

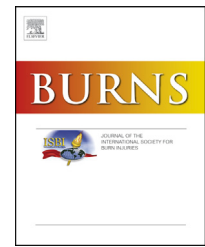


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# Role of angiotensin-converting enzyme (ACE) and ACE2 in a rat model of smoke inhalation induced acute respiratory distress syndrome

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## ABSTRACT

Smoke inhalation induced acute respiratory distress syndrome (ARDS) has become more and more common throughout the world and it is hard to improve the outcome. The present research was to investigate possible roles of angiotensin-converting enzyme (ACE) and ACE2 in lung injury resulted from smoke exposure. Rats were exposed to dense smoke to induce ARDS. Histological changes, blood gases, bronchoalveolar lavage fluids (BALF) and wet-to-dry weight were analyzed to evaluate lung injury after smoke inhalation; beside, we also measured the expression of ACE and ACE2 at different time points to explore the possible mechanism of those changes. The results showed that pH of arterial blood, partial blood oxygen (PaO<sub>2</sub>) and blood oxygen saturation (SO<sub>2</sub>) decreased after smoke inhalation at different time points ( $P < 0.01$ ); while, partial blood carbon dioxide (PaCO<sub>2</sub>), wet-to-dry weight ratio, leukocytes count, protein concentration and inflammatory cytokines in BALF increased after smoke exposure ( $P < 0.01$ ). More importantly, both immunohistochemical staining and Western blot results showed that ACE and ACE2 expression in lungs from the experimental groups significantly increased compared with that of the control group ( $P < 0.05$ ). This study indicated that inflammation pulmonary edema and histological changes resulted from smoke inhalation induced lung injury were possibly attributed to abnormal expression of ACE and ACE2 related pathway.

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## 1. Introduction

Acute respiratory distress syndrome (ARDS) is a common and devastating complication after acute illness or injury, and it results in high morbidity, mortality, and health care costs [1]. Despite use of lung-protective ventilation, overall ICU and hospital mortality of ARDS patients is still higher than 40% [2].

In addition to sepsis, trauma and pneumonia, a large epidemiologic study suggested the relationship with evidence of a dose-response effect between cigarette smoking and ARDS [3], including approximately 50% of ARDS cases associated with cigarette smoking [4]. Smoke is a complex and harmful substance comprising a variety of noxious gases, irritants and asphyxiants [5]. Numerous investigations have been conducted and found that smoke may directly or

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indirectly damage lung endothelia and alveolar epithelia and lead to the change of pulmonary hemodynamics. The possible mechanism maybe associated with large inflammatory cells, including neutrophils, monocytes, lymphocytes and platelets, and proinflammatory mediators, including cytokines, proteases, oxidants neuromediator, cyclooxygenase and procoagulant factors [6]. However, large-scale trials on ARDS have not found effective pharmacologic therapy for improving the clinical outcome.

The renin–angiotensin system (RAS) plays a key role in maintaining blood pressure homeostasis, as well as fluid and salt balance [7]. Angiotensin-converting enzyme (ACE) and ACE2 share homology in their catalytic domain. ACE activity triggers vasoconstriction by generating angiotensin II from angiotensin I, while ACE2 counterbalances with ACE and functions as a negative regulator of the renin–angiotensin system (RAS) [8]. Previous reports indicate that the RAS also plays a critical role in acute lung diseases, especially acute respiratory distress syndrome (ARDS). Researches showed that ACE2 knock-out mice could be protected from SARS infection [9]. Besides, researches in acid-aspiration-induced ARDS, endotoxin-induced ARDS and peritoneal sepsis-induced ARDS showed that ACE, angiotensin (Ang) II type I (AT 1) receptor, acts as lung injury-prompting factor, whereas ACE2 protects against that kind of lung injury [10,11]. Recent report that ACE2 could mediate the severe acute respiratory distress syndrome induced by influenza A (H7N9) virus infection in an experimental mouse model [12]. These findings suggested that RAS may play a critical role in the pathogenesis of ARDS and might facilitate the development of a novel drug target for ARDS.

In order to address whether the RAS is activated in smoke inhalation-induced ARDS, the present study was designed, and, the two key members, ACE and ACE2, involved in the classical RAS, were detected and assessed at different time points after smoke inhalation. It was hoped that the results would enhance the understanding of the role of RAS in ARDS induced by smoke inhalation, and provide valuable data for developing pharmaceutical therapies of ARDS.

## 2. Materials and methods

### 2.1. Animals

Thirty healthy adult Sprague-Dawley rats, weighing 200–220 g each, were obtained from the Experimental Animal Center of the Fourth Military Medical University. The rats were given an adaptive feeding with standard rat chow and water and were housed in a temperature and light controlled (20–25 °C; 12-h light/dark cycle) environment for two weeks. All procedures have been reviewed and approved by the Animal Care Committee of the Fourth Military Medical University for use in this study.

### 2.2. Smoke exposure

Thirty rats were randomly divided into five groups: control group (the rats were free from smoke exposure); 1 h group (the rats were sacrificed at the first hour after exposure); 4 h group

(the rats were sacrificed at the 4th hour after exposure); 10 h group (the rats were sacrificed at the 10th hour after exposure); and 24 h group (the rats were sacrificed at the 24th hour after exposure). There were 6 rats in each group. Smoke inhalation induced lung injury models were built according to the method described by Zhu et al. [13]. Briefly, 150 g of dry wood shavings and 30 ml of kerosene were mixed thoroughly in a pot. This material was then heated to produce smoke. Using an air blower, the smoke was wafted from the pot to a 20 cm × 20 cm × 20 cm holding cage. This cage was kept at a temperature of 35 ± 2 °C and was equipped with transparent glass on the top with a light (bulb) in each of the four corners. The transparent glass provided visibility for the determination of the optimum smoke density, which, for the purposes of this experiment, was characterized as the point at which the holding cage was full of white smoke that was thick enough to blur the bottom of the holding cage. After the cage was filled with smoke, 24 rats were placed in the holder for 10 min, and subsequently taken out of the holder for 5 min. This cycle was repeated three times. Animals were given a daily dose of buprenorphine (0.1 mg/kg, i.p.) for analgesia starting immediately after the injury. The rats were anesthetized with 25 mg/kg of 1% pentobarbital by intraperitoneal injection and were sacrificed by cervical dislocation at the end of the experimental time period.

