

# The protective effect of lidocaine on lipopolysaccharide-induced acute lung injury in rats through NF- $\kappa$ B and p38 MAPK signaling pathway and excessive inflammatory responses

L.-J. CHEN<sup>1</sup>, Y.-B. DING<sup>2</sup>, P.-L. MA<sup>3</sup>, S.-H. JIANG<sup>4</sup>, K.-Z. LI<sup>2</sup>, A.-Z. LI<sup>2</sup>, M.-C. LI<sup>2</sup>, C.-X. SHI<sup>2</sup>, J. DU<sup>2</sup>, H.-D. ZHOU<sup>5</sup>

<sup>1</sup>Operating Room, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, China

<sup>2</sup>Department of Anesthesiology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, China

<sup>3</sup>Department of Anesthesiology, Qingdao Hiser Medical Center, Qingdao, China

<sup>4</sup>Muping District Center for Disease Control and Prevention, Yantai, China

<sup>5</sup>Department of Anesthesiology, Zhejiang Cancer Hospital, Hangzhou, China

*Lin-Jing Chen and Yong-Bo Ding contributed equally to this work*

**Abstract.** – **OBJECTIVE:** Acute lung injury is a severe disease with a high rate of mortality, leading to more important illness. We aimed at exploring the protective role and potential mechanisms of lidocaine on lipopolysaccharide (LPS)-induced acute lung injury (ALI).

**MATERIALS AND METHODS:** Sprague Dawley (SD) rats were randomly assigned to control group receiving 0.9% saline solution, LPS group treated with 4 mg/kg LPS i.p., LPS + lidocaine (treated with 4 mg/kg LPS i.p. followed by giving 1 mg/kg, 3 mg/kg, 5 mg/kg of lidocaine i.v.). Lung specimens and the bronchoalveolar lavage fluid (BALF) were collected for histopathological examination and biochemical analyze 12 h after LPS induction. The cytokines expression of TNF- $\alpha$ , IL-6 and MCP-1 was measured by ELISA. In addition, the malondialdehyde (MDA) content, the activities of total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) in lung tissues were also detected using ELISA. The protein expressions of p38, p-p38, p65, p-p65 and I $\kappa$ B were analyzed by Western blot.

**RESULTS:** The results indicated that after lidocaine treatment was able to decrease significantly wet-to-dry (W/D) ratio and ameliorate the histopathologic damage. Additionally, total protein content and the number of leukocytes in BALF significantly decreased. ELISA result indicated that the levels of TNF- $\alpha$ , IL-6 and MCP-1 in BALF were markedly suppressed. Meanwhile, the activities of T-AOC and SOD in lung tissues significantly increased, while the content of MDA significantly decreased after treatment with lidocaine. Moreover, Western blot suggested that li-

docaine inhibited phosphorylation of NF- $\kappa$ B p65 and p38 MAPK.

**CONCLUSIONS:** Therefore, lidocaine could ameliorate the LPS-induced lung injury via NF- $\kappa$ B/p38 MAPK signaling and excessive inflammatory responses, providing a potential for becoming the anti-inflammatory agent against lung injury.

*Key Words:*

Acute lung injury, Lidocaine, Lipopolysaccharide, NF- $\kappa$ B, p38 MAPK.

## Introduction

Acute lung injury (ALI) is the damage of structure and serious oxidation disorders caused by various pathogens, exhibited clinical syndrome, such as respiratory distress and dispersive lung infiltration<sup>1</sup>. ALI and its more severe form, acute respiratory distress syndrome (ARDS), are characterized by severe hypoxemia, pulmonary edema and neutrophil accumulation in the lung, which are the prominent source of morbidity and mortality in critically ill patients<sup>2,3</sup>. Sepsis is a major cause of ALI, and lipopolysaccharide (LPS), a primary component of outer membrane of gram-negative bacteria and predominant inducer of inflammatory responses to these bacteria, can reproduce the features of human ALI in mice<sup>4,5</sup>. Therefore, intratracheal administration of

LPS into lungs has been extensively accepted as an ideal pharmacological research model of ALI.

Inflammation and oxidative stress are known to play a vital effect in the pathogenesis of ALI. Various inflammatory mediators, such as monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin 16 (IL-16), have been implicated in LPS-induced ALI<sup>6</sup>. The previous study<sup>7</sup> showed that release of the mediators mentioned above was involved in the activation of NF- $\kappa$ B signaling pathway. In addition, inflammatory mediator production was also associated with activation of the p38-mitogen activated protein kinase (p38 MAPK) pathway<sup>8</sup>. Since the expression of pro-inflammatory mediators is tightly regulated at a transcriptional level via the MAPK and NF- $\kappa$ B signaling pathways<sup>9,10</sup>, the targeted inhibition of the activity of NF- $\kappa$ B and p38 MAPK has been identified as a new therapeutic method for inflammatory diseases<sup>11</sup>. Many studies have exhibited the anti-inflammatory effect of lidocaine. Lahat et al<sup>12</sup> reported that they have proved that lidocaine could suppress the expression of the pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-2 in T-cell. Previous researches<sup>13,14</sup> have reported that lidocaine, a common local anesthetic drug, has notable anti-inflammatory properties in addition to their direct anesthetic activity *in vitro*. In 2008, Gu et al<sup>15</sup> demonstrated that lidocaine could significantly decrease the phosphorylation of p38 MAPK in the activated microglia. Wang et al<sup>16</sup> reported that lidocaine exhibited anti-inflammatory activity in LPS-activated primary microglia, and the underlying mechanism might be involved in the inhibition of the NF- $\kappa$ B signal pathway. Therefore, we found that the anti-inflammatory effects of lidocaine treatment may be due to the inhibition of NF- $\kappa$ B and p38 MAPK signaling pathways in the previous works. We examined the effects of lidocaine in ALI, hypothesizing that lidocaine prevented LPS-induced ALI via suppression of p38 MAPK and NF- $\kappa$ B signaling. We observed that lidocaine inhibited ALI by anti-inflammation and anti-oxidative stress after LPS challenge. These results might demonstrate the potential of lidocaine in the treatment of ALI.

