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Construction and evaluation of a graded rat model for smoke inhalation lung injury

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ABSTRACT

Objective: To address the critical impact of smoke inhalation lung injury on burn patient prognosis and the lack of animal models simulating varying injury severity, we established a graded rat model to facilitate research into its pathological mechanisms and treatments.

Methods: Eighteen male Wistar rats were divided into a control group and groups exposed to smoke for 5, 10, 15, 30 minutes ($n=3$ per group). A custom smoke chamber and specific materials (50 g pine wood chips + 10 mL kerosene) were used. Post-exposure assessments included lung function, lung coefficient (wet weight), histopathology (H&E staining), and leukocyte count and IL-6 levels in alveolar lavage fluid.

Results: Lung function declined significantly in all exposure groups versus controls, with progressive worsening over time. The lung coefficient increased significantly in the 15- and 30-minute groups. Histology revealed intact structure at 5 and 10 minutes, alveolar rupture and neutrophil infiltration at 15 minutes, and hyaline membranes at 30 minutes. Leukocyte count and IL-6 levels rose markedly after 15 and 30 minutes of exposure. Statistical comparisons confirmed progressive injury severity with longer exposure.

Conclusions: Graded rat models of mild, moderate, and severe inhalation lung injury were successfully established following 5, 15, and 30 minutes of smoke exposure, respectively. Injury and inflammation intensified significantly with exposure duration, providing a valuable foundation for further research.

KEYWORDS: Animal models; Inhalation lung injury; Lung function; Lung coefficient; IL-6

Summary

Question: What is a feasible method for establishing a graded animal model of smoke inhalation lung injury that simulates varying levels of severity in rats?

Findings: Graded rat models of smoke inhalation lung injury were successfully established based on exposure duration (5, 15, and 30 minutes). Injury severity, as assessed by lung function decline, increased lung coefficient, histopathological changes (including alveolar rupture, neutrophil infiltration, and hyaline membranes), and elevated leukocyte count and IL-6 levels in alveolar lavage fluid, progressed significantly with longer exposure time.

Meaning: This study provides a reproducible and severity-graded animal model that simulates mild, moderate, and severe smoke inhalation lung injury, which can serve as a valuable tool for investigating underlying pathological mechanisms and testing potential therapeutic interventions.

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1. Introduction

Smoke inhalation injury, a prevalent and severe trauma in fire accidents[1,2], can directly lead to serious complications such as acute respiratory distress syndrome, airway obstruction, and pulmonary edema. Moreover, it may trigger systemic inflammatory response syndrome and multiple organ dysfunction[3,4], thereby significantly increasing mortality among burn patients[5]. According to statistical data, approximately 50%–80% of fire-related fatalities are associated with smoke inhalation injury[6].

Harmful components in smoke—including carbon monoxide, cyanide, aldehydes, and particulate matter—can damage the airway mucosal structure, disrupt alveolar-capillary barrier function, and activate inflammatory cascades. These effects initiate a range of pathological processes such as oxidative stress, neutrophil infiltration, and apoptosis[7,8]. The severity of inhalation injury is closely associated with the duration of exposure, concentration, and chemical composition of the smoke[9]. Depending on exposure conditions, pathological manifestations vary mild cases typically present with airway inflammation, whereas severe cases can rapidly progress to pulmonary fibrosis and even respiratory failure.

Currently, the clinical management of smoke inhalation injury continues to face multiple challenges, including the absence of standardized early intervention protocols and considerable variability in treatment responses depending on injury severity[10]. Therefore, it is essential to develop animal models that can accurately replicate different degrees of smoke inhalation injury. Such models will help systematically elucidate the dynamic molecular and cellular changes in mild, moderate, and severe cases, provide a theoretical basis for targeted therapies, and enable comparative evaluation of the efficacy of various interventions—such as anti-inflammatory agents, antioxidants, and mechanical ventilation—across different injury levels. Furthermore, these models will contribute experimental support for the development of tailored treatment strategies, including airway management and fluid resuscitation protocols.

Based on the objectives, this study selected a mixture of wood chips and kerosene—highly similar to actual fire smoke—as the fuel source. The resulting combustion smoke contained high concentrations of carbon monoxide, irritating gases (including aldehydes and sulfur dioxide), and polycyclic aromatic hydrocarbon (PAH) particulate matter. By controlling the quantity of combustible material and combustion duration, both smoke inhalation time and concentration were precisely regulated, enabling the establishment of rat models exhibiting mild,

moderate, and severe inhalation lung injury. This model will not only facilitate in-depth investigation of the pathophysiological mechanisms underlying smoke inhalation injury but also serve as a critical tool for developing precise therapeutic strategies.

