coli 0111:B4, catalogue#L2630) were procured from Sigma-Aldrich (St. Louis, MO). MIF ELISA kit (catalogue#DY1978) was procued from R&D systems (MN, USA). Mouse TNF-α, IL-6, IL-1β, IL-10, IL-13 were obtained from eBioscience (CA, USA). Whereas, the TLR4 (catalogue#14358), anti-NF-κBp65 (catalogue#6956), anti-p-NF-κBp65 (catalogue#3036), anti-IκBα (catalogue#7543), anti-p-IκBα (catalogue#9246) and β-actin (catalogue#3700) monoclonal antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). The myeloperoxidase determination kit was obtained from the Jiancheng Bioengineering Institute of Nanjing (Nanjing, Jiangsu, China).

LPS-induced ALI mouse model

Seventy-two Male BALB/c mice were randomly divided into six groups taking twelve mice in each group. The groups have been classified as follows, Control group, LPS group, LPS+CM (10, 20 and 40 mg/kg) group and LPS + VGX-1027 (0.5mg/mouse) group (standard). The LPS was instilled to BALB/c mice were via the intranasal route (i.n.) (10 µg in 50 µL PBS per mouse) to induce lung injury. Whereas, the test drug,CM (10, 20 and 40 mg/kg) and VGX-1027 (0.5mg/mouse) were given intraperitoneally (i.p.) 1 h after LPS treatment. The control mice receive PBS (i.n.) without LPS. After 6 h, a collection of bronchoalveolar lavage fluid (BALF) was carried for subsequent analysis.

Histopathological studies of lung

A light microscope was used to observe the pathological changes of the lung. In the current histological examination, the lungs were harvested, fixed in 10% formalin solution and embedded in paraffin.The 5 µm-sections were cut and stained with hematoxylin and eosin (H&E) stainingand were subsequently observed under a light microscope instrument at × 200 magnification (9).

Lung Wet/Dry weight ratio

The Lung Wet/Dry weight ratio was used as an index for estimation of edema in lungs. The left lungs were collected, and weighed to measure the 'wet' weight. The lungs were then dried in an oven at 80°C for 48 hours and re-weighed as dry weight. The lung Wet/Dry weight ratio was calculated by dividing the wet by the dry weight (9).

Myeloperoxidase(MPO) activity

The lungs were harvested, homogenized, and centrifuged. The resulting supernatant were used for the determination of myeloperoxidase activity using the MPO activity assay kit (Nanjing Jiancheng Bioengineering Institute, China).All procedures were performed according to the manufacturer's protocol (9).

Cells counts in bronchoalveolar lavage fluid (BALF)

The BAL samples were collected and centrifuged at 3000g for 7min. Then the sedimented cells were re-suspended in PBS solution and subjected to determine the total cell counts. Different cell counts were determined by a Wright-Giemsa stained kit (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Enzyme-Linked Immunosorbent Assay

Inflammatory cytokines levels (TNF-α, IL-1β,IL-6, IL-10, IL-13) were measured in the BAL samples using an Enzyme-Linked Immunosorbent Assay (ELISA) kit. All procedures were performed according to the manufacturer's instructions.

Western Blotting analysis for Total Protein Analysis

Total proteins obtained from left lung tissues were extracted by T-PER, Pierce. Bi-cinchoninic acid assay method was used to determine the protein concentration. Equal small fractions of protein (40µg) were separated gel electrophoresis. After electrophoresis, the proteins were blotted to polyvinylidene fluoride membranes and were incubated with TLR4, NF-κB, and β-actin for 12 hours at 4°C. After the incubation for 2 h in 5% nonfat milk the secondary antibodies horseradish peroxidase-labeled (1:10000) were added and incubated for 1h at room temperature. ECL plus Western blotting detection system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) was used for detection of the blots in this analysis.

