Original Paper

Acute Respiratory Distress Syndrome Induced by White Smoke Inhalation: a Potential Animal Model For Evaluating Pathological Changes and Underlying Mechanisms

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Key Words

White smoke inhalation • Acute respiratory distress syndrome • NF-κB • Therapeutic target • Animal model

Abstract

Background/Aims: White smoke inhalation (WSI) is an uncommon but potentially deadly cause of acute respiratory distress syndrome (ARDS). However, no clinical treatment protocol has been established for the treatment of WSI-induced ARDS. Therefore, it is necessary to investigate the effects of WSI in ARDS and the mechanisms underlying the effects of WSI to determine a novel therapeutic target. **Methods:** On the basis of the duration of continued inhalation of white smoke (3 min, 5 min, and 7 min), rats were divided into three groups (WSI-3 min, WSI-5 min, and WSI-7 min). The survival rate, pathological change, and computed tomography (CT) score were evaluated to determine the modeling conditions. In the established WSI-5 min models, evaluations were performed to evaluate the following: arterial blood gas levels, lung wet/dry weight ratio, the expression of inflammatory cytokines, and the effect of NF-κB signaling pathway. **Results:** The survival rate of rats at 72 h post-WSI in the WSI-3 min, WSI-5 min, and WSI-7 min groups was 83.33%, 75%, and 25%, respectively. Results from evaluation of H&E staining, CT scan, arterial blood gas levels, and lung wet/dry weight ratio suggest that the pathological changes in the rat in the WSI-5 min and WSI-7 min groups are very similar to those in patients with ARDS induced by WSI. Additionally, the expression

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of INF- γ , TGF- β 1, TNF- α , and IL-1 β were increased, and the NF- κ B signaling pathway was activated in the WSI-5 min group. **Conclusion:** The rat model of WSI-5 min can be used as a WSI-induced ALI model for further experiments. The NF- κ B signaling pathway may be a potential therapeutic target for the treatment of WSI- induced ARDS.

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Introduction

Smoke pots, widely used in fire drills, can produce white smoke seconds after ignition, and the entire smoking time lasts for 6-7 min [1]. On exposure to high concentration of white smoke, especially in a confined space, white smoke is inhaled into the lung through the respiratory tract, which triggers inhalation injury [2], including acute lung injury (ALI), acute respiratory distress syndrome (ARDS) and pulmonary fibrosis, even leading to death [3-5]. However, no clinical treatment protocol has been established for the treatment of white smoke inhalation (WSI)-induced ARDS. Major components of the white smoke generated from a smoke pot include zinc chloride, hexachloroethane, oxides (zinc oxide, aluminum oxide, iron oxide, etc.), and carbon dust [6]. Additionally, some intermediate products, such as tetrachlorethylene, carbon tetrachloride, carbon monoxide, and carbon dioxide, are present mixed with the main components [7]. Cigarette smoking induces alveolar enlargement, endoplasmic reticulum stress, DNA damage, led to impairment of lung function [8, 9]. The components of white smoke differ from the components of smoke generated by the combustion of tobacco or wood dust; the former, in addition to carbon and carbon oxides, also contains other highly corrosive, irritant and toxic substances, including halogenated hydrocarbons and zinc chloride, which increase the severity of inhalation injury [10]. In the past few decades, many studies have been reported on ARDS caused by the individual or combined components of white smoke including zinc chloride and hexachloroethane [11, 12]. However, the effects of all components of white smoke on ARDS, and the mechanisms underlying these effects, remain unclear.

Previous reports of ARDS caused by WSI were mainly based on analysis of clinical cases. Owing to the effects of the treatment, it is difficult to determine the pathophysiological changes and pathogenesis of ARDS caused by WSI. Therefore, an animal model of WSI-induced ALI is needed to understand the pathophysiological changes and pathogenesis of ARDS caused by WSI, which will aid in determining strategies for timely diagnosis and finding a novel therapeutic target for this condition. In this study, we explored the conditions for developing an animal model with WSI-induced ALI based on the survival rate for investigated the pathophysiological changes of ARDS caused by WSI. Furthermore, the effect of inflammatory cytokines and NF- κ B signaling pathway on WSI-induced ALI model were evaluated.

