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Original Article

The influence of lung ischemia-reperfusion injury on myocardium

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Abstract: Object: To investigate the effect of lung Ischemia-reperfusion injury on cardiac muscle. Methods: SD rats were randomly divided into the following four groups: sham operation group (TS group); experimental control group (TG group); curcumin group (TC group); dexamethasone group (TM group). After 4 h and 24 h of lung ischemia-reperfusion, the cardiac muscle was collected. We measured the concentrations of TNF-α, IL-6, caspase-3, antioxidant capacity and neutrophil infiltration in myocardial tissue. Results: After lung ischemia-reperfusion 4 h, TNF-α, IL-6 levels, MDA and neutriphils infiltration increased in myocardial tissue in control group, total antioxidant capacity decreased, and caspase-3 exhibited no significant changes. However, 24 h after reperfusion, there were no significant difference between the sham group and experimental group. Conclusion: Lung ischemia-reperfusion injury affected the function of cardiac muscle, especially 4 h after reperfusion. Curcumin and dexamethasone effectively protected myocardial injury after lung ischemia-reperfusion.

Keywords: Lung ischemia, cardiac muscle, cytokines, oxidation, neutrophil infiltration

Introduction

Ischemia-reperfusion injury is a phenomenon of pathophysiology, when tissue restored blood flow after a period of ischemia, tissue damage increases. This kind of phenomenon can occur in multiple organs, seen in clinical transplantation, shock, extracorporeal circulation in heart surgery and stop cycle, is a complex pathophysiological process, its precise mechanism has not been fully elucidated. In recent years, with the wide application of extracorporeal circulation technology, especially the development of lung transplant technology, the ensuing lung ischemia-reperfusion injury is an inevitable pathologic process [1, 2]. Ischemia-reperfusion injury mechanisms include a series of cellular structure and function result from no reflow, calcium overload in intracellular calcium homeostasis disorders, energy metabolism disorders, gathered a large number of white blood cells infiltration and its effect on the release of various cytokines, and the function of the free radical and lipid peroxidation damage [3]. The vascular endothelial cell injury, is considered to be one of the key initial segment, as well as the activation of neutrophils [4]. Lung ischemia-reperfusion injury of the important reasons is the formation of oxygen free radicals, oxygen free radical is a kind of high molecular reaction, can cause lipid peroxidation of lung tissue to strengthen, impaired gas exchange [5]. In addition, the damage can cause damage to the intracellular calcium overload, protein structure, and further aggravate the damage of DNA injury and apoptosis [6].

Ischemia-reperfusion injury mechanism caused by the generation of oxygen free radicals, calcium overload, leukocyte activation, etc., can lead to heart, brain, liver, lung, kidney and so on multiple organ injury [7-9]. Ischemia-reperfusion injury mechanism caused by the generation of oxygen free radicals [10], calcium overload [11], leukocyte activation [12], etc., can lead to heart, brain, liver, lung, kidney and so on multiple organ injury [13].

This study focused on whether harmful substances produced from lung ischemia-reperfu-

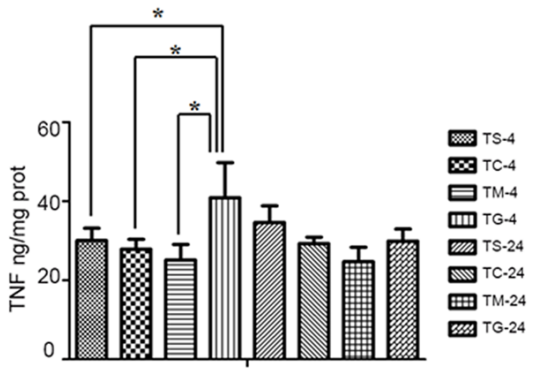

Figure 1. Content of TNF-α after ischemia-reperfusion in myocardial cell.