### 2.3. Blood gas analysis

Carotid artery intubation was carried out during spontaneous respiration. Two milliliter of arterial blood was collected with a syringe containing heparinized saline. Blood gas analysis was immediately conducted with an IL 1640 pH/blood gas/electrolytes analyzer (Instrumentation Laboratory, Lexington, MA) after blood sample were collected.

### 2.4. Bronchoalveolar lavage fluids

Lungs were lavaged with an endotracheal tube holding 2 ml cold phosphate-buffered saline solution for three times immediately after anesthetic satisfaction. Total leukocytes numbers in one aliquot of the pooled bronchoalveolar lavage fluids (BALF) were counted with a haemocytometer under a microscope, following the instructions of the manufacture. The remaining BALF sample was prepared by the centrifugation with 500 × *g* for 15 min at room temperature. The resulting supernatant was stored at –70 °C until use for determination of the protein concentration and cytokine level. Inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6), were determined using enzyme-linked immunosorbent assay (ELISA) kits (Sigma, St. Louis, MO). The protein concentration in BALF was quantified with Bradford assay.

### 2.5. Wet-to-dry weight ratio

The lungs were immediately removed from the thoracic cavity and washed extensively with normal saline to remove all contaminating blood. Gravimetric methods were used for evaluating the degree of pulmonary edema according to Ali etc. recommendation [14]. Briefly, the upper lobe of the left

lung tissue was excised and weighed (wet weight) with a precision balance. Samples were then heated at a 70 °C oven for 48 h, and weighed again (dry weight). Wet-to-dry weight ratio (WDR) was calculated using the following formula:  $WDR = (\text{wet-dry weight}) \times 100/\text{dry weight}$ .

## 2.6. Lung pathology

After fixing with 10% formalin for 24 h, lung tissues were dehydrated in ascending series of ethanol solutions (70%, 80%, 95% and 100% alcohol). The tissue samples were then embedded in paraffin and sectioned with 5 μm thickness. The slides were stained with hematoxylin and eosin and mounted. Two experienced pathologists in a blinded manner evaluated the tissue features by a microscopic examination. Ten fields of each lung section were examined (magnification ×400). The severity of lung injury was scored by the criteria of edema, neutrophil infiltration, hemorrhage, bronchiole epithelial desquamation, and hyaline membrane formation. The degrees of lung injury were ranged from 0 to 4, briefly, with 0 indicating absent, 1 mild, 2 moderate, 3 severe, and 4 the most severe.

## 2.7. Immunohistochemistry

Immunohistochemical staining for ACE and ACE2 expression was carried out by an indirect, two-step method. 5-μm thick paraffin-embedded sections of lung tissue were mounted on charged slides, fixed in cold acetone (Sigma), and immersed in 3% (v/v) hydrogen peroxide in PBS for 10 min to prevent endogenous peroxidase activity. The antigens were then activated by microwaving and incubated overnight with a polyclonal goat anti-ACE and anti-ACE2 antibody (2 μg/ml; Santa Cruz Biotechnology) at 4 °C, respectively. The slides were further incubated for 30 min with a secondary HRP-En Vision IgG antibody (Zhongshan Biotech, China) for 30 min at room temperature. Specific staining was detected by the two-step streptavidin-peroxidase method (Non-Biotin HRP Direction System). ACE and ACE2 were visualized by incubating the sections with a solution of 3, 3'-diaminobenzidine for 10 min. The primary antibody with normal goat sera served as a negative control. Then counterstaining was performed with hematoxylin. The immunoreactive cells were counted in at least eight fields and expressed as the positive cell ratio to the length of alveolar septa.

## 2.8. Western blotting

The lung tissues were homogenized and centrifuged at  $1000 \times g$  (4 °C) for 30 min. The protein samples (50 mg) were run on 10% SDS-PAGE, transferred onto nitrocellulose filters (Santa Cruz) at 200 mA for 90 min, and then blocked with 10% skim milk powder in TBS. The membrane was washed with 1% BSA/TBST and incubated with polyclonal goat antibodies that recognize ACE, ACE2 (both at 1 μg/ml; Santa Cruz Biotechnology), and β-actin (0.2 μg/ml; Santa Cruz Biotechnology) overnight at 4 °C. After washing again with TBST, the membranes were blotted with a secondary antibody conjugated with horseradish peroxidase at a 1:10 000 dilution for 1 h, and then detected with enhanced chemiluminescence

(Pierce, Rockford, IL, USA) for 1 min. After washing with 1% TBS/Tween (3 × 15 min), the membrane were incubated with a secondary antibody conjugated with horseradish peroxidase (Zhongshan Biotech, China) at a 1:3000 dilution for 1 h, and then washed (3 × 15 min) with TBST. The signal was detected using enhanced chemiluminescence plus kit (Amersham).

## 2.9. Statistic analysis

All data are presented as the mean ± S.D. Statistical comparisons among different time points were made using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls post-hoc test.  $P < 0.05$  was regarded as statistically significant.

