Protective effect of Shenfu injection preconditioning on lung ischemia-reperfusion injury

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Abstract. Lung ischemia-reperfusion injury remains a problem in thoracic surgery, as minimal progress has been made concerning its prevention and control. In the present study, the protective effects and the underlying mechanism of Shenfu injection preconditioning on a rat lung ischemia-reperfusion model was investigated. Shenfu injection is a well-known Chinese traditional medicine, which is composed of Red Radix Ginseng and Radix *Aconitum carmichaelii*, with ginseng saponin and aconitum alkaloids as the active ingredients. A total of 72 specific pathogen-free, healthy male Wistar rats were randomly divided into control, model and Shenfu injection (10 ml/kg injection prior to injury) groups and were assessed at the following points: Ischemia 45 min; reperfusion 60 min; and reperfusion 120 min. Blood collected from the aorta abdominalis was cryopreserved at -70°C for the analysis of malondialdehyde (MDA) and superoxide dismutase (SOD) activity. Lung tissues were divided into three equal sections in order to assess the wet-to-dry (W/D) lung ratio, tumor necrosis factor (TNF)-α expression levels, myeloperoxidase (MPO) activity, alveolar damage, total protein and hematoxylin and eosin staining. The results demonstrated that the lung W/D weight ratio, TNF-α expression levels and SOD activity in the Shenfu group were significantly lower at 120 min reperfusion (P<0.05), as compared with the model group. MPO and MDA activity significantly decreased following reperfusion at 60 and 120 min (P<0.05), as compared with the model group. In addition, the degree of alveolar damage in the Shenfu group was significantly decreased (P<0.05), as compared with the model group. In addition, compared with the model group, the degree of alveolar damage in the Shenfu group was significantly lower (P<0.05); however, no significant changes in total protein were observed. The extent of alveolar structural damage and the proportion of interstitial neutrophils and alveolar and interstitial red blood cells were lower in the Shenfu group, as compared with the model and control groups. Therefore, the results of the present study suggested that Shenfu injection may have protective effects on lung ischemia-reperfusion injury.

Introduction

Cell metabolic disorders and structural damage are aggravated in the viscera when the blood flow blocking is removed and the reperfusion recovered. This damage further aggravates ischemic injury following blood reperfusion, and is known as ischemia-reperfusion injury The mechanism underlying ischemia-reperfusion injury in the lungs remains controversial; however, the majority of previous studies (1-3) agree with the following statements: At the early stage of lung ischemia-reperfusion injury, a number of precursor proteins in vascular endothelial cells are activated and these cells release various cell adhesion factors, promote neutrophil adhesion and aggregation, and platelet deposition, and subsequently induce microvascular congestion. With continued reperfusion, the expression of cell adhesion molecules on the surface of neutrophils and endothelial cells is increased. Subsequently, the cells adhere and release chemotactic factors, such as tumor necrosis factor (TNF), which increase cell adhesion, promote the narrowing of capillaries and block blood perfusion (4). As various vascular systolic materials are released, the microvascular function is altered, resulting in aggregated cell injury (5)

Shenfu injection is a well-known traditional Chinese medicine, which is composed of Radix *Ginseng rubra* and Radix *Aconitum carmichaelii*, with ginseng saponin and aconitum alkaloids active ingredients. Previous studies have demonstrated that treatment with Shenfu injection has a curative effect during shock and resuscitation (6), can elevate blood pressure and improve microcirculation to aid in an inflammatory reaction, protect ischemic myocardial cells (7) and prevent acute kidney ischemia-reperfusion injury (8). Furthermore, shenfu injection treatment has also been shown to exhibit good curative effects on transient focal cerebral ischemic injury (9-11), reduce pathological and structural damage in the lungs and liver (12), inactivate anti-oxygen free radical

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xanthine oxidase, improve lung blood flow, adjust the function of immune, system protect vascular endothelial cells, improve hypoxia tolerance and protect from endotoxic shock-induced lung injury (13-15). However, there is a lack of published information investigating the use of Shenfu injection for the treatment of lung ischemia-reperfusion injury.

The present study used a rat model of lung ischemia-reperfusion injury to investigate the role and underlying mechanism of Shenfu injection for the treatment of lung ischemia-reperfusion injury. The following properties were evaluated: Lung wet dry weight (W/D) ratio, tumor necrosis factor-α (TNF-α) expression levels, myeloperoxidase (MPO), malondialdehyde (MDA) and superoxide dismutase (SOD) activity, and microscopic lung damage.