Materials and Methods

Preparation of experimental rats

All animal protocols were approved by the ethics committee of the No. 181 Hospital of the People's Liberation Army (PLA) and the disposal methods followed animal ethics standards. Specific pathogen-free (SPF) male Sprague-Dawley (SD) rats weighing 200-220 g were used. All rats were purchased from the animal center of Guilin medical college. The rats were allowed free access to water and food and were maintained at 20-25°C; the room was continuously ventilated, with 12 hours of illumination and 12 hours of darkness. The rats were adapted to the environment for 7 days before establishing the WSI model.

Establishment of the WSI model

The experimental steps for establishing the WSI model are shown in Fig. 1. First, rats were placed in a rat cage, with an isolation network to separate each rat, so that the impact of white smoke could be observed individually. Then, the rat cage was placed in the center of the 2-m² homemade laboratory equipment that is used for research on smoke-induced injury (Patent No.: 201720002689.0), and the smoke pot was

located 60 cm below the rat cage. Additionally, the smoke pot was ignited and allowed to burn, and it gradually produced smoke. The duration of WSI (WSI-time) was calculated from the beginning of smoke generation. After WSI, the rats were fed under normal conditions as described in 2.1.

Experimental groups

Thirty-six rats were divided into 3 groups randomly: WSI-3 min group (WSI-time was 3 mins); WSI-5 min group (WSI-time was 5 mins); and WSI-7 min group (WSI-time was 7 mins). The survival rate of the rats was observed at 24 h, 48 h, and 72 h post-WSI to screen the modeling conditions. Then, rats induced by WSI were observed at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI.

Histomorphological features of the lung tissues

The histomorphological features of the lung tissues were evaluated by hematoxylin and eosin (H&E) staining. The diaphragmatic leaves of the right lung from rats in each group were fixed with 4% formalin for 48 h, washed, dehydrated, hardened, cleared, and infiltrated with wax; the samples were then embedded in paraffin, and 4- μ m sections were obtained. Hematoxylin and eosin (H&E) staining was performed according to the manufacturers' instructions (Solarbio, Beijing, China). Histomorphological features of the lung tissues were observed under an optical microscope (Olympus, Japan). The degree of lung injury was graded as described previously [13].

CT Scan evaluation

Rats were fixed to a foam board in supine position under general anesthesia using chloral hydrate. Then, rat breast was scan was performed by a GE LightSpeed VCT (GE Healthcare, Little Chalfont, UK) 64-slice CT at 80 kV tube voltage, 150 mA tube current, and medium bowtie filter (0.625-mm half-value layer). CT images were scored according to the Radiologist's score (RADS) method as previously described [14].

Analysis of arterial blood gas levels

One-mL of anti-coagulated blood sample was collected from the abdominal aorta of each rat. Then, the PaO_2 , PaCO_2 , and $\text{PaO}_2/\text{FiO}_2$ were analyzed by blood gas analyzer (Radiometer, Denmark).

Wet/dry weight ratio

After anesthetic overdose and exsanguination (by severing of the inferior vena cava and the abdomen aorta), all the excised lung lobes per mouse were weighed (wet weight), placed in an oven and weighed daily until its weight unchanged (dry weight). The wet/dry weight ratio was then calculated.

Inflammatory cytokines were measured by ELISA

In each rat, the right lung was removed followed by ligation of the right main bronchus. Injection of 1 ml of iced saline from the end of the trachea was performed, and the lavage was repeated three times. Then, 2.6 ml of bronchoalveolar lavage fluid was collected and centrifuged at 350 g for 10 min at 4 ° C. TGF- β 1, TNF- α , IL-1 β , and INF- γ levels in the supernatant of the bronchoalveolar lavage fluid were evaluated using ELISA kits (Multi science, Hangzhou, Zhejiang, China).

Western blot analysis

Western blot analysis was used to measure protein expression in the lung tissue. Briefly, a protein lysis buffer was added in the minced lung tissue and homogenized. Then, samples were centrifuged at 12000 rpm and 4°C. The protein concentrations in the supernatant were quantified using the BCA protein quantification kit (Tiangen, Beijing, China), and subsequently aliquoted into samples. Then, 50 μ g of protein

Fig. 1. Schematic of the establishment white smoke inhalation (WSI) model.

