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Tanshinone IIA ameliorates acute lung injury by inhibition of the NLRP3 inflammasome

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Abstract: Tanshinone IIA is the phenanthrenequinone derivative extracted from the perennial plant *Salvia miltiorrhiza* Bunge (red sage). We investigated whether inhibition of the nucleotide-binding oligomerization domain (NOD)-like receptor family protein 3 (NLRP3) inflammasome mediates the protective effect of tanshinone IIA in acute lung injury (ALI) induced in rats by oleic acid (OA) injection. Compared with the control treatment, OA injection induced pulmonary histological impairment, increased the lung wet/dry weight ratio (7.0±1.1 vs 4.3±0.6) and CO_2 partial pressure ($PaCO_2$) (52±6.4 vs 40±3.6 mmHg), decreased arterial O_2 partial pressure (PaO_2) (63±8.4 vs 100±3.0 mmHg), and increased tumor necrosis factor α (TNFα) (8.8±2.3 vs 5.2±1.5 pg/mL), monocyte chemoattractant protein-1 (MCP-1) (36.1±4.9 vs 25.2±6.6 pg/mL) and interleukin-1β (IL-1β) (15.9±3.2 vs 4.6±1.3 pg/mL) in the bronchoalveolar lavage (BAL) fluid. Tanshinone IIA provided protection against ALI, observed as a reduction in the lung wet/dry weight ratio and CO_2 partial pressure, and increased O_2 partial pressure. The cytokine increase was also prevented. Tanshinone IIA attenuated increased protein levels of NLRP3, caspase-1 and IL-1β in pulmonary tissues, suggesting that it ameliorates ALI by preventing NLRP3 inflammasome activation.

Keywords: tanshinone IIA; acute lung injury; inflammation; NLRP3; inflammasome

INTRODUCTION

Acute lung injury (ALI) is a clinical disorder responsible for one of the major causes of in-hospital hypoxemic respiratory insufficiency, resulting in morbidity and mortality [1]. ALI features impairs the permeability of the alveolar-capillary barrier, followed by pulmonary edema with fluid containing a large amount of proteins, resulting in hypoxemia [2]. Despite marked improvement in the treatment of ALI, ALI-caused mortality is high [3]. Therefore, investigating the mechanism of pulmonary damage in ALI should help improve the clinical treatment.

The main pathology in ALI is observed as a disturbed balance between pro- and anti-inflammatory processes, accompanied by stimulation of apoptosis [4,5]. IL-1β is one of the early inflammatory cytokines induced in ALI that causes the secretion of other cytokines [6]. IL-1β is activated by the NLRP3 inflammasome [7]. Inflammasome, comprised of NLRP3, apoptosis-associated speck-like protein and procaspase-1, is an important intracellular multiprotein complex of the innate immune system, which is increased in pulmonary tissue. Upon stimulation, the NLRP3 inflammasome cleaves procaspase-1 to produce caspase-1, which mediates the generation of the biologically active form of IL-1β from pro-IL-1β [8-10]. Numerous studies have demonstrated the essential role of inflammasome in ALI [11-14]. Prevention of the activation of the NLRP3 inflammasome is a potential target for treating ALI [15,16].

Tanshinone IIA is the phenanthrenequinone derivative extracted from *Salvia miltiorrhiza* Bunge that is extensively used in Chinese traditional medicine for treating several diseases. Tanshinone IIA has various biological effects, such as anti-inflammatory and antioxidant effects. The protective effect of tanshinone IIA against ALI has been verified in animal models of ALI induced by lipopolysaccharide (LPS) [17-20], seawater exposure [21, 22] and paraquat [23]. However, the mechanism of the ameliorative effect of tanshinone IIA on ALI has not been fully investigated.

Tanshinone IIA was found to inhibit activation of inflammasome and to ameliorate cardiac damage in canines with myocardial ischemia-reperfusion injury [24]. These results suggested an attenuating effect of tanshinone IIA on inflammasome. In view of the major role of NLRP3 in ALI progression, we hypothesized that tanshinone IIA might ameliorate ALI by inhibiting inflammasome. We used the ALI model induced by oleic acid (OA) in rats to examine the effect of tanshinone IIA on inflammasome.