Materials and methods

Reagents and kits. Shenfu injection (Z51021920; 100 ml/ampulla; batch no. 100602) was purchased from Ya'an San-Jiu Pharmaceutical Co., Ltd. (Ya'an, China). TNF-α enzyme-linked immunosorbent assay (ELISA; cat no. MTA00B) MDA kits (cat no. A003-1) were provided by Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China). MPO (cat no. A044) and SOD (cat no. A001-1) kits were produced by the Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

Animals. A total of 72 specific pathogen-free healthy male Wistar rats, weighing 250-350 g (age, 60±3 days), were provided by the Lanzhou University Medical College Animal Experiment Center (license no. SCXK 2009-0004). Rats were maintained in $0.063m^2$ cages under regulated temperature and humidity (temperature, 19-24°C; humidity, 55-60%) with a 12-h dark:light cycle and *ad libitum* access to water and standard feed.

Drug delivery and groups. Rats were randomly divided into three groups with 24 rats in each group. In the control group, intraperitoneal injection of 0.9% sodium chloride (3 ml/kg/, i.p., 10 sec) was administered daily for 2 days; after 30 min, the right hilar was divided following thoracotomy without blocking the lung door. In the model group, intraperitoneal injection of 0.9% sodium chloride (3 ml/kg/, i.p., 10 sec was administered daily for 2 days; after 30 min, the lung ischemia-reperfusion model was established. In the Shenfu group, the Shenfu injection was diluted 3.7 times with 0.9% sodium chloride prior to use and intraperitoneal injection of Shenfu (3 ml/kg, i.p., 10 sec) was administered daily for 2 days; after 30 min, the lung ischemia-reperfusion model was established.

Lung ischemia-reperfusion model. An *in vivo* rat model of lung ischemia-reperfusion was established using the improved Eppinger method (16,17). Anesthesia was induced in rats by an intraperitoneal injection of 10% chloral hydrate (Lanzhou Chunsheng Chemical Reagent Co., Ltd. (Lanzhou, China; 3 ml/kg). Rats were fixed in a supine position on a variable temperature carpet (36±0.1°C) and the left chest fur was clipped and disinfected with 75% alcohol and tincture of iodine. Rats were intubated following tracheotomy, and the lower left pulmonary ligament was divided and ventilated

with inhaled air. The following parameters were maintained: $PetCO_2$, 35-45 mmHg; respiratory frequency, 60 times/min; unilateral lung ventilation tidal volume, 8-10 mg/kg; bilateral lung ventilation, 15-20 mg/kg; and inspiration and expiration ratio, 1:2. Following 30 min ventilation, the left hilus pulmonis was divided using forceps without damaging the artery. Subsequent to the administration of heparin sodium (50 units) to the dorsal vein of the penis was administered and 5 mins later the left lung door was clamped noninvasively. The clamping procedure included the left primary bronchus and the left pulmonary artery and vein. Using a straw, the lung surface was subsequently flushed with a small quantity of cold saline and the wound was covered with sterile warm saline-infiltrated gauze. The vascular clamp was released after 45 min to allow for reperfusion. At each of the three time points (ischemia 45 min, reperfusion 60 min and reperfusion 120 min), six rats were sacrificed by arterial bleeding. The percentage of alveolar damage (the number of alveoli containing ≥2 red blood cells and white blood cells/200) in the lung following reperfusion was calculated at 120 min.

Bronchial lavage was performed at the reperfusion was performed at the 120 min time point in another six rats, and the collected broncho-alveolar lavage fluid was stored for cryopreservation at -70°C for the assessment of alveolar damage and total protein. Blood samples collected from the aorta abdominalis were cryopreserved at -70°C for subsequent MDA and SOD analyses. Lung tissue samples were divided into three equal parts, as follows: Upper, a third of the specimen was used to measure the lung W/D ratio; middle, a third of the specimen was fixed in 10% neutral buffered formalin, embedded in paraffin and subjected to hematoxylin and eosin (HE) staining; and under, a third of the specimen was stored at -70°C for TNF-α and MPO analyses.

W/D ratio. The lung surface was flushed with physiological saline, and the blood was removed. The upper third of the lung tissue and the dry blood on the surface were obtained, weighed, and recorded as wet weight. The dry weight was measured after incubating the samples at 100°C for 48 h. Lung W/D ratio (%) was calculated as W/D (100%).

