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RESEARCH ARTICLE

A dynamic smoke generation and nose-only inhalation exposure system for rats: preliminary results from studies of selected transportation materials

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Abstract

Context: Smoke inhalation injury is the main cause of fatalities for fire victims. Understanding in the pathophysiology of the injury has not been fully explored in recent years. To further explore the pathophysiological mechanism, a dynamic and controllable animal model is necessary.
Objective: To develop a rat model of smoke inhalation injury to simulate human victims in air-restricted vehicle cabin fires.
Materials and methods: Smoke concentration, including CO, O_2, VOCs and smoke temperature under different combustion conditions, were detected. Levels of COHb, respiratory function, lung wet-to-dry weight ratio and protein concentration in BALF and blood were measured. Pathological evaluations of lung in tissues were conducted at 1, 6, 24 and 48 h post-exposure.
Results: Smoke concentration rose with the increase of combustion temperature and decrease of oxygen flow. Further, 215 kinds of VOCs in the smoke were detected, and the concentrations of benzene, methylbenzene, ethylbenzene, dimethylbenzene, phenylethylene and trimethylbenzene was 32.93, 402.06, 764.03, 113.73, 1006.61 and 89.28 mg/m^3, respectively. Significant hypoxemia and CO poisoning occurred in rats. The FCOHb after exposure for 14 min immediately rose to (44.2 ± 12.3) % and then gradually decrease to a normal level at 300 min post-exposure. At 24 h post-exposure, Penh increased significantly ($p < 0.05$), and high pulmonary vascular permeability and significant lung edema ($p < 0.05$) were observed in the smoke inhalation group.
Discussion and conclusion: In summary, the novel rat model of smoke inhalation injury system used in the study is dynamic and controllable, and appropriate for use in smoke inhalation injury studies of air-restricted cabins in vehicles.

Keywords

Air-restricted cabin, animal model, nose-only, rat, smoke inhalation injury

History

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Introduction

In recent years, more and more vehicles have been produced and used due to the rapid development of economies in many countries. And, road accidents are becoming a global problem that is facing all countries with motorized forms of transport and are on the increase in many of the still developing countries (Wang, 2014), especially in China because of that country's large convenient expressway network and the rapid traffic system China has established in recent years (Esmale et al., 2013).

Road accidents cost countries between 1 and 3% of their annual Gross Domestic Product (GDP), and road traffic accidents are forecasted to rise to become the 5th leading cause of death by 2030 from its 9th ranking in 2009 (WHO, 2009). Driver or passenger cabins of these motorized vehicles are typically air restricted. Once a crash happens, dense smoke is emitted rapidly into the cabins of the vehicles, and victims trapped in these enclosed spaces are more likely to suffer serious lung injuries from smoke inhalation (Lee & Mellins, 2006), the leading cause of fatalities from fires (Stefanidou et al, 2008). Over the past few years, major advances have been made in the treatment of burns, but advances in smoke inhalation treatment are still limited (David et al., 2009; Lange et al., 2010; Li et al., 2013), and smoke inhalation injuries need more effective research efforts.

Animal models are the intermediate step taken for research between laboratory studies and trials in humans. To explore the effect and mechanism of smoke inhalation injury further, several kinds of smoke producing and inhalation systems have been designed and manufactured (David et al., 2009; Esechie et al., 2008; Lange et al., 2010; Li et al., 2013; Murakami et al., 2002a,b), as well as animal models for smoke inhalation injury. Researches based on these systems and models have

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provided abundant information and accomplishment in pathophysiology mechanism and the treatment of smoke inhalation injury. However, imperfections in these systems and models still persist and may be the reason for some inconformity and contradiction within these studies.

First, in past studies, most of the smoke used in the models comes from combustion of a single material related to fire disasters (Esechie et al., 2008; Lange et al., 2010), which could not mimic the real circumstance of fires in structure, motor vehicle and other venues. Second, the rate and temperature of non-metal material combustion could rarely be controlled, and the oxygen flow delivered into a combustion system was seldom monitored (Zhu et al., 2012), which could impact and determine the concentration of the smoke in the exposure system to a great extent (Chaturvedi, 2010; Hartzell, 1996). Third, the temperature of smoke inhaled cannot be controlled and scarcely ever detected (Matthew et al., 2001), which is most important in separating smoke inhalation injury from a combined injury in fire. Fourth, animals who suffer smoke are commonly anaesthetized (Esechie et al., 2008; Murakami et al., 2002a) before smoke inhalation, which is not similar to the real physiological status of humans who are suffering in fires. Finally, animals suffering from smoke in other studies were free to roam in the exposure boxes without anaesthetization and they could freely tussle, huddle and flee in the smoke, so the smoke inhaled can hardly be similar for each animal (Zhu et al., 2012). In addition, different types of combustion chambers and inhalation styles that were used in models lacked a constant monitoring of smoke concentration (Matthew et al., 2001; Murakami et al., 2002a,b). These animal models did not duplicate the real situation of smoke inhalation in enclosed spaces or injury conditions similar to victims of actual fires.

The interest of our team focused on smoke inhalation injury in air-restricted circumstance sustained for quite a long time, especially in the cabins of vehicles. Therefore, in this study, a new kind of smoke system was introduced, and its stability and repeatability was then closely evaluated. Further still, we develop a rat model of nose-only inhalation with the smoke exposure system to simulate the real smoke inhalation injury in a closed space. The goal was to improve the understanding of pathophysiological aspects underlying these injuries and evaluate the effects of various treatment strategies so as to improve survival rates in humans.

Materials and methods

Smoke system

The smoke system designed and constructed by Engineer Weilin Fan at Hope Industry and Trade Co., Ltd. of China consists of an air cleaning subsystem, flow control subsystem, combustion control subsystem, pressure buffer subsystem, smoke inhalation subsystem, environmental monitoring subsystem and exhaust treatment subsystem. The air cleaning subsystem has an air compressor, air dehumidifier and oil water separator, which was used to obtain and store clean dry and compressed air (Figure 1A). Clean and dry air pre-treated by the air cleaning subsystem was delivered into the combustion control subsystem or smoke inhalation subsystem directly from two different separated pipelines (Figure 1A). The flow of the air delivered into the combustion control subsystem ranged from 0 to 5.5 m^3/h, precisely controlled by the flow control subsystem with an error of 0.01 m^3/h.

The combustion control subsystem consists of a quartz tube, hoop heater, stepping motor and combustion temperature controller (Figure 1C). Non-metal combustion material was placed into a quartz tube, which was encircled by a hoop heater shaped as a hollow cylinder (Figure 1C). The hoop heater was fixed on the top of a stepping motor, which then moved at a constant velocity. The burning rate of the material, determined by the velocity of the stepping motor ranged from 0 to 10.0 cm/min, was controlled exactly by the central processing unit of the smoke system, with an error of 0.005 cm/min. The combustion temperature ranged from 0 to 999 °C was controlled precisely by the combustion control subsystem with an error of 1 °C.