Statistical analysis

All data were presented as mean ± standard deviation (SD). Statistically significant differences between groups were analyzed using one way ANOVA followed by Student-Newman-Keuls post-hoc test using the GraphPad Prism version 5.0. P value <0.05 was considered to indicate a statistically significant difference.

Results

Effects of CM on LPS induced histopathologic changes

In the present study we have used VGX-1027 as standard, which is an orally active isoxazoline compound ((S,R)-3-phenyl-4, 5-dihydro-5-isoxazoleacetic acid). It is a new class of immune modulators that inhibits the production of several proinflammatory cytokines responsible for the damaging effects in inflammatory diseases such as RA.We examined the effects of CM on histopathologic changes in LPS induced ALI mice. Hematoxylin and eosin (H&E) staining were used to determine the pathological changes in the present study. Here, we observedthe normal intact lung structure with clear pulmonary alveoli in the control group. Whereas, the LPS-treated group clearlysuggests thesignificant pathological changes containing the alveolar wall thickening inflammatory cell infiltration, fibrosis with collapse of air alveoli, interstitial edema, and some patchy hemorrhage. However, treatment with CM markedly attenuated the LPS induced pathological changes in the lung tissues as shown in Figure1.

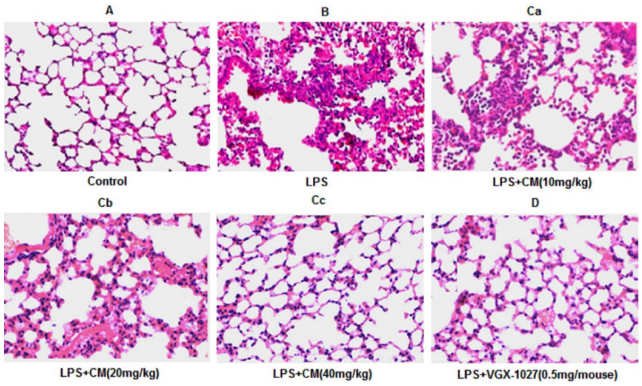

Figure 1. CM effects on lung tissues in LPS-induced ALI mice. Mice received an intra-peritoneal (i.p.) injection of CM (10, 20 and 40 mg/kg) 1h after lipopolysaccharide (LPS). Lungs tissues sections were stained with hematoxylin and eosin (H&E) processed for histological evaluation from each experimental group at 6 h after LPS challenge. The figure represented (x200)from each experimental group; (A): Control group, (B): LPS group, (Ca-Cc): LPS+ CM (10, 20 and 40 mg/kg) group, (D): LPS+VGX-1027 (0.5mg/mouse).

CM inhibited LPS-induced Pulmonary Edema

LPS-induced pulmonary edema was assessed by lung Wet/Dry weight ratio. As results shown in Figure 2, the lung W/D ratio was markedly higher in the LPS group than the control group. CM (10, 20, and 40 mg/kg) significantly reduced the LPS-induced lung Wet/Dry weight ratio.Whereas, the VGX also significantly-decreased Wet/Dry weight ratio.

CM reduced MPO activity induced by LPS

In the present study, Myeloperoxidase (MPO) activity was measured to determine the activation and infiltration of neutrophilsand macrophage in the lung tissues. After 6 h of LPS challenge, the MPO activity was remarkably increased in comparison to the control group. However, this MPO activity was markedly reduced after the initiation oftreatment with CM (10, 20, and 40 mg/kg) and VGX treatments as shown Figure3.