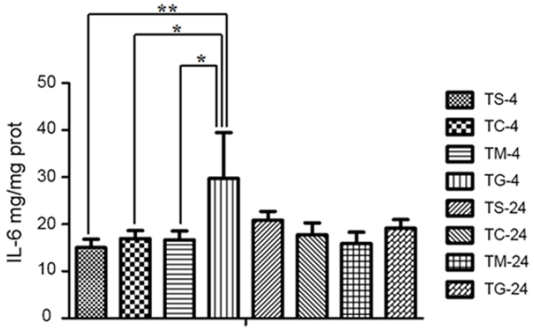

Figure 2. Content of IL-6 after ischemia-reperfusion in myocardial cell.

sion injury will produce impact on important organs myocardial by blood circulation. And whether drug treatment, such as curcumin and dexamethasone which can protect lung injury in lung ischemia reperfusion, can protect myocardial.

Materials and methods

Experimental animal

Study design is approved by fudan university animal care committee. Animal use and handling in accordance with the national department of health issued "laboratory animal care and use guidelines". The male SD rats (experimental animal center of fudan university, Shanghai, China) used in the experiment weigh about 250-300 g, with preoperative fasting 12 h, but offering water.

Experimental drugs and reagents

iNOS, MPO and MDA, T-AOC detection kits, bought from Nanjing Jian Cheng biological engineering research institute; Curcumin (CUR) bought from the Sigma company; Dexamethasone sodium phosphate injection from Tianjin Pharmaceutical Co., Ltd. Jiaozuo; TNF alpha and IL-6 ELISA kits were from R&D company; rat anti-rabbit caspase-3 polyclonal antibody bought from CST company in the United States. Total antioxidant capacity assay kit; Malondialdehyde (MDA) assay kit (TBA method); Total antioxidant capacity assay kit; Nitric Oxide Synthase (NOS) assay kit (Colormetric method).

Animal model preparation and grouping

Intraperitoneal injection sodium pentobarbital at 50 mg per kilogram of body weight to anesthesia, 0.2 mg atropine intramuscular injection, skin preparation, fix rat at supine position, insert size 14 venous indwelling needle casing in the rat trachea, connect to Harvard breathing machine (model 683) and ventilate. Breathing machine parameter settings: inhaled oxygen concentration 60% (with oxygen), positive end-expiratory pressure at 2 cm water column, breathing rate 75 times/min, tidal volume at 10 ml/kg, intubation at the right carotid artery monitoring flesh and blood. Dilute with ringer's solution of sodium pentobarbital to 8 mg/ml concentration. Trace injection pump via tail vein with continuous rehydration and maintenance of anesthesia at the rehydration rate of 2 ml/kg/h. Turn animal at right side-lying position, the knife is entered into the chest between the left side of the fifth rib. After lungs exposure, blunt separate bottom left pulmonary ligament. Five minutes after injection 50 U heparin (two heparin dissolved in 250 ml of normal saline, injection volume is 0.5 ml) via penis intravenous, blood drawing from right carotid artery to monitor blood gas before ischemia. Clip hilum of the left side of lung at air condition with no damage vascular clamp, including left main bronchus, arteries and veins. Cover the slit with gauze wetting with saline water. During the period of ischemia, covered with brine after wet gauze slit, external use plastic mulch, reduce moisture to evaporate. Cover the gauze with plastic film, in order to reduce evaporation. Use incandescent bulbs to heat to maintain anal temperature at 37-38°C in rats. 90 min later, loosen vascular clamp, reperfusion 4 h. After reperfusion 4 h, Blood right aorta and left pulmonary vein and measure blood gas. Each animal blood 3 ml from carotid, cryogenic centrifugal, take supernatant, repackaging, save to -80°C refrigerator, under test. Kill animals by carotid artery bleed-