Smoke was generated by a controlled infra-red combustion or oxidation of non-metal materials used in transport vehicles (cars and trucks, etc.). And, the materials within the quartz tube were irradiated by the hoop heater to a temperature which was measured at some point. The smoke was delivered into the smoke inhalation subsystem by a plastic tube 100 cm long with the inside diameter of 0.8 cm. The flow rate of the smoke in the tube was determined by the flow control subsystem and was the same with the smoke flow in the combustion control subsystem. Before each combustion experiment, the plastic tube was change to a new one with the same size. The smoke inhalation subsystem was a 0.9 m^3 hermetic cylindrical cabin. As designed initially, the rats were set in the cabin and allowed to move freely before the smoke exposure. However, to ensure a balance and consistency of the smoke inhaled, we designed and has patented a nose-only smoke inhalation box (Patent No.: ZL 201220710470.3) with 12 animal cages on both sides (Patent No.: ZL 201130413022.8). This box was a cuboid made of acrylic plexiglass. The length was 20 cm, the width was 10 cm and the height was 36 cm. Twelve holes were placed adequately on both sides of the box with 12 cylindrical acrylic plexiglass cages jointed in the holes. A rat was set in each cage with its nose exposed to the box through the hole. The tails of the rats were left outside the box through the tail boards at the bottoms of the cages. The temperature, concentration, humidity and pressure of the smoke in the inhalation box were detected by the environmental monitoring subsystem (Figure 1D and E).

Animal preparation

This study was approved by the Animal Care and Use Committee of the Third Military Medical University (TMMU). All animals were conducted according to the guidelines of the National Institutes of Health and the Chinese Physiology Society for the care and use of laboratory animals. Adult male Sprague–Dawley rats, weighing 200–220 g, were purchased from experimental animal center at TMMU and then kept in individual cages in a temperature-controlled room with a 12 h light/dark cycle and free access to food and water.

Experimental design

In Part 1, the flow of the air was set at 0.2, 0.5 and 1.5 m^3/h and the combustion temperatures were set at 200, 300, 400,

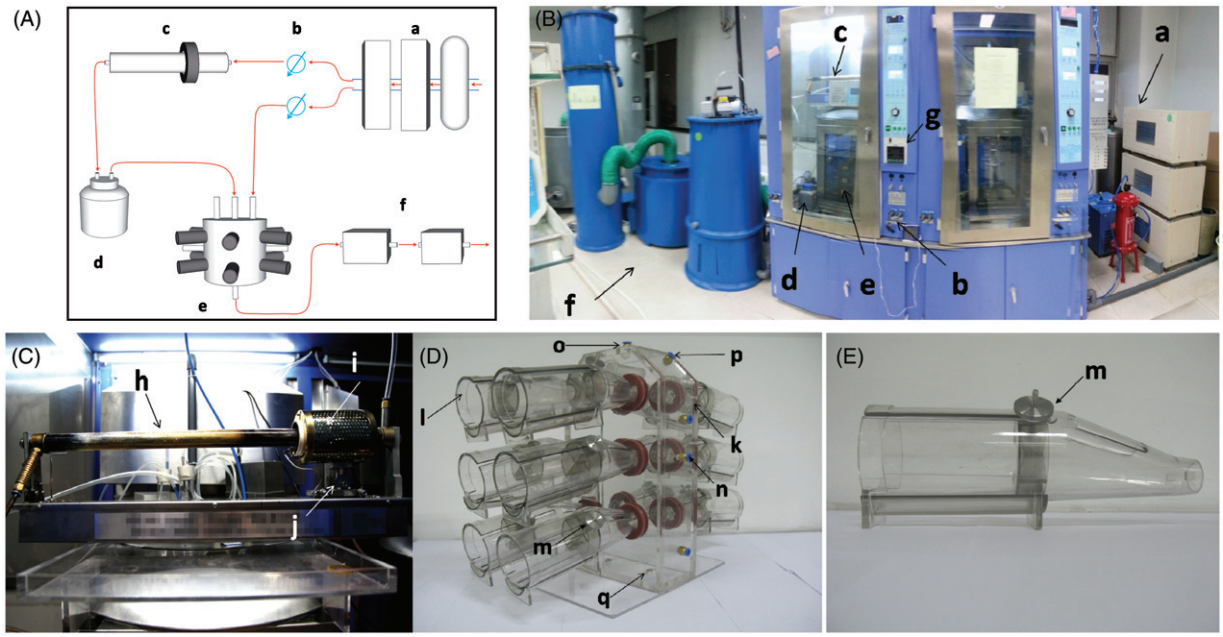


Figure 1. Smoke generation and exposure system. (A) Schematic representation of (a) air cleaning subsystem; (b) flow control subsystem; (c) combustion control subsystem; (d) pressure buffer subsystem; (e) smoke inhalation subsystem; (f) exhaust treatment subsystem. (B) *The smoke system*: (g): combustion temperature controller. (C) *Combustion control subsystem*: (h) the quartz tube used to pad with non-metal combustion material; (i): the hoop heater; (j): the stepping motor used to control the motion speed of the hoop heater. (D) *The sketch of nose-only smoke inhalation box*: (k): The smoke exposure box made with acrylic plexiglass with 12 holes on both sides adequately; (l): cylindrical acrylic plexiglass cage jointed in the hole; (m): the tail boards at the bottoms of the cages; (n): the sampling hole of smoke; (o): inlet of inhaled smoke; (p): inlet of clean air; (q): outlet of exhaust smoke. (E) The nose-only smoke inhalation cage.

500, 600 and 700 °C, respectively, with a total of 18 different combustion conditions. Only the concentration and temperature of the smoke in the nose-only smoke inhalation box were detected for each condition in this part of the experiment.

In Part 2, 60 rats were randomly divided into six groups ($n = 10$) with 12, 14, 16, 18, 20 and 22 min of smoke exposure time. The combustion condition was set as follow: flow of the air was 0.5 m^3/h, combustion temperature was 500 °C and burning rate of the material was 1.0 cm/min. The loss of total combustion material occurred in the tube was (443 ± 37) mg/min ($n = 6$). The carboxyhemoglobin (COHb) levels for the vein blood of each rat's tail were determined immediately at the end of the smoke inhalation exposure. Then animal mortalities were recorded from the smoke exposure time point to a subsequent seven days. No other parameters were measured in these rats.

In Part 3, 30 rats were randomly divided into two groups: a control group (fresh air exposure, 6 rats and C group) and an injury group (smoke inhalation for 14 min, 24 rats and I group). After the smoke exposure, six rats were randomly selected to sacrifice in I group at 1, 6, 24 and 48 h by an overdose of pentobarbital sodium (100 mg/kg body weight), respectively.