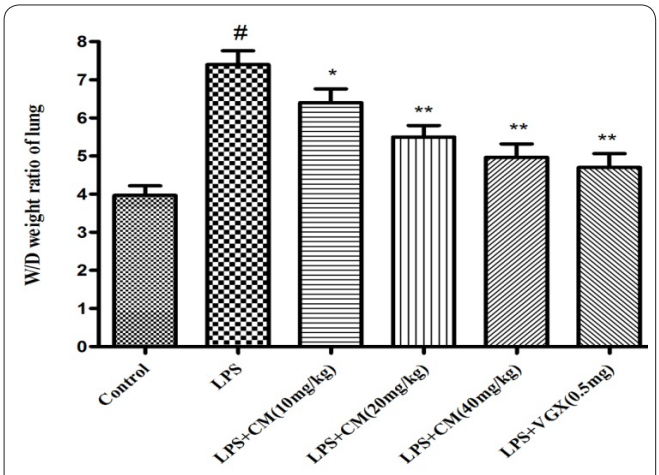

Figure 2. Effects of CM on the lung W/D weight ratio in LPS-induced ALI mice. The lung wet/dry weight ratio was measured after 6 h of LPS administration. The data showed as means ± S.D mean ± S.D (n=12 mice/group). P# <0.01 compared to that of control group, P*<0.05, P**<0.01compared with LPS group.Statistically significant differences between groups were analyzed using one-way ANOVA followed by Student-Newman-Keuls post-hoc test using the GraphPad Prism version 5.0.

CM Inhibited LPS-Induced inflammatory cell infiltration in the bronchoalveolar lavage fluid (BALF)

In the current study, anti-inflammatory action of CM was further evaluated on LPS induced neutrophils, macrophages and total cell number in bronchoalveolar lavage fluid (BALF). As shown in Figure4, neutrophils, macrophages and numbers of total cells were increased in the LPS group compared to the control group. Moreover, CM and VGX-1027 significantly reduced the neutrophils (A), macrophages (B) and total cell number (C) in the BALF (Figure 4).

Effects of CM on inflammatory mediators in BALF

The anti-inflammatory effects of CM were measured on LPS-induced inflammatory cytokines. Here, the level of TNF-α, IL-1β, IL-6, IL-10, IL-13, and MIF concentrations were determined using ELISA kits. As results shown in Figure 5, LPS-induced inflammatory mediators TNF-α (A), IL-1β (B), IL-6 (C), IL-13 (E), and MIF(F) production were significantly inhibited by CM and VGX-1027. However, CM also raised the IL-10 (D) in BALF (Figure 5).

Effects of CM on LPS-induced TLR4 expression and NF-κBactivation

To study the anti-inflammatory mechanism of CM, TLR4 and NF-κB expressions were assessed in the lung tissues of LPS induced ALI mice. As shown in Figure 6, LPS significantly increased TLR4 and c expressions (P#<0.01). However, CM significantly suppressed the increased TLR4(B) and NF-κBp65(C) expression and IκBα degradation (D) (Figure 6).

Discussion

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)are the critical illness syndrome

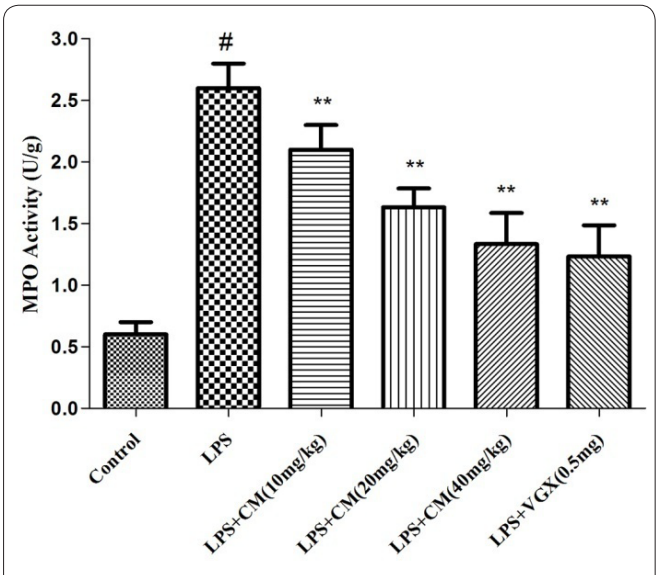

Figure 3. CM effects on MPO activity in LPS-induced ALI mice. MPO activity was measured in lung homogenate at 6 h after LPS administration. The data presented as mean ± S.D (n=12 mice/group). P#<0.01 compared with the control group, P*<0.05, P**<0.01 compared with LPS group.Statistically significant differences between groups were analyzed using one-way ANOVA followed by Student-Newman-Keuls post-hoc test using the GraphPad Prism version 5.0.