was electrophoresed using an 10% SDS-PAGE and transferred onto a nitrocellulose membrane (BioTrace, USA). The membranes were incubated overnight with antibodies against phosphorylated (p)-I κ B- α , I κ B- α , p-p65 NF- κ B, p65 NF- κ B, and GAPDH (Cell Signaling Technology, 1:1000 dilution) at 4°C on a rotating shaker. Subsequently, the membranes were washed with TBST three times for 5 min each, followed by incubation with horseradish peroxidase-labeled goat anti-rabbit IgG (Beijing ZSGB Bio, 1: 5000 dilution) for 1 h at 25°C on a horizontal shaker. Then, the membranes were washed with TBST twice for 5 min each time, followed by chemiluminescent detection. The protein band areas were quantified using the Image Lab 6.0 software, and the GAPDH antibody was used as the internal reference.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) v19.0 software (SPSS Inc, Chicago, IL, USA). All data are expressed as mean $\pm$ standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc tests of least significant difference (LSD) for multiple pairwise comparisons. A P value < 0.05 was considered statistically significant.

Results

Effect of WSI time on the survival rate of rats

The survival rate of rats after WSI-induced ARDS is shown in Table 1. The results showed that the survival rate of rats in WSI-3 min, WSI-5 min, and WSI-7 min groups decreased gradually. Compared with the survival rate at 0 h post-WSI, the survival rate of rats at 24 h, 48 h, and 72 h post-WSI in WSI-3 min group showed no significant reduction; the survival rate of rats showed no significant reduction at 24 h but had significantly reduced at 48 h and 72 h post-WSI in WSI-5 min group; and the survival rate of rats had significantly reduced at 24 h, 48 h, and 72 h post-WSI in the WSI-7 min group.

WSI is associated with histomorphological change in the lung tissue in rats

Histomorphological changes in the lung tissue were evaluated by H&E staining, and the results are shown in Fig. 2. At 6 h and 12 h post-WSI, lung tissue in the WSI-3 min and WSI-5 min groups exhibited congestion of airway mucosa, edema, bleeding, and slightly enlarged alveolar space. At 24 h post-WSI, lung tissue in the WSI-3 min and WSI-5 min groups exhibited neutrophil infiltration and shedding and necrosis of epithelial cells. At 48 h post-WSI, lung tissue in the WSI-3 min and WSI-5 min groups exhibited alveolar edema and diffuse bleeding. At 72 h post-WSI, lung tissue in the WSI-5 min group exhibited alveolar wall thickening and alveolar wall collapse, but lung tissue in the WSI-3 min group exhibited decreased edema and reduced neutrophil infiltration. Lung tissue in the WSI-7 min group exhibited neutrophil infiltration, edema, and bleeding at 6 h and 12 h post-WSI. With the extension of observation time, lung tissue in the WSI-7 min group exhibited obviously enlarged alveolar space, thinner alveolar septum, and destroyed alveolar walls. The results also showed that the pathological score in WSI-3 min group gradually increased at 6 h, 12 h, 24 h, and 48 h post-WSI, followed by reduction at 72 h post-WSI; the pathological score in the WSI-5 min and WSI-7 min groups gradually increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI. Compared with the control group, the pathological score in the WSI-3 min, WSI-

Table 1. Effect of the duration of white smoke inhalation (WSI) on the survival rate of rats. *P<0.05, **P<0.01, ***P<0.001 vs. 0 h in same group. #P<0.05, ##P<0.01, ###P<0.001 vs. WSI-3 min group at same time-point post-WSI

Time post WSI	WSI-3 min	WSI-5 min,	WSI-7 min
0 h	100% (12/12)	100% (12/12)	100% (12/12)
24 h	100% (12/12)	83.33% (10/12)	58.33% (7/12)**
48 h	91.66% (11/12)	75% (8/12)*	41.66% (5/12)***
72 h	83.33% (10/12)	75% (8/12)*	25% (3/12)*****

Fig. 2. Histomorphological features of the lung tissue injury due to white smoke inhalation (WSI) were evaluated by Hematoxylin and Eosin staining in the WSI-3 min, WSI-5 min, and WSI-7 min groups at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI and in the control group. The data of the pathological score are represented as mean $\pm$ SD. * P <0.05, ** P <0.01, and *** P <0.001 vs. Control. # P <0.05, ## P <0.01, and ### P <0.001 vs. WSI-3 min. & P <0.05 and && P <0.01 vs. WSI-5 min.