Smoke inhalation

Twelve rats without anesthesia were placed into the cages separately before each smoke exposure and allowed to adapt to the cage environment for 10 min. We selected two kinds of untreated commercial non-metal materials, including floor glue and heat insulator (the main ingredients were rubber and polyurethane flexible foam, respectively) (Figure 2A and B). These typical materials for a certain kind of heavy trucks were cut into small uniform rectangular pieces (Figure 2C and D). The cross-section of the two non-metal material pieces was 5 mm × 5 mm, and the mass ratio for unit length was 2.6:1 in accordance with the actual use ratio in the same type commercial heavy diesel truck. Smoke was generated continuously by the combustion control subsystem and then delivered into the smoke inhalation subsystem when the smoke exhausted steadily. The rats were exposed to 12–22 min periods of smoke successively without separation by exposure to ambient air. After these defined periods of smoke inhalation, fresh air was delivered directly into the inhalation box and smoke inside was eliminated within several seconds. The rats were then removed from cages for follow-up detections. The control rats were also placed in the nose-only smoke inhalation box with successive exposure to fresh air by the air cleaning subsystem, but without exposure to smoke.

Detection of smoke composition

The smoke samples were collected from holes on the side of the smoke inhalation box during the smoke exposure. Concentrations of oxygen and carbon monoxide in the smoke were measured by a portable SUMMIT-708 combustion analyzer (Summit Company Limited, South Korea). The chemical compositions of the smoke were detected using gas chromatography-mass spectroscopy (General Electrics, Groton, CT).

Fraction carboxyhemoglobin measurements

After removal from the smoke inhalation box, blood from the tail vein of each rat was immediately collected in a

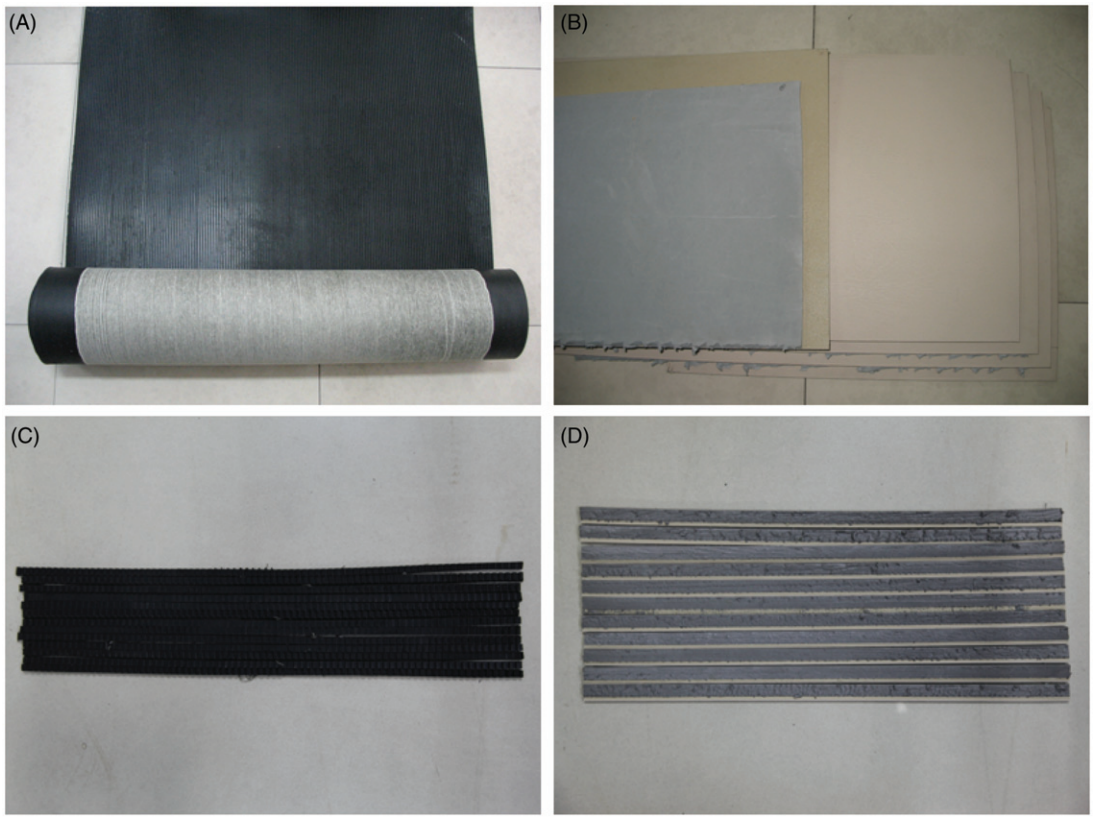


Figure 2. Non-metal materials used in the smoke generation system. (A) Floor glue and its main ingredient is rubber. (B) Heat insulator, including surface layer and inner layer, and the main ingredient is rubber and polyurethane flexible foam, respectively. (C) Floor glue cut into small uniform rectangular pieces with the cross-section of 5 mm × 5 mm. (D) Heat insulator cut into small uniform rectangular pieces with the cross-section of 5 mm × 5 mm.

heparinized tube. Fraction carboxyhemoglobin (FCOHb) was determined, using the double wave length ultraviolet spectrophotometric method (Ramieri et al., 1974). Blood samples (10 µl) were collected every 60 min after smoke exposure until the COHb levels returned to normal.

Measurements of respiratory function

Whole-body plethysmography of the conscious rats was carried out to monitor the airway function using a plethysmography chamber (Buxco Electronics, Wilmington, NC) connected to a computer-assisted data acquisition system (PowerLab 8SP; AD Instruments, Australia). The animals were acclimatized to the chamber circumstance for 10 min before each measurement. Before smoke exposure (0 h), lung function parameters, viz., respiratory rate (breaths per minute), tidal volume, minute volume, inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, pause and enhanced pause (Penh) were recorded for 10 min. After each recording, the rats were taken out of the chamber. Recordings of coughed, sneezed or movement of rats were not taken into consideration. The pressure difference between the reference chamber and the rat chamber was detected with two pressure transducers attached to the system. The resultant waveform was analyzed using spirometry chart software (AD Instruments, Australia).

The respiratory rate was recorded as breaths per minute (BPM) derived from the spirometry flow (1 s^{-1}). Penh, known as enhanced pause, is a calculated parameter that reflects bronchoconstriction and presented as a percentage change from a baseline. After fresh air and smoke exposure, recordings were taken at 0, 1, 6, 24 and 48 h of either fresh air or smoke exposure. Recordings were taken from the same six rats before and defined hours post-exposure. And, recordings taken from rats before exposure (fresh air exposure) were defined as the baseline.

Assessment of pulmonary vascular permeability

Pulmonary permeability index (PPI), as an indicator of pulmonary vascular permeability, is usually used to estimate lung injury. PPI was measured here as previously described (Liu et al., 2012) and calculated from the ratio of protein concentration in bronchoalveolar lavage fluids (BALF) to that in plasma. The protein concentrations of plasma and BALF were measured using the Coomassie brilliant blue method following manufacturer's instructions (Nanjing Jiancheng Corp., China).