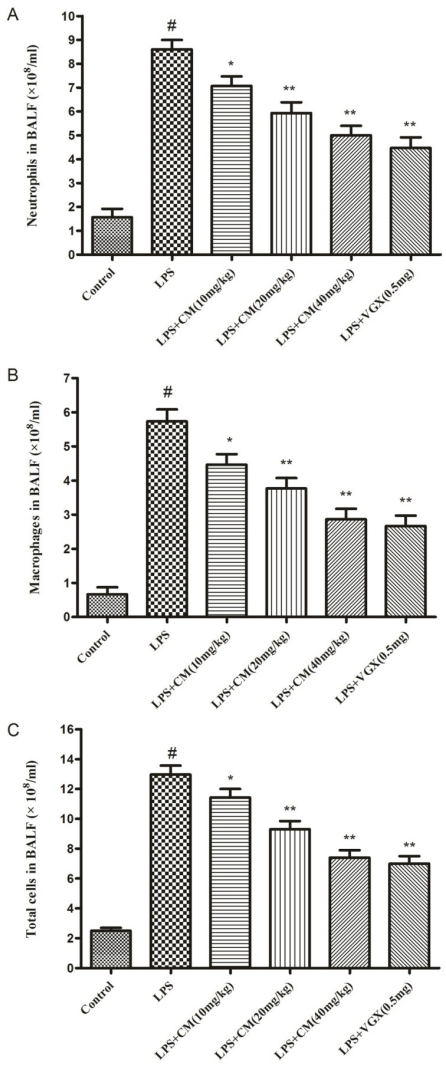

Figure 4. Effects of CM on LPS induced inflammatory cells infiltration in the BALF.The data values expressed as mean ± S.D (n=12 mice/group). P#<0.01 compared with control group, P*<0.05, P**<0.01 compared with LPS group.Statistically significant differences between groups were analyzed using one-way ANOVA followed by Student-Newman-Keuls post-hoc test using the GraphPad Prism version 5.0.

