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Original Article

Experimental studies on animal models of acute lung injury in guinea pigs occurring after the application of high-temperature gas

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Abstract: Background: Experimental study on animal model of acute lung injury induced by high temperature gas in guinea pig. Methods: 20 male guinea pigs were randomly divided into 2 groups: the control group N and the injury group I which made into the model of acute lung injury by inhaling high-temperature gas, and then after 4 hours, simultaneously measuring lung wet/dry weight ratio (W/D) and arterial blood gas analysising, another HE staining of lung tissue, observing the pathological changes of lung tissue by light endoscopic. Results: The W/D of lung in group I was higher weigh than in group N; Both between the group I and the group N have significant differences for pathological changes. Conclusion: This study is to design high temperature gas which supply guinea pigs could be successfully achieved to make by experimental animal models of ALI, the model to fulfill the requirements of ALI, simulation and high performance, good repeatability, high stability, for the future study of experimental animal models of guinea pig with ALI provide a reliable guarantee, use of worthy of promotion.

Keywords: Experimental animal models, acute lung injury (ALI), guinea pig, experimental study

Introduction

This study has conducted Acute Lung Injury (ALI) experimental animal models by supplying high-temperature gas for guinea pigs to inhale. Observations of the guinea pigs showed the following symptoms: coughing, dyspnoea, respiratory distress and symptoms associated with progressive hypoxaemia, caused by high temperature thermal effects. Then the guinea pigs were euthanisedand the entire lungs removed in order to get the wet and dry weight of the lungs of the guinea pigs. Then histopathological and cytological exanimation was made ofthe right lung, the arterial blood gas and the variance of cytokine levels were analysed, so as to explore the pathogenesis of ALI in the guinea pigs and to study the success rate of making ALI in an animal model, with the research results reported below.

Material and method

Laboratory equipment and materials

Purchase 20 healthy male guinea pigs from the Experimental Animal Centre in Anhui Provincial Hospital Affiliated to Anhui Medical University with an individual weight of 203±19 g each.

Purchase Elisa reagent of TNF-α and elastase produced by Shanghai Yuanye Biotechnology Co. Ltd; the high-temperature gas test platform was designed by the State Key Laboratory of Fire Science in Chinese Scientific and Technical University; use a Multiskan MK2 Enzyme-labelled meter produced by the Finnish Labsystem Company to take measurements and analyse; use a Well wash 4 MK2 microplate washer produced by the Finnish Labsystem company; use a TGL-16GB centrifuge produced by the Shanghai Anting Scientific Instrument

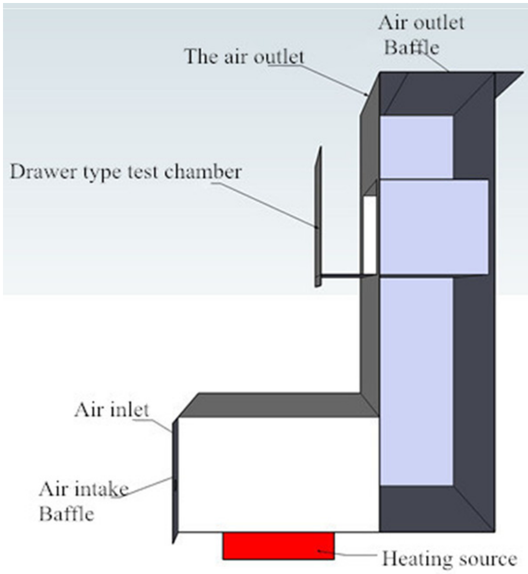

Figure 1. Three-dimensional figure of experiment equipment of high temperature gas.

Factory; application transferpettor produced by Germany's Eppendorf; use an HH.B11.600 electric thermostat box produced by the Guangzhou Huruiming Laboratory Equipment Company; utilise an electric air drying oven produced by Shanghai Fuma Laboratory Instrument Co. Limited; use a GEM Premier 3000 arterial blood gas analyser produced by Kairon International Industry Co. Ltd.

Development method for the experimental model

Devised high temperature gas is 150°C supplies to group I in guinea pigs for 5 minutes, and the guinea pig made into animal model of ALI, and then, the guinea pigs of two groups were killed, taking lung gross of guinea pig, weighing guinea pig lung wet/dry weight ratio (W/D). selected respectively, N groups of guinea pig characterized by HE staining, using the optical microscope observation of two groups of pathological changes in lung tissue and documenting the results of the pathological changes in time; Arterial blood gas analysis and determination of guinea pigs were statistically compared.