MATERIALS AND METHODS

Animals and experimental procedure

Male Sprague-Dawley (SD) rats (250±10 g) were obtained from Vital River (Beijing, China). The rats were housed under standard conditions with 12-h light/dark cycles (lights on at 6:00), controlled temperature (28~30°C) and free access to food and tap water. All animal procedures were in compliance with the Animal Management Rule of the Ministry of Health, People's Republic of China (documentation No. 55, 2001) and the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the Animal Care Committee of Taizhou People's Hospital.

SD rats were randomly divided into four groups as follows: control, tanshinone IIA, OA, OA+tanshinone IIA. Control rats were intravenously injected with physiological saline (1 mL/kg body weight); OA rats were intravenously injected with OA to induce lung injury (125 μL/kg body weight in 1 mL saline; Sigma-Aldrich, St Louis, MO, USA). Tanshinone IIA (10 mg/kg body weight; Xian Guanyu Bio-tech, Xian, China) was intravenously injected or injected 30 min before the injection of OA. The rats were killed 6 h after OA treatment.

Blood and lung tissue sampling

Arterial blood samples (300 μL) were collected from the abdominal aorta of rats after sacrifice into a heparinized syringe for examining arterial PaO_2 and $PaCO_2$ in the lung by blood gas analysis. The lungs were excised, the right lung was weighed to measure the lung wet/dry (W/D) weight ratio to assess edema, and the left lung was cut into two parts. The first part was fixed in 10% formalin to observe lung injury by hematoxylin-eosin (H&E) staining by light microscopy. The second part was stored at -80°C for protein extraction.

Bronchoalveolar lavage (BAL) preparation

After blood collection, a cannula was inserted into the trachea. The lungs were lavaged three times with 5 mL phosphate buffered saline (PBS; pH 7.4) to obtain BAL fluid. The protein content of BAL fluid was estimated [25], and the presence of cytokines (TNFα, MCP-1 and IL-1β) using a Bio-Plex Kit (Bio-Rad, Hercules, CA) was determined.

Lung histology

Specimens fixed in 10% formalin were dehydrated, cut into 5-μm-thick sections and stained with H&E for routine histology.

Lung W/D weight ratio

The wet weight of right lungs was recorded immediately after removal. The lungs were then placed in an incubator at 60°C for 72 h and the dry weight was measured. The W/D lung ratio was presented as W/D lung mass, which shows the fraction of wet lung weight caused by water.

Western blot analysis

Pulmonary tissue homogenate was centrifuged at 12000 ×*g* for 15 min and the supernatant was mixed with 5 × loading buffer (Applygen Technologies Inc. Beijing, CN). The mixture was boiled for 10 min and cooled to room temperature. Protein concentration was estimated with a Bradford protein assay kit. Equal amounts of protein (20~100 μg) were loaded on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels (PAG), transferred to nitrocellulose membranes and incubated with NLRP3, caspase-1 and IL-1β (all 1:1000, Abcam, Cambridge, UK). Secondary antibodies (Kirkegaard & Perry Laboratories Inc., Gaithersburg, US) were used at 1:5000 dilution. Chemiluminescence was exposed to X-ray film (Kodak Scientific) and the blots were scanned and quantified using NIH ImageJ.

Statistical analysis

Statistical analysis involved the use of GraphPad Prism ver. 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Data are expressed as the mean±SD. Comparisons among more than 2 groups were analyzed by one-way ANOVA followed by the Newman-Keuls test. P<0.05 was considered statistically significant.

RESULTS

Effect of tanshinone IIA on the histological indicators of injury in OA-treated rats

Controls and tanshinone IIA-treated rats showed normal lung architecture, open alveoli and thin alveolar walls (Fig. 1A and B). Rats with OA-induced ALI showed widespread inflammatory infiltration, thickened alveolar walls and septa, with hemorrhaging and a proteinaceous exudate filling some alveoli (Fig. 1C). The pathogenesis of lung injury was ameliorated with tanshinone IIA treatment, with reduced infiltration (Fig. 1D).