2. Methods

2.1. Animals and experimental intervention

A total of 18 six- to eight-week-old male Wistar rats (weighing 220–250 g) were obtained from SPF (Beijing) Biotechnology Co., Ltd. [SCXK (Beijing) 2024-0001] and housed at Contec Medical Laboratory Service Hebei Co., Ltd. [SYXK (Ji) 2021-006]. Throughout the study, the rats were provided with standard maintenance feed from the same supplier and had access to water *ad libitum*. The animals were maintained under a 12-hour light/dark cycle, with humidity controlled at 40%–60% and temperature maintained at 22–24 °C.

To establish a model of mild, moderate, and severe inhalation-induced lung injury, 18 male Wistar rats were randomly assigned to a control group and five experimental groups (exposed to smoke inhalation for 5, 10, 15, and 30 minutes, respectively), resulting in five groups with three rats per group. The rats were placed in a smoke exposure chamber. The control group breathed normal air, while the five experimental groups underwent smoke inhalation for 5, 10, 15, and 30 minutes, respectively. Rats in the other four experimental groups and the control group underwent pulmonary function tests. After euthanasia, bilateral lung tissues were collected for wet weight measurement. The right lung tissue was used for pathological examination, and the left lung tissue was cryopreserved in liquid nitrogen for subsequent research.

2.2. Reagents and instruments

KT-YOSB-0634 Smoking Box (Research Institute of Tsinghua University, Shenzhen, China); SD-M2000A Animal Anesthesia Machine (sdhealth, China); Madlab-4C/501H Biosignal Collector (Zhongshi Dichuang, China); and an Upright Fluorescence Microscope (Leica, DM3000, Japan).

2.3. Establishment of a model of smoke inhalation lung injury

A custom-designed smoke exposure chamber was constructed for modeling purposes in this study. The smoke box consists of a combustion chamber and an enclosed smoke chamber. Measuring

60×50×135 cm, the unit features the combustion chamber at the bottom, which can be loaded with various combustible materials for smoke generation. Above it is the sealed smoke chamber, divided into four compartments to house experimental rats. The smoke chamber is fitted with ventilation holes connected to the combustion chamber and an exhaust fan at the top. For the experiment, the smoking material used was a mixture of 50 g of dried pine wood chips and 10 mL of kerosene. The mixture was ignited and allowed to burn for 10 minutes after the smoke had stabilized.

2.4. Pulmonary Function Tests

The rats in each group were connected to a Madlab-4C/501H biosignal collector (following the manufacturer's instructions), and the recorded indicators included forced vital capacity (FVC), the volume exhaled in the first 0.3 seconds (FEV0.3), and the ratio of FEV0.3 to FVC (FEV0.3/FVC) *etc.*

2.5. Lung coefficient calculation

Lung coefficient is a key indicator for assessing pulmonary edema following smoke inhalation injury[11]. Prior to the smoke inhalation experiment, each of the 18 rats was weighed and the values were recorded for subsequent analysis. After pulmonary function testing, the airway and thoracic cavity were opened to extract lung tissue, and the wet lung weight was measured. The lung coefficient was calculated using the following formula: lung coefficient (%)=[wet lung weight (g)/body weight (g)]×100%.

2.6. Measurement of leukocyte count and IL-6 expression in alveolar lavage fluid

Following the pulmonary function test, rats from different groups were anesthetized via intraperitoneal injection of 3% pentobarbital sodium and placed in a supine position. An incision was made along the midline of the neck. After separating the muscle tissue, the trachea was fully exposed. A small incision was made between the tracheal cartilage rings, and a lavage catheter was inserted into the right main bronchus, then ligated and secured. Pre-warmed (37°C) sterile saline (2 mL per aliquot) was slowly infused into the lung through the catheter. After 1 minute, the alveolar lavage fluid was slowly withdrawn, achieving a recovery rate of approximately 20%. This procedure was repeated three times. The collected alveolar lavage fluid was centrifuged (4 °C, 2000 rpm, 10 minutes). The supernatant was collected, and the level of IL-6 was determined using the ELISA method. The cell pellet was resuspended for leukocyte counting. A 10 μ L aliquot of the cell suspension was drawn using a micropipette and slowly loaded into a hemocytometer chamber. After allowing the cells to settle, all leukocytes within the four corner

squares were counted under a microscope. The total leukocyte count per milliliter of the original suspension was calculated using the following formula: (Total count÷4)×Dilution factor×10.