Grouping of experimental animals

Select 20 healthy male guinea pigs, dividing randomly into 2 groups with 10 in each group. Healthy male guinea pigs are set as Control Group N, with ALI Group as Group I.

ALI animal model making

Jointly develop a high-temperature gas model device for animal experiments with the State Key Laboratory of Fire in the Chinese Scientific and Technical University, design a computer integrated module to automatically control the space radiant source of heat increase and variable output power within the entire device structure, make a regulatory control on the changes of air flow and temperature inside the experimental device with the variable adjustable temperature range of 0°C~200°C.

Separately select 10 guinea pigs from Group N and Group I, weigh each of them and timely record the weight data of each guinea pig, and administer anaesthesia by injecting into the abdominal cavity of the guinea pigs with 400 mg/kg of 10% chloral hydrate. Anaesthesia requirements: avoid the occurrence of guinea pigs lagging in response and getting scorched because of too much anaesthesia, but also avoid the occurrence of guinea pigs reacting strongly and striking the inner wall causing death because of too shallow anaesthesia. After anaesthesia is complete, place the guinea pig on the platform of the experimental apparatus, start the ignition, adjust the variable temperature to 150°C inside the experimental model device and supply the high-temperature gas to guinea pigs for inhaling, and control the entire ALI experimental time to 3 minutes. When the ALI experiment on the guinea pig is finished, quickly adjust the high temperature gas to room temperature inside the experimental device, observe the respiration and activities of the guinea pigs, and timely record coughing, dyspnoea, respiratory distress and other clinical manifestations exhibited.

After the guinea pigs have been observed for 4 hours, draw the blood of the abdominal aorta for arterial gas analysis.

After finishing the animal model for the ALI experiment on the guinea pig, carry out dissection on experimental guinea pigs and take out the lung tissue, weigh up lung W/D, make histopathological and cytological exanimation with the right upper lung of the guinea pigs.

Design of the ALI experimental model device

ALI animal experimental model device: As is shown in the **Figures 1-3** of the high-tempera-

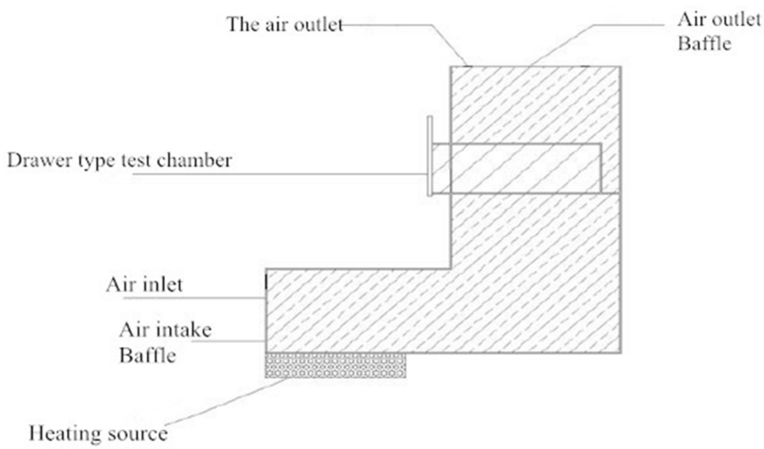

Figure 2. Floor plan of high temperature gas experiment platform.

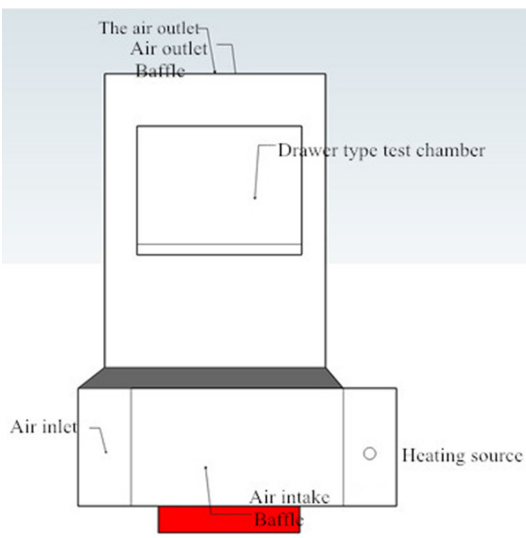

Figure 3. Positive and floor plan of experiment equipment of high temperature gas.

ture gas experimental device, the model device consists of three sections. The first part is the cabin structure with its main structural features being: 1. Here are air outlets and the baffle plates of the air outlets on both sides of top compartment; 2. Vertical cabinet stays upright and the bottom of the horizontal cabinet touches the ground, vertical and horizontal cabinets are at right-angles to each other, and the air space in both cabinets can mingle with each other; 3. There are drawer-style test platforms inside the vertical cabinet for placing the guinea pigs to make ALI, and there are set air inlet ports and baffle plates of the air inlets in the front side of the horizontal cabinet with the heat source in the bottom of its horizontal cabinet to supply high-temperature gas and the switch to adjust the gas temperature.