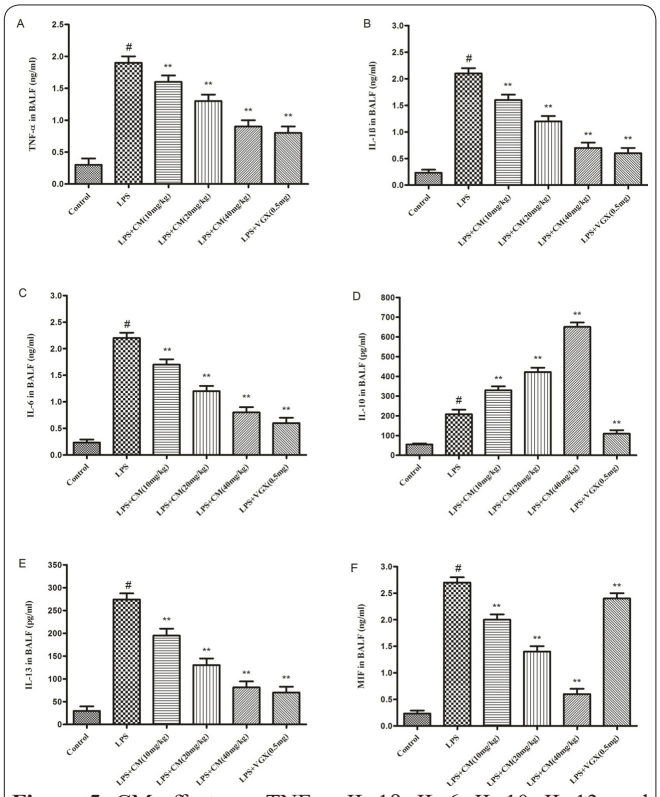

Figure 5. CM effects on TNF-α, IL-1β, IL-6, IL-10, IL-13, and MIF in the BALF of LPS-induced ALI mice.TNF-α, IL-1β, IL-6, IL-10, IL-13, and MIF in the BALF were analyzed by ELISA. The data presented as mean ± S.D (n=12 mice/group. P#<0.01 compared with control, P*<0.05, P**<0.01 compared with LPS. Statistically significant differences between groups were analyzed using one-way ANOVA followed by Student-Newman-Keuls post-hoc test using the GraphPad Prism version 5.0.

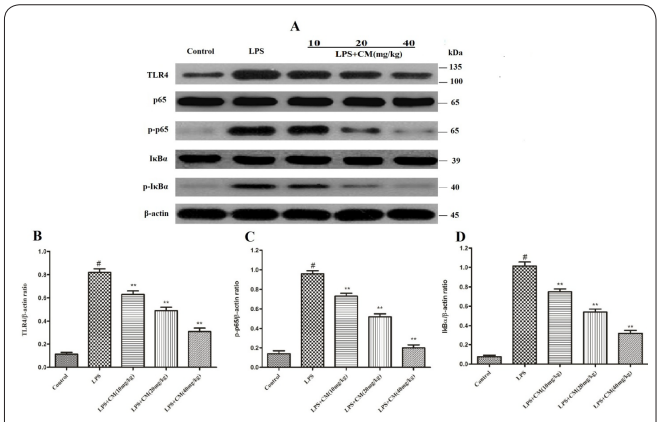

Figure 6. CM Inhibits the LPS-induced TLR4 expression, NF-κB activation and IκBα degradation. The data presented as mean ± S.D (n=12 mice/group). P#<0.01 compared with those in the control group, P*<0.05, P**<0.01 compared with LPS group.Statistically significant differences between groups were analyzed using one-way ANOVA followed by Student-Newman-Keuls post-hoc test using the GraphPad Prism version 5.0.

of acute respiratory failure, that is resulted from sepsis, multiple transfusions, trauma, and severe pulmonary infections (1). It is mainly associated with bilateral alveolar infiltrates, disruption of the alveolar epithelium, protein-rich fluid edema and hypoxemia (17). Despite presence of many advance supportive treatments (like protective ventilation and nutritional supports), the incidence and mortality are still very high (30%-50%) (5, 6, 7). Due to absence of effective pharmacological treatment for ALI, more research is still required to discover a potential therapeutic agent to treat ALI/ARDS.

Cinnamaldehyde is anactiveconstituent from volatile oil ofcinnamon, isolated from the stem bark of *Cinnamomum cassia*, which exhibit many biological effects such asanti-bacterial, anti-inflammatory, anti-oxidant, anti-ulcer, anti-diabetic, and anti-tumor effects (13, 14). Cinnamaldehyde is associated with its inhibitory properties on cytokines products (such as TNFα, IL-6, IL-1β, and IL-8)induced inflammation. Many studies also support its antioxidant action via suppression of NF-κB activation (14, 15). It is confirmed that Cinnamaldehydehad many therapeutics effects with low toxicity.However, there are still no researches that Cinnamaldehyde may have therepeutic effects on inflammation after acute lung injury. Thus, in current study, we investigated an anti-inflammatory effect of cinnamaldehyde in LPS-induced lung injury.

LPS is one of the major potent bioactivator of macrophagesthat induce the acute lung injury (8). LPSstimulates the TLR4 which activates NF-κB signaling pathway and increase the release of pro-inflammatory

mediators (9, 10). The release of tumor necrosis factor (TNFα), interleukin IL-6, IL-1β, and IL-8 are the key inflammatory mediators and play a critical role in inflammation-induced lung injury (9, 10). LPS-induced ALI mice model was used to evaluate the protective effects of CM on the inflammatory mediators; edema and histopathology changes in lungs.