Lung pathology

Lung tissues were taken 0, 1, 6, 24 and 48 h after injury for histological analysis, respectively. The right upper lung lobe was harvested and fixed in 10% formalin for 24 h, and standard dehydration and paraffin-wax embedding procedures were used. Hematoxylin and eosin-stained slides were prepared using standard methods. Light microscopic analysis of the lungs was performed by blinded observation to

evaluate pulmonary architecture, tissue edema formation and infiltration of the inflammatory cells. Thirty areas of lung parenchyma were graded on a scale of 0 to 4, that is, 0 = absent and appears normal; 1 = minimal (<25%); 2 = mild (25–50%); 3 = moderate (50–75%); 4 = severe abnormalities (>75%) for congestion, edema, inflammation and hemorrhage (Murakami et al., 2002b). A mean score for each of the parameters was calculated. An experienced pathologist blinded to the treatment conditions made all histological assessments.

Measurement of lung wet-to-dry weight ratio

The rats were anesthetized with isoflurane and sacrificed at each time point after the smoke exposure. The middle lobe of right lung tissues was taken for measurement of the wet-to-dry weight ratio, which is an estimate of the level of lung water content (Yamamoto et al., 2012). The lung tissues were weighted and then dried to a constant weight in an oven at 65 °C for 2 days (Tsan et al., 1999). The wet-to-dry weight ratio was obtained by dividing the wet weight by the final weight of the dried lungs.

Assay of T-AOC in lung and serum

The lower lobe of the right lung tissues and serum were harvested for a measurement of total antioxidative capacity (T-AOC). The tissue homogenates were prepared in a 0.1 g/ml wet weight of ice-cold isotonic physiological saline. The samples were centrifuged at 3000 rpm at 4 °C for 10 min, and the supernatants were used to detect the T-AOC level. All biochemical parameters were measured according to the assay kit manual, and T-AOC was expressed as unit per milligram protein.

Statistical analysis

Data were presented as means ± SD, and analyzed by SPSS for windows, Version 19.0 (SPSS Inc., Chicago, IL). Results were analyzed using one-way ANOVA. A Kaplan–Meier analysis was used for survival data assessment. A p value <0.05 was considered to be statistically significant.

Results

Assessment of the stability of the smoke exposure system

Different combustion conditions for smoke generation

In this study, the ambient temperature was 22.9 ± 1.6 °C, and the humidity was 46.6 ± 1.2%. As the combustion temperature increased, CO concentration elevated, and O_2 concentration declined significantly with different air flow into the combustion subsystem compared to those at 200 °C ($p < 0.05$). However, as the combustion temperature exceeded 500 °C, the concentration of CO and O_2 seemed to reach a plateau. As the air flow in the combustion subsystem increased, CO concentration decreased, and O_2 concentration increased significantly at different temperatures of combustion ($p < 0.05$). Smoke temperature increased slightly as the combustion temperature increased; however, the difference was not significant ($p > 0.05$; Figure 3).

Time course changes of smoke composition

Taking certain conditions of combustion for example, a heating temperature of 500 °C, an air flow of 0.5 m^3/h and a burning rate of 1.0 cm/min, the concentration of CO increased, and O_2 decreased significantly with combustion time compared to the same levels at 0 min. After 6 min of combustion, the concentration of CO and O_2 reached the extreme level and remained steady (Figure 4). This data were collected from the results of three combustions. A qualitative analysis of the chemical composition of the smoke was conducted and the results show that there are 215 different kinds of chemicals in the smoke. Those details are shown in Table 1. The main chemical compositions of the smoke, such as the benzene series, were also measured, and Table 2 lists these concentrations of benzene series found in the smoke.

Assessment of systemic toxicity for animals exposed to smoke

Effects of different exposure times on survival

No rats were found dead from exposure to 12 min of smoke. For an observation duration of 168 h, 14 min exposure resulted in 10% mortality and 22 min exposure produced 90% mortality. Further, nearly 90% of the deaths in total groups occurred within 24 h after smoke inhalation (Figure 5).

Changes in FCOHb after smoke inhalation

The FCOHb of the control rats was 0.0 ± 0.4%. It immediately increased to a high level at the end of smoke exposure and rose with the prolongation of the exposure periods (Figure 6A). However, the FCOHb began to decrease and returned to a normal level at 5 h post-smoke inhalation (Figure 6B).

Assessment of pulmonary toxicity effect on animals exposed to smoke

Effects of smoke exposure on respiratory function

Time-course changes of the multiple respiratory parameters following smoke inhalation were examined at 1, 6, 24 and 48 h post-exposure in an ancillary study (Table 2). Significant differences to the baseline could be demonstrated in all of injury groups in the before–after study. A comparison of multiple endpoints that characterize the control of respiration is depicted in Table 3. Penh, which was a most sensitive endpoint to probe for changes in respiratory function, increased in all injury groups at 1, 6, 24 and 48 h post-exposure, and significance of the difference was demonstrated in the group of 24 and 48 h post-exposure compared to baseline.

Lung pathology

Within 48 h after smoke inhalation injury, hemorrhage, congestion, septal thickening and edema were observed in the histological sections of lung (Figure 7). Table 4 shows the quantitatively analyzed histopathological changes of lung tissue for congestion, hemorrhage, infiltration and edema. A total histology score was calculated as an index to evaluate overall tissue injury. Smoke inhalation injury significantly