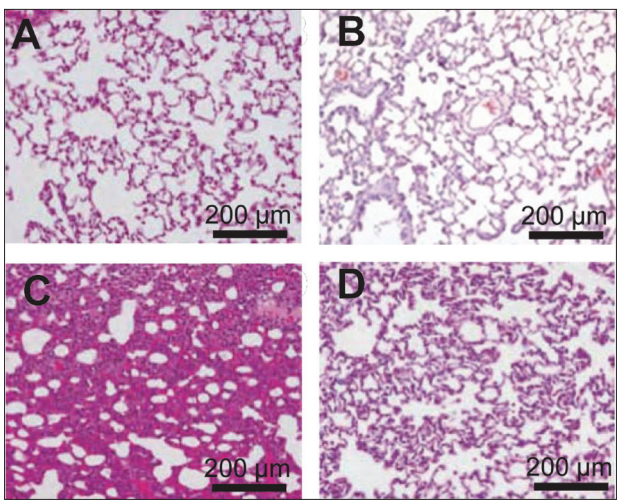

Fig. 1. Effect of tanshinone IIA on histological injury in acute lung injury (ALI) induced by oleic acid (OA) in rats. Tanshinone IIA significantly ameliorated OA-induced pulmonary histological pathology. Representative HE staining (200×) of lung tissue in rats: control group (**A**), tanshinone IIA (Tan) group (**B**), OA group (**C**) and OA+Tan group (**D**); n = 8 in each group.

Effect of tanshinone IIA on pulmonary impairment in OA-induced ALI

As compared to the controls, ALI rats showed a significantly increased ratio in W/D lung weight (Fig. 2A), decreased PaO_2 (Fig. 2B) and increased $PaCO_2$ (Fig. 2C) (all p<0.05). Compared to the group administered OA alone, in the OA+tanshinone IIA group the ratio of W/D lung weight was significantly decreased (Fig. 2A), the PaO_2 was increased (Fig. 2B) and the $PaCO_2$ was decreased (Fig. 2C) (all p<0.05). The ratio of W/D lung weight (Fig. 2A), PaO_2 (Fig. 2B) and $PaCO_2$ (Fig. 2C) were similar in the tanshinone IIA-treated and control groups (all p>0.05).

Effect of tanshinone IIA on inflammatory cytokines in BAL fluid

As compared with the controls, the OA treatment significantly increased the levels of TNFα, MCP-1 and IL-1β in the BAL fluid (Fig. 3, p<0.05). The tanshinone IIA treatment reversed the OA-induced protein levels (Fig. 3, p<0.05). The levels of TNFα, MCP-1 and IL-1β were similar in tanshinone IIA-treated and control groups (all p>0.05).

Tanshinone IIA reduced OA-activation of the NLRP3 inflammasome in rat lung

Compared with the controls, the OA treatment significantly increased the protein levels of NLRP3, caspase-1 and IL-1β (Fig. 4, $p < 0.05$). Tanshinone IIA treatment reduced the OA-induced elevation of NLRP3, caspase-1 and IL-1β (Fig. 4, p<0.05). Administration of tanshinone IIA alone in rats had no effect on protein levels of NLRP3, caspase-1 and IL-1β (Fig. 4, p<0.05).

DISCUSSION

We report on the ameliorative effect of tanshinone IIA in OA-induced ALI in rats, which may be mediated by the inhibition of inflammasome activation. Tanshinone IIA significantly ameliorated OA-induced pulmonary histological pathology, edema, impaired gas exchange, and it increased the levels of cytokines in BAL fluid. The protein levels of NLRP3, caspase-1 and IL-1β in pulmonary tissues were increased in OA-treated rats and attenuated by tanshinone IIA treatment.