During our histological evaluation, characteristic severe pathological changes were noted after 6 h of LPS challenge, including alveolar wall thickening, inflammatory cells infiltration, hyaline membrane formation, fibrosis, interstitial edema, and some patchy hemorrhage. We found that CMmarkedly attenuated inflammatory cells infiltration and thereby improvedtypical and severe pathological alterations in the lung tissues (Figure1). ALI and ARDS are generallymanifested by pulmonary protein-rich fluid edema, and increased permeability of alveolar capillary barrier in the lung (18). In present study, we measured the wet/dry weight ration as an index of pulmonary edema. We examinedthe lung edema by measuring the lung W/D weight ratio. The results revealed that CM significantly reduced LPS-induced pulmonary edema in lung of mice(Figure2). Myeloperoxidase (MPO) activity was measured to determine the activation and infiltration of neutrophils and macrophage in the lung tissues. After 6 h of LPS challenge, the MPO activity was significantly increased in comparison to the control group. However, this MPO activity was markedly reduced by CM (10mg, 20mg, and 40mg/kg) as shown Figure3.

Various studies confirmed that over-productions or infiltration of inflammatory cells (specifically neutrophils and macrophages) could cause directly or indirectly alveolar epithelium and microvascular endothelium injuries in LPS induced ALI (19, 20, 21, 22). Our results showed that LPS significantly increased the neutrophils, macrophages and total cells in BALF, though these inflammatory cells count remarkably attenuated by CM (Figure4).

It has been reported thatALI is provoked by pro-inflammatory cytokines,including TNF-α, IL-1β, IL-6 and Macrophage migration inhibitory factor (MIF) production, which consider to play key role in the pathogenesisof ALI/ARDS (23). Tumor Necrosis Factor (TNF-α) is generally produced by monocytes and macrophages, which triggers the pro-inflammatory signaling cascades, to lead damage of vascular endothelial cells (24, 25, 26). It is known to propagate the extension inflammatory process.Macrophage migration inhibitory factor (MIF) is rapidly expressed by monocytes and macrophages in response to bacterial toxins and pro-inflammatory mediators that promotes inflammatory responses. It is also widely accepted that MIF is implicated in the pathogenesis of ALI/ARDS (27, 28). The active cytokines IL-6 and IL-1β areancrucial endogenous mediator, associated with LPS-induced inflammation (29, 30). IL-10 is an immuno-modulatory, prevents the LPS-induced endotoxemia via suppressing the TNF-α production (29, 30, 31). It is considered to show a protective action against the LPS induced ALI mice.

Our current finding demonstrated thatrelease of TNF-α, IL-1β, IL-6, IL-13 and MIF production markedly inhibited byCM inBALF of LPS-induced mice. Furthermore, the release of anti-inflammatory cytokines IL-10 also regulated by CM treatment (Figure5). Therefore, our dataconfirm the protective effects of CMbyup-regulation of the pro-inflammatory cytokines in the lungs.

Nuclear transcription factor (NF-κB), is a nuclear protein, considered to be a regulator of inflammatory process. It is well confirmed that NF-κB stimulates the transcription of inflammatory cytokines and plays a crucial role in pathogenesis of lung inflammation (32). NF-κB(p50/p65) is normally located in the cytoplasm as inactive form, and considered to be associated with an inhibitor of IκB protein (32, 33). The LPS activated NF-κB,leads phosphorylation-mediated degradation of IκB. The phosphorylation induced degradation of IκB enables the translocation ofNF-κBinto nucleus and activate gene transcription of inflammatory cytokine (32, 33).