5 min, and WSI-7 min groups were significantly increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI (P <0.05). Compared with the WSI-3 min group, the pathological score in the WSI-5 min and WSI-7 min groups were significantly increased at the same time post-WSI (P <0.05). Compared with the WSI-5 min group, the CT score in the WSI-7 min group was significantly increased at 48 h and 72 h (P <0.05), but no significant changes were observed at 6 h, 12 h, and 24 h post-WSI (P >0.05).

WSI is associated with enhanced CT score of lung tissue in rats

Fig. 3 shows the results of the chest CT scan of rats in the normal control group and in the WSI-3 min, WSI-5 min, and WSI-7 min groups after 6 h, 12 h, 24 h, 48 h, and 72 h after WSI. The results showed that the lung marking, ground-glass opacities, and consolidation of lung in the WSI-3 min, WSI-5 min, and WSI-7 min groups showed different degrees of increase compared with the control group. The results also showed that the CT score in the WSI-3 min group gradually increased at 6 h, 12 h, 24 h, and 48 h post-WSI, followed by reduction at 72 h post-WSI; the CT scores in WSI-5 min and WSI-7 min groups were gradually increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI. Compared with control group, the CT score in WSI-3 min, WSI-5 min, and WSI-7 min groups were significantly increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI (P <0.05). Compared with the WSI-3 min group, the CT score in WSI-7 min group was significantly increased at the same time post-WSI (P <0.001), and the CT score in WSI-5 min were significantly increased at 24 h, 48 h, and 72 h (P <0.001) but had no significant changes at 6 h and 12 h post-WSI (P >0.05). Compared with the WSI-5 min group, the CT score in the WSI-7 min group were significantly increased at 6 h, 12 h, and 24 h (P <0.001), but no significant changes were observed at 48 h and 72 h post-WSI (P >0.05).

Fig. 3. Lung injury induced by white smoke inhalation (WSI) was tested by CT scan in the WSI-3 min, WSI-5 min, and WSI-7 min groups at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI and in the control group. The data of the CT score were represented as mean $\pm$ SD. The CT score in control group was 0. *** P <0.001 vs. Control. ### P <0.001 vs. WSI-3 min. &&& P <0.001 vs. WSI-5 min.

WSI-5 min is associated with changes in arterial blood gas in rats

The results of evaluation of PaO_2 , PaCO_2 , and $\text{PaO}_2/\text{FiO}_2$ in the control group and at 6 h, 12 h, 24 h, 48 h, and 72 h after WSI-5 min are shown in Fig. 4. PaO_2 and $\text{PaO}_2/\text{FiO}_2$ gradually decreased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min. The level of PaCO_2 gradually increased at 6 h, 12 h, 24 h, and 48 h followed by reduction at 72 h post-WSI-5 min. The results also showed that the levels of PaO_2 and $\text{PaO}_2/\text{FiO}_2$ were significantly decreased (P <0.001), while the levels of PaCO_2 were significantly increased (P <0.01) at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min compared with those in the control group.

WSI-5 min is associated with increased wet/dry weight ratio in rat lung

Fig. 5 shows the results of evaluation of wet/dry weight ratio in the control group and at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min. Wet/dry weight ratio gradually increased at 6 h, 12 h, 24 h, and 48 h, followed by reduction at 72 h post-WSI-5 min, and the wet/dry weight ratio was significantly increased in the WSI-5 min group compared with the control group (P <0.001).

WSI-5 min is associated with increase in the levels of inflammatory cytokines in rats

The levels of TGF- β 1, TNF- α , IL-1 β , and INF- γ in the bronchoalveolar lavage fluid were measured by ELISA (Fig. 6). The results showed that the levels of TGF- β 1, TNF- α , IL-1 β ,

Fig. 4. PaO_2 (mmHg), PaCO_2 (mmHg), and $\text{PaO}_2/\text{FiO}_2$ were evaluated at 6 h, 12 h, 24 h, 48 h, and 72 h in the white smoke inhalation (WSI)-5 min and the control group. Values for PaO_2 (mmHg), PaCO_2 (mmHg), and $\text{PaO}_2/\text{FiO}_2$ are presented as mean $\pm$ SD. ** $P < 0.01$, and *** $P < 0.001$ vs. Control.

and $\text{INF-}\gamma$ were significantly increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min compared with the control group ($P < 0.05$). Additionally, the levels of $\text{TGF-}\beta 1$ was gradually increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min. The levels of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ gradually increased at 6 h, 12 h, 24 h, followed by reduction at 48 h and 72 h post-WSI-5 min. The levels of $\text{INF-}\gamma$ were increased at 6 h, followed by reduction at 12 h, 24 h, 48 h, and 72 h post-WSI-5 min.