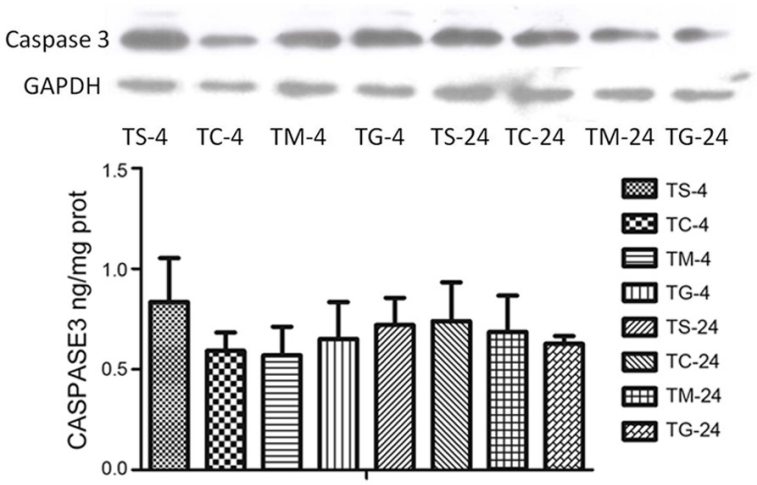

Figure 3. Content of caspase3 after ischemia-reperfusion in myocardial cell.

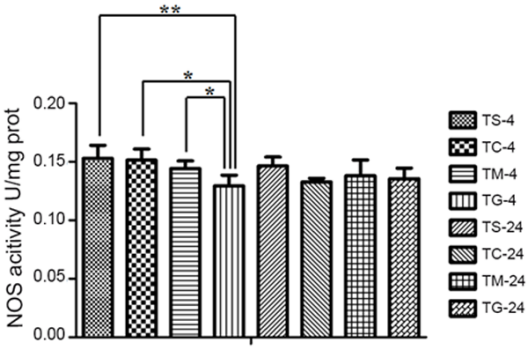

Figure 4. Content of iNOS after ischemia-reperfusion in myocardial cell.

ing. Cardiopulmonary blocks were quickly removed and analysis was performed according to the following step. Take surgical operation and histologic analysis according to the blind method.

Animals were randomly divided into 4 experimental group: 12 rats per group, the total number of rats is 48. The treatment group received curcumin (CUR) or dexamethasone (DXM). Experiment of drug usage and dose reference Ghoneim AI, *etc* [14].

Control group (sham) marked as TS. Rats with 50 mg/kg curcumin intraperitoneally marked as TC group. Rats intraperitoneally injection with 5 mg/kg DXM marked as TM group. Operation control group marked as TG group.

LISA and western blot analysis

In accordance with the kit instructions, we performed the analyses of iNOS, MPO and MDA, TAOC and other indicators.

Detect IL-6, TNF-α content in myocardial tissue of rats with ELISA test kit, in strict accordance with the kit instructions.

Caspase3 was measured with western blot: protein was separated on polypropylene acyl ammonia gel electrophoresis, transfered to PVDF membrane, blocked nonspecific site with 5% skimmed milk. Dilute the first antibody according to 1:1000 and incubation at 4°C overnight. Wash away first antibodies and add the second antibody which also diluted by skimmed milk at 1:1000, and incubate the second antibody for 1 h at room temperature. Wash away the second antibody, electro-chemi luminescence immunoassay.

Statistical analysis

The values involved in this study were represented as means ± standard error for each group and analyzed by The Student *t*-test.

Result

Effects of lung ischemia-reperfusion on myocardial cell inflammation and apoptosis

It is generally believed that the mechanism of ischemia reperfusion injury has close relationship with cytokines and oxygen free radicals [15]. So we measured the concentration of TNF-α and IL-6 in myocardial cell. Compare with TS and TG groups, the levels of TNF-α and IL-6 significantly increased ($P < 0.01$) at ischemia reperfusion 4 h, and IL-6 level return to normal after 24 h reperfusion (**Figures 1** and **2**). Treatment with dexamethasone and curcumin can significantly inhibit the rise of IL-6 levels ($P < 0.05$).