The second part is the air inlet and outlet device, with the main function to control the air flow, gas temperature and adjust the high-temperature gas. The third part in the bottom of the model is the ignition control switch for enabling or disabling the heat, with its main function being to immediately start ignition by turning on the switch and stop ignition by turning off the switch.

Temperature parameters: Input voltage: 220~380 V, output current: 0~20 A, output power: 0~5 kw, output temperature range: room temperature ~200°C, high temperature is basically set in constant temperature of about 150°C, ±2°C.

Moulding process of ALI animal experiments: For the moulding of ALI animal experiments, the process of using a radiant heat source rapidly heating the air is achieved by the structure of the ALI experimental device. The overall mould structure is made by welding together designed steel plates with a thickness of 1 mm. The outside of the device is wrapped with fire retardant insulation material and aluminum foil, which aims to protect the experimental model device from cooling due to heat radiation and to reduce radiant heat loss from the experimental apparatus as a whole, so as to achieve the desired adjustment of effectively controlling the temperature from room temperature to high temperature. In the process of using the ALI animal model, the air enters through the inlet, the baffle plate of the air inlet plays a role in controlling intake capacity, and the computer automatically adjusts the output power of the outside radiant heat source to heat the air. Make use of the rule of air convection flow, gradually increasing the overall air temperature in the test cabin so as to reach the desired test temperature. The upper and lower test sections of the experimental model testing have been separately equipped with a thermocouple, it therefore could enable real-time dynamic data acquisition and monitoring during the process of temperature control and rising with the computer integrated module, giving real-time dynamic monitoring of the temperature change process. After reaching the experiment's required temperature during the testing for experiment moulding, dynamically change the output power of the radiant heat source through the computer so as to make the temperature during the trial period of the moulding experiment maintain the required proper temperature for ALI moulding experiments. Adjusting and controlling the high temperature, time and dosage of anaesthesia to the guinea pigs are critical to make the moulding.

Anaesthetise the guinea pigs before the experiment, put the anaesthetised guinea pig into a small cage after completion of the anaesthesia, open the drawer of the cabin door for the test of moulding of the experiments, put the small cage with the anaesthetised guinea pigs for the moulding experiments into the test cabin, and shut the door. Start the ignition switch, make ignition and combustion last for 3 minutes after reaching the desired temperature for the testing in the modelling experiment which requires controlling the temperature fluctuations in the moulding process of the experiment at 150°C. When the experimental device has little effect on the overall temperature changes during the entire moulding process, the experimental success rate is high. On the contrary, when the experimental device has large temperature fluctuations during the entire moulding process, the success rate is low and fails in experiments.

Measurement and treatment of the test sample

The process of extraction and preparation for test sample: Guinea pigs were observed for four hours after making the modelling, anaesthetise each group of guinea pigs with 10% chloral hydrate with anaesthetic doses described above. Fix each guinea pig on the surgical operating table, open the abdominal cavity, draw 4 ml blood through the abdominal aortic, instantly carry out arterial blood gas analysis. Then extracted 4 ml abdominal venous blood, inject into the test tube, place inside the centrifuge, perform a high speed centrifugation with 3000 r/min for 20 minutes, allow to stand for 2 minutes, extract the supernatant, put inside the low temperature refrigerators at -20°C, and leave for testing the content of TNF-α and elastase.

Prepare and calculate the wet/dry weight (W/D): Select each cavy's right lung tissue from Group I and Group N. Place the extracted specimen on standard filter paper, gauge the wet weight after drying the surface liquid, record each parameter value of wet weight for each cavy's right lung tissue in time. Then, put it into the convection constant temperature drying oven and dry for 48 hours with a temperature of 100°C. After completely drying the lung tissue, weigh the dry weight, record each parameter value of dry weight for each cavy's right lung tissue in time. At last, figure out the W/D ratio and record the two group's parameter values in due course.