WSI-5 min is associated with activation of the NF- κ B signaling pathway in rats

The expression of p-I κ B- α , I κ B- α , p-p65 NF- κ B, and p65 NF- κ B were analyzed by western blotting (Fig. 7). The results showed that the expression of p-I κ B- α , p-p65 NF- κ B, and p65 NF- κ B were significantly increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min compared with the control group ($P < 0.05$). Additionally, p-p65 NF- κ B and p65 NF- κ B were gradually increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI-5 min. The expression of p-I κ B- α gradually increased at 6 h, 12 h, 24 h, and 48 h, followed by reduction at 72 h post-WSI-5 min.

Discussion

WSI is an accidental event, but it may cause ALI, ARDS, and even death. Previous reports on ARDS caused by WSI were mainly based on analysis of clinical cases. However, studies on WSI-induced ARDS using animal models are rare.

In this study, we found that the survival rates of rats in the WSI-3 min, WSI-5 min, and WSI-7 min groups were gradually reduced with the extension of feeding time (at 24 h, 48 h, and 72 h). The survival rates of rats at 72 h post-WSI in the WSI-3 min, WSI-5 min, and WSI-7 min groups were

Fig. 5. Wet/dry weight ratio was evaluated in the white smoke inhalation (WSI)-5 min group at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI and in the control group. Values for wet/dry weight ratio are presented as mean $\pm$ SD. *** $P < 0.001$ vs. Control.

Fig. 6. Levels of TGF- β 1, TNF- α , IL-1 β , and INF- γ in the bronchoalveolar lavage fluid were measured by ELISA at 6 h, 12 h, 24 h, 48 h, and 72 h in the white smoke inhalation (WSI)-5 min and the control groups. The data are presented as mean $\pm$ SD. * P <0.05, ** P <0.01, and *** P <0.001 vs. Control.

Fig. 7. The expressions of p-I κ B- α , I κ B- α , p-p65 NF- κ B, and p65 NF- κ B were analyzed by western blotting at 6 h, 12 h, 24 h, 48 h, and 72 h in the WSI-5 min group. The data are presented as mean $\pm$ SD. * P <0.05, ** P <0.01, and *** P <0.001 vs. Control.

83.33%, 75%, and 25%, respectively. The results suggest that due to the low survival of rats in the WSI-7 min group, further experiment using those rats is impossible; thus, the rats in the WSI-3 min and WSI-5 min groups may be used as a WSI model for further experiments.

In this study, histopathological examination of the lungs of the rats revealed congestion, edema, bleeding, and neutrophil infiltration in the airway mucosa, with alveolar edema and diffuse bleeding, alveolar wall thickening, and alveolar wall collapse post-WSI. The pathological scores gradually increased at 6 h, 12 h, 24 h, 48 h, and 72 h post-WSI, and all scores were significantly higher than those in normal rats, similar with the pathological characteristics of patients induced by WSI. The pathological characteristics of patients induced by WSI include pulmonary edema, bleeding, inflammatory infiltration, diffuse alveolar damage, and pulmonary fibrosis [15-17]. In our study, the results also revealed that the pathological scores were reduced at 72 h post-WSI in the WSI-3 min group and were gradually increased after WSI in the WSI-5 min and WSI-7 min groups; the pathological scores in the WSI-5 min and WSI-7 min groups were significantly higher than those in the WSI-3 min group, suggesting that the degree of lung injury increased in a time-dependent manner in the WSI-5 min and the WSI-7 min groups, but the lung injury may have resolved spontaneously at 72 h post-WSI in the WSI-3 min group. Additionally, the lung marking, ground-glass opacities, and consolidation of lung showed different degrees of increase after WSI. The CT features were similar to the characteristic features of pulmonary vascular lesions in the adult respiratory distress syndrome caused by inhalation of zinc chloride smoke [18]. Similar to the pathological scores, the CT scores also suggest that the degree of lung injury was increased in a time-dependent manner in the WSI-5 min and WSI-7 min groups, but the

lung injury may have spontaneously resolved at 72 h post-WSI in the WSI-3 min group. The results of H&E staining and CT scanning suggest that the pathological changes in the rats in the WSI-5 min and WSI-7 min groups are very similar to those observed in humans and can simulate the pathological changes in patients with lung injury induced by WSI. But the lung injury may have spontaneously resolved at 72 h post-WSI in the WSI-3 min group, so the rats of the WSI-3 min group can not be used as WSI models. Thus, based on our study results obtained by evaluation of the survival rate, H&E staining, and CT scan, the rats of the WSI-5 min group can be used as WSI models for further experiments.