TNF-α expression levels. In order to assess the levels of TNF-α in the lung homogenates among the groups at three time points, absorbance was determined at 450 nm wavelength [optical density (OD) value] using a spectrophotometer (Beckman DU-640 Spectrophotometer; Beckman Coulter Commercial Enterprise (China) Co., Ltd.). Preparation of the 10% lung homogenate with PBS involved specimen and standards for anti-TNF-α monoclonal antibody in an ELISA plate, which was washed. TNF-α expression levels were detected according to the method by Song *et al (*18) The proportion between TNF-α and OD450 was determined using a standard curve.

MPO activity. Blood vessels and fascia were harvested from the lung tissues and weighed. Using an MPO kit (cat no. A044) produced by the Nanjing Jiancheng Biological Engineering Research Institute (Jiangsu, China) the homogenate medium (number two reagent) was added to lung tissues according to the weight volume ratio of 1:9, homogenized in ice water for 5 min

and transformed into 10% tissue homogenate. Subsequently, 0.9 ml 10% tissue homogenate was mixed with 0.1 ml of number three reagent from the MPO kit. Following thorough mixing, the solution was placed in a water bath at 37°C for 15 min prior to analysis. The production of A was measured by colorimetry at 460 nm wavelength, and MPO activity was calculated as follows: MPO activity (U/g) = (testing tube OD value - control tube OD value) / 11.3 x sampling weights (g).

SOD activity. Following 10 min blending after the addition of the contents of the SOD kit. The colorimetric analysis was performed using a 1 cm optical path and 550 nm wavelength. Distilled water was used for the zero setting. Plasma SOD activity (NU/ml) was calculated as follows: Plasma SOD activity = (control absorbance - test absorbance) / control absorbance / 0.5 x reaction quantity / sample quantity.

MDA activity. Abdominal aortic blood (2.0 ml) were collected quiescent in an anticoagulant tube for 60 min, and centrifuged at 1,000 x g for 10 min to isolate the plasma. MDA was evaluated using a glucosinolate barbituric acid method and calculated as follows: MDA = (test tube absorbance - blank test tube absorbance) / (standard tube absorbance - blank standard tube absorbance) x standard sample concentration x number of sample dilution before the test.

Alveolar damage and total protein (TP)/alveolar damage in rats. Using the Belaidi method (3), alveolar white blood cells were counted in ten fields of vision using an MM-800 Nikon microscope (magnification, x200; Nikon Corporation, Tokyo, Japan), and these cells was considered to indicate alveolar leukocyte aggregation. Based on the Muratan method (19), >200 alveoli were continuously observed using under x20 and x10 magnification microscopy. Alveoli that contained ≥2 red blood cells and white blood cells were considered as damaged alveolar and the following equations ere used for calculations: Percentage of alveolar damage = number of damage alveolar / 200 x 100%. Protein content (μg/g) = [C x volume after centrifugation (ml)] / sample weight (g). As such, these blood cells were used to identify the quantity of alveolar damage. The concentration of TP in broncho-alveolar lavage fluid was determined using Coomassie brilliant blue staining (Lanzhou Chunsheng Chemical Reagent Co., Ltd.). For HE (Lanzhou Chunsheng Chemical Reagent Co., Ltd.) staining, lung tissues were fixed in formalin for >24 h and embedded in paraffin. Samples were then cut (5 μm) and subsequently stained with HE.

HE staining. Lung tissues were fixed in 10% formalin for >24 h and embedded in paraffin. Subsequently, they were embedded into wax blocks on a microtome, sliced into 5-μm thick sections, drying was at 45°C in a constant temperature oven, the wax was removed with xylene, dehydrated, and finally distilled water was added, and subsequently stained with HE (hematoxylin dye for 5 min, separated in the acid and ammonia water for 30 sec, dehydrated in alcohol, stained with eosin dye for 2-3 min. Canada balsam was added dropwise in sections after dehydration with alcohol and treatment with xylene and finally, the cover glass was added. The stains were viewed using a MM-800 Nikon microscope (magnification, x200).

Statistical analysis. Statistical analysis was performed using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) via a normal distribution test (Kolmogorov Smirnov test) and homogeneity test for variance (Levene's t-test). Normally distributed data were presented as the mean ± standard deviation. Between-group comparisons and heterogeneity of variance were analyzed using Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Lung W/D ratio. The lung W/D ratio was not significantly different at the three time points in the control group, whereas the lung W/D ratios of rats in the model and Shenfu groups significantly increased with prolonged reperfusion (P<0.05). The model and Shenfu groups were not significantly different at the ischemia 45 min time point, as compared with the control group, but were significantly increased at the reperfusion 60 min and reperfusion 120 min time points (P<0.05). Lung W/D ratios in the Shenfu group were decreased at reperfusion 60 min and 120 min, compared with the model group, however, there were no significant differences (P>0.05). Thus the results revealed that Shenfu injection can reduce the W/D ratios at reperfusion times (Fig. 1).