Inhal Toxicol, 2014; 26(14): 897–907

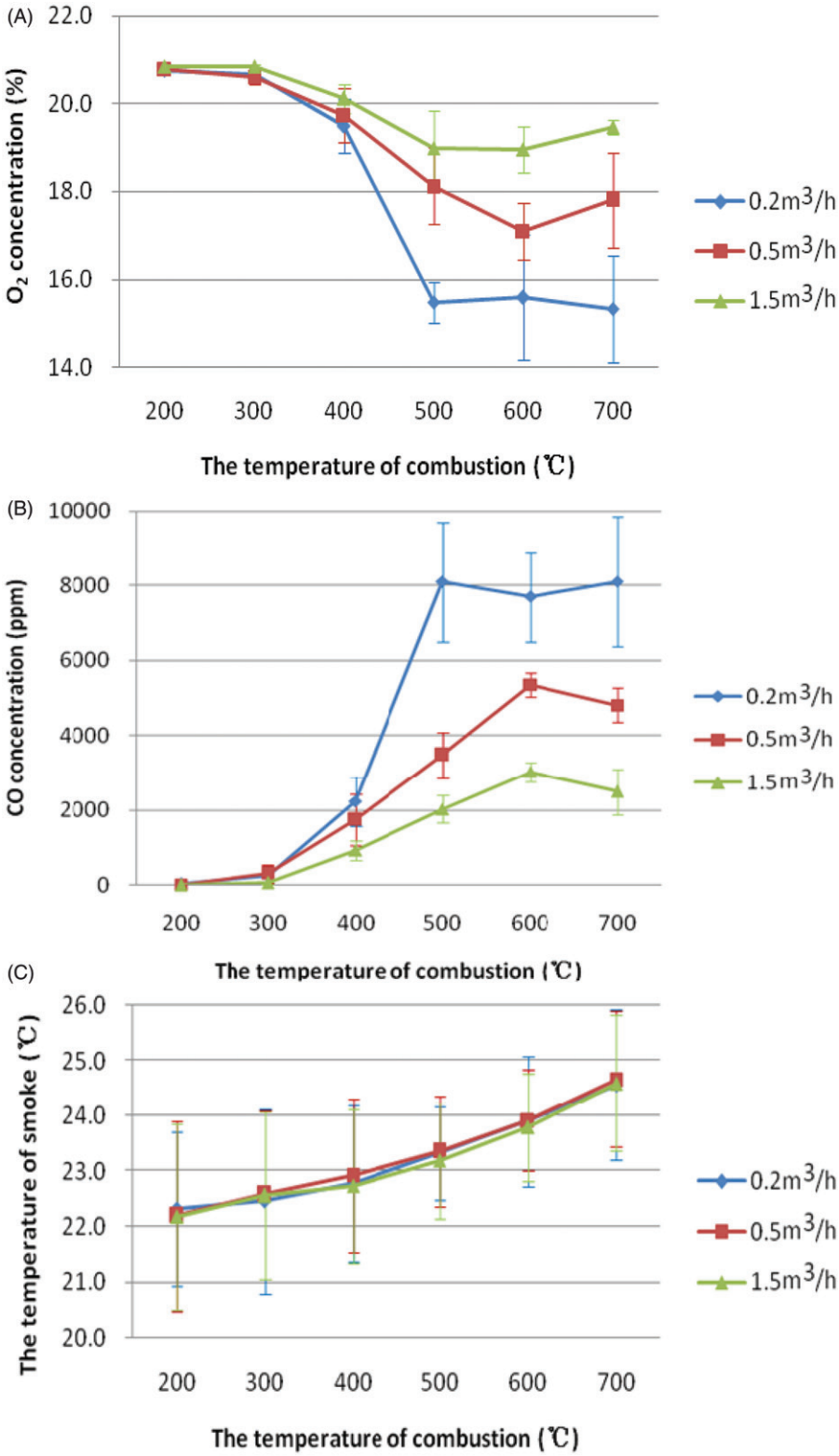


Figure 3. Different combustion conditions for smoke generation. (A) Effect of different combustion conditions on the concentration of O_2 in smoke. (B) Effect of different combustion conditions on the concentration of CO in smoke. (C) Effect of different combustion conditions on the temperature of smoke.

increased the total scores compared to sham injury ($p < 0.01$), while injury at 24 h post-exposure was most serious for the observation period.

Measurements for the lung wet-to-dry weight ratio

The lung wet-to-dry weight ratio was higher in the injury group than the sham group after smoke inhalation. And, the difference was significant at the 1 and 24 h time points after smoke inhalation injury ($p < 0.05$; Figure 8).

High pulmonary vascular permeability in response to smoke exposure

Smoke inhalation resulted in a significant increase in capillary leakage. Total protein concentrations in BALF and

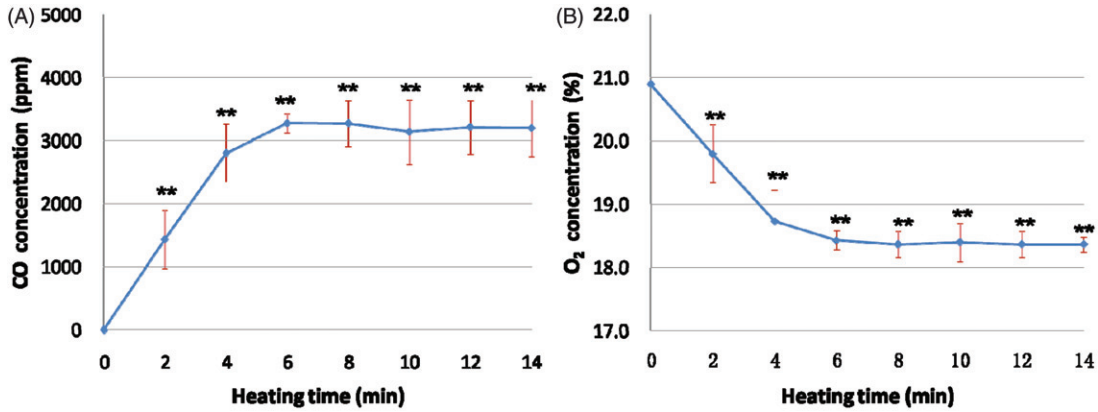


Figure 4. Time course changes of the main inorganic gases in smoke ($n = 3$). (A) Changes of CO concentration in smoke with time of combustion. (B) Changes of O_2 concentration in smoke with combustion time. **: Compared with pre-heating time point group, $p < 0.01$.

Table 1. Qualitative analysis of the chemical composition in the smoke produced by combustion of non-metal material used in certain type of heavy trucks.

Species	Number
Alkane	15
Halogenated alkane	6
Olefin	60
Halogenated olefin	1
Alkyne	3
Naphthene	14
Cyclic olefin	31
Aromatic hydrocarbon	56
Aldehyde	1
Alcohol	1
Ketone	5
Nitrile	6
Terpene	2
Heterocyclic compounds	13
Carbonyl sulfide	1
Total	215

the pulmonary permeability index (PPI) increased significantly in the injury groups at 1, 6, 24 and 48 h post-exposure ($p < 0.05$). At 24 h post-exposure, the increase reached a peak value for the observation period (Table 5).

Measurement of T-AOC in tissues

Lung tissue levels of T-AOC increased significantly at 1 h post-injury ($p < 0.05$) and then declined to normal levels at 6, 24 and 48 h compared to the control group. T-AOC in serum decreased after smoke inhalation and a significance of difference was observed only in the group of 24 h post-injury compared to the control group ($p < 0.05$; Table 6).

Discussion

In the last few decades, considerable research into smoke and burn injury has been undertaken, and a pathology outline has been partially described. Although a better understanding of the pathophysiology of smoke-induced lung injury has been revealed and extensive research has focused on treatment of this lung injury, relatively little of this effort has successfully translated into human intensive care (Sterner et al., 2009). Sometimes, treatment proven effective in one animal model has been contradicted in another (Sterner et al., 2009). The efficacy of these potential therapies in animal studies thus cannot easily be compared due to the major differences in the manner of injury and the animal models and a lack of smoke inhalation quantification.