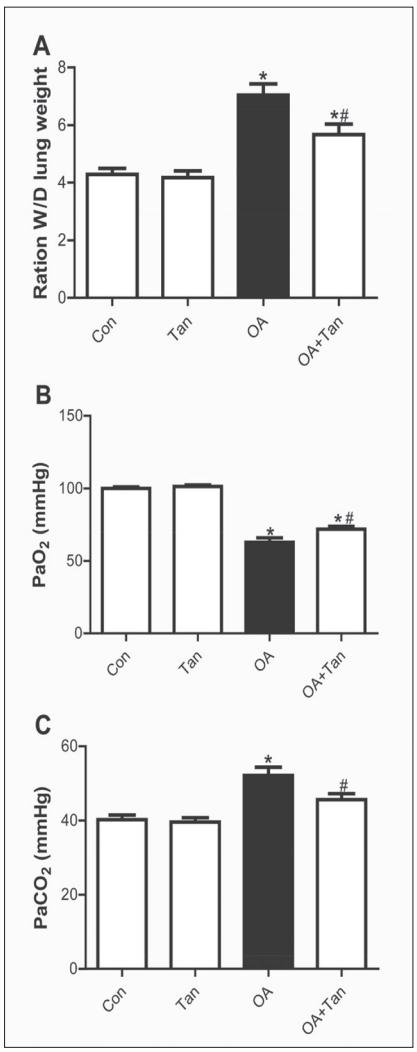

Fig. 2. Effect of tanshinone IIA on pulmonary impairment in OA-induced ALI. Tanshinone IIA significantly ameliorated OA-induced pulmonary edema and impairment of gas exchange. **A** – Ratio of W/D lung weight; **B** – PaO_2; **C** – $PaCO_2$; Con – control, Tan – tanshinone IIA, OA – OA and OA+Tan – OA plus tanshinone IIA groups; *, p<0.05 vs. control; #, p<0.05 vs. OA; n=8 in each group.

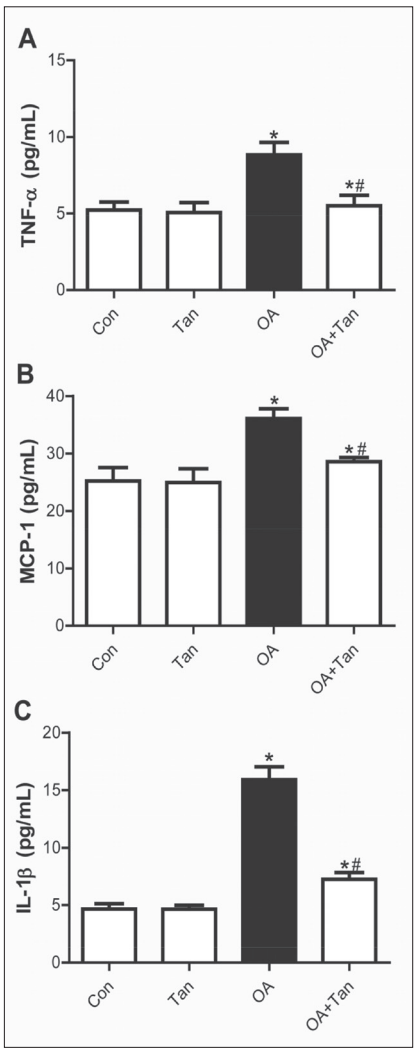

Fig. 3. Effect of tanshinone IIA on inflammatory cytokines in bronchoalveolar lavage (BAL) fluid. Tanshinone IIA significantly ameliorated OA-induced increased levels of cytokines in BAL fluid. **A** – TNF-α; **B** – MCP-1; **C** – IL-1β; *, p<0.05 vs control; #, p<0.05 vs. OA; n=8 in each group.

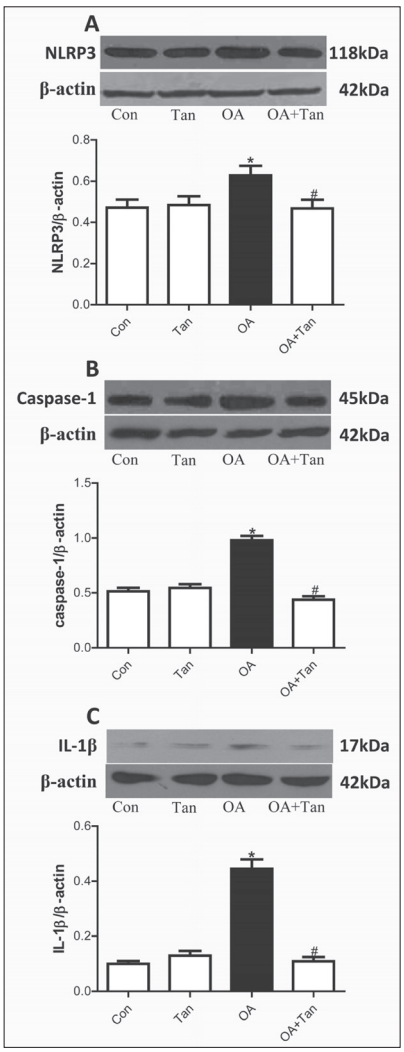

Fig. 4. Tanshinone IIA prevented OA-activated NLRP3 inflammasome in rat lung. The protein levels of NLRP3, caspase-1 and IL-1β detected by Western blotting of pulmonary tissues were increased in OA-treated rats and were attenuated by tanshinone IIA treatment. **A** – NLRP3; **B** – caspase-1; **C** – IL-1β. *, p<0.05 vs control; #, p<0.05 vs OA; n=8 in each group.