TNF-α. Lung TNF-α expression levels were not significantly different in the control group at each time point. However, TNF-α expression levels were significantly elevated at the reperfusion at the 120 min time point, compared with the ischemia 45 min time point in the model group and Shenfu injection group (P<0.05). TNF-α expression levels significantly increased at reperfusion 120 min in the Shenfu group (P<0.05), as compared with the ischemia 45 min and reperfusion 60 min time points. In addition, TNF-α expression levels were significantly elevated at all three time points in the model and Shenfu group, as compared with the control group (P<0.05). However, TNF-α levels were significantly lower at the reperfusion 120 min time point in the Shenfu group, as compared with the model group (P<0.05; Fig. 2). The present results reveal that Shenfu injection is able to decrease lung TNF-α expression levels at the reperfusion 120 min time point

MPO. No significant differences in serum MPO activity levels were detected among the time points in the control group. MPO activity levels were significantly elevated at the reperfusion 60 min and reperfusion 120 min time points, as compared with the ischemia 45 min time point in the model and Shenfu groups (P<0.05). In addition, MPO activity significantly increased at each time point in the model and Shenfu group, as compared with the control group (P<0.05). No significant differences in MPO activity levels were detected at the ischemia 45 min time point in the Shenfu group (P>0.05), but MPO activity levels significantly decreased at the reperfusion 60 min and reperfusion 120 min time points, as compared with the model group (P<0.05; Fig. 3). Thus, the present results reveal that Shenfu injection can decrease lung MPO activity levels during reperfusion times.

SOD. No significant differences in SOD activity were detected among the time points in the control group. SOD activity

significantly decreased at the reperfusion 60 min and reperfusion 120 min time points, as compared with the activity at ischemia 45 min in the model group (P<0.05). In addition, SOD activity was significantly decreased at the reperfusion 60 min time point, as compared with ischemia 45 min in the Shenfu group (P<0.05). SOD activity was not significantly different at the reperfusion 60 min and reperfusion 120 min time points in the Shenfu group, as compared with the control group. However, SOD activity was significantly reduced at reperfusion 120 min in the Shenfu group, as compared with the model group (P<0.05; Fig. 4). The results indicate that Shenfu injection can reduce serum SOD activity at the stated reperfusion times.

MDA. No significant differences in serum MDA activity were detected among the time points in the control group. MDA activity was significantly higher at the reperfusion 60 min and reperfusion 120 min time points, as compared with ischemia 45 min in the model and Shenfu group (P<0.05). In addition, MDA activity significantly increased at reperfusion 60 min and reperfusion 120 min in the model and Shenfu group, as compared with the control group (P<0.05). MDA activity was not significantly different at ischemia 45 min in the Shenfu group, as compared with the model group. However, MDA activity was significantly reduced at the reperfusion 60 min and reperfusion 120 min time points (P<0.05; Fig. 5), as compared with the model group. Thus the results revealed that Shenfu injection is able to reduce serum MDA activity at certain reperfusion times

Alveolar damage and TP. The model and Shenfu groups exhibited varying degrees of alveolar damage and TP concentration increases at the reperfusion 120 min time point, as compared with the control group (P<0.05; Fig. 6). The degree of alveolar damage in the Shenfu group was significantly decreased, as compared with the model group (P<0.05). Although no significant changes in TP were detected between the model and Shenfu groups, a marked decrease in TP content was detected in the Shenfu group, as compared with the model group. The results reveal that Shenfu injection can decrease the alveolar damage at the reperfusion 120 min time-point

Light microscopy. The control group exhibited no signs of alveolar damage, with visible organization of alveolar wall integrity, and no interstitial lung edema and neutrophil infiltration. The model group exhibited severely damaged alveolar structures, a large quantity of alveoli, congestive alveolar walls, severe pulmonary interval thickening, wide alveolar spaces, interstitial edema, serous effusion, a large quantity of red blood cells and neutrophil infiltration. Notably, the extent of the damage in the alveolar structure, interstitial neutrophils, and alveolar and interstitial red blood cells was reduced in the Shenfu group, as compared with the control and model groups (Fig. 7). The results indicated that Shenfu injection may relieve the degree of alveolar structure damage.