Cell apoptosis is a process regulated by the genes of cascade, the most important of which is Caspases cascade. Caspase-3, as a common downstream target of the cascade key factor, executs the death program. Detection of Caspase-3 content in myocardium can reveal the apoptosis of myocardial cells. Comparing the differences of Caspas-3 content in TS group

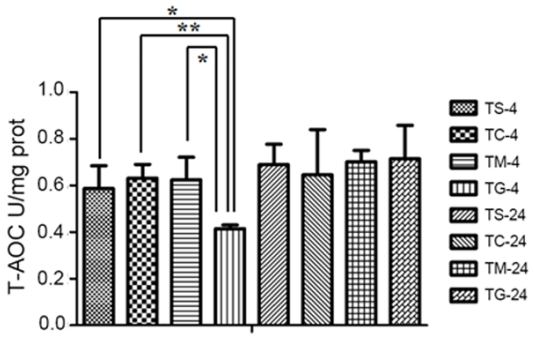

Figure 5. Content of T-AOC after ischemia-reperfusion in myocardial cell.

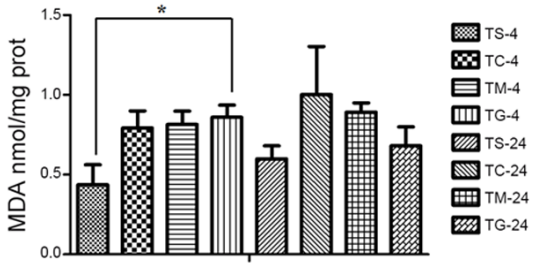

Figure 6. Content of MDA after ischemia-reperfusion in myocardial cell.

and TG group, the myocardial cell did not exhibit significant apoptosis phenomenon (P > 0.05) in lung ischemia-reperfusion after 4 h and 24 h (**Figure 3**).

Effect of lung ischemia reperfusion on myocardial iNOS

In healthy person's heart, the level of iNOS is low or even undetectable, but high levels of iNOS can be detected in the cardiac myocyte in a heat failure [16]. A high concentration of NO can induce myocardial cell apoptosis. After 4 h of lung ischemia-reperfusion 4 h, iNOS in TG group significantly reduced (P < 0.05) when compared with TS group, and dexamethasone and curcumin significantly reversed iNOS level (P < 0.05). The iNOS level remained stable (P > 0.05) after 24 h of lung ischemia reperfusion (**Figure 4**).

Effects of lung ischemia-reperfusion on myocardial cell antioxidant capacity

Comparison of total antioxidant capacity in myocardial cells in TS and TG group (T-AOC) after 4 h lung ischemia-reperfusion indicated that lung ischemia reperfusion reduced myocardial antioxidant capacity (P < 0.05) (**Figure 5**). MDA, as an important marker of lipid peroxide, reflects the intensity of peroxidation. Lung ischemia reperfusion resulted in significantly increased myocardial MDA (P < 0.05) (**Figure 6**). This suggests that the lung ischemia reperfusion affects lipid peroxidation of myocardial cells in mice, has bad effect on removal system of free radicals and reactive oxygen, thus affecting heart tissue. Treatment with dexamethasone (P < 0.05) or curcumin (P < 0.01) can effectively increase myocardial antioxidant capacity.

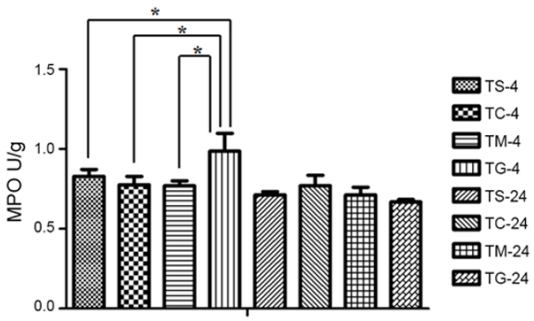

Figure 7. Content of MPO after ischemia-reperfusion in myocardial cell.

Effects of lung ischemia-reperfusions on myeloperoxidase

The activity of MPO is direct associated with the number of neutrophils. MPO activity of myocardial cells increased significantly (P < 0.05) 24 h after lung ischemia-reperfusion (**Figure 7**), namely, after lung ischemia-reperfusion neutrophils infiltrating degree increase in myocardial cells, and inflammation enhanced.