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## 3. Results

### 3.1. Clinical manifestations

The rats showed rapid and shallow breathing after smoke inhalation and they then became irritable, tachypnea and cyanosis. Secretion from their mouth and the nasal cavity increased. The rats experienced respiratory distress and dyspnea. The moist rales and stridor in both lungs could be heard from the injured rats. The most significant feature was that the symptoms and signs of injury in the rats in response to smoke inhalation became more and more severe as time passed.

### 3.2. Blood gas analysis

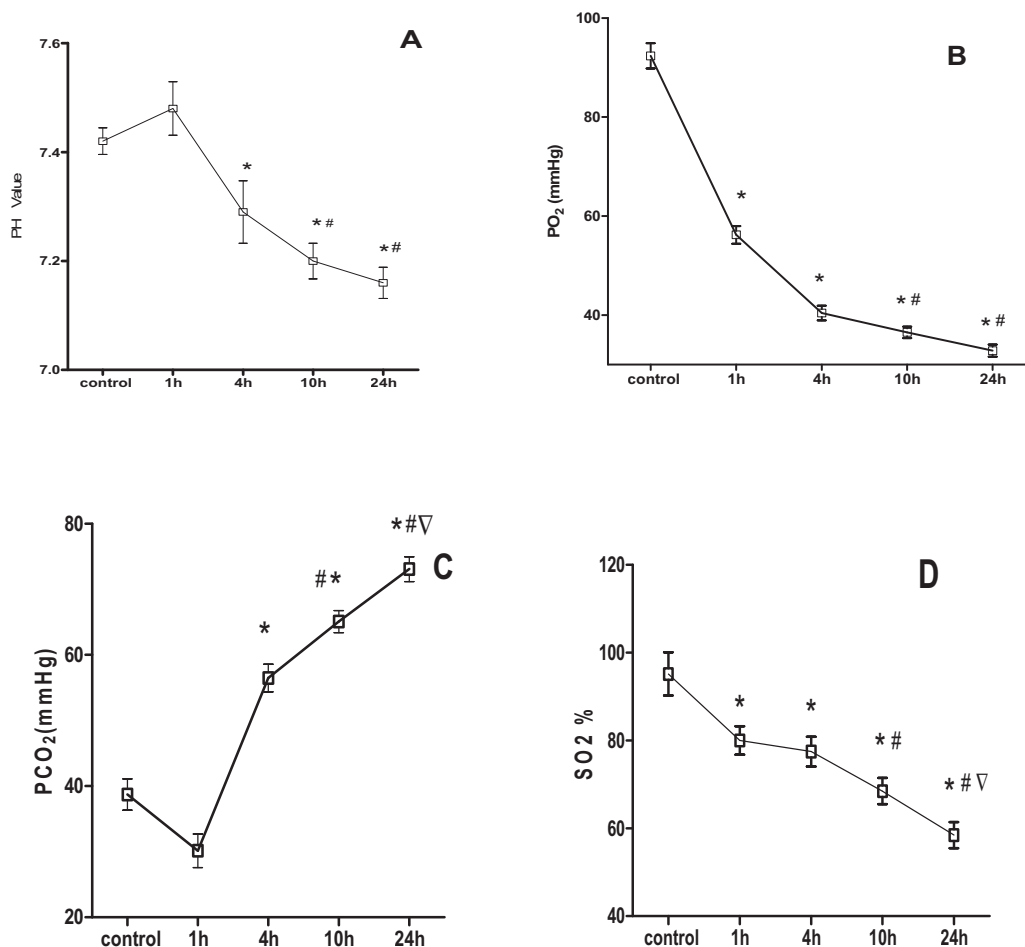
After smoke inhalation, arterial blood gas of the injured rats, including pH, partial blood oxygen ( $\text{PaO}_2$ ), partial blood carbon dioxide ( $\text{PaCO}_2$ ) and blood oxygen saturation ( $\text{SO}_2$ ) had significant changes. We found that the rats at 1 h after exposure developed type I respiratory failure with decreased  $\text{PaO}_2$  and  $\text{SO}_2$  compared with the control group. Hypoxemia and hypercapnia were exhibited in the injured rats 4th hour after smoke exposure. The degree of type II respiratory failure was aggravated without therapeutic measures implemented. Analysis of arterial blood gas at the 10th hour and the 24th hour after exposure showed severely acidemia with pH 7.20 and 7.16, respectively (Fig. 1A–D).

### 3.3. Protein content and leukocytes count in BALF

To evaluate the severity of lung injury, protein content and total cell count in BALF were probed. The results demonstrated that there was an obvious elevated tendency at different time point. Compared with the control, protein content and leukocytes count in other experiment groups showed significant difference ( $P < 0.05$ ). Moreover, there was also significant difference among experiment groups ( $P < 0.05$ ) (Fig. 2A and B).

### 3.4. Inflammatory cytokines

We found that TNF-α and IL-6 in BALF were expressed slightly in the control group rats. Expression of TNF-α and IL-6 dramatically increased after exposure to smoke. TNF and IL-6 in the injured



**Fig. 1 – Analysis of arterial blood at different time point after smoke exposure. Data presented were means ± SD. \* Denotes  $P < 0.01$  vs control group, # denotes  $P < 0.01$  vs 1 h group, ∇ denotes  $P < 0.01$  vs 4 h group. (A) PH; (B) PO<sub>2</sub>; (C) PCO<sub>2</sub>; (D)SO<sub>2</sub>.**

rats rose significantly, at least 5 times as high as that of the control rats (Fig. 3A and B).

### 3.5. Wet-to-dry weight ratio

The data indicated that the lung tissue from the normal group had a wet-to-dry ratio value of  $3.36 \pm 0.73$ . At 1 h after smoke inhalation, there was a significant increase (25%, 0.86) as compared to control ( $P < 0.05$ ). The WDR value continued to elevate with time, which was  $4.41 \pm 0.39$ ,  $4.83 \pm 0.81$ , and  $5.75 \pm 0.53$  at 4 h, 10 h, and 24 h, respectively (Fig. 4).