Discussion

Emphysema, lung fibrosis, silicosis and bronchial lung expansion are serious end-stage lung diseases that can cause chronic
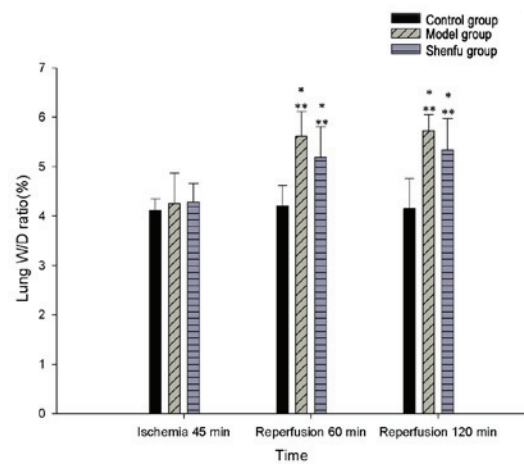

Figure 1. Lung W/D ratio among the groups at three time points. *P<0.05 vs. the control; **P<0.05 vs. the ischemia time point. W/D, weight/dry weight.
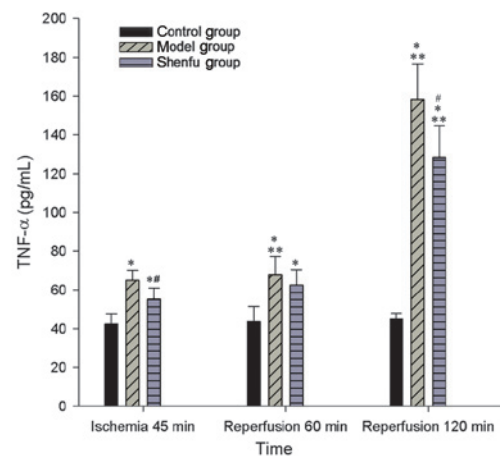

Figure 2. TNF-α levels in lung homogenates among the groups at three time points. *P<0.05 vs. the control group; **P<0.05 vs. the ischemia time point; #P<0.05 vs. the model group. TNF-α, tumor necrosis factor-α.

respiratory function failure and result in patients requiring a breathing machine. To date, lung transplantation remains the only effective treatment method for end-stage lung disease (20). Lung transplantations have been performed for >30 years in China and statistical analyses have demonstrated that one-year patient survival percentage is 70%. Lung transplant failure is predominantly a result of lung ischemia-reperfusion injury, which has a mortality rate of 25% (21,22). Ischemia-reperfusion is also a risk factor for chronic rejection following transplantation, which affects the long-term survival of patients.

The mechanism underlying lung ischemia-reperfusion injury is yet to be fully elucidated; although increased pulmonary vascular permeability and dysfunction, and further lung damage following lung ischemia-reperfusion are thought to be associated. Microscopy has been used to observe damaged endothelial cells following the apoptosis and necrosis of pulmonary capillary and alveolar epithelial cells following ischemia-reperfusion injury (1,2). To date, three theories have been proposed to explain the mechanism underlying lung ischemia-reperfusion injury. The neutrophil theory indicates that numerous chemical chemotaxes, including
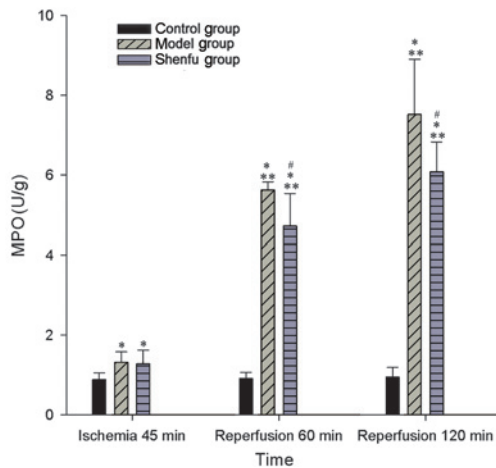
Figure 3. MPO activity levels in lung tissue among the groups at three time points. *P<0.05 vs. the control group; **P<0.05 vs. the ischemia time point; #P<0.05 vs. the model group. MPO, myeloperoxidase.
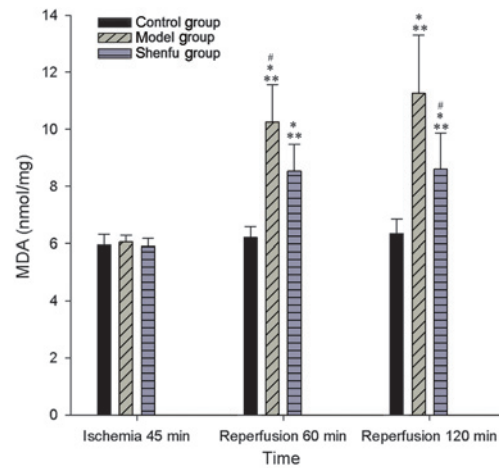