2.7. Pathological observations

Following pulmonary function testing, the airway and thoracic cavity were opened to permit macroscopic examination of the lungs, which was followed by radiographic imaging. Subsequently, the right lung tissue was collected and fixed in 10% formaldehyde solution. Conventional methods of paraffin embedding, sectioning, and HE staining were performed to evaluate pathological alterations in the lung tissue. The left lung was preserved in liquid nitrogen for subsequent analysis.

2.8. Statistical analysis

The data were analyzed by Prism10. Data are presented as the mean±standard deviation. One-way analysis of variance was used for comparison between multiple groups. Statistical significance for comparisons among multiple groups was defined as $P<.05$.

3. Results

3.1. Lung function results of rat model with graded smoke inhalation lung injury

In the graded smoke inhalation lung injury model, compared with the control group, lung function was significantly impaired in all other experimental groups (30-minute group: $P=.001$; 15-minute group: $P=.001$; 10-minute group: $P=.006$; 5-minute group: $P=.01$) (Supplementary Figure 1). Intergroup comparisons among the experimental groups revealed no significant difference in lung function between the 5-minute and 10-minute smoke inhalation groups ($P=.97$). The 15-minute group exhibited a significant reduction in lung function compared with both the 10-minute group ($P=.01$) and the 5-minute group ($P=.008$). Furthermore, the 30-minute group showed the most pronounced decline in lung function, with statistically significant differences compared to the 15-minute ($P=.01$), 10-minute ($P=.001$), and 5-minute groups ($P=.001$) (Figure 1).

3.2. Lung coefficient results of rat model of graded smoke inhalation lung injury

The lung coefficient was 0.5% in the 15-minute smoke inhalation group and 0.9% in the 30-minute group, both showing statistically

significant differences compared with the control group (0.2%) ($P=.003$ and $P=.001$, respectively). In contrast, the 5- and 10-minute smoke inhalation groups exhibited lung coefficients of 0.3%, which did not differ significantly from the control group ($P>.99$ and $P=.99$, respectively) (Supplementary Figure 2). Intergroup comparisons revealed a statistically significant difference between the 30-minute and 15-minute groups ($P=.03$), as well as between the 15-minute and 10-minute groups ($P=.02$). Significant differences were also observed between the 30-minute group and the 10-minute group ($P=.001$), between the 15-minute group and the 5-minute group ($P=.01$), and between the 5-minute group and the 30-minute group ($P=.001$) (Figure 2). However, no significant difference was found

between the 5-minute and 10-minute groups ($P=.98$). These results suggest that the severity of lung injury is dependent on the duration of smoke inhalation, with longer exposure times leading to more severe lung injury.

3.3. Pathological detection results of HE staining in rat model of graded smoke inhalation lung injury

Histopathological examination of lung tissue by H&E staining revealed that, compared with the control group, no significant structural damage was observed in rats subjected to 5 or 10 minutes of smoke inhalation. In the 15-minute smoke inhalation group, alveolar rupture and neutrophil infiltration were detected

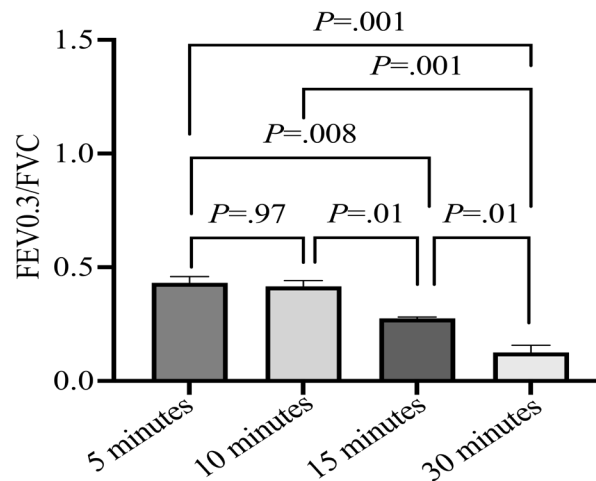


Figure 1. FEV0.3/FVC comparison results of different smoke inhalation time groups.

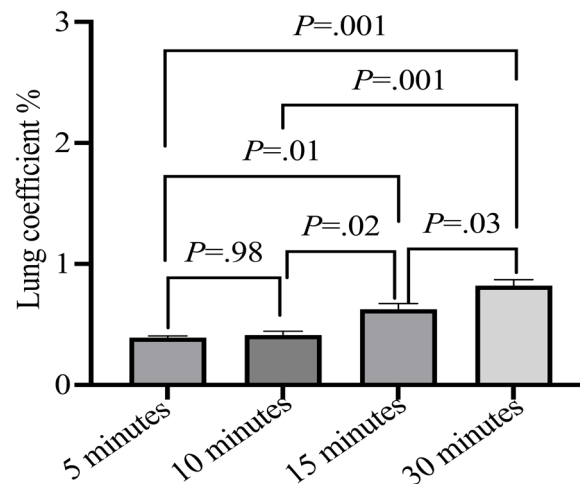


Figure 2. Lung coefficient comparison results of different smoke inhalation time groups.

under microscopy. After 30 minutes of smoke inhalation, hyaline membrane formation became apparent (Figure 3).