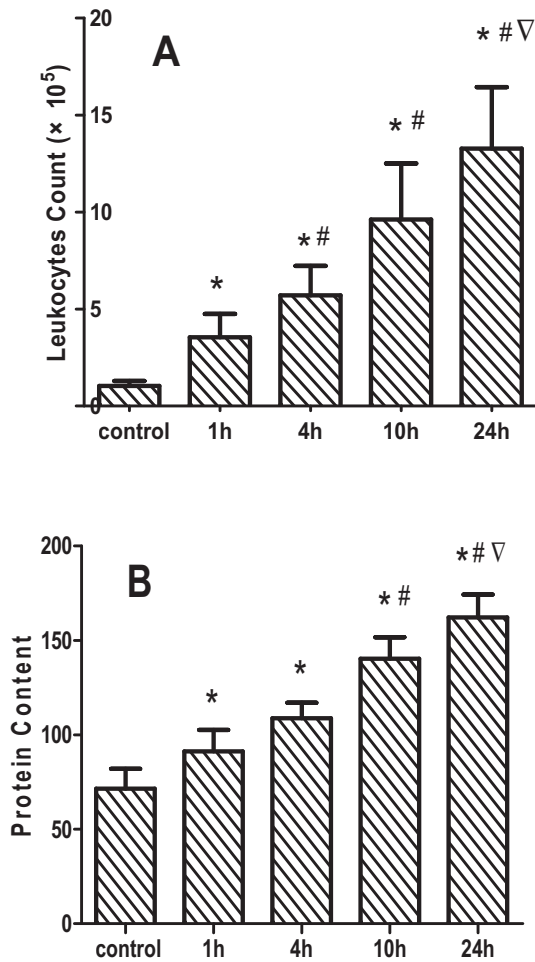
### 3.6. Lung pathology

Based on gross appearance, the volume of the lungs in the injured rats slightly increased compared with that of the untreated rats. The surface of the injured lungs became darkish and reddish and there were hemorrhages under the lung amicula. An examination with a light microscope revealed that the control group rats had a distinct framework, complete alveolar walls and interstitium without exfiltration. The first hour group rats showed mild edema, moderate neutrophil infiltration and mild hemorrhage. The 4th hour group rats

displayed mild edema, moderate neutrophil infiltration, moderate hemorrhage and mild bronchiole epithelial desquamation. The 10th hour group rats showed moderate edema, severe neutrophil infiltration, severe hemorrhage, mild bronchiole epithelial desquamation and a little hyaline membrane formation. The 24th hour group rats showed severe edema, severe neutrophil infiltration, severe hemorrhage, moderate bronchiole epithelial desquamation and a little hyaline membrane formation (Table 1). The lungs in the 10 h and 24 h groups revealed indications of more severe injury compared with the lungs from rats in the 1 h and 4 h groups (Fig. 5).

### 3.7. Immunohistochemistry

Immunohistochemical staining was used to determine the localization of ACE and ACE2 in the rat lungs. The investigation revealed that the major sites of ACE2 protein expression were in alveolar type I epithelium, alveolar type II epithelium, bronchiolar epithelium, endothelium and smooth muscle cells of pulmonary vascular structure. No obvious signal was detected in the bronchiolar smooth muscle cells. ACE expression was evident in the lung endothelium and lung parenchyma, but not in the vascular smooth muscle (Figs. 6 and 7).



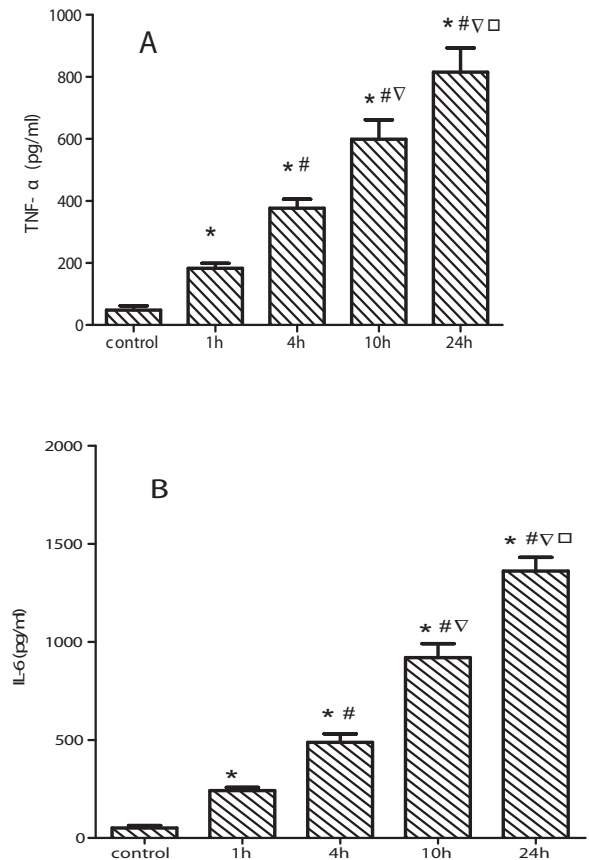
**Fig. 2 – Protein content and leukocytes count in BALF at different time point after smoke exposure. Data were means  $\pm$  SD. \* Denotes  $P < 0.01$  vs control group, # denotes  $P < 0.01$  vs 1 h group, ▽ denotes  $P < 0.01$  vs 4 h group. (A) Protein content;(B) leukocytes count.**