## Materials and Methods

### Animal Group

Male Sprague Dawley (SD) rats weighting 250-280 g (aged 7-8 weeks) were purchased from Ex-

perimental Animal Center of Suzhou Aiermaite Technology Co. Ltd. (SPF grade, Certificate No. SCXK20150004). All animals were treated in accordance with the Care and Use of Laboratory Animals. Rats were randomly divided into the following five groups (n=10): control group, LPS group, LPS + lidocaine (1 mg/kg, 3 mg/kg, and 5 mg/kg) groups. According to guidelines and the terms of all relevant local legislation, the LPS-induced ALI model was prepared according to the method of Heidemann and Glibetic<sup>17</sup>. In this study, the dose selected was based on the preliminary experiments. Firstly, rats were anesthetized with 10% chloral hydrate (350 mg/kg, *i.p.*) followed by receiving *i.p.* 4 mg/kg of lipopolysaccharide (2 mg/ml, Sigma-Aldrich, St. Louis, MO, USA). The 10 rats in control group received the same volume of 0.9% saline solution. Then, the rats in the LPS + lidocaine group were injected lidocaine (Sigma-Aldrich, St. Louis, MO, USA) of 1 mg/kg, 3 mg/kg, and 5 mg/kg through the tail vein 30 min after LPS administration. Meanwhile, the rats in control and LPS groups were given an equal volume of 0.9% saline solution. After LPS administration for 12 h, the rats were anaesthetized with 10% chloral hydrate and sacrificed. Then, the lungs of rats were harvested and stored in liquid nitrogen for later use.

### Bronchoalveolar Lavage Fluid (BALF) Collection and Measurement of BALF Cytokines

12 h after LPS administration, rats were anesthetized and provided with a plastic cannula inserted to the trachea. BALF was obtained by washing the airway five times with 0.7 ml ice-cold phosphate-buffered saline (PBS). The total number of leukocyte in BALF was counted using a hemocytometer. Subsequently, BALF samples were centrifuged at 1500 rpm, 4°C for 10 min, and cytospin samples were prepared from the cell pellet. The supernatant samples were used to quantify the cytokines levels of rats tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1) levels in BALF using ELISA according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Co., Nanjing, China).

### Lung Wet/Dry Ratio

Lungs were harvested 12 h after LPS challenge and weighed immediately to obtain the wet weight value. After that, lungs were dried in an incubator at 65°C for 72 h to obtain the dry weight. Finally,

the ratio of lung-wet weight to dry weight was calculated and recorded.

### **Histological Evaluation**

Lung tissues were inflated under a pressure of 23 cm H<sub>2</sub>O with 4% (v/v) paraformaldehyde and then embedded in paraffin. 4- $\mu$ m thick sections were prepared using a microtome, stained with hematoxylin and eosin (HE) solution, respectively, and observed under light microscopic. Lung injury was graded by ALI scoring system as described previously<sup>4,18</sup>. Three categories including infiltration of inflammatory cells, thickness of the alveolar walls and hemorrhage were graded in a blinded manner according to a five-point scale: 0 = minimal damage; 1 = mild damage; 2 = moderate damage; 3 = severe damage; 4 = maximal damage. The total score was calculated by adding up the individual scores of each category.

### **Quantification of T-AOC, SOD activity and MDA Content in Lung Tissue**

The malondialdehyde (MDA) content, the activities of total antioxidant capacity (T-AOC), and superoxide dismutase (SOD) in lung tissues were measured strictly using ELISA kits (Nanjing Jiancheng Co., Nanjing, China) according to the manufacture's instructions.

### **Western Blotting Analysis**

Lung tissues were harvested at 12 h after LPS administration; then, non-pulmonary tissues were cleared and frozen at -80°C. Tissue samples were homogenized with RIPA lysis buffer (SolarBio, Beijing, China) and centrifuged at 800 g, 4°C for 10 min. The protein concentration was detected through bicinchoninic acid (BCA) protein assay kit (Chengdu Must Biotechnology Co., Ltd., Chengdu, China). The equivalent amounts of protein (80  $\mu$ g) were subsequently separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, and transferred onto a polyvinylidene difluoride (PVDF) membrane (Wuhan Boster Biological Technology, Ltd., Wuhan, China). The membranes were blocked by 5% non-fat dry milk at room temperature for 2 h and incubated overnight at 4°C with primary antibodies p38 and p-p38 (1:1000, Cell Signaling Technology, Danvers, MA, USA), p65 and p-p65 (1:500, Cell Signaling Technology Danvers, MA, USA) and  $\beta$ -actin (1:1000, Cell Signaling Technology, Danvers, MA, USA) antibody. Subsequently, the membranes were incubated with secondary antibody (1:1000, Cell Sig-

nalizing Technology, Danvers, MA, USA), followed by detection with chemiluminescence and visualization by enhanced chemiluminescence (ECL) kit (Amersham, Little Chalfont, UK). The protein bands were analyzed by LabImage version 2.7.1 (Kapelan GmbH, Halle, Germany).

### **Statistical Analysis**

Statistical analysis was implemented using SPSS18.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean  $\pm$  SD; differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range method or Newman-Keuls test.  $p < 0.05$  was considered as statistically significant.