The protective effect of tanshinone IIA on lung injury has been confirmed in different animal models, including lung injury induced by LPS [17-20], seawater exposure [21, 22] and paraquat [23]. In the OA-induced ALI model in rats, we demonstrated the ameliorative effect of tanshinone IIA on ALI, confirmed by an alleviation of pathological histological indicators, pulmonary edema and gas exchange, and decreased levels of inflammatory cytokines in BAL fluid. From our and previous results, tanshinone IIA could be an effective agent against ALI.

To further investigate the mechanism of tanshinone IIA in ameliorating ALI, we examined the protein levels of NLRP3, caspase-1 and IL-1β in pulmonary tissues, which showed activation of inflammasome. The essential role of the NLRP3 inflammasome in ALI has been investigated [11-14], and it has been considered a novel target for ALI treatment [15,16]. We found that

tanshinone IIA significantly reduced the OA-increased protein levels of NLRP3, caspase-1 and IL-1β in pulmonary tissues, suggesting that tanshinone IIA might ameliorate ALI by inhibiting inflammasome activation.

One limitation of our work was the inability to confirm the causal role of inflammasome involved in the ameliorative effect of tanshinone IIA. Hu et al. [24] also reported that tanshinone IIA inhibited the increase in inflammasome in the myocardium induced by ischemia-reperfusion, which might mediate the cardioprotective effect of tanshinone IIA. Therefore, further research should investigate the direct inhibitory effect of tanshinone IIA on the NLRP3 inflammasome and whether the protective effect of tanshinone IIA against ALI can be blunted when controlling inflammasome activation by genetic methods or agonist treatment.

CONCLUSION

Our results demonstrate that tanshinone IIA significantly ameliorated OA-induced ALI. OA-activated inflammasome in pulmonary tissues was attenuated by the tanshinone IIA treatment. Considering the essential role of the NLRP3 inflammasome in the progression of ALI, tanshinone IIA might ameliorate ALI by inhibiting inflammasome. These results point to a novel strategy for ALI therapy.

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Author contributions: Tianyu Chen designed and performed the experiments, analyzed the data and wrote the manuscript; Shaoyun Qin and Ying Dai designed and performed the experiments.

Conflict of interest disclosure: The authors declare no conflicts of interest.