3.8. Western blotting

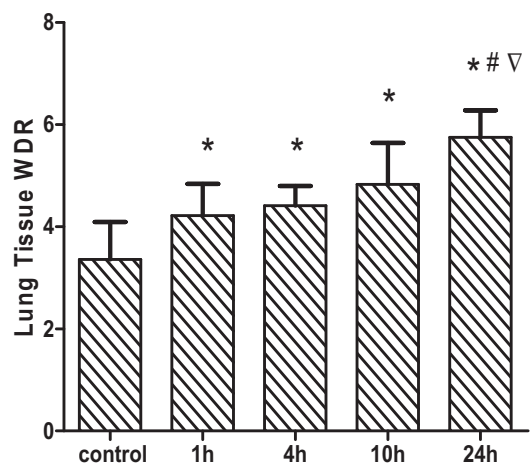
ACE and ACE2 expression levels in rat lungs were quantified by Western blot analysis. As shown in Fig. 8, immunoreactive bands of approximately 195 kDa and 90 kDa were seen, respectively. The ACE and ACE2 content in the lungs was significantly different among the different experimental groups. The contents of ACE and ACE2 protein in lungs increased 4 h after from smoke inhalation compared to that of normal lungs ( $P < 0.05$ ), more importantly, ACE and ACE2 content much more dramatically increased in the lungs 24 h after injury compared with that in lungs 4 h after from smoke inhalation ( $P < 0.05$ ).

4. Discussion

Smoke is known to have more than 200 kinds of components, including aldehydes, nitrogen oxides, phosgene, hydrogen chloride and other halogenated hydrogen compounds, such as



**Fig. 3 – Inflammatory cytokines TNF- $\alpha$  and IL-6 in BALF at different time point after smoke exposure. Data were means  $\pm$  SD. \* Denotes  $P < 0.01$  vs control group, # denotes  $P < 0.01$  vs 1 h group, ▽ denotes  $P < 0.01$  vs 4 h group, □ denotes  $P < 0.01$  vs 10 h group. (A) TNF- $\alpha$ ; (B) IL-6.**



**Fig. 4 – WDR value of lung tissue at different time point after smoke exposure. Data were means  $\pm$  SD. \* Denotes  $P < 0.01$  vs control group, # denotes  $P < 0.01$  vs 1 h group, ▽ denotes  $P < 0.01$  vs 4 h group.**



**Table 1 – the resulting scores of injured lung pathology.**

Group	Edema	Neutrophil infiltration	Hemorrhage	Epithelial desquamation	Hyaline membranes
Control group	0.2 ± 0.4	0.4 ± 0.5	0 ± 0	0.1 ± 0.1	0 ± 0
1 h group	1.1 ± 0.2 <sup>*</sup>	2.2 ± 0.6 <sup>*</sup>	1.5 ± 0.4 <sup>*</sup>	0.5 ± 0.5 <sup>*</sup>	0 ± 0
4 h group	1.5 ± 0.3 <sup>*</sup>	2.6 ± 0.4 <sup>*</sup>	1.8 ± 0.6 <sup>*</sup>	0.8 ± 0.3 <sup>*</sup>	0 ± 0
10 h group	1.8 ± 0.8 <sup>*#</sup>	2.8 ± 0.7 <sup>*#</sup>	2.3 ± 0.5 <sup>*#</sup>	1.2 ± 0.4 <sup>*#</sup>	0.2 ± 0.5 <sup>*#</sup>
24 h group	2.4 ± 0.6 <sup>*#∇</sup>	3.1 ± 0.4 <sup>*#</sup>	2.7 ± 1.1 <sup>*#∇</sup>	1.5 ± 0.8 <sup>*#∇</sup>	0.3 ± 0.4 <sup>*#∇</sup>

The severity of lung injury was scored by the criteria of edema, neutrophil infiltration, hemorrhage, bronchiole epithelial desquamation, and hyaline membrane formation. The degrees of lung injury were ranged from 0 to 4, briefly, 0 indicating absent, 1 mild, 2 moderate, 3 severe, 4 the most severe. Data were means ± SD.

<sup>\*</sup> Denotes  $P < 0.01$  vs control group.

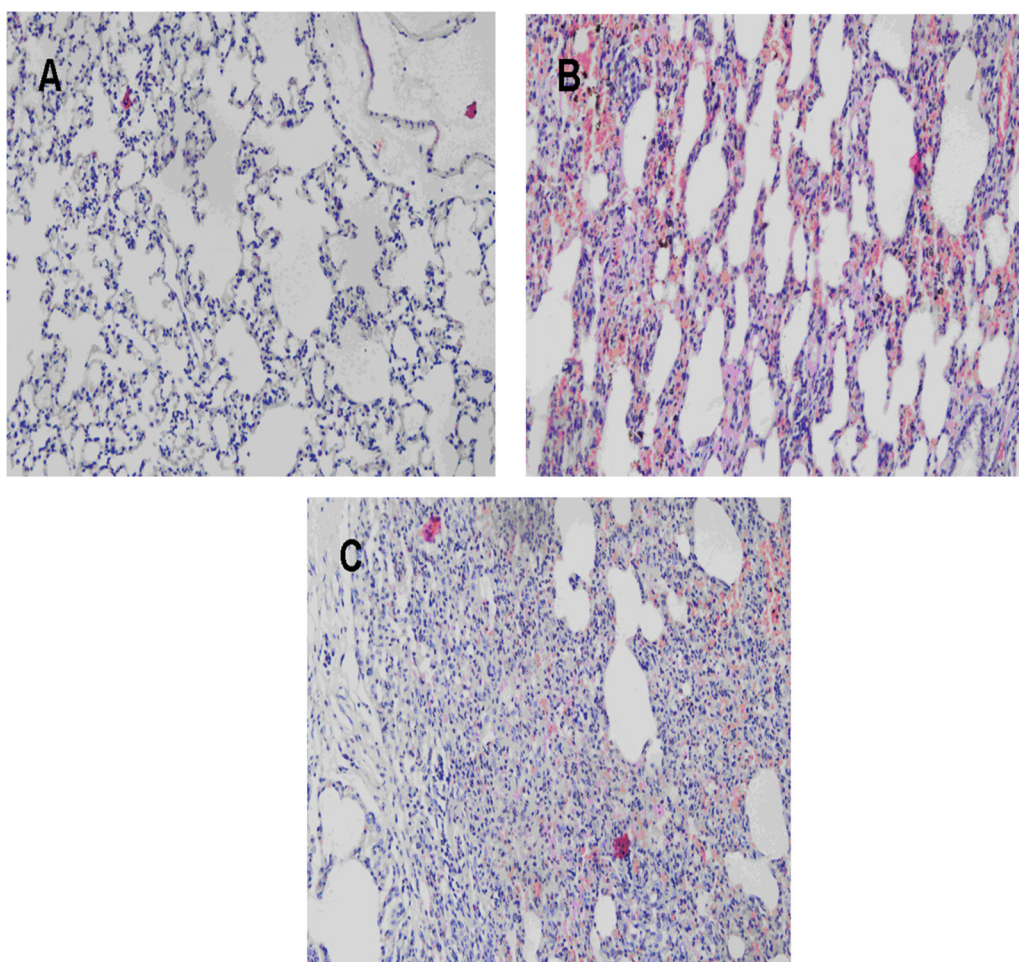
<sup>#</sup> denotes  $P < 0.01$  vs 1 h group.

