Comparison of Protective Effects of Safflower Injection and Extract of Ginkgo Biloba on Lung Ischemia/Reperfusion Injury in Rabbits

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ABSTRACT Objective: To observe the protective effects of safflower injection (SI) and extract of Ginkgo biloba (EGB) on lung ischemia-reperfusion injury (LIRI) and investigate its mechanism. Methods: In vivo rabbit model of LIRI was reconstructed. Forty rabbits were randomly and equally divided into four groups: sham-operation group (sham group), ischemia-reperfusion group (model group), ischemia-reperfusion plus SI group (safflower group) and ischemia-reperfusion plus EGB injection group (EGB group). Malondialdehyde (MDA) content, superoxide dismutase (SOD) and xanthine oxidase (XO) activity in serum were measured. The wet/dry weight ratio (W/D) of the lung tissue and activity of myeloperoxidase (MPO) were also tested. Ultrastructure change of the lung tissue was observed by the electron microscope. The expression of intercellular adhesion molecule-1 (ICAM-1) was measured by immunohistochemistry (IHC). Results: In the model group, MDA and XO increased and SOD decreased in serum compared with the sham group (P<0.01). The values of W/D, MPO and ICAM-1 of the model group were higher than those of the sham group (P<0.01), but those of the safflower group and EGB group were significantly lower than those of the model group (P<0.01). The IHC demonstrated that ICAM-1 expression in lung tissue of the model group was significantly higher than those of the safflower group (P<0.01), compared with safflower group, in the EGB group MDA, XO, MPO decreased, SOD and ICAM-1 expression increased (P<0.05), but the change of W/D was not statistically significant (P>0.05). Conclusions: SI and EGB may attenuate LIRI through antioxidation, inhibition of neutrophil aggregation and down-regulation of ICAM-1 expression. But EGB had more effect on the antioxidation, while SI did better on regulating ICAM-1 expression.

KEYWORDS Safflower Injection, Ginkgo biloba extraction, lung, ischemia-reperfusion injury, intercellular adhesion molecule-1

Ischemia-reperfusion (I/R) injury is commonly seen in lung transplantation, pulmonary artery sleeve lobectomy and extracorporeal circulation and other surgeries, and it is the main reason for patients' pulmonary arterial hypertension, pulmonary edema and respiratory failure, while satisfactory treatment was not found currently. The exact mechanism of occurrence of lung ischemia-reperfusion injury (LIRI) is not very clear, and it involves many aspects including the generation of a large number of oxygen-derived free radicals, calcium overload, endothelial cell damage, platelet aggregation and inflammatory cytokine release. Safflower and other traditional blood-activating and stasis-dissolving Chinese herbs have antagonistic effects on LIRI, while the extract of Ginkgo biloba (EGB) has the protective effect on I/R injury,\(^1\) \(^3\) and the protective effects of these two traditional Chinese herbs may be related to scavenging free radicals and lipid peroxidation. In this study, in vivo rabbit LIRI model was constructed to detect indicators related to lung injury, the change in expression of intercellular adhesion molecule-1 (ICAM-1) in lung tissue was measured by immunohistochemistry (IHC), and the protective effects of safflower and EGB pretreatment on LIRI were compared, which provides a theoretical basis for clinical prevention and treatment of LIRI.

METHODS

Animals and Main Reagents

Forty healthy Japanese white rabbits of either gender weighing 1.7–2.3 kg were provided by Laboratory Animal Center of Medical College,
Zhejiang University [SYXK (Shaan) 2009-001]. Safflor Injection (Lot 010904, 10 mL/ampoule) was purchased from Hubei Minkang Pharmaceutical Co., Ltd., China. Ginaton Injection (each ampoule containing 17.5 mg EGB with 4.2 mg ginkgo flavone glycoside) was purchased from Germany YWillmar Schwabe Dr.Pharmaceutical. All assay kits of malondialdehyde (MDA), superoxide dismutase (SOD), xanthine oxidase (XO) and myeloperoxidase (MPO) were provided by Nanjing Jiancheng Bioengineering Institute, China. ICAM-1 antibody test kit was purchased from Wuhan Boster Biological Engineering Co., Ltd., China.

Model Construction

In vivo LIRI rabbit model was reconstructed according to the method introduced by Sekido, et al:

1. atropine (0.1 mg) for intramuscular injection,
2. 25% urethane (5 mL/kg) for anesthesia intravenous injection, the rabbit was done tracheostomy and then connected with animal ventilator to assist respiration with the respiratory rate of 20–30 times/min, the tidal volume of 10 mL/kg, the oxygen concentration in inspired air being 100%, and the electric heater maintaining body temperature; left jugular vein intubation, physiological saline keeping for intravenous drip with 0.5–1.5 mL/min; cutting off the third, fourth and fifth ribs along the left sternal border, leaving blocking after being free from left pulmonary hilar, and using the band-blockade to ligate the left pulmonary artery, pulmonary vein and left main bronchus at the end-expiratory left pulmonary hilar, making the left lung ischemia; opening and restoring blood supply and ventilation for the left lung reperfusion.

Experimental Groups and Sample Collection

In the experiment, the rabbits were randomly divided into four groups. The sham-operation group (sham group, n=10): mechanical ventilation for 4 h after thoracotomy, and except leaving the left pulmonary hilar pass the band-blockade and not for ligation, no other treatments were given. The I/R group (model group, n=10): blocking the left pulmonary hilar for 1 h after thoracotomy, and then opening for 3 h of reperfusion. The I/R plus Safflor Injection group (safflor group, n=10): intravenous Safflor Injection (2.0 mL/kg) 20 min before ischemia, and the remaining steps were the same with model group. The I/R plus EGB injection group (EGB group, n=10): intravenous Ginaton injection (200 mg/L) 20 min before ischemia, and the remaining steps were the same with the model group. After the experiment was ended, 5 mL blood from the nape artery and part of the lung tissue were taken from the rabbits of the 4 groups for experimental use.

MDA Content as Well as SOD and XO Activity Assay in Serum

MDA content was measured with barbituric acid, SOD activity was detected by xanthine oxidase technique, XO activity was measured with chemical colorimetry, and these were operated according to kit instructions.

Lung Wet/Dry Weight Ratio and MPO Activity Assay

Part of the lung tissue was taken for sufficient physiological saline rinsing, and excessive moisture was blotted with filter paper and the wet weight was measured. It was dried in a constant temperature electric blast drying oven at 70 °C for 24 h and the dry weight was measured, and the ratio of the two was the wet/dry weight ratio (W/D). The lung tissue homogenate was taken for MPO activity assay with spectrophotometry according to the instruction.

Measurement of ICAM-1 in Lung Tissue by IHC

The experimental steps were referred to the recommended method on the kit (conventional strpt avidin biotin complex method, diaminobenzidine staining), and the expression was positive if the staining was brown. The absorbance (A) was read with the absorbance analysis software developed by East China University of Science and Technology, which was regarded as the relative content of ICAM-1.

Statistical Analysis

The experimental data were represented by mean ± standard deviation, and were analyzed by SPSS 11.0 statistical software. The multi-group comparison was analyzed by analysis of variance (ANOVA), and the pairwise comparison was adopted q test; the comparison between each group and sham group was tested by Dunnett-t, and the difference was significant when P<0.05.

RESULTS

Changes of MDA Content as Well as SOD and XO Activities in Serum

Compared with the sham group, SOD significantly decreased in serum, and MDA and XO obviously increased in the model group (P<0.01).