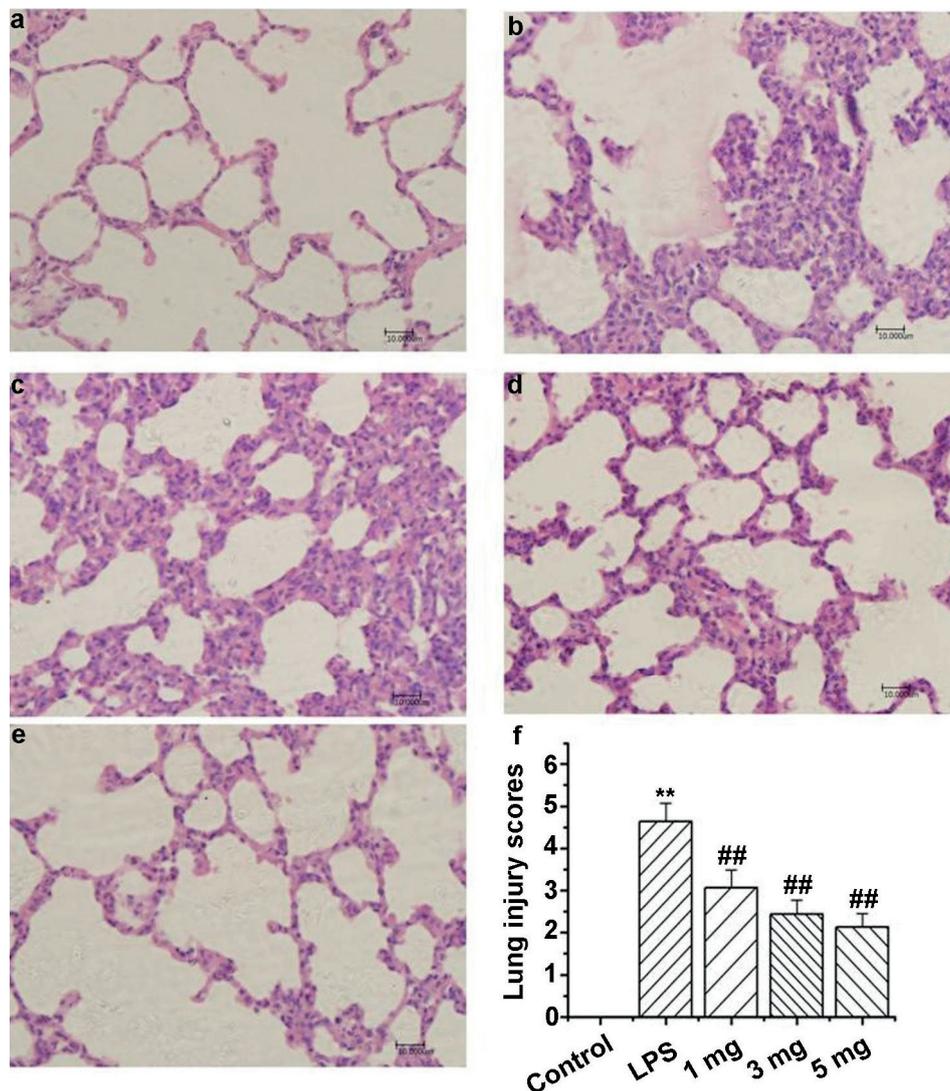
## **Results**

### **Effects of Lidocaine on Lung Histopathologic Changes**

To assess the effect of lidocaine on LPS-induced ALI rats, HE staining was applied to observe the lung histologic changes with or without lidocaine. Lung tissues in the LPS group were significantly damaged, mainly exhibited inflammatory infiltration, interstitial edema, thickening of the alveolar wall, and hemorrhage (Figure 1B). However, the treatment of lidocaine after LPS challenge ameliorated the pulmonary injury (Figure 1C-E). Additionally, the lung injury score was also evaluated. The lung injury score of 4.7 in LPS group was significantly higher than that of LPS+lidocaine group ( $p < 0.01$ ), and the scores showed a markedly downward trend with the gradual increase of lidocaine concentration (Figure 1F).

### **Lidocaine Ameliorated LPS-induced Acute Lung Injury**

As for the assessment of non-cardiogenic pulmonary edema, the lung W/D ratio is a critical feature of ALI. As shown in Figure 2A, W/D ratio in LPS group significantly increased compared with the control group (5.6 vs. 4.3,  $p < 0.01$ ). However, lidocaine administration (1, 3 or 5 mg/kg) significantly reduced the W/D ratio compared with LPS group ( $p < 0.01$  or  $p < 0.05$ ). The W/D ratio was approximately 4.9, 4.7, and 4.5, corresponding to 1 mg/kg, 5 mg/kg and 5 mg/kg of lidocaine, respectively. Meanwhile, the ameliorations in pulmonary were revealed in a dose-dependent manner. In addition, cellular counts and proteins are also important feature to assess the inflammatory response. The number of leukocyte and the total protein were about  $6.5 \times 10^7/l$



**Figure 1.** Lidocaine improved LPS-caused ALI 12 h after treatment of LPS ( $\times 200$ ). 4- $\mu\text{m}$  thick sections of lung tissue were analyzed by HE staining. (A) Control group; (B) LPS group; (C) LPS+1 mg/ml lidocaine group; (D) LPS+3 mg lidocaine group; (E) LPS+5 mg lidocaine group; (F) Lung injury scores. Data were expressed as mean  $\pm$  SD. \*\* $p < 0.01$  vs. control group; ## $p < 0.05$  vs. LPS group.

and 133  $\mu\text{g}/\text{ml}$  in LPS group, respectively, exhibiting remarkable increase in comparison with control group ( $p < 0.05$ ) (Figure 2B-C). However, lidocaine treatment decreased both number of leukocyte and total protein than those in LPS group ( $p < 0.05$ ), especially in 5 mg/kg of LPS. The number of leukocyte and total protein was about  $4.9 \times 10^7/\text{l}$  and 75  $\mu\text{g}/\text{ml}$ , respectively, which were lower than other LPS + lidocaine groups.

#### **Lidocaine Down-Regulates TNF- $\alpha$ , IL-6 and MCP-1 in BALF**

To identify the anti-inflammatory property of lidocaine, the level of inflammatory mediators in-