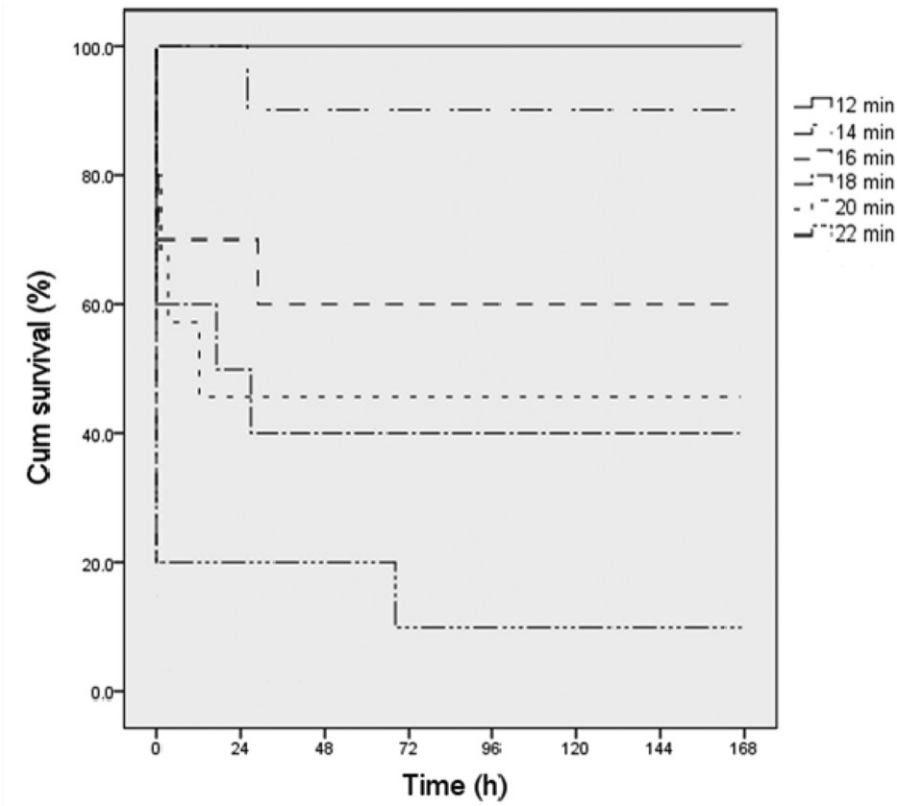
This study observed the effect of smoke on drivers and passengers in the air-restricted cabins of heavy trucks or coaches following fires in heavy traffics. Thus, a suitable smoke-producing system and a suitable animal model were both primary and important. The traditional research has utilized two different animal models. For small animals like mice, rats and guinea pigs, smoke is delivered into a sealed chamber and inhaled spontaneously. Smoke inhalation injury for large animals like sheep, goats, dogs and baboons is generated by conveyance of cooled smoke from burning cotton or wood bark chips delivered directly into pulmonary region via a tracheostomy tube (Westphal et al., 2005). These models, however, lack a suitable quantifying of both smoke production and inhalation, and so were not suitable to use in the current exact and quantitative research effort.

Thus, a new smoke inhalation system was designed to simulate a real smoke situation when heavy truck fires occur. The ratio of the volume of the overall smoke system to animals was exactly the same as that of trucks to passengers. The non-metal materials used to produce the smoke were samples taken from chairs, wires, rubber flooring and other materials used in heavy trucks. The amount of these non-metal materials used in each experiment was strictly kept in line with the ratio mentioned above. The smoke was delivered via a long plastic pipe running between the combustion subsystem and the smoke inhalation subsystem. The data collected from this experiment show that the difference between the temperature of the smoke inhaled and environment outside the smoke inhalation system was not significant. It proved that the smoke inhalation was the only injury factor in our traffic fire models, which was most important for the subsequent experiments. In our smoke inhalation system, the combustion temperature, burning rate, oxygen flow and smoke concentration were exactly detected and controlled, which guaranteed good stability and repeatability in multiple experiments for each batch of smoke inhalation injury.

Table 2. Time course changes of main benzene series in smoke produced by non-metal material used in certain type of heavy trucks.

	Benzene (mg/m^3)	Methylbenzene (mg/m^3)	Ethylbenzene (mg/m^3)	Dimethylbenzene (mg/m^3)	Phenylethylene (mg/m^3)	Trimethylbenzene (mg/m^3)
0 min	0.0020	0.0214	0.0101	0.0320	0.0011	0.0507
5 min	17.51	276.06	560.61	76.46	759.48	53.70
10 min	23.06	316.45	627.57	91.56	837.98	61.55
15 min	32.93	402.06	764.03	113.73	1006.61	89.28

Figure 5. Effects of different exposure times on survival rate of rats.



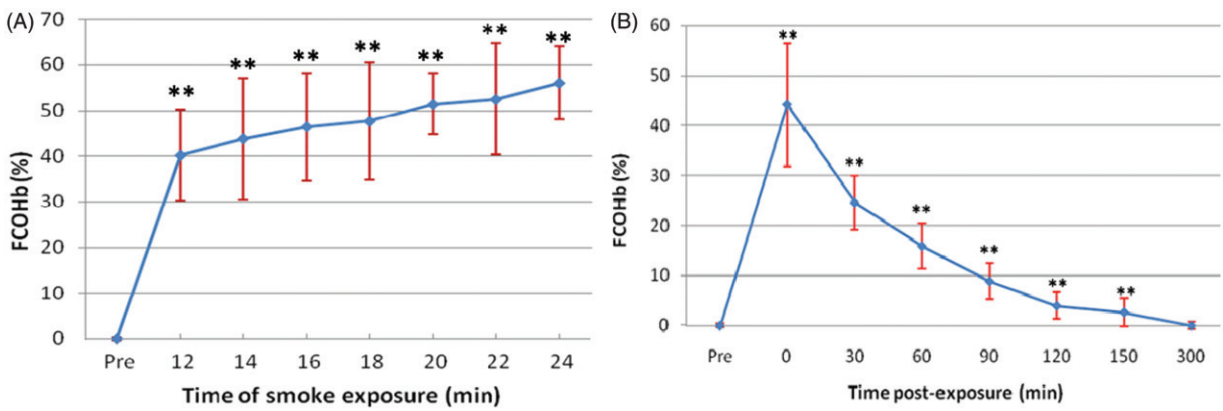


Figure 6. Changes in FCOHb after smoke inhalation. (A) Effects of smoke inhalation on fraction carboxyhemoglobin following different exposure periods. (B) Changes of FCOHb after smoke exposure for 14 min with the certain combustion condition. $^{**}p < 0.01$ versus rats pre-exposure.

This study detected smoke concentration in several different combustion conditions, a factor rarely reported in the similar research efforts. The results prove that the toxicity of smoke is most precisely determined by fire ventilation and combustion temperature. Smoke concentration was quite stable after it reached to its peak value. With this system we were able to simulate a wide range of smoke conditions in heavy truck fires, a process quite suitable for use in smoke

Table 3. Changes of pulmonary function in rats with 1, 6, 24 and 48 h post-exposure of smoke for 14 min.