Pathological examination for experimental sample: Select the left upper lung tissue. The selected left upper lung tissue shall be fixed in a 10% formaldehyde solution, paraffin-embedded, sectioned. Then, conduct the HE staining (haematoxylin-eosin staining) for pathologic histology and cytology examination.

Statistical treatment

The measurement data used in the two groups' experimental parameters shall be expressed as average ± standard deviation ($\overline{x}\pm s$). Statistical software SPSS11.5 shall be used for comparing the one-way analysis of variance for each group. The SNK method shall be used for comparison between the groups, $P < 0.05$ is the difference which has the statistical significance.

Results

Pathological examination result refer to ***Figures 4*** *and* ***5***

Figure 4 shows the lung anatomical tissues from a normal and health cavy. **Figure 5** shows the sacrificed cavy which is under anaesthesia and has been put into the high temperature environment for 3 minutes. After dissection of the cavy lung tissue, fractured alveolar wall, fusion and increased alveolar space, broadened and damaged interstitial tissue and anatomical disorder can be observed clearly. Distinct congestion and oedema and a large number of inflammatory cell invasions can be found in the alveolar wall blood capillary.

Measurement result for the two groups of W/D and arterial blood gas analysis refer to **Table 1**.

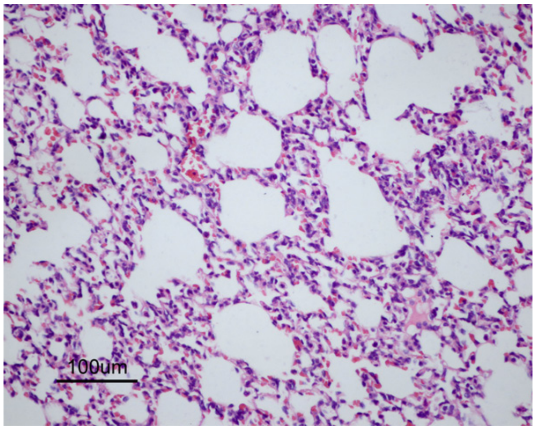

Figure 4. Controlled group of normal and healthy guinea pigs (N group).

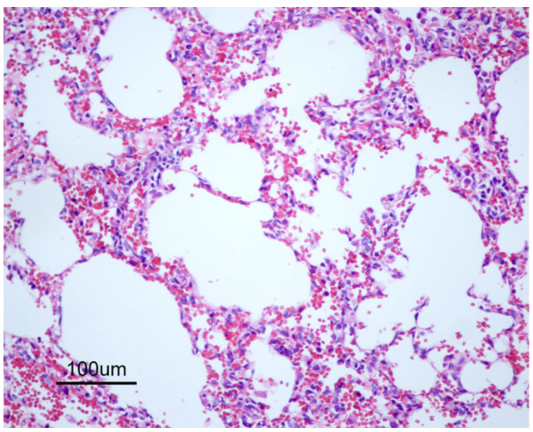

Figure 5. Group of acute lung injury (group I).

Discussion

During modern warfare the flamethrower, which is often used and burning material created by various fires ensuing its use will release massive high temperature and thermal energy instantly and cause the surrounding air temperature to rise rapidly. If the human body inhales high temperature air, it can cause airway trauma and human body Acute Lung Injury (ALI). The aim of this experiment is to simulate the high temperatures which are caused by flamethrowers or fire and is inhaled by the cavy, making the animal model and experiment on and research on the designed device. According to the guiding principle of performance-based design for fire control, it points out that [1] the heat tolerance limit of human bodies in a thermal radiation hazard environment, such as a smoke layer, shall be 2.5 kW/m^2. It means that the temperature of the upper smoke layer is approximately 180°C~200°C. When heat radiation intensity reaches < 2.5 kW/m^2, the tolerance time of the human body is >5 minutes; When heat radiation intensity is >2.5 kW/m^2, the tolerance time of the human body is >30 seconds. In addition, the relationship between the tolerance time limit of the human body and the temperature in high temperature conditions (Crane formula) can be represented as $t_c(T) = \frac{3.28 \times 10^8}{T^{3.61}}$ (herein, T is the environmental temperature, in degree centigrade, the unit of $t_c(T)$ is minute). The device for this experiment is improved and updated based on this theory [1]. The flame temperature shall be controlled at 150°C, duration shall be 3 minutes. Make the animal model for the cavy ALI and experiment on and research the designed device. The result from **Table 1** shows that the lung W/D ratio from Group I is significantly increased compared with Group N. The significant difference can be observed in comparative statistics among groups (P < 0.05). It suggests that ① pulmonary oedema can be observed distinctly in Group I. The pulmonary oedema can cause a significant increase in lung volume weight, while compliance of lung tissue is significantly decreased; ② the experiment and research result for the flame simulation device above shows that after inhaling the high-temperature air, Acute Lung Injury (ALI) occurred in the cavy and the result is very pronounced.