Figure 5. MDA activity levels in blood samples among the groups at three time points. *P<0.05 vs. the control group; **P<0.05 vs. the ischemia time point; #P<0.05 vs. the model group. MDA, malondialdehyde.
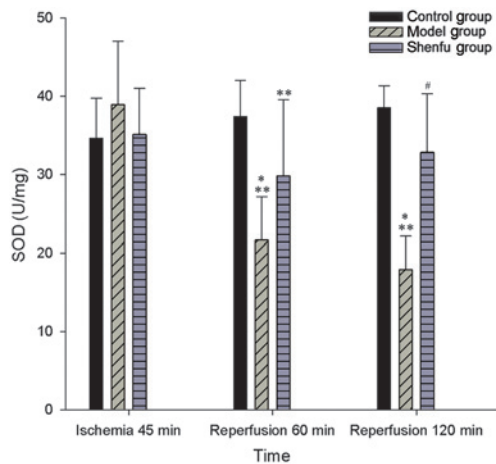

Figure 4. SOD activity levels in blood samples among the groups at three time points. *P<0.05 vs. the control group; **P<0.05 vs. the ischemia time point; #P<0.05 vs. the model group. SOD, superoxide dismutase.
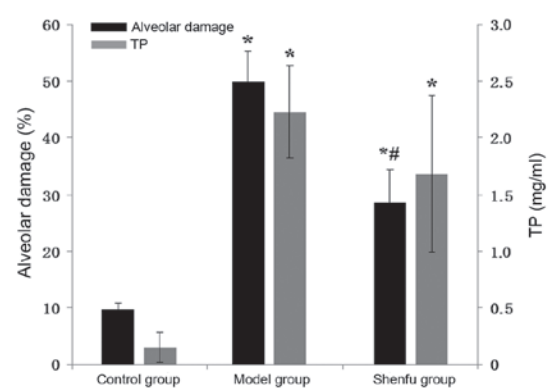

Figure 6. Alveolar damage and TP after reperfusion for 120 min among the groups. *P<0.05 vs. the control group; #P<0.05 vs. the model group. TP, total protein.

leukotriene and TNF, are released into the blood following ischemia-reperfusion. The free radical theory states that when ischemia occurs in pulmonary capillary endothelial cells, xanthine dehydrogenase is converted into xanthine oxidase, which exists in the endothelium. Adenosine is degraded by xanthine oxidase and produces a large quantity of oxygen free radicals following reperfusion to mediate lipid peroxidation, inhibit protein function, and induce endothelial cell membrane damage, endothelial edema and jam capillary (23). Free radicals are also capable of promoting inflammatory factors and neutrophil aggregation and activation, thereby resulting in lung tissue damage and the decline of lung function (24). The calcium overload theory states that following lung ischemia, the adenosine triphosphate concentration in cells decreases, the activity of sodium pumps decreases, intracellular Na^+ ion concentration increases and cellular edema occurs. Following reperfusion, the high intracellular Na^+ ion content activates Na^+/Ca^{2+} exchange proteins, and Na^+ is extracellularly transported. A large quantity of Ca^{2+} ions simultaneously enter the cells, thereby causing calcium overload. Subsequently,

the damaged cell membrane, mitochondria, and sarcoplasm retina increase the permeability of the membrane, resulting in endothelial edema (25). This condition may interfere with the oxidative phosphorylation of the mitochondria, thereby reducing ATP production, promoting free radical generation, and damaging lung tissues (23). A number of drugs prescribed to treat moderate or reduced ischemia-reperfusion injury are available in clinics, including: SOD and calcium antagonists, auxiliary medication with prostaglandin to dilate blood vessels, and sodium nitrate to improve arterial blood oxygenation (26).

Based on the suggested mechanisms underlying lung ischemia-reperfusion injury, targeted therapy drugs and data from animal experiments, clinical experience, and related pervious studies, it can be speculated that Shenfu injection may reduce lung ischemia-reperfusion injury. As studies investigating Shenfu injection and its effect on lung ischemia-reperfusion injury are lacking (12,27,28), the present study aimed to examine the role of Shenfu injection in a rat model of lung ischemia-reperfusion to determine the mechanisms underlying its protective effects.