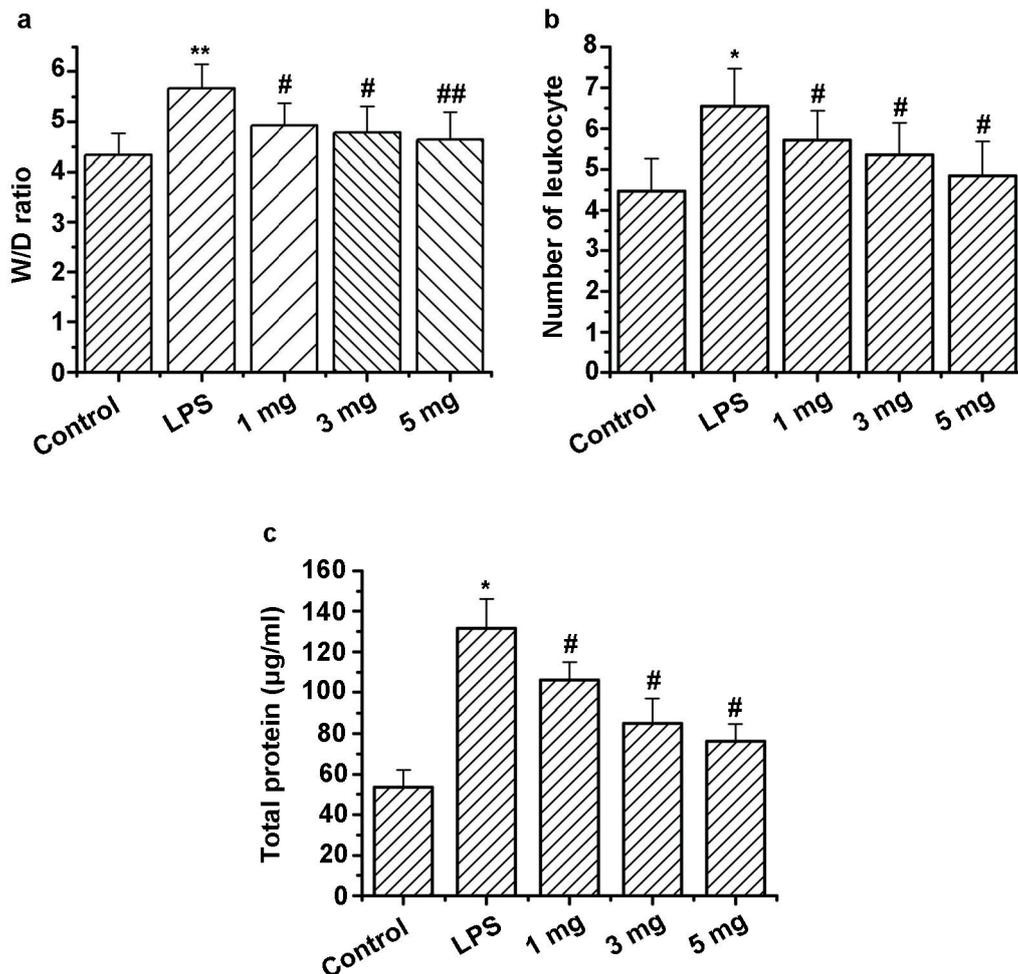
cluding TNF- $\alpha$ , IL-6 and MCP-1 in BALF was measured by ELISA method. The concentration of TNF- $\alpha$ , IL-6, and MCP-1 of LSP groups in BALF were markedly higher than control group (155 pg/ml vs. 50 pg/ml, 425 pg/ml vs. 65 pg/ml, 410 pg/ml vs. 75 pg/ml, respectively,  $p < 0.05$  or  $p < 0.01$ ) (Figure 3). The expression of these mediators after lidocaine injection was decreased than those in LPS group ( $p < 0.05$  or  $p < 0.01$ ). Additionally, the effect of lidocaine on pulmonary inflammatory showed a dose-dependent manner. At dose of 5 mg/kg lidocaine, the expression levels of TNF- $\alpha$ , IL-6, and MCP-1, were approximately 85 pg/ml, 225 pg/ml, and 260 pg/ml.

**Effect of Lidocaine on T-AOC, SOD Activity and MDA Content in Lung Tissue**

Oxidative stress has been recognized as a critical factor of pathophysiology in LPS-induced ALI. In this study, the activities of SOD and MDA were examined. As shown in Figure 4, the activity of T-AOC and SOD in LPS group (0.4 U/g and 63 U/g) were significantly declined compared with the control group ( $p < 0.01$ ), along with the noticeable rise of MDA content (2.6 U/g,  $p < 0.01$ ). Administration of lidocaine markedly increased the activity of T-AOC and SOD ( $p < 0.05$  or  $p < 0.01$ ), and decreased the content of MDA ( $p < 0.05$  or  $p < 0.01$ ) with a dose-dependent manner.

**Effect of Lidocaine on NF- $\kappa$ B Expression in Lung Tissue**

Phosphorylation of NF- $\kappa$ B prompts the transcription of most pro-inflammatory cytokines, playing a pivotal role in the pathogenesis of ALI<sup>19</sup>. As shown in Figure 5A and C, LPS stimulation significantly increased p65 phosphorylation up to around 4.5 ( $p < 0.01$ ), while lidocaine significantly reduced the p-p65 induction by LPS, especially in 5 mg/kg lidocaine. In parallel with serine phosphorylation of NF- $\kappa$ B and p65, the effect of lidocaine on I $\kappa$ -B degradation (Figure 5B) was also examined. The result showed that lidocaine significantly inhibited I $\kappa$ -B degradation in a dose-dependent manner.



**Figure 2.** The inhibitory effects of lidocaine on LPS-induced acute lung injury. Male Sprague Dawley rats received lidocaine with a dose of 1 mg/kg, 3 mg/kg, and 5 mg/kg 30 min after LPS administration. (A) Levels of lung W/D ratio after lidocaine injection via tail vein. (B) Number of leukocyte in BALF after treatment of LPS for 12 h. (C) The expression of total protein in lung tissues. After treatment of LPS for 12 h, the lung tissues were collected and analyzed by Western blot. Data are presented as mean  $\pm$  SD (n=10). \* $p < 0.05$ , \*\* $p < 0.01$  vs. control group; # $p < 0.05$ , ## $p < 0.01$  vs. LPS group.