The pathological examination result from **Figures 1** and **2** presents that the volume of cavy lung tissue from Group I is increased, the alveolar wall is fractured, the alveolar space is fused and increased, interstitial tissue is broadened and damaged and anatomical disorder can be observed clearly. Distinct congestion and oedema and a large number of inflammatory cells invasion can be found in the alveolar wall blood capillary. Therefore, the experiment verifies that the cavy has the typical ALI. The animal model for cavy ALI for the experiment and research in this article is similar to literature and report [2-4].

According to the analysis of the arterial blood gas of the guinea pig in **Figure 1**, the pH value of group I decreased dramatically compared with group N, accompanied by arterial blood PaO_2 which dropped greatly, and $PaCO_2$ appar-

Table 1. The changes of W/D and arterial blood gas of guinea pig lung of two groups ($\bar{x}\pm S$)

Group	The number of rats	Lung tissue (W/D)	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
N	10	4.88±0.11	7.38±0.04	177.30±24.70	31.10±8.77
I	10	5.72±0.31▲	7.02±0.12▲	98.80±62.60▲	74.70±23.23▲

Note: Comparison between I and N ▲P < 0.05.

ently raised, the statistics between the two groups was significantly different (P < 0.05). It revealed that the guinea pigs breathing in type II suffered with severe respiratory acidosis. It can be extrapolated that the simulated fire experiment device of this study possessed favourable ALI model making effect. The experimental research on guinea pigs, the ALI animal model in this paper is similar to the literature report [5, 6].

In summary, the application of this project developed an animal model experimental device for high temperature gas inhalation, and can simulate the heat of a flame thrower inhaled by the guinea pig and can control the temperature and the duration of high temperature gas and high temperature gas flow, via the study of the guinea pig, it can be concluded that: (1) the anatomy of the weight observation of the guinea pig lung W/D ratio increased significantly; (2) the measurement of arterial blood gas showed a significant drop in pH, accompanied by arterial blood PaO_2 dropping significantly, and $PaCO_2$ increased markedly; (3) pathological examination under an optical microscope revealed the lung tissue volume to be significantly enlarged, the alveolar ruptured, fusion chamber expanded, lung cells of blood vessels revealed hyperaemia oedema, a large number of inflammatory cells infiltrated and pulmonary interstitial broadening, structure disordered, etc. The experiments proved that: the success of making a guinea pig ALI animal study, further explained the research on animal model experiment equipment with very good effect to build an experimental animal ALI model. Using this study to design an animal model experimental device for high temperature gas inhalation, the effect on the experimental research of simulating the ALI animal model is significant, the model meets the requirements of ALI, with high simulation and performance, fine repeatability, strong stability which provides a reliable guarantee for the future study of guinea pig ALI experimental animal models and is well worth promotion and application.

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Disclosure of conflict of interest

None.

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References

[1] Luscombe DW. Fire safety in health care premises. Health Estate J 1990; 44: 2-4.
[2] Fang Y, Xu P, Gu C, Wang Y, Ni T, Yu WR. Ulinastatin improves pulmonary function in severe burn-induced acute lung injury by attenuating inflammatory response. J Trauma 2011; 71: 1297-1304.
[3] Liu J, Wu S, Hao J. Tanshinone II Sodium Sulfonate Acid Sodium Treatment Firearm Wound A Guinea Pig Acute Lung Injury and Cytokine Regulation Research. Chinese Journal of Clinical Physicians (electronic) 2013; 7: 150-153.
[4] Huang HR, Zhang L, Li DM, Wu HW, Yi J, Shen Y, et al. The Experiment on Research of Rat Lung Injury After Extracorporeal Circulation. Journal of Southeast Defence 2014; 113-116.
[5] Huang HB, Shen J, Zhang L, He DK. The expression of the Protective Effects of Related Factors of Ulinastatin on Rat Acute Lung Injury. Chinese Journal of Emergency Medicine 2010; 12: 37-42.
[6] Chang YJ, Wu S, Hao J, Liu J, Li SW, Qian J, etc. The influence of Different Doses of Ulinastatin on the High Temperature Gas to Guinea Pigs Caused by Acute Lung Injury. J Clin Lung 2011; 781-783.