Shenfu injection originates from the traditional medicine Shenfu decoction, which is extracted from red ginseng and black *aconitum carmichaelii*. The primary ingredients of Shenfu
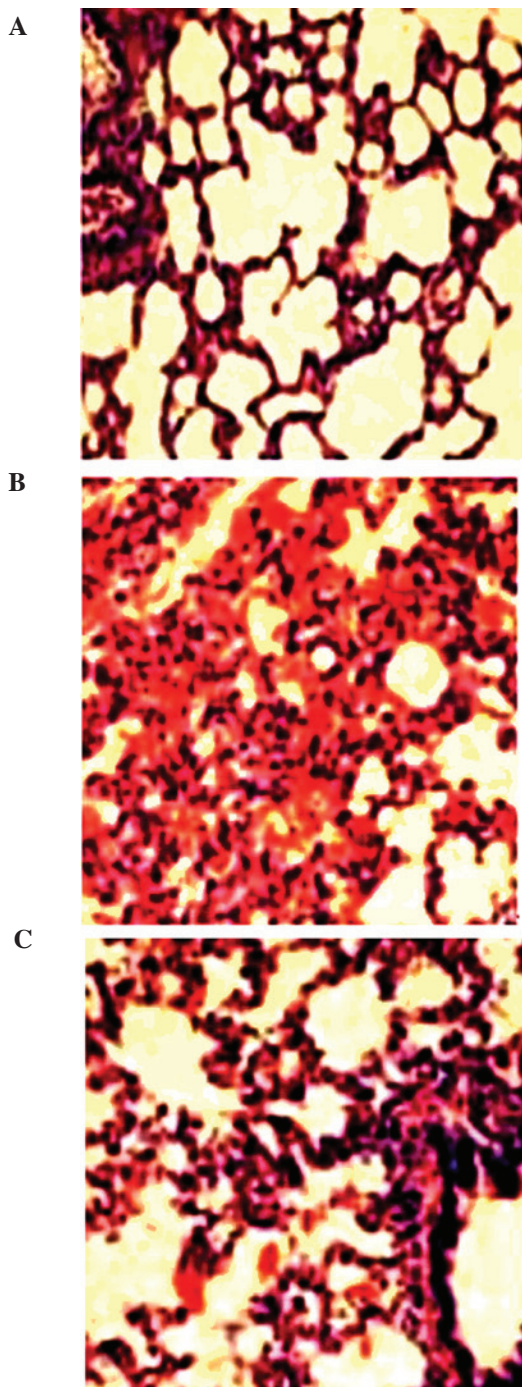
A

B

C

Figure 7. Hematoxylin and eosin staining of (A) the control, (B) model and (C) Shenfu groups. No signs of alveolar damage were detected in the control group; whereas the model group exhibited severely damaged alveolar structures, serous effusion, a large quantity of red blood cells and neutrophil infiltration. The extent of the damage to the alveolar structure, interstitial neutrophils, and alveolar and interstitial red blood cells was lower in the Shenfu group compared with the control and model groups.

injection include ginseng saponin Rb1 (≥0.5 g/l) and aconitum alkaloids (≤0.1 g/Ul). Previous studies have demonstrated that ginseng saponins exert protective effects against myocardial ischemia-reperfusion injury (29). Furthermore, Shenfu injection is capable of increasing the activity of SOD, decreasing MDA levels, removing oxygen free radicals, protecting against lipid peroxidation, antagonizing Ca^{2+} and producing a glucocorticoid-like effect (25). It has also been demonstrated