REFERENCES

1. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD. Incidence and outcomes of acute lung injury. N Engl J Med. 2005;353:1685-93.
2. Bhattacharya J, Matthay MA. Regulation and Repair of the Alveolar-Capillary Barrier in Acute Lung Injury. Annu Rev Physiol. 2013;75:593-615.
3. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF, Ranieri M, Rubenfeld G, Thompson BT, Wrigge H, Slutsky AS, Pesenti A; LUNG SAFE Investigators; ESICM Trials Group. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. JAMA. 2016;315:788-800.
4. Malhotra A. Low-tidal-volume ventilation in the acute respiratory distress syndrome. N Engl J Med. 2007;357:1113-20.
5. Ware LB. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Semin Respir Crit Care Med. 2006;27:337-49.
6. Ganter MT, Roux J, Miyazawa B, Howard M, Frank JA, Su G, Sheppard D, Violette SM, Weinreb PH, Horan GS, Matthay MA, Pittet JF. Interleukin-1β causes acute lung injury via αvβ5 and αvβ6 integrin-dependent mechanisms. Circ Res. 2008;102:804-12.
7. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002;10:417-26.
8. Gross O, Thomas CJ, Guarda G, Tschopp J. The inflammasome: an integrated view. Immunol Rev. 2011;243:136-51.
9. Schenten D, Medzhitov R. The control of adaptive immune responses by the innate immune system. Adv Immunol. 2011;109:87-124.
10. Hosseinian N, Cho Y, Lockey RF, Kolliputi N. The role of the NLRP3 inflammasome in pulmonary diseases. Ther Adv Respir Dis. 2015;9:188-97.
11. Grailer JJ, Canning BA, Kalbitz M, Haggadone MD, Dhond RM, Andjelkovic AV, Zetoune FS, Ward PA. Critical role for the NLRP3 inflammasome during acute lung injury. J Immunol. 2014;192:5974-83.
12. Jones HD, Crother TR, Gonzalez-Villalobos RA, Jupelli M, Chen S, Dagvadorj J, Arditi M, Shimada K. The NLRP3 inflammasome is required for the development of hypoxemia in LPS/mechanical ventilation acute lung injury. Am J Respir Cell Mol Biol. 2014;50:270-80.
13. Liu Z, Zhao H, Liu W, Li T, Wang Y, Zhao M. NLRP3 inflammasome activation is essential for paraquat-induced acute lung injury. Inflammation. 2015;38:433-44.
14. Han S, Cai W, Yang X, Jia Y, Zheng Z, Wang H, Li J, Li Y, Gao J, Fan L, Hu D. ROS-Mediated NLRP3 Inflammasome Activity Is Essential for Burn-Induced Acute Lung Injury. Mediators Inflamm. 2015;2015:720457.
15. Liu T, Zhou Y, Li P, Duan JX, Liu YP, Sun GY, Wan L, Dong L, Fang X, Jiang JX, Guan CX. Blocking triggering receptor expressed on myeloid cells-1 attenuates lipopolysaccharide-induced acute lung injury via inhibiting NLRP3 inflammasome activation. Sci Rep. 2016;6:39473.
16. Zhang Y, Li X, Grailer JJ, Wang N, Wang M, Yao J, Zhong R, Gao GF, Ward PA, Tan DX, Li X. Melatonin alleviates acute lung injury through inhibiting the NLRP3 inflammasome. J Pineal Res. 2016;60:405-14.
17. Shi XM, Huang L, Xiong SD, Zhong XY. Protective effect of tanshinone II A on lipopolysaccharide-induced lung injury in rats. Chin J Integr Med. 2007;13:137-40.
18. Xu M, Dong MQ, Cao FL, Liu ML, Wang YX, Dong HY, Huang YF, Liu Y, Wang XB, Zhang B, Zhao PT, Luo Y, Niu W, Cui Y, Li ZC. Tanshinone IIA reduces lethality and acute lung injury in LPS-treated mice by inhibition of PLA2 activity. Eur J Pharmacol. 2009;607:194-200.

19. Xu M, Cao F, Liu L, Zhang B, Wang Y, Dong H, Cui Y, Dong M, Xu D, Liu Y, Zhao P, Niu W, Li Z. Tanshinone IIA-induced attenuation of lung injury in endotoxemic mice is associated with reduction of hypoxia-inducible factor 1α expression. Am J Respir Cell Mol Biol. 2011;45:1028-35.
20. Xu M, Cao FL, Zhang YF, Shan L, Jiang XL, An XJ, Xu W, Liu XZ, Wang XY. Tanshinone IIA therapeutically reduces LPS-induced acute lung injury by inhibiting inflammation and apoptosis in mice. Acta Pharmacol Sin. 2015;36:179-87.
21. Li J, Xu M, Fan Q, Xie X, Zhang Y, Mu D, Zhao P, Zhang B, Cao F, Wang Y, Jin F, Li Z. Tanshinone IIA ameliorates seawater exposure-induced lung injury by inhibiting aquaporins (AQP) 1 and AQP5 expression in lung. Respir Physiol Neurobiol. 2011;176:39-49.
22. Li JH, Xu M, Xie XY, Fan QX, Mu DG, Zhang Y, Cao FL, Wang YX, Zhao PT, Zhang B, Jin FG, Li ZC. Tanshinone IIA suppresses lung injury and apoptosis, and modulates protein kinase B and extracellular signal-regulated protein kinase pathways in rats challenged with seawater exposure. Clin Exp Pharmacol Physiol. 2011;38:269-77.
23. Wang Y, Wu H, Niu W, Chen J, Liu M, Sun X, Li Z. Tanshinone IIA attenuates paraquat-induced acute lung injury by modulating angiotensin-converting enzyme 2/angiotensin-(1-7) in rats. Mol Med Rep. 2018;18:2955-62.
24. Hu Q, Wei B, Wei L, Hua K, Yu X, Li H, Ji H. Sodium tanshinone IIA sulfonate ameliorates ischemia-induced myocardial inflammation and lipid accumulation in Beagle dogs through NLRP3 inflammasome. Int J Cardiol. 2015;196:183-92.
25. Yoshii C,NagataN, TaoY, Suematsu R,NikaidoY,KidoM. Relationship between inflammatory cells in bronchoalveolar lavage fluid and pathologic changes in the lung interstitium. Respiration 1998;65:386–92.