3.4. The Smith Score evaluates the pathology of lung tissue in rats with graded smoke inhalation lung injury

Previous studies have demonstrated that the Smith Score serves as a quantitative measure for assessing pulmonary edema, alveolar inflammation, and interstitial inflammation in rats, thereby enabling evaluation of pathological lung tissue damage in cases of inhalation injury with varying severity^[12,13] (Table 1). In this study, a total of 12 pathological images were analyzed, encompassing four experimental groups (exposure durations: 5, 10, 15, and 30 minutes) with three images per group. The analysis focused on the following parameters: the presence and area proportion of pulmonary hemorrhage, the presence and extent of inflammatory cell infiltration, the presence of pulmonary edema, and the formation of hyaline membranes. Subsequently, a semi-quantitative scoring system was applied based on established criteria from previous research: 1 point for involvement of $\leq 25\%$ of the area, 2 points for 26%-50%, 3 points for 51%-75%, and 4 points for $>75\%$. The Smith Score did not differ significantly after 5 minutes of smoke inhalation ($P=.28$) but was significantly higher in the 10-minute ($P=.02$), 15-minute ($P=.001$), and 30-minute ($P=.001$) groups (Supplementary Figure 3). Intergroup comparisons among the experimental groups revealed the 30-minute group had the highest Smith Score, significantly greater than the 15-minute ($P=.001$), 10-minute ($P=.001$), and 5-minute ($P=.001$) groups. The score of the 15-minute group was also significantly higher than that of the 10-minute ($P=.007$) and 5-minute ($P=.001$) groups. No significant difference was observed between the 5-minute and 10-minute

groups ($P=.37$) (Figure 4).

3.5. Evaluation of inflammatory response via leukocyte count and IL-6 expression in alveolar lavage fluid from rats with gradient smoke inhalation injury

To evaluate lung inflammation in rats with graded smoke inhalation injury, alveolar lavage fluid was collected. The leukocyte count was $2.03 \times 10^4/\text{mL}$ (control), $5.8 \times 10^4/\text{mL}$ (5-min), $10.2 \times 10^4/\text{mL}$ (10-min), $18.7 \times 10^4/\text{mL}$ (15-min), and $35.7 \times 10^4/\text{mL}$ (30-min), respectively. Compared to the control group, Leukocyte count was significantly increased in the 15-min ($P=.001$) and 30-min ($P=.001$) groups but not in the 5-min ($P=.53$) and 10-min groups ($P=.06$) (Supplementary Figure 4A). Among the inhalation groups, the 30-min group had the highest count, significantly greater than the 15-min ($P=.004$), 10-min ($P=.001$), and 5-min ($P=.001$) groups. The 15-min group count was also significantly higher than that of the 5-min group ($P=.02$). However, no significant differences were observed between the 5-min and 10-min groups ($P=.57$) or between the 10-min and 15-min groups ($P=.12$) (Figure 5A).

Interleukin-6 (IL-6), a key marker for assessing inflammatory intensity, has been established to correlate positively with both local and systemic inflammation. Its level not only reflects the extent of tissue damage but also predicts disease progression risk and serves as a dynamic indicator for monitoring treatment response^[14]. In this study, IL-6 level in the alveolar lavage fluid were 0.13 pg/mL in the control group and 2.6, 4.7, 13.3, and 25.5 pg/mL in the 5-, 10-, 15-, and 30-minute smoke inhalation groups, respectively (Supplementary Figure 4B). Compared with the control, IL-6 levels were significantly elevated in the 15-minute ($P=.001$) and 30-minute ($P=.001$) groups, but not in the 5- ($P=.53$) and 10-minute

Table 1. Smith Score of different groups.