### Effect of Lidocaine on p38 MAPK Expression in Lung Tissue

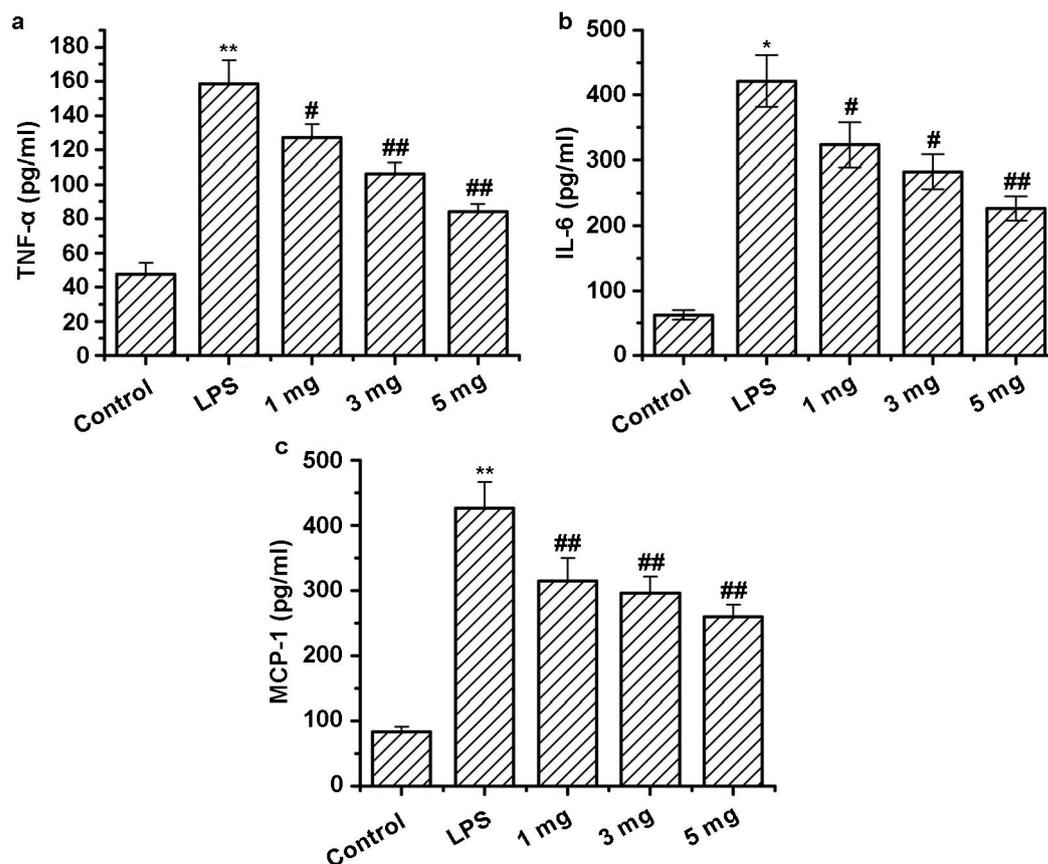
p38 MAPK is independent of its phosphorylation state, and has been reported to participate in inflammation and the activation of NF- $\kappa$ B<sup>20</sup>. As illustrated in Figure 5D, compared with control group, p38 phosphorylation evidently increased after LPS challenge ( $p < 0.01$ ). The ratio of p-p38/p38 was about 4.6. Giving a different dose of lidocaine, we found that lidocaine significantly reduced p38 phosphorylation compared with the LPS group ( $p < 0.05$  or  $p < 0.01$ ).

### Discussion

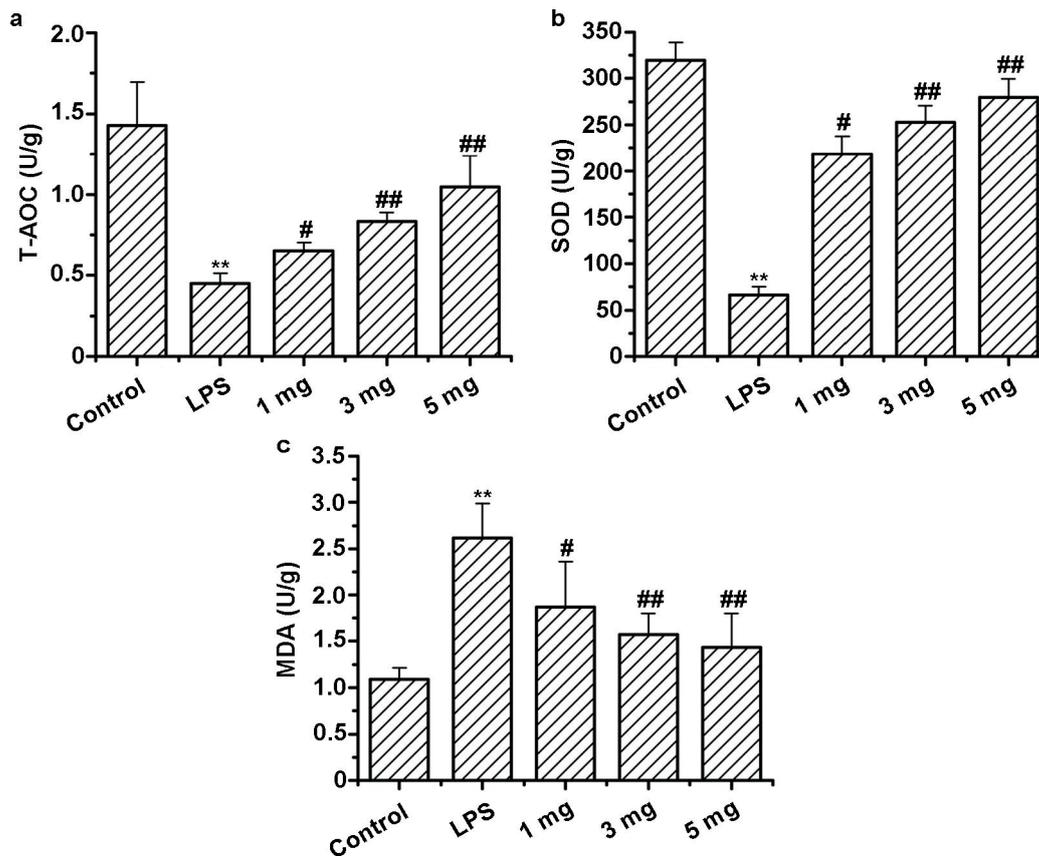
ALI, a devastating disease, is responsible for significant morbidity and mortality; nevertheless, there are still few effective drugs in the clinic<sup>2,21</sup>. LPS was recognized as one of the mainly pro-in-

flammatory reaction factors in infection diseases<sup>19</sup>. Previous studies<sup>6</sup> demonstrated that LPS possesses strong biological activities that could induce the inflammatory response and even result in ALI. We uncovered that administration of lidocaine attenuated LPS-induced ALI in mice. Lidocaine inhibited the deterioration of pulmonary edema, oxidative stress damage and suppressed the expression of pro-inflammatory mediators such as TNF- $\alpha$ , IL-6, and MCP-1 via two different pathways of NF- $\kappa$ B and p38 MAPK.