	1 h		6 h		24 h		48 h	
PF index	Baseline	Smoke	Baseline	Smoke	Baseline	Smoke	Baseline	Smoke
F	104.1 ± 8.7	100.6 ± 16.6	95.5 ± 14.9	132.9 ± 25.8[a]	106.1 ± 13.2	174.5 ± 26.9[a]	109.1 ± 7.4	121.2 ± 11.0
TV	1.74 ± 0.28	1.34 ± 0.19[a]	1.52 ± 0.15	1.39 ± 0.19	1.65 ± 0.29	1.23 ± 0.17[a]	1.79 ± 0.25	1.27 ± 0.14[a]
MV	180.0 ± 35.0	132.7 ± 22.5[a]	145.4 ± 28.8	184.2 ± 52.7	171.8 ± 26.7	209.1 ± 18.1[a]	195.0 ± 28.4	150.7 ± 18.2[a]
PIF	10.37 ± 1.90	7.87 ± 1.77[a]	8.66 ± 1.69	11.03 ± 3.48	10.15 ± 1.80	13.78 ± 2.49[a]	12.50 ± 1.72	9.10 ± 1.75[a]
PEF	9.44 ± 2.11	9.20 ± 4.11	8.65 ± 3.61	11.39 ± 4.66	9.12 ± 1.64	12.74 ± 1.93[a]	10.02 ± 1.66	8.56 ± 1.45
Ti	0.26 ± 0.03	0.29 ± 0.05	0.31 ± 0.05	0.22 ± 0.05[a]	0.26 ± 0.02	0.15 ± 0.03[a]	0.24 ± 0.02	0.24 ± 0.03
Te	0.32 ± 0.03	0.34 ± 0.07	0.34 ± 0.06	0.26 ± 0.04[a]	0.32 ± 0.06	0.21 ± 0.04[a]	0.32 ± 0.02	0.29 ± 0.03
PAU	1.00 ± 0.11	1.22 ± 0.33	1.04 ± 0.33	1.47 ± 0.47[b]	0.98 ± 0.09	1.68 ± 0.59[a]	0.97 ± 0.10	1.29 ± 0.27[b]
Penh	1.08 ± 0.22	1.46 ± 0.53	1.17 ± 0.64	1.68 ± 0.51	1.03 ± 0.17	1.82 ± 0.84[b]	0.93 ± 0.15	1.44 ± 0.50[b]

[a]Compared with baseline, $p < 0.01$.
[b]Compared with baseline, $p < 0.05$.

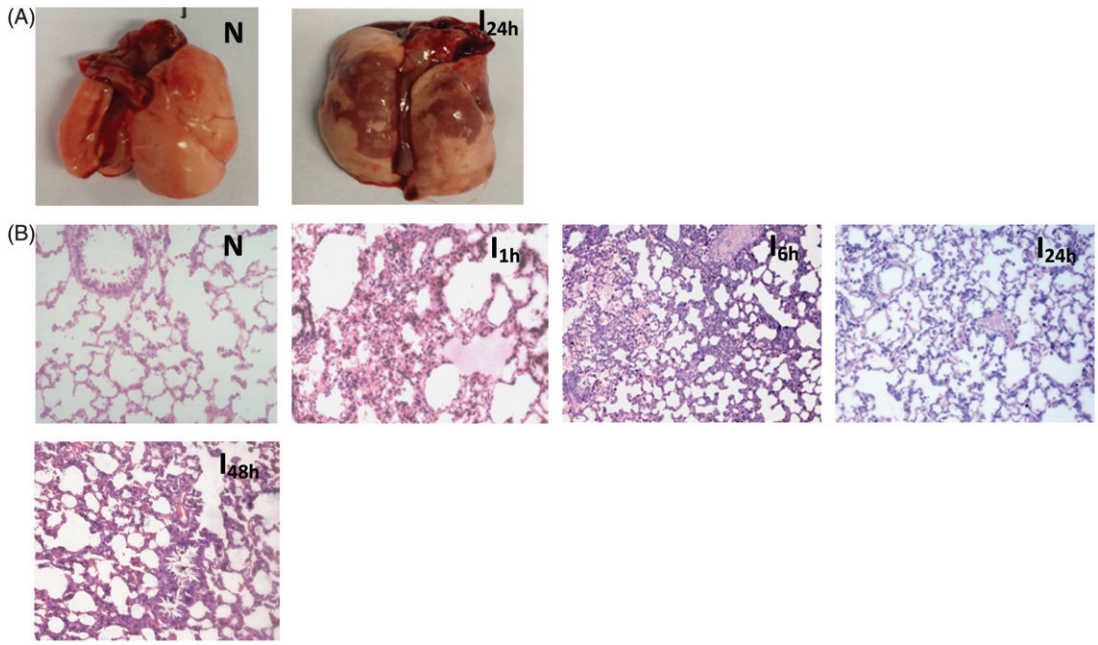


Figure 7. Histopathology of the lung tissues. (A) Gross examination of the rat lungs in control gtoup and 24 h post-exposure group; (B) Hematoxylin and eosin staining of the lungs at 1, 6, 24 and 48 h post-smoke inhalaiton.

Table 4. Differences of histological changes between normal group and injury groups with 1, 6, 24 and 48 h post-smoke exposure.

Group	Hemorrhage	Edema	Infiltration	Total score
Normal	0.73 ± 0.26	0.38 ± 0.18	0.89 ± 0.33	2.00 ± 0.59
1 h	3.03 ± 0.39[a]	0.60 ± 0.32	1.28 ± 0.37[b]	4.92 ± 0.45[a]
6 h	2.90 ± 0.67[a]	2.11 ± 0.34[a]	2.19 ± 0.47[a]	7.20 ± 1.03[a]
24 h	3.56 ± 0.36[a]	1.93 ± 0.74[a]	3.01 ± 0.44[a]	8.50 ± 1.24[a]
48 h	2.31 ± 0.33[a]	1.90 ± 0.27[a]	2.98 ± 0.46[a]	7.19 ± 0.74[a]

[a]Compared with normal group, $p < 0.01$.
[b]Compared with normal group, $p < 0.05$.

inhalation injury research on air-restricted cabins. Since there is a lack of fire data on heavy truck traffic, a moderate combustion condition was chosen as the best example for a smoke inhalation injury experiment, namely, a heating temperature of 500 °C, an air flow of 0.5 m^3/h and a burning rate was 1.0 cm/min. These certain conditions may not represent the overall perspective of smoke in truck fires, but they can provide us with enough information about the smoke inhalation effect on drivers and passengers. We also monitor the smoke concentration, especially the concentrations of O_2 and CO, to establish the stability of the exposure system. The data was collected from three independent experiments having the same combustion condition. The coefficient of variation in the concentration of O_2 and CO during smoke exposure is 11.8 and 1.1%, respectively (Figure 4).

This result is similar to the data from Zhu et al.'s (2012) research, in which the smoke exposure system was confirmed to be stable. To the best of our knowledge, this is the only reference to explore the details of O_2 and CO concentrations during smoke exposure over last decade. Although further comparison and confirmation cannot be achieved due to the limitations of relative references, the stability of the exposure system in our research could still be concluded from the details of CV in the concentration of O_2 and CO.