<sup>∇</sup> Denotes  $P < 0.01$  vs 4 h group.

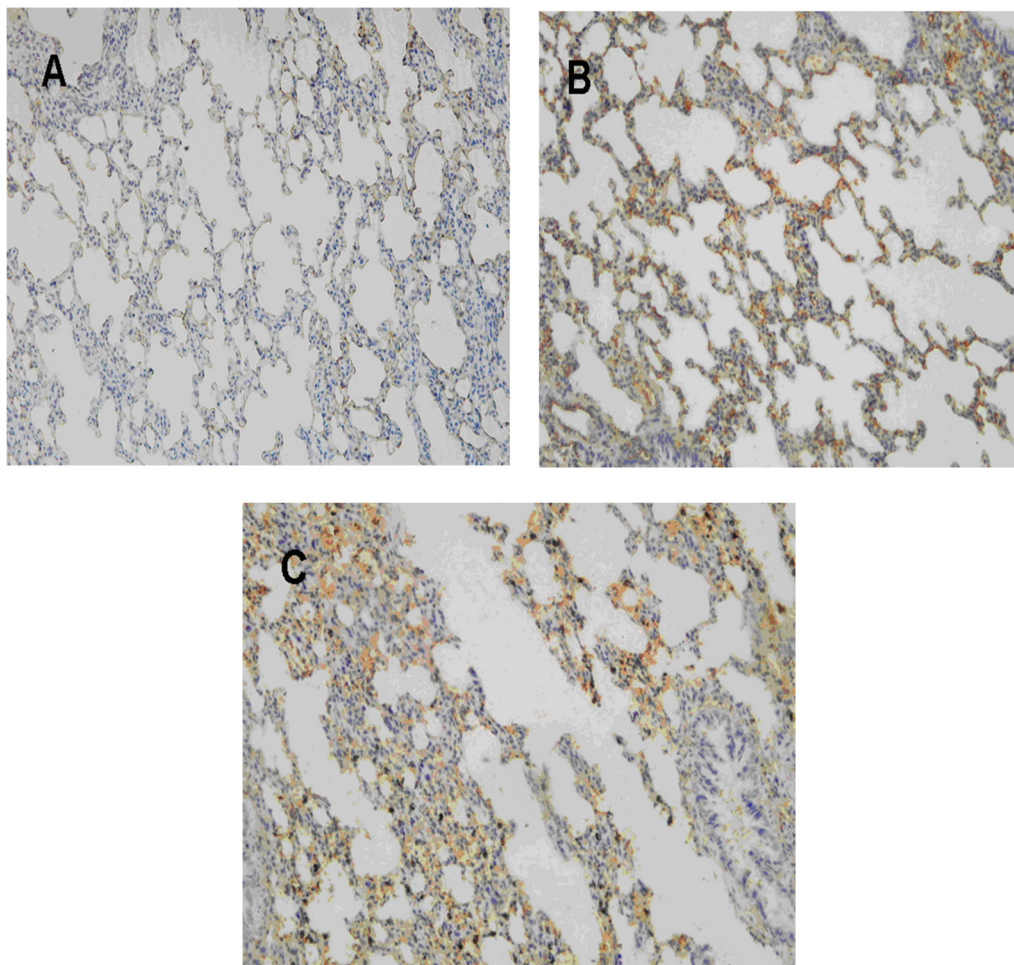
<sup>□</sup> Denotes  $P < 0.01$  vs 10 h group.

sulfur dioxide, ammonia and so on [15]. Exposure to wood smoke, which is composed primarily of aldehydes in general and aldehyde C in particular, causes protein denaturation in airway mucosa, induces inflammation, injures cilia, reduces

the vitality of alveolar macrophages, and damages alveolar capillary membranes to increase permeability, leading to pulmonary edema. In the current research, we successfully established smoke-induced acute respiratory distress in adult



**Fig. 5 – Pathology of lung tissue at different time point.** Examination with a light microscope revealed that the rats without smoke exposure had a distinct framework, complete alveolar walls and interstitium without exfiltration; the 1 h group rats showed mild edema, moderate neutrophil infiltration and mild hemorrhage. The 4 h group rats displayed mild edema, moderate neutrophil infiltration, moderate hemorrhage and mild bronchiole epithelial desquamation. The 24 h group rats showed severe edema, severe neutrophil infiltration, severe hemorrhage, moderate bronchiole epithelial desquamation and a little hyaline membrane formation. The lungs in the 1 h and 24 h groups revealed indications of more severe injury compared to the lungs from rats in the 1 h and 4 h groups. (A) control group; (B) 4 h group; (C) 24 h group.