Pulmonary edema, the permeability change, and neutrophils infiltration, are the most important pathological findings in ALI<sup>22</sup>. For quantifying the magnitude of pulmonary edema, the lung W/D ratio was evaluated. We found that the lung W/D ratio in LPS + lidocaine group was notably lower than the LPS group, which indicated that lidocaine attenuated the development of pulmonary edema. In addition, we observed a signi-



**Figure 3.** LPS-caused changes of inflammatory cytokines in BALF. (A) TNF- $\alpha$ , (B) IL-6, (C) and MCP-1 in BALF after LPS challenge. ELISA analysis of TNF- $\alpha$ , IL-6 and MCP-1 after *i.p.* injection of LPS for 12 h. Data are presented as mean  $\pm$  SD.  $n=10$  each group. \* $p < 0.05$ , \*\* $p < 0.01$  vs. control group; # $p < 0.05$ , ## $p < 0.01$  vs. LPS group.



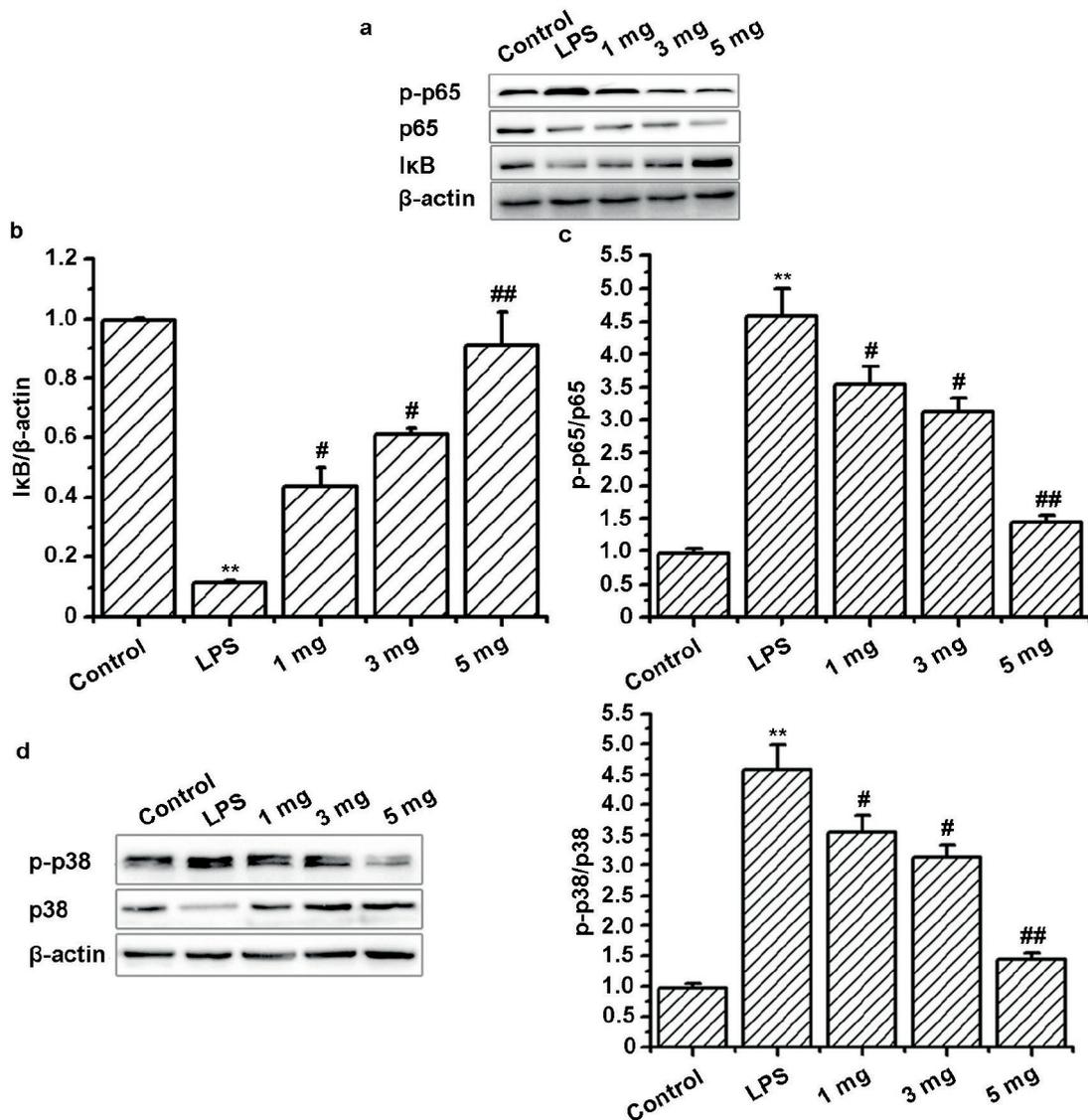
**Figure 4.** Lidocaine ameliorates the LPS-induced decreased level of T-AOC. (A) SOD activity and (B) MDA content (C) in lung tissue analyzed by ELISA method. Data are presented as mean  $\pm$  SD. \*\* $p < 0.01$  vs. control group; # $p < 0.05$ , ## $p < 0.01$  vs. LPS group.

ficant decrease in the number of leukocyte and the total protein after lidocaine administration. The result showed that lidocaine decreased the lung vascular permeability and neutrophils infiltration. Furthermore, these results were supported by lung histological evaluation, which showed that lidocaine markedly attenuated the histological changes including inflammatory infiltration, interstitial edema, thickening of the alveolar wall and hemorrhage. These results demonstrated the protective effects of lidocaine against LPS-induced ALI.