In present study, we found that increased TLR4 and NF-κB p65 expression and IκBα degradation, were significantly suppressedby CM in LPS induced ALI mice, and thereby CM showed preventive action on translocation of NF-κB into the nucleus of the lung (Figure6). TLR4 is important receptor of LPS, can activate NF-κB signaling pathway andsubsequent regulates production of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-6 and IL-1β (11, 12). Various studies shown that TLR4 triggers the NF-κB signaling pathway to release of inflammatory mediators, associated with acute lung injury (ALI) induced by LPS (11,12,34,35). It is widely accepted that TLR4–NF-κB signaling pathway is implicated in the pathogenesis of ALI/ARDS(9, 10). Therefore, we assessed the effects of CM on TLR4 expression in LPS induced ALI mice. The results revealed that CM inhibited TLR4 expression in LPS induced ALI mice (Figure6).

In conclusion, our finding demonstrated the anti-inflammatory and protective effect of Cinnamaldehyde on lipopolysaccharide induced acute lung injury in mice.Theanti-inflammatory mechanism of Cinnamaldehyde was reported through suppression of TLR4-dependent NF-κBactivation.The results revealed that Cinnamaldehyde could be potential drug in acute lung injury.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. UdobiKF, ChildsE, Touijer K. Acute respiratory distress syndrome. AmFam Physician 2003; 67: 315-322.

2. AshbaughDG, BigelowDB, PettyTL, LevineBE. Acute respiratory distress in adults. Lancet 1967; 2: 319-323.

3. BernardGR, ArtigasA, BrighamKL, CarletJ, Falke K, HudsonL, LamyM, LeGallJR, MorrisA, Spragg R. Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. The Consensus Committee. Intensive Care Med 1994; 20: 225-232.

4. Rubenfeld GD, CaldwellE, PeabodyE, WeaverJ, MartinDP, NeffM, SternEJ, Hudson LD. Incidence and outcomes of acute lung injury. N Engl J Med 2005; 353: 1685-1693.

5. Ketoconazole for early treatment of acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. The ARDS Network. JAMA 2000; 283: 1995-2002.