Group	Pulmonary edema	Alveolar and interstitial inflammation	Alveolar and interstitial hemorrhage	Atelectasis and the formation of a transparent membrane	Smith Score
Control	0	0	0	0	0
	0	1	0	0	1
	0	0	0	0	0
5 minutes	0	0	1	0	1
	0	1	0	0	1
	0	1	1	0	2
10 minutes	0	2	1	0	3
	0	1	1	0	2
	0	1	1	0	2
15 minutes	1	3	2	0	6
	1	2	2	0	5
	1	2	1	0	4
30 minutes	2	4	2	1	9
	1	3	3	1	8
	2	4	3	0	9

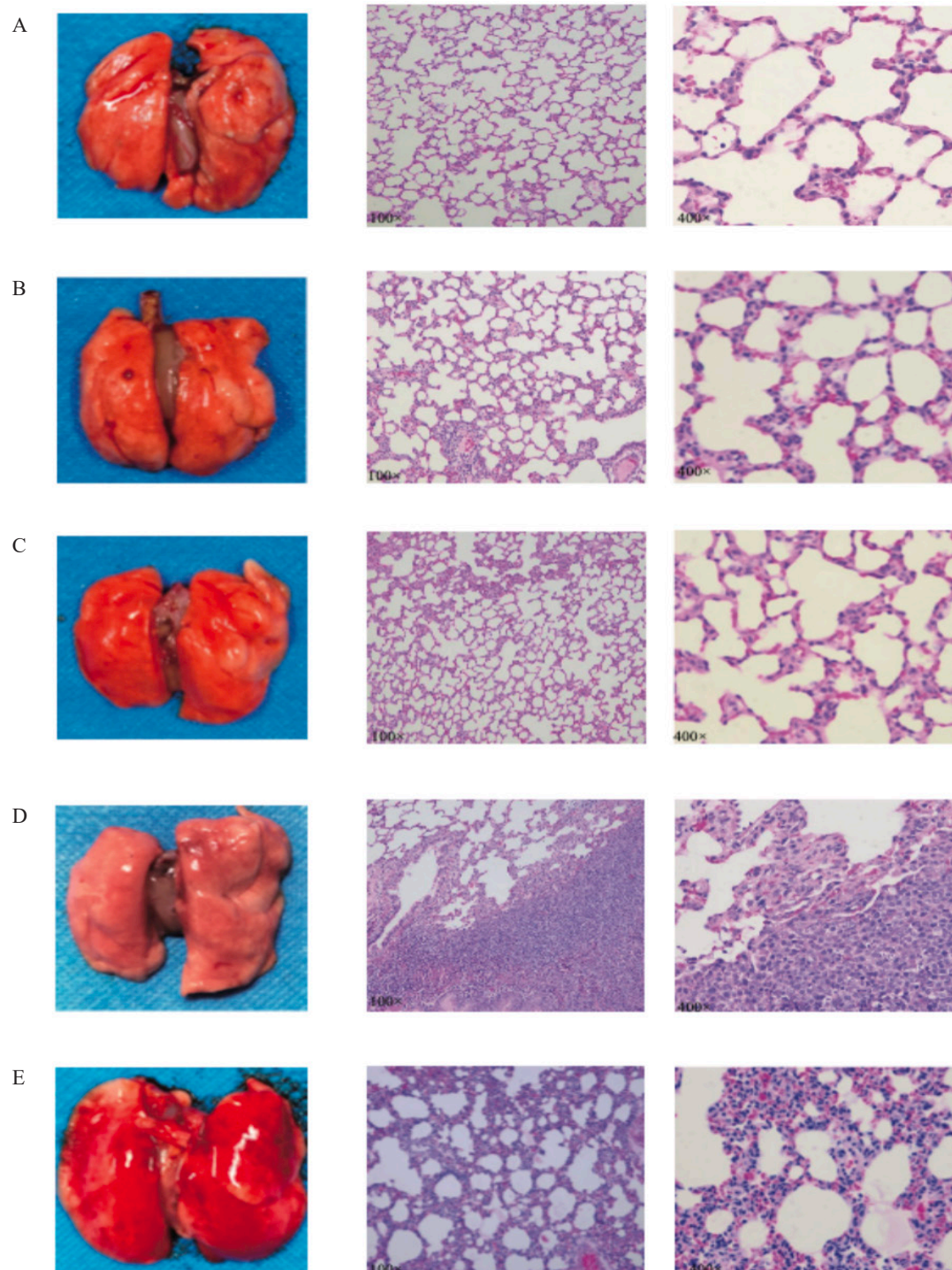


Figure 3. Pathological gross and microscopic photographs of lung tissues of rats in different groups (H&E staining, $\times 100$ in the middle and $\times 400$ in the right figure in each group). A: Control group; B: Smoke inhalation group for 5 minutes; C: Smoke inhalation group for 10 minutes; D: Smoke inhalation group for 15 minutes; E: Smoke inhalation group for 30 minutes.