**Fig. 6 – The ACE protein expression at different time point. Immunohistochemical staining revealed that the major sites of ACE protein expression were was evident in the lung endothelium and lung parenchyma, but not in vascular smooth muscle. (A) control group; (B) 4 h group; (C) 24 h group.**

rats as evidenced by the clinical symptoms and signs, significant changes in arterial blood gases, the remarkable increase of W/D ratio, protein content and leukocytes count in BALF. There is a wide range in the type and severity of lung injury due to the different source of smoke and duration of exposure. In order to evaluate the initial and dynamic inflammatory mechanism in the acute stage, we examined the concentrations of proinflammatory cytokines such as TNF- $\alpha$  and IL-6 in BALF. The results showed that cytokine-mediated inflammatory cascade was likely to play a key role in the pathogenesis of ARDS [16]. Moreover, the degree of inflammation gradually exacerbated at different time points in the first 24 h. Based on those findings, we assumed that the phenotypes of lung injury in smoke inhalation were partly related to the accumulation of inflammatory factor [17].

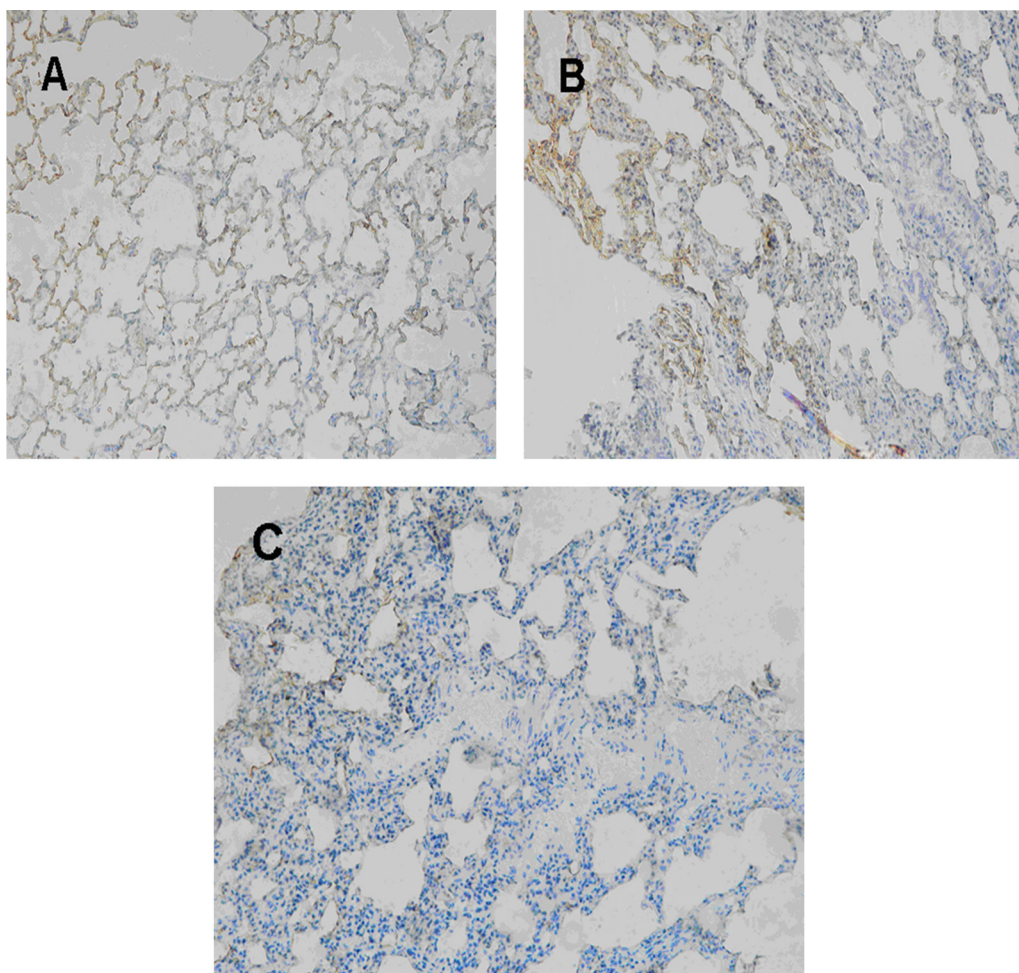
Pathology testing showed that, in smoke inhalation-induced injury, the initial harm was mostly to the trachea and bronchi, characterized by mucosal hyperemia and increased microvascular permeability, exfoliation of the epithelial lining, mucous secretion, mucous plugging, and an acute inflammatory cell influx [18]. There was an increase

in microvascular pressure and permeability to both large (proteins) and small (electrolytes, water, and glucose) molecules after smoke inhalation [19]. As it progresses, ARDS may result in widespread pulmonary vascular damage, which commonly produces pulmonary hypertension [20]. There was also a marked loss of functional pulmonary vessels resulted from a combination of intense vasoconstriction, interstitial edema and thromboembolism [20].

Even though rapid release of chemical mediators in the airway was a suggested mechanism for lung injury related to smoke inhalation, there was limited clarification on the underlying processes which lead to progressive lung injury. In recent decades, RAS has been thought to be essential in maintaining blood pressure homeostasis, as well as fluid and salt balance [21]. However, the latest evidence suggested that activation of a pulmonary RAS might influence the pathogenesis of ARDS via such mechanisms as inflammation, vascular permeability, vascular tone, and fibroblast activity [22].