6. Kollef MH, SchusterDP. The acute respiratory distress syndrome. N Engl J Med 1995; 332: 27-37.
7. MilbergJA, DavisDR, SteinbergKP, HudsonLD. Improved survival of patients with acute respiratory distress syndrome (ARDS): 1983-1993. JAMA 1995; 273: 306-309.
8. RoncoC. Lipopolysaccharide (LPS) from the cellular wall of Gram-negative bacteria, also known as endotoxin, is a key molecule in the pathogenesis of sepsis and septic shock. Preface. Blood Purif 2014; 37: 1
9. JiangQ, YiM, GuoQ, WangC, WangH, MengS, LiuC, FuY, JiH, ChenT. Protective effects of polydatin on lipopolysaccharide-induced acute lung injury through TLR4-MyD88-NF-kappaB pathway. IntImmunopharmacol 2015; 29: 370-376.
10. BeduschiMG, GuimaraesCL, BussZS,DalmarcoEM. Mycophenolatemofetil has potent anti-inflammatory actions in a mouse model of acute lung injury. Inflammation 2013; 36: 729-737.
11. TauseefM, KnezevicN, ChavaKR, SmithM, SukritiS, GianarisN, ObukhovAG, VogelSM, SchraufnagelDE, DietrichA, BirnbaumerL, MalikAB, MehtaD. TLR4 activation of TRPC6-dependent calcium signaling mediates endotoxin-induced lung vascular permeability and inflammation. J Exp Med 2012; 209: 1953-1968.
12. JohEH, GuW, KimDH. Echinocystic acid ameliorates lung inflammation in mice and alveolar macrophages by inhibiting the binding of LPS to TLR4 in NF-kappaB and MAPK pathways. BiochemPharmacol 2012; 84: 331-340.
13. KhoddamiA, WilkesMA, RobertsTH. Techniques for analysis of plant phenolic compounds. Molecules 2013; 18: 2328-2375.
14. RaoPV, GanSH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med 2014; 2014: 642942.
15. ChaoLK, HuaKF, HsuHY, ChengSS, LinIF, ChenCJ, ChenST, ChangST. Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. Food ChemToxicol 2008; 46: 220-231.
16. LinCC, WuSJ, ChangCH, NgLT. Antioxidant activity of Cinnamomum cassia. Phytother Res 2003; 17: 726-730.
17. JohnsonER, MatthayMA. Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv 2010; 23: 243-252.
18. MatthayMA, ZimmermanGA. Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. Am J Respir Cell MolBiol 2005; 33: 319-327.
19. JanzDR, Ware LB. Biomarkers of ALI/ARDS: pathogenesis, discovery, and relevance to clinical trials. SeminRespirCrit Care Med 2013; 34: 537-548.
20. WareLB. Autopsy in ARDS: insights into natural history. Lancet Respir Med 2013; 1: 352-354.
21. ZhuT, WangDX, ZhangW, LiaoXQ, GuanX, BoH, SunJY, HuangNW, HeJ, Zhang, YK, TongJ, LiCY. Andrographolide protects against LPS-induced acute lung injury by inactivation of NF-kappaB. PLoS One 2013; 8: e56407.
22. JinLY, LiCF, Zhu GF, WuCT, WangJ, YanSF. Effect of siRNA against NF-kappaB on sepsisinduced acute lung injury in a mouse model. Mol Med Rep 2014; 10: 631-637.
23. BozzaM, SatoskarAR, LinG, LuB, HumblesAA, GerardC, DavidJR. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. J Exp Med 1999; 189: 341-346.
24. MukhopadhyayS, HoidalJR, MukherjeeTK. Role of TNFalpha in pulmonary pathophysiology. Respir Res 2006; 7: 125.
25. PatelBV, WilsonMR, O'DeaKP,TakataM. TNF-induced death signaling triggers alveolar epithelial dysfunction in acute lung injury. J Immunol 2013; 190: 4274-4282.
26. CollinsT, ReadMA, NeishAS, WhitleyMZ, ThanosD, ManiatisT. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. FASEB J 1995; 9: 899-909.
27. MawhinneyL, ArmstrongME, O' ReillyC, BucalaR, LengL, Fingerle-RowsonG, FayneD, KeaneMP, TynanA, MaherL, CookeG, LloydD, ConroyH, DonnellySC. Macrophage migration inhibitory factor (MIF) enzymatic activity and lung cancer. Mol Med 2014; 20: 729-735.
28. KorsgrenM, Kallstrom L, UllerL, BjerkeT, SundlerF, PerssonCG, KorsgrenO. Role of macrophage migration inhibitory factor (MIF) in allergic and endotoxin-induced airway inflammation in mice. Mediators Inflamm 2000; 9: 15-23.
29. GoodmanRB, PuginJ, LeeJS, MatthayMA. Cytokine-mediated inflammation in acute lung injury. Cytokine Growth Factor Rev 2003; 14: 523-535.
30. CrossLJ, MatthayMA. Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury. Crit Care Clin 2011; 27: 355-377.
31. GerardC, BruynsC, MarchantA, AbramowiczD, VandenabeeleP, DelvauxA, FiersW, GoldmanM, VeluT. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 1993; 177: 547-550.
32. ZhangY, YanR, HuY. Oxymatrine inhibits lipopolysaccharide-induced inflammation by down-regulating Toll-like receptor 4/nuclear factor-kappa B in macrophages. Can J PhysiolPharmacol 2015; 93: 253-260.
33. Rahman I, MacNeeW. Role of transcription factors in inflammatory lung diseases. Thorax 1998; 53: 601-612.
34. KaishoT, AkiraS. Toll-like receptor function and signaling. J Allergy ClinImmunol 2006; 117: 979-987.
35. YangHZ, WangJP, MiS, LiuHZ, CuiB, YanHM, YanJ, LiZ, LiuH, HuaF, LuW, HuZW. TLR4 activity is required in the resolution of pulmonary inflammation and fibrosis after acute and chronic lung injury. Am J Pathol 2012; 180: 275-292.