Discussion

Studies have shown that inflammatory mediators play an important role in the process of myocardial cell hypoxia injury [17]. Inflammation factors produced by lung ischemia-reperfusion enter into the heart muscle, leading to increased TNF-α and IL-6 in muscle cells significantly [18]. The increased expression of TNF-α can affect cardiac function and myocardial damage through various ways [19]. As the initiating link in the cytokine cascade, TNF-α can not only activate nuclear transcription factor NF-κB [20], but also interact with former inflammatory IL-1, IL-6, etc [21]. Under the stimulus of these former inflammatory factors, neutrophil respirato-

ry bursts, further aggravating the local tissue injury. Further, TNF-α can induce myocardial apoptosis by sphingosine-dependent mechanism [22]. A large amount of former inflammatory factors produced by lung ischemia-reperfusion, can continue to produce form inflammatory factors by activating the corresponding receptors, results in a vicious circle and increased myocardial injury. However, we did not detect changes in concentrations of Caspase-3 in myocardial cells. One possible reason is injury caused by lung ischemia-reperfusion is not enough to make the myocardial apoptosis.

Nitric oxide (NO) is a free radical, and can turn into cytotoxicity medium in certain circumstances, at this time NO plays an important part in ischemia-reperfusion injury process [23]. NO, as a kind of gas molecular messenger, however, has many physiological functions, including forceful blood vessels regulation and immune control [24, 25]. Nitric oxide synthase (NOS), the limited enzyme in the synthesis process of NO, exists in the body with a variety of forms. Induced nitric oxide synthase (iNOS) was found in many cells, such as macrophages, epithelial cells, endothelial cells [26]. NO can reduce the vascular tension, prevent neutrophil and platelet adhesion. Endogenous NO decreased after ischemia reperfusion in the human body and animal experiments [24]. Therefore NO as a double-edged sword, plays a certain role in ischemia-reperfusion injury. Our experiment results show that the lung ischemia-reperfusion can reduce iNOS and make NO decrease in the cell. While after drug treatment, the iNOS activity increased again, and the treatment can also promote intracellular NO production, regulate vascular tone, inhibit PMN, platelet activation and aggregation and adhesion to the inner surface of blood vessels [27]. Drug treatment has played an important role in inhibiting apoptosis in our experiment.

Within normal system, ROS production system and removal system tend to balance. MDA is the end product of the lipid peroxidation mediated by free radical. MDA is the most classic and effective indicators to reflect the degree of oxidative damage in body, which can cause macromolecular, such as nucleic acid and protein, crosslinking and polymerization, and has cytotoxicity [28]. As end product of lipid peroxidation, MDA content and cell damage degree were positively correlated [29]. MDA can not only reflect the degree of lipid peroxidation, also can indirectly reflect the generation of oxygen free radicals of the body and the degree of cell damage [30]. In order to understand the lung ischemia-reperfusion effects on myocardial cell oxidation system, we tested MDA and T-AOC content in myocardial cells. We found their content significantly changes in myocardial cells after lung ischemia-reperfusion, the antioxidant ability of myocardial cells decreased, and increased reactive oxygen species. This may be because oxidation intermediate produced in the process of lung ischemia-reperfusion injury to the lungs flow into the heart through the circulation of the blood, that is why we detected the significant changes of MDA and T-AOC in myocardial cells after lung ischemia reperfusion. And the use of these two drugs can effectively increase the myocardial antioxidant ability, protecting myocardial cells.

MPO mainly exists in azurophilic granules in neutrophils [31]. Thus MPO activity can be used as neutrophils markers to quantify the number of white blood cells infiltrating, is the reliability index to evaluate the degree of neutrophil infiltration in the tissue [32]. We detected increased neutrophil infiltration in myocardial tissue 4 h after lung ischemia-reperfusion, which boosting the inflammatory reaction, the reaction became weaker after 24 h. The use of dexamethasone and curcumin can restrain the infiltration of neutrophils, thus protecting myocardium.

Our experimental results show that the lung ischemia-reperfusion influences myocardial cell functions, including cytokines increases, antioxidant capacity weakens, oxidation degree and the inflammation infiltration increases, while using curcumin and dexamethasone treatment can protect myocardium.

Disclosure of conflict of interest

None.

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