Evidence indicated pro-inflammatory mediators, including TNF- $\alpha$ , IL-6, and MCP-1 participate in the early development of inflammation and play a crucial role in ALI<sup>21,23</sup>. Both TNF- $\alpha$  and IL-6 are the main markers of acute inflammatory response. MCP-1, a chemokine mainly expressed by alveolar macrophages and pulmonary vascular endothelial cells in the lung, was quickly up-regulated by inflammation stimuli,

leading to the recruitment of more inflammatory cells, particularly neutrophils and macrophages, infiltrating into the damaged tissues<sup>23,24</sup>. Our study showed that the TNF- $\alpha$ , IL-6, and MCP-1 level increased in BALF after LPS challenge. However, the releases of these pro-inflammatory mediators were inhibited by lidocaine administration and its effect was in a dose-dependent manner. These results suggested that the amelioration of LPS-induced ALI by lidocaine might be, at least in part, associated with the reduction of TNF- $\alpha$ , IL-6, and MCP-1 production.

Additionally, NF- $\kappa$ B activation plays a key role in the production of inflammatory cytokines<sup>25</sup>. Wright and Christman<sup>26</sup> reported that the inhibition of NF- $\kappa$ B activation can reduce its downstream inflammatory cytokine levels and contributes to ameliorate ALI. Under normal physiological conditions, NF- $\kappa$ B is sequestered in the cytoplasm by its inhibitor I $\kappa$ B. Stimulated by LPS induces the phosphorylation of p65, ente-



**Figure 5.** NF- $\kappa$ B and p38 MAPK expression induced by LPS and its protective by lidocaine (1 mg/kg, 3 mg/kg, and 5 mg/kg) in the lung of rats. (A) Western blotting of p65, p-p65 and I $\kappa$ B protein expressions in lung tissue,  $\beta$ -actin was used as an internal control; (B-C) Bands on Western blot were analyzed by LabImage version 2.7.1 software. The data were expressed the ration of I $\kappa$ B/ $\beta$ -actin and p-p65/p65. (D) The protein band of p38 and p-p38 performed by Western blot and the quantitative result of the relative expression of p-p38. Data were displayed as the ratio of p-p38/p38.  $\beta$ -actin was used as an internal control. The data were expressed as mean  $\pm$  SD. \*\* $p < 0.01$  vs. control group; # $p < 0.05$ , ## $p < 0.01$  vs. LPS group.

ring the nucleus and regulating the expression of inflammatory mediators<sup>27</sup>. We demonstrated that LPS stimulation significantly increased the phosphorylation of p65 and I $\kappa$ B $\alpha$  degradation. However, lidocaine treatment inhibited I $\kappa$ B $\alpha$  degradation and p65 phosphorylation. Moreover, MAPK, including ERK, JNK and p38 MAPK, have been demonstrated to play a crucial role in signal transduction pathways, regulation of cytokine release, and participation in the activation of NF- $\kappa$ B

in LPS-induced ALI<sup>28,29</sup>. Among all of those, p38 MAPK pathway is reported to be activated by pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-6. In this study, the result showed p38 MAPK was activated in LPS-induced lung injury. However, lidocaine treatment markedly suppressed LPS-induced p38 phosphorylation. These results indicated that blocking p38 MAPK and NF- $\kappa$ B signaling pathways might be the main reasons of lidocaine abatement of pulmonary inflammation.

Furthermore, excessive oxidative damage also plays a proven role in the pathogenesis of ALI<sup>30</sup>. T-AOC is an anti-oxidative biomarker representing the level of enzyme and non-enzyme original antioxidant in the body<sup>31</sup>. SOD is also extensively used as a biochemical indicator of pathological states associated with oxidative stress. In addition, MDA is a degradation product of the oxygen-derived free radicals and lipid oxidation, which reflects the damage caused by reactive oxygen species<sup>32</sup>. We demonstrated that treatment with lidocaine raised the activation levels of T-AOC and SOD, while reduced the content of MDA. These observations suggested that lidocaine is capable of reducing serious lung damage through inhibition oxidative stress.

### Conclusions

The protective mechanism of lidocaine may be attributed partly to suppress the p38 AMPK/NF-κB signaling pathway. Lidocaine attenuated LPS-induced ALI in mice by the inhibition of inflammation and oxidative stress, which could be a new therapeutic agent for the prevention of acute lung injury.

### Ethical Committee Approval

The research was conducted in accordance with the Declaration of Helsinki and Ethics Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University.

### Conflict of Interest

The Authors declare that they have no conflict of interest.

### References

- LV O. Glucocorticoid combined with hyaluronic acid enhances glucocorticoid receptor activity through inhibiting p-38MAPK signal pathway activation in treating acute lung injury in rats. *Eur Rev Med Pharmacol Sci* 2016; 20: 3920-3929.
- HAN SC, CAI WX, YANG XK, 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.
- YU JL, ZHANG XS, XUE X, WANG RM. Patchouli alcohol protects against lipopolysaccharide-induced acute lung injury in mice. *J Surg Res* 2015; 194: 537-543.
- SAKAGUCHI M, MARUTANI E, SHIN HS, CHEN W, HANAOKA K, XIAN M, ICHINOSE F. Sodium thiosulfate attenuates acute lung injury in mice. *Anesthesiology* 2014; 121: 1248-1257.
- ZHANG X, LIU F, LIU H, CHENG H, WANG W, WEN Q, WANG Y. Urinary trypsin inhibitor attenuates lipopolysaccharide-induced acute lung injury by blocking the activation of p38 mitogen-activated protein kinase. *Inflamm Res* 2011; 60: 569-575.
- JING W, CHUNHUA M, W. Effects of acteoside on lipopolysaccharide-induced inflammation in acute lung injury via regulation of NF-κB pathway in vivo and in vitro. *Toxicol Appl Pharmacol* 2015; 285: 128-135.
- LI W, HUANG H, NIU X, FAN T, HU H, LI Y, YAO H, LI H, MU Q. Tetrahydrocoptisine protects rats from lps-induced acute lung injury. *Inflammation* 2014; 37: 2106-2115.
- LIU MW, SU MX, ZHANG W, WANG YQ, CHEN M, WANG L, QIAN CY. Protective effect of Xuebijing injection on paraquat-induced pulmonary injury via down-regulating the expression of p38 MAPK in rats. *BMC Complement Altern Med* 2014; 14: 498.
- XIAOFENG Y, QINREN C, JINGPING H, XIAO C, MIAOMIAO W, XIANGRU F, XIANXING X, MEIXIA H, JING L, JINGYUAN W, XINXIN C, HONGYU L, YANHONG D, LANXIANG J, XUMING D. Geniposide, an iridoid glucoside derived from *Gardenia jasminoides*, protects against lipopolysaccharide-induced acute lung injury in mice. *Planta Med* 2012; 78: 557-564.
- LV H, ZHU C, LIAO Y, GAO Y, LU G, ZHONG W, ZHENG Y, CHEN W, CI X. Tenuigenin ameliorates acute lung injury by inhibiting NF-κB and MAPK signalling pathways. *Respir Physiol Neurobiol* 2015; 216: 43-51.
- ZHONG WT, WU YC, XIE XX, ZHOU X, WEI MM, SOROMOU LW, CI XX, WANG DC. Phyllirin attenuates LPS-induced pulmonary inflammation via suppression of MAPK and NF-κB activation in acute lung injury mice. *Fitoterapia* 2013; 90: 132-139.
- LAHAT A, BEN-HORIN S, LANG A, FUDIM E, PICARD O, CHOWERS Y. Lidocaine down-regulates nuclear factor-kappaB signaling and inhibits cytokine production and T cell proliferation. *Clin Exp Immunol* 2008; 152: 320-327.
- FENG G, LIU S, WANG GL, LIU GJ. Lidocaine attenuates lipopolysaccharide-induced acute lung injury through inhibiting NF-κB activation. *Pharmacology* 2008; 81: 32-40.
- YUAN T, LI Z, LI X, YU G, WANG N, YANG X. Lidocaine attenuates lipopolysaccharide-induced inflammatory responses in microglia. *J Surg Res* 2014; 192: 150-162.
- GU YW, SU DS, TIAN J, WANG XR. Attenuating phosphorylation of p38 MAPK in the activated microglia: a new mechanism for intrathecal lidocaine reversing tactile allodynia following chronic constriction injury in rats. *Neurosci Lett* 2008; 431: 129-134.
- WANG HL, ZHANG WH, LEI WF, ZHOU CQ, YE T. The inhibitory effect of lidocaine on the release of high mobility group box 1 in lipopolysaccharide-stimulated macrophages. *Anesth Analg* 2011; 112: 839-844.