In this study, the survival experiment indicated that mortality was increased with duration of the smoke inhalation.

As 14 min of smoke inhalation resulted in the lowest mortality rate in our experiment designed, we chose 14 min for the following-up experiments (Figure 5). In the preliminary experiment, we tested the effect of injury at 24, 48, 72 and as long as 168 h post-exposure. The preliminary data showed that 24 h was the peak effect time point of lung pathology, lung wet-to-dry weight ratio, the expression of NF-κB and p38 (these data have not been published yet), which is in accordance with other reports (Murakami & Traber et al., 2003; Schweitzer et al., 2011). As these data were preliminary and do need to be explored further, the injury effect at 1, 6, 24 and 48 h post-exposure in this study was observed and became the first of a series reports on smoke inhalation injury from the combustion of selected transportation materials.

To evaluate the degree of carbon monoxide poisoning, the study measured the COHb levels of blood in rats as the most reliable index of the amount of carbon monoxide in their bodies after exposure (Dolan, 1985). These results show that the COHb level of blood increased intensively and quickly to nearly 40% after smoke exposure and then quickly declined to a normal level in less than 300 min. That finding means that people in the same situation may suffer from severe carbon monoxide poisoning and show tachycardia, tachypnea, neurological symptoms and even coma and confusion. These are all adverse symptoms that will prevent these victims from escaping a fire.

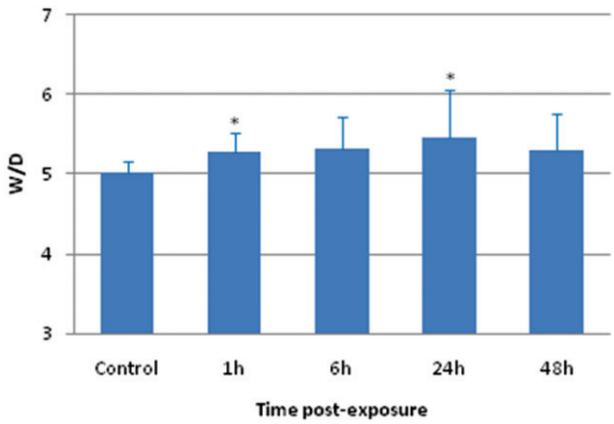


Figure 8. Changes of lung water content from control and smoke treated rats at 1, 6, 24 and 48 h post-exposure. *Compared with control group, $p < 0.05$.

In smoke inhalation injury, airway obstruction is one of the major causes of progressively worsening pulmonary gas exchange. Obstructive cast material occludes the lumen of the airway, resulting in hypoventilation or focal loss of ventilation and a reduction of respiratory function (Enkhbaatar & Traber, 2004). Most of the studies have attempted to prove the effect of smoke inhalation on the respiratory function focused on pulmonary pathological section, but with no direct pulmonary function test. In this study, we used a non-invasive measurement method, namely a whole-body plethysmography, on conscious rats. The results indicated that Penh, which is a most sensitive endpoint to probe for changes in respiratory function, increased significantly in rats exposed to smoke. Penh reached its peak at 24 h after smoke exposure and directly proved that smoke inhalation can depress the pulmonary function, especially at 24 h post-exposure.

Numerous studies have been reported previously that smoke injury triggers the transcriptional activation of several inflammatory genes, which led to increased cellular levels of reactive oxygen species and reactive nitrogen species, and also a large numbers of inflammatory mediators, such as cytokines, chemokines and enzymes (Shimoda et al., 2003; Westphal et al., 2006). These active mediators can cause an activation of neutrophils and increase in pulmonary vascular permeability and lung edema (Murakami & Traber, 2003). In this study, extensive lung parenchymal damage was observed in those rats who were subjected to smoke inhalation, including changes in lung tissue related to congestion, hemorrhage, septal thickening and edema. The total scores of lung histological changes in the smoke injury groups were significantly higher than those in sham injury group.

In order to explore the early pathological progression of lung injury in rats, we also observed the histopathology characteristics at 1, 6, 24 and 48 h after smoke inhalation. These results show that the degree of lung injury was increased progressively after injury and reached the top degree at 24 h time point. The lung wet-to-dry weight ratio and pulmonary vascular permeability results show the same progression characteristic as the lung histopathology. At 24 h post-exposure, lung edema and pulmonary permeability were

Table 5. Changes of PPI in groups of control and smoke treated rats at 1, 6, 24 and 48 h post-exposure.

	Control	1 h	6 h	24 h	48 h
BALF (mg/ml)	0.173 ± 0.042	0.387 ± 0.142[a]	0.664 ± 0.160[a]	0.679 ± 0.328[a]	0.557 ± 0.193[a]
Serum (mg/ml)	39.4 ± 7.9	35.9 ± 2.7	37.5 ± 3.5	36.1 ± 4.9	36.3 ± 5.6
PPI	0.0045 ± 0.0012	0.0108 ± 0.0037[a]	0.0178 ± 0.0043[a]	0.0192 ± 0.0098[a]	0.0153 ± 0.0042[a]

[a]Compared with control group, $p < 0.01$.

Table 6. Changes of T-AOC from control and smoke treated rats at 1, 6, 24 and 48 h post-exposure.

	Control	1 h	6 h	24 h	48 h
Lung tissue (U/mg protein)	0.78 ± 0.06	1.24 ± 0.52[a]	0.85 ± 0.09	0.82 ± 0.04	0.86 ± 0.21
Serum (U/mg protein)	15.56 ± 2.36	14.01 ± 2.47	13.52 ± 3.18	13.28 ± 2.36[a]	13.84 ± 3.38

[a]Compared with control group, $p < 0.05$.

elevated to their peak, which means that the degree of lung injury becomes the severest at 24 h following smoke inhalation.

There are numerous oxidants, such as oxygen-free radicals and volatile aldehydes contained in smoke, that are probably the major causes of damage to biomolecules in lungs exposed to smoke. However, organism has an antioxidant defense system that counters the toxic activities of radical species formed by antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px; Giuca et al., 2010). In this study, we investigated T-AOC in the lungs and serums of the rat model. Lung tissue levels for T-AOC declined to normal level at 6, 24 and 48 h following a short increase at 1 h post-injury, and T-AOC in serum decreased continuously after smoke inhalation. These results revealed that systemic inflammatory response may play an important role in the progress of smoke inhalation injury and anti-inflammatory drugs may play a future therapeutic role in the treatment of smoke-induced injuries.

Conclusion

This research developed a rat model of smoke inhalation injury to simulate the situation of victims in heavy truck smoke disasters. The application of the smoke inhalation system described in this study produced stable smoke and developed expected injuries with good stability, easy replication and exact mortality during inhalation procedures. We believe that this novel model of rats following smoke inhalation injury will help researchers explore mechanistic details underlying these kinds of injuries and further still lead to better screening effective medical treatments to prevent the death of victims in large vehicle fire disasters.

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Declaration of interest

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