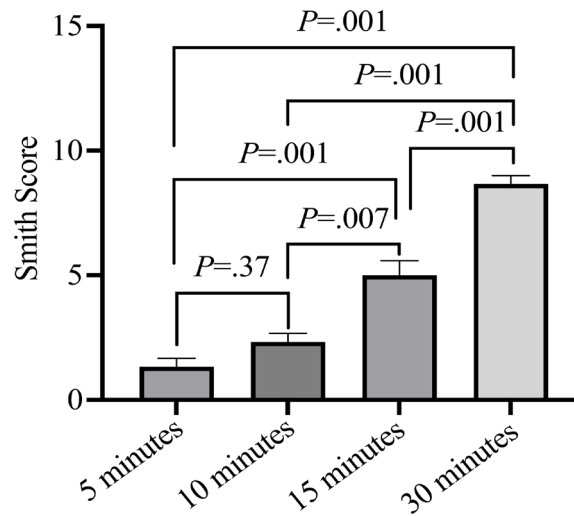


Figure 4. Smith Score comparison results of different smoke inhalation time groups.

($P=.11$) groups. Comparisons among the experimental groups revealed that the 30-minute group had the highest IL-6 level, being significantly greater than the 15-minute ($P=.002$), 10-minute ($P=.001$), and 5-minute ($P=.001$) groups. The level in the 15-minute group was also significantly higher than that in the 5-minute ($P=.005$) and 10-minute ($P=.02$) groups. No significant difference was observed between the 5-minute and 10-minute groups ($P=.77$) (Figure 5B).

3.6. Establishing a graded rat model of smoke inhalation lung injury through multi-parameter evaluation

Integrating data from lung function, lung coefficient, HE staining, Smith Score, alveolar lavage fluid leukocyte count, and IL-6 level, the 5- and 10-minute smoke inhalation groups showed decreased lung function compared to the control group, but without significant changes in lung tissue structure, lung coefficient, Smith Score, or inflammatory markers. In contrast, significant and progressive lung damage was observed in the 15- and 30-minute groups across all measured parameters. Notably, the 30-minute group exhibited more severe injury than the 15-minute group, and the 60-minute exposure was lethal. Based on these graded responses, a rat model of smoke inhalation lung injury was successfully established using 5, 15, and 30 minutes of exposure to represent mild, moderate, and severe injury, respectively, with smoke concentration controlled by a consistent fuel quantity.

4. Discussion

Smoke inhalation-induced lung injury is a critical determinant of prognosis in burn patients, characterized by complex pathological mechanisms and a lack of effective individualized treatments[15,16]. To simulate the progression of inhalation injury of varying severity, a rat model of graded lung injury—mild, moderate, and severe—was successfully established by controlling smoke exposure duration. Experimental results demonstrated that different exposure times induced a continuum of pathological changes, ranging from mild inflammation to severe lung injury. These alterations were consistently reflected in lung function parameters, lung coefficients, and histopathological findings.

In the modeling process, a closed smoke exposure system was employed to regulate the duration of exposure to combustion-derived aerosols from burning materials (pine sawdust and kerosene). Exposure times were set at 5 minutes for mild injury, 15 minutes for moderate injury, and 30 minutes for severe injury, in order to establish varying degrees of inhalation-induced lung injury. The key indicators evaluated by the model included the ability to objectively reflect pathological changes in the gold standard for airway and lung tissue injury, as well as lung coefficient, pulmonary function test results, Smith Score, leukocyte count and IL-6 level in bronchoalveolar lavage fluid. It was observed that the decline in pulmonary function and the increase in lung coefficient was highly consistent with the extent of pathological injury,