- 17) HEIDEMANN SM, GLIBETIC M. Heat stress protects against lung injury in the neutropenic endotoxemic rat. *Inflammation* 2005; 29: 47-53.
- 18) PARSEY MV, TUDER RM, ABRAHAM E. Neutrophils are major contributors to intraparenchymal lung IL-1 beta expression after hemorrhage and endotoxemia. *J Immunol* 1998; 160: 1007-1013.
- 19) YEH CH, YANG JJ, YANG ML, LI YC, KUAN YH. Rutin decreases lipopolysaccharide-induced acute lung injury via inhibition of oxidative stress and the MAPK-NF- $\kappa$ B pathway. *Free Radic Biol Med* 2014; 69: 249-257.
- 20) PETR V, LEGI S, VOJTECH N. Andrographolide protects against LPS-induced acute lung injury by inactivation of NF- $\kappa$ B. *PLoS One* 2013; 8: e56407.
- 21) SU ZQ, MO ZZ, LIAO JB, FENG XX, LIANG YZ, ZHANG X, LIU YH, CHEN XY, CHEN ZW, SU ZR, LAI XP. Usnic acid protects LPS-induced acute lung injury in mice through attenuating inflammatory responses and oxidative stress. *Int Immunopharmacol* 2014; 22: 371-378.
- 22) LUCAS R, VERIN AD, BLACK SM, CATRAVAS JD. Regulators of endothelial and epithelial barrier in integrity and function in acute lung injury. *Biochem Pharmacol* 2009; 77: 1763-1772.
- 23) XIAO M, ZHU T, ZHANG W, WANG T, SHEN YC, WAN QF, WEN FQ. Emodin ameliorates LPS-induced acute lung injury, involving the inactivation of NF- $\kappa$ B in mice. *Int J Mol Sci* 2014; 15: 19355-19368.
- 24) XIE K, YU Y, HUANG Y, ZHENG L, LI J, CHEN H, HAN H, HOU L, GONG G, WANG G. Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis. *Shock* 2012; 37: 548-555.
- 25) LI YX, LI XH, LIU G, SUN R, WANG L, WANG J, WANG H. Fucosterol attenuates lipopolysaccharide-induced acute lung injury in mice. *J Surg Res* 2015; 195: 515-521.
- 26) WRIGHT JG, CHRISTMAN JW. The role of nuclear factor kappa B in the pathogenesis of pulmonary diseases: implications for therapy. *Am J Resp Med* 2003; 2: 211-219.
- 27) RAN X, CHAO S, JUN-GANG Z, YUN H, KUAN-BING C, WEN-JUN S. Protective effect of veratric acid on lipopolysaccharide-induced acute lung injury in mice. *Eur J Pharmacol* 2014; 740: 227-232.
- 28) CHEN J, WANG JB, YU CH, CHEN LQ, XU P, YU WY. Total flavonoids of *Mosla scabra* leaves attenuates lipopolysaccharide-induced acute lung injury via down-regulation of inflammatory signaling in mice. *J Ethnopharmacol* 2013; 148: 835-841.
- 29) LI KC, HO YL, HSIEH WT, HUANG SS, CHANG YS, HUANG GJ. Apigenin-7-glycoside prevents LPS-induced acute lung injury via downregulation of oxidative enzyme expression and protein activation through inhibition of MAPK Phosphorylation. *Int J Mol Sci* 2015; 16: 1736-1754.
- 30) CHEN XJ, ZHANG B, HOU SJ, SHI Y, XU DQ, WANG YX, LIU ML, DONG HY, SUN RH, BAO ND, JIN FG, LI ZC. Osthole improves acute lung injury in mice by up-regulating Nrf-2/thioredoxin 1. *Respir Physiol Neurobiol* 2013; 188: 214-222.
- 31) XIAO N, WANG XC, DIAO YF, LIU R, TIAN KL. Effect of initial fluid resuscitation on subsequent treatment in uncontrolled hemorrhagic shock in rats. *Shock* 2004; 21: 276-280.
- 32) QIAN H, LIU D. The time course of malondialdehyde production following impact injury to rat spinal cord as measured by microdialysis and high pressure liquid chromatography. *Neurochem Res* 1997; 22: 1231-1236.