The PaO_2 and $\text{PaO}_2/\text{FiO}_2$ were significantly decreased ($P < 0.001$) while PaCO_2 was significantly increased ($P < 0.01$) in the WSI-5 min group compared with the control group, suggesting that the rats in the WSI-5 min group develop respiratory insufficiency, which is exacerbated in a time-dependent manner. Additionally, we found that the wet/dry weight ratio gradually increased in the WSI-5 min group, and it was significantly increased compared with control group, suggesting that the degree of pulmonary edema increased in the WSI-5 min group in a time-dependent manner. This finding is similar to the findings of previous studies that showed the degree of pulmonary edema was increased after smoke inhalation in ovine model [19]. The results of the evaluation of arterial blood gas levels and lung wet/dry weight ratio further indicate that the pathological changes in the rat from the WSI-5 min group are very similar to those in humans and can simulate the pathological changes in patients with lung injury induced by WSI.

To identify the potential mechanisms underlying the effects of WSI, the levels of TGF- β 1, TNF- α , IL-1 β , and INF- γ in the bronchoalveolar lavage fluid and the expression of p-I κ B- α , I κ B- α , p-p65 NF- κ B, and p65 NF- κ B in lung tissues were assessed. IL-1 β , INF- γ , TNF- α and TGF- β 1 levels were closely related to lung injury, inhibiting those expression can reduce lung injury [20]. TGF- β 1 after acute respiratory distress syndrome can regulate lung injury repair, especially the regulation of repair in pulmonary fibrosis in the advanced stages of the disease [21, 22]. TNF- α activates the inflammatory response and plays an important role in lung injury [23]. TGF- β 1 and TNF- α levels were reported to be increased after WSI during military training [24]. IL-1 β suppresses cigarette smoke-induced pulmonary inflammation in mice [25]. In the early stage of ARDS, IL-1 β expression was enhanced and promoted alveolar epithelial repair [26]. The results showed that the levels of TGF- β 1, TNF- α , IL-1 β , and INF- γ were significantly increased in the WSI-5 min group compared with the control group, suggesting that early inflammatory cytokines were rapidly produced in response to WSI. NF- κ B, an important nuclear transcription factor, were activated by various inflammatory cytokines such as TGF- β 1, TNF- α , and IL-1 β . NF- κ B activation can in turn promote the expression of inflammatory cytokines such as INF- γ , TGF- β 1, TNF- α , and IL-1 β [27, 28]. A previous study showed that NF- κ B activation occurred in the lungs of patients with ARDS and may contribute to the increased expression of immunoregulatory cytokines and other proinflammatory cytokines [29]. NF- κ B activation promotes cigarette smoke-induced lung inflammation and airway epithelial cell apoptosis [30]. In this study, we found that the expressions of p-I κ B- α , p-p65 NF- κ B, and p65 NF- κ B were significantly increased at 6 h, 12 h, 24 h, 48 h, and 72 h in the WSI-5 min group compared with the control group. These results suggest that the NF- κ B signaling pathway was activated by WSI, which may promote the expression of INF- γ , TGF- β 1, TNF- α , and IL-1 β , increasing the severity of lung injury. Thus, the NF- κ B signaling pathway may be a potential therapeutic target for the treatment of WSI-induced ARDS.

In conclusion, we found that the pathological changes in the rat model of WSI-5 min are very similar to those in patients with ARDS induced by WSI. Additionally, the NF- κ B signaling pathway was activated by WSI, which may promote the expression of INF- γ , TGF- β 1, TNF- α , and IL-1 β . Thus, the NF- κ B signaling pathway may be a potential therapeutic target for the treatment of WSI-induced ARDS. The rats in the WSI-5 min group can be used as the WSI-induced ALI models for further experiments.

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Disclosure Statement

The authors who have taken part in this study declare that they have no conflicts of interest with respect to this paper.

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