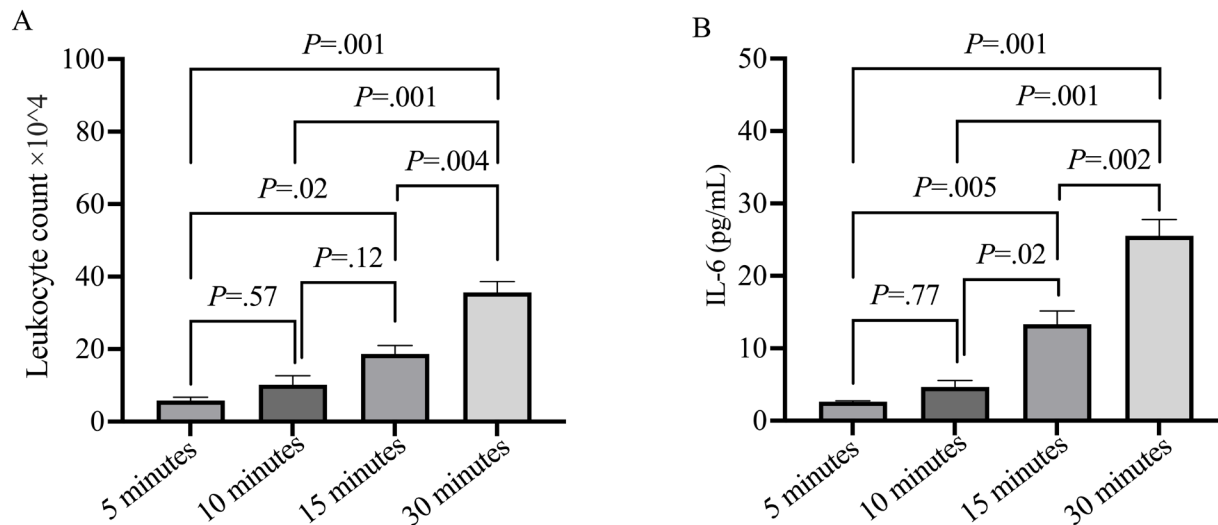


Figure 5. Total leukocyte counts and IL-6 concentrations in broncho-alveolar lavage fluid across different smoke inhalation time groups. After smoke inhalation, the rats underwent endotracheal intubation for right lung lavage under adequate anesthesia. A. Leukocyte count comparison results of different smoke inhalation time groups; B. IL-6 level comparison results of different smoke inhalation time groups.

collectively reflecting the dynamic progression of lung damage. Brief exposure (5–10 minutes) resulted in only mild injury, with lung tissue structure largely intact, suggesting that this stage is characterized primarily by reversible acute inflammation. When exposure was extended to 15 minutes, destruction of alveolar structure and neutrophil infiltration occurred, indicating progression to a moderate lung injury stage marked by intensified inflammatory responses leading to parenchymal damage and irreversible injury. A 30-minute exposure triggered pathological changes characteristic of pulmonary fibrosis, including hyaline membrane formation and typical features of acute respiratory distress syndrome. These findings indicate that the alveolar-capillary barrier was severely damaged, progressing to irreversible lung injury with further aggravated severe damage.

This model offers several distinct advantages. First, it enables controlled induction of graded lung injury. By precisely adjusting exposure parameters, it can simulate smoke inhalation injuries of varying severity as encountered in clinical settings, making it suitable for mechanistic investigations and dose-response studies of therapeutic interventions. Second, it exhibits high reproducibility in pathophysiological features. Key processes such as airway damage, alveolar edema, and oxidative stress are consistently replicated, providing a reliable platform for in-depth molecular mechanism analysis. Furthermore, the model holds considerable translational value: it can be utilized to evaluate novel treatments (e.g., anti-

inflammatory agents, lung-protective ventilation strategies) and supports robust preclinical efficacy assessment. In terms of the association between the model and clinical practice, the model reproduces the common pathological characteristics of patients such as pulmonary capillary barrier damage, which provides an experimental basis for the study of disease mechanism. At the same time, the model can be used for the validation of treatment strategies, such as the response to glucocorticoids or antioxidants in severe lung injury, which can indirectly predict clinical efficacy and help optimize the individualized protocol of critically patients. In addition, in first aid and rehabilitation research, the model can also be used to evaluate the impact of different oxygen therapy modalities, such as high-flow oxygen therapy and mechanical ventilation, on prognosis, thereby guiding clinical decision-making.

Although the model demonstrates high reliability, several limitations should be noted. Currently, the observation period is relatively short, which limits the accurate simulation of long-term outcomes, such as pulmonary fibrosis and other chronic complications. Extending the follow-up duration would improve the assessment of long-term prognosis. Furthermore, given that smoke inhalation lung injuries in clinical settings are often accompanied by burns in other body regions, future studies could develop a combined "burn with inhalation injury" model to enhance the clinical relevance and applicability of the research. In summary, this study successfully established a rat model of smoke inhalation-

induced lung injury with graded severity, the pathological features of which closely recapitulated clinical manifestations. This model provides a valuable tool for in-depth investigation of disease mechanisms and for screening effective interventions. At the mechanistic level, it enables precise delineation of molecular events specific to distinct injury phases, such as the initiation of inflammation, tissue destruction, and the repair or fibrosis stage. In therapeutic exploration, the model offers a standardized platform for objectively evaluating the efficacy of various drugs or treatments—including anti-inflammatory agents, pulmonary surfactants, and ventilation strategies—across mild, moderate, and severe disease conditions. Thus, the graded smoke inhalation lung injury model developed herein demonstrates clear differentiation and reliability, serving as an effective experimental tool for relevant translational research.