ACE and ACE2 have been known as the key members in the classical renin-angiotensin system. The lungs are major organs that express Ang II and ACE in human beings and





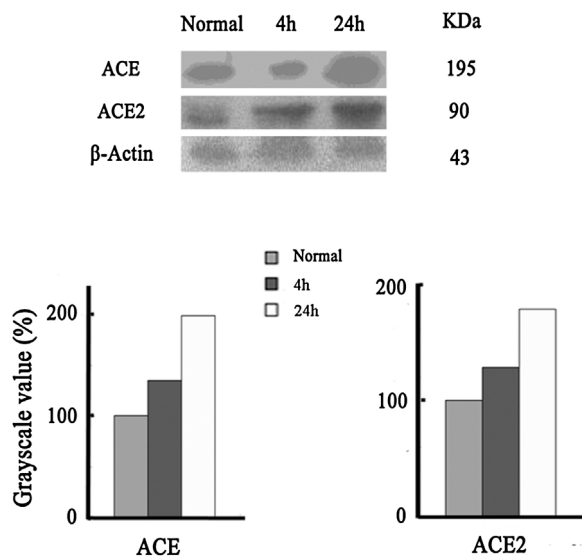
**Fig. 7 – The ACE2 protein expression at different time point. Immunohistochemical staining revealed that the major sites of ACE2 protein expression were in alveolar type I epithelium, alveolar type II epithelium, bronchiolar epithelium, endothelium and smooth muscle cells of pulmonary vascular structure. No obvious signal was detected in the bronchiolar smooth muscle cells. (A) control group; (B) 4 h group; (C) 24 h group.**

mice. ACE2 is the first human homolog of ACE discovered and isolated from human cDNA libraries prepared from ventricular and lymphoma tissues [23,24]. The enzyme of ACE2 Exhibits 42% sequence identity and 61% sequence similarity to ACE. Like ACE, ACE2 is membrane-bound, but it is a monocarboxypeptidase that generates Ang (1-9) from the decapeptide Ang I and generates Ang (1-7) from AngII[20]. ACE2 plays dual roles by both degrading the vasoconstrictor Ang II and producing the vasodilator Ang (1-7) [25]. Moreover, Su et al. [26] have reported that Ang (1-7) antagonizes Ang II-mediated cell signaling and limits Ang II-induced expression of transforming growth factor- $\beta$ 1.

The importance of RAS in ARDS has been widely emphasized since ACE2 was first demonstrated to be a functional receptor for the SARS coronavirus (SARS-CoV) in vitro and in vivo by binding to the receptor-binding domain (RBD, amino acids 319–510) of the SARS-CoV S protein [27,28]. Idell et al. [29] found that ARDS patients had elevated ACE levels in bronchoalveolar lavage. Orfanos [30] have also reported that the pulmonary capillary endothelium-bound ACE activity

correlated with the severity of lung injury in the patients with acute respiratory distress syndrome. Recent retrospective epidemiological studies demonstrated that pretreatment of an ACE inhibitor was associated with decreased mortality in patients hospitalized with community-acquired pneumonia [31]. Besides, Imail et al. [11] have investigated the role of ACE2 in ARDS using ace2 knockout mice. Loss of ACE2 expression in mutant mice also resulted in enhanced vascular permeability, increased lung edema, accumulation of neutrophil, and worsened lung function. The negative regulation of Ang II levels by ACE2 accounts, in part, for the protective function of ACE2 in ARDS. Additionally, there were large differences in plasma ACE concentrations between individuals but are similar concentrations within families [32], which suggested the genetic susceptibility of ACE to ARDS.

In the present study, we observed that ACE and ACE2 protein levels increased obviously in rat lung tissue after a short time of smoke inhalation. The fact that ACE increased immediately evidenced by the ACE levels in the lungs of rats sacrificed 1 h after smoke inhalation injury. ACE2 increased



**Fig. 8 – ACE and ACE2 expression levels in rat lungs. immunoreactive bands of approximately 195 kDa and 90 kDa were seen, corresponding to rat ACE and rat ACE2, respectively. The ACE and ACE2 content were dramatically increased in rat lungs immediately after smoke inhalation injury as compared to normal lungs.**

notably in the lungs of rats 1–4 h after smoke inhalation injury. The levels of ACE and ACE2 remained high for the remainder of our observed time. We concluded that the lung RAS participated in this injury process. From these results, we also concluded that, because of the noxious substances that were inhaled, the lung injury became much more severe with the time, and then ACE in the lung was generated and delivered to the periphery unceasingly, which would provoke a gradual increase in the Ang II in the lung. Ang II accumulation in the pulmonary capillary wall plays an important dual role in hemodynamics and unhemodynamics. In hemodynamics, up regulated Ang II may cause pulmonary vasoconstriction, pulmonary arterial pressure upgrading, and the increase of pneumoangiogram permeability. In Ang II unhemodynamics, in addition to taking part in the extracellular constituent accumulating in the local lung, Ang II may promote the infiltration of monocytes/macrophages and encourage transforming growth factor-1 (TGF-1) secretion, leading to more severe lung injury and the formation of pulmonary hypertension and fibrosis. In order to improve these reactions, the body increases the levels of ACE2 and vasodilating Ang (1–7) to restrain Ang II action in vivo. The ACE level is proportional to the amount of tissue Ang II available, and ACE2 may counterbalance the Ang II-promoting effects of ACE in tissue.

## 5. Conclusion

The ACE and ACE2 expression in rat lungs after smoke inhalation injury may play an important roles in the pathogenesis of ARDS. In order to explore novel therapeutic targets for lung injury, it is necessary in the future experiment to evaluate the treatment effect by utilizing pharmacological agents such as ACE2 agonists, ACE inhibitors or Ang II AT

1 receptors to pre- and/or post-treat rats with smoke inhalation injury.

## Conflict of interest statement

Authors declared that they have no conflict of interest.

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