that Shenfu injection can stabilize the cell membrane, reduce myocardial enzyme release and protect ischemic myocardial cells (7). A previous study, which used an adult rabbit model of acute kidney ischemia-reperfusion, demonstrated that Shenfu injection can protect SOD activity. SOD is an endogenous oxygen free radical scavenger that directly inactivates oxygen free radicals, increases nitric oxide, inhibits leukocyte adhesion and Na^+ in-flow, and prevents acute ischemia-reperfusion injury (8). Zheng *et al* (7) demonstrated that different doses of intraperitoneal Shenfu injection (5, 10 and 20 ml/kg) have a dose-dependent protective effect on transient focal cerebral ischemia. Previous clinical studies have demonstrated that Shenfu injection exhibits good curative effects on bronchial asthma and chronic obstructive pulmonary disease (6,8,11). Furthermore, modern pharmacological studies have confirmed that Shenfu injection is capable of inhibiting the activation of nuclear transcription factor κB and decreasing the expression levels of TNF-α and interleukin (IL)-6, thereby reducing pathological damage in the lungs and liver and protecting the structures of these organs (12). Shenfu injection directly inactivates the anti-oxygen free radical xanthine oxidase, improve lung blood flow, adjust immune function (30). Shenfu injection also protects vascular endothelial cells, functions as an anti-inflammatory and anti-endotoxin compound, improves hypoxia tolerance, and protects against lung injury induced by endotoxic shock (13-15) Therefore, we hypothesize that Shenfu injection at 10 ml/kg dosage can exert protective effects on cells when applied prior to lung ischemia-reperfusion injury.

Following lung ischemia-reperfusion, blood flow and ventilation rapidly increase, oxygen levels in lung tissues increase sharply, and oxygen free radicals that attack unsaturated fatty acids in biological membranes are released; thus, lipid peroxidation occurs and forms lipid peroxides, which induce fluid loss from the cell membrane and tissue edema, increase permeability and aggravate lung injury (31-33). Lung W/D ratio is an index of lung water content which is used to assess the degree of lung edema and reflect the degree of lung injury. In the present study, the rat lung W/D ratio significantly increased at the ischemia 45 min and reperfusion 120 min time points, indicating the occurrence of high lung water content and edema following lung ischemia-reperfusion. The W/D ratio significantly decreased in the Shenfu injection group, as compared with the model group, indicating that Shenfu injection can inhibit edema and reduce lung injury. This finding implied that Shenfu injection may increase the blood flow in the ischemia/non-ischemia area and stabilize the hemodynamics. Hanci *et al* (34) have identified that the effects of Shenfu injection are correlated with its dosage.

TNF, which is an important pre-inflammatory factor, exists in two forms, namely TNF-α and TNF-β. TNF-α is secreted by activated monocytes/macrophages and has a molecular weight of 17 kDa. TNF-α promotes immunity and inflammatory reactions. During ischemia-reperfusion, TNF-α concentration rapidly increases and exhibits higher sensitivity than other cytokines (23). TNF-α can activate and promote humoral inflammatory reactions which may induce a series of pathological responses in the body, including the degranulation of neutrophils,and the expression of CD11b/CD18 adhesion molecules. TNF-α also triggers the adhesion of CD11b/CD18 on vascular endothelial cells, amplifies low stimulation reaction,

damages vascular endothelial cells, increases vascular permeability, reduces circulation resistance, induces the release of other humoral inflammatory factors, including IL-8, leukotriene and platelet activating factor, and increases the phagocytosis of macrophages (35,36). Therefore, TNF-α can be used as an inflammatory indicator in the body and TNF-α expression levels are associated with the degree of damage.

SOD, which is a primary oxidase and super-organism oxygen free radical scavenger, has been demonstrated to reflect the ability of the body to remove free radicals. SOD disproportionates the lipid peroxide (as MDA) for hydrogen peroxide and water, thereby protecting cells from damage. Previous animal studies have demonstrated that SOD levels decrease in response to the large quantity of oxygen free radicals following reperfusion; such a decrease in SOD concentration damages the structure and function of the endothelial cells in lung tissues (37,38). The current study demonstrated that Shenfu injection may reduce SOD levels in plasma following reperfusion.

MDA, which is a metabolic product of lipid peroxidation, is important in lipid peroxidation of membrane lipid failure, and can reflect the degree of lipid peroxidation in the body as an indirect index of the degree of lung reperfusion injury (39). The present study demonstrated that high levels of MDA were expressed during lung injury in rats following lung ischemia-reperfusion, which confirmed that the present rat model of lung ischemia-reperfusion was successful. The results demonstrated that Shenfu injection improved resistance to oxygen free radical damage and reduced lung ischemia-reperfusion injury by inhibiting the oxidative stress reaction.

In conclusion, the results of the present study suggested that Shenfu injection may reduce the lung W/D ratio and the concentration of TNF-α and MPO in rats. Furthermore, Shenfu injection also reduced plasma SOD and MDA expression levels and TP content in broncho-alveolar lavage fluid. Therefore, Shenfu injection may reduce the physical injury of the lung, and to an extent protects against ischemia-reperfusion injury. As a result, we recommend that further investigation into the clinical application of Shenfu injection prior to ischemia-reperfusion injury is performed.

Acknowledgements

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