Conflict of interest statement

The authors report no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

Study concept and design: ZYQ, ZYZ; Analysis and interpretation of data: SF, ZY, DYY, XYH; Drafting of the manuscript: ZYQ, SF, XYH; Critical revision of the manuscript for important intellectual content: ZYZ, ZJL, GYP; Statistical analysis: ZYQ, SF, XYH.

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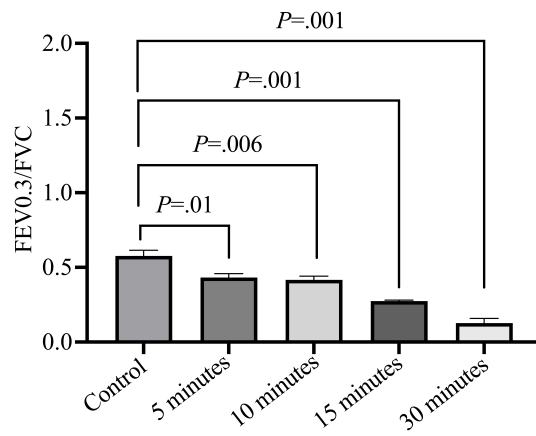
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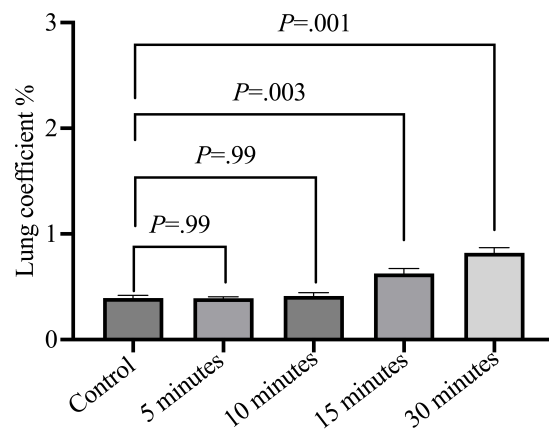
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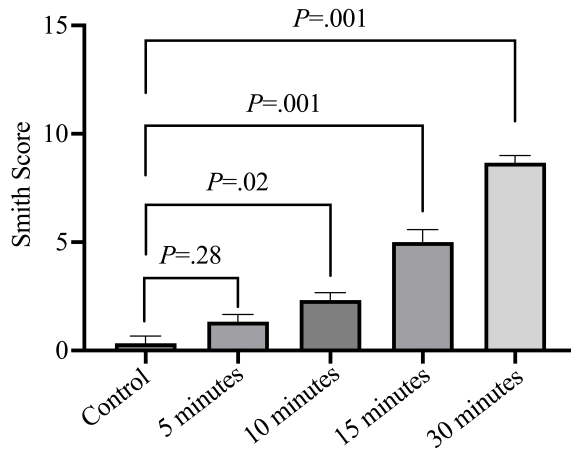
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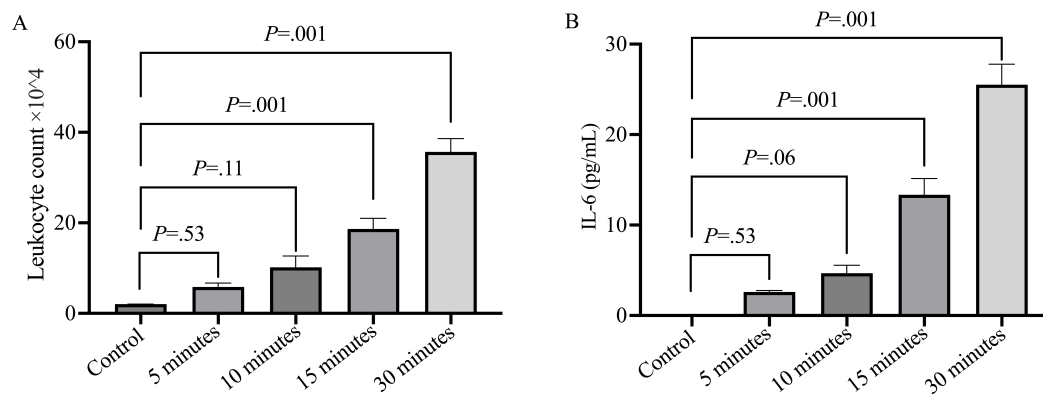
Supplementary Figure 1. FEV0.3/FVC comparison results of different groups.



Supplementary Figure 2. Lung coefficient comparison results of different groups.



Supplementary Figure 3. Smith score comparison results between different groups.



Supplementary Figure 4. Total leukocyte counts and IL-6 concentrations in broncho-alveolar lavage fluid across different groups. A. Leukocyte count comparison results between different groups; B. IL-6 level comparison results between different groups.