European Review for Medical and Pharmacological Sciences 2013; 17: 2207-2217

Pre-treatment with Cobra venom factor alleviates acute lung injury induced by intestinal ischemia-reperfusion in rats

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Abstract. – BACKGROUND: Previous studies have shown that complement activation is required for intestinal ischemia-reperfusion (IIR)-induced tissue damage. Cobra venom factor (CVF), a structural and functional homolog to the activated form of C3 (the central component of the complement system), can cause exhaustive activation of the alternative pathway and deplete the complement components.

AIM: This study aims to investigate the effect of CVF pretreatment on acute lung injury induced by IIR in rats.

MATERIALS AND METHODS: Lung injury was induced by clamping superior mesenteric artery (SMA) for 60 min followed by 4 h of reperfusion. CVF was given via the tail vein 24 h before the operation.

RESULTS: Histological results as well as lung edema determination and permeability assay showed the severe damages were induced in the lungs of rats in the IIR group, accompanying with the increases in the levels of pulmonary malondialdehyde (MDA), myeloperoxidase (MPO) activity, intercellular adhesion molecule-1 (ICAM-1), interleukin (IL)-8. Remarkably, CVF pretreatment significantly attenuated the morphological lung injury, lung edema and lung permeability, reduced the increase of the levels of MDA, MPO, ICAM-1 and IL-8 induced by IIR. In addition, the severe damage of intestinal and elevation of plasma diamine oxidase activity in the IIR rats were significantly alleviated by CVF pretreatment.

CONCLUSIONS: CVF pretreatment could significantly reduce the acute lung injury induced by IIR. The mechanism might include, at least in part, the inhibition of oxidant generation, infiltration of neutrophils, ICAM-1 expression and IL-8 release. CVF might be an efficient reagent for preventing the IIR injuries in clinical condition.

Key Words:
Cobra venom factor, Acute lung injury, Intestinal ischemia-reperfusion, Complement depletion.

Introduction

Intestinal ischemia-reperfusion (IIR) is one of the most serious and common clinical events which can occur in different pathophysiologic conditions, including acute blood loss, shock, disseminated intravascular coagulopathy (DIC), ileus and multiple trauma. Generally, IIR might result in sepsis, systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS)[1]. IIR induces not only the intestinal damage, but also the remote organ injury. Acute respiratory distress syndrome (ARDS) induced by lung injury is one of the most serious complications[2,3]. The lung injury caused by IIR is a complex process and the exact pathological mechanism remains unclear. It was reported that the complement activation was one of the important initiating causes that mediate intestinal mucosal barrier injury[4]. Furthermore, there is evidence that the complement activation is also responsible for the pathogenesis of acute lung injury induced by IIR[5].

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The complement system plays a major role in immunological protection by providing for opsonization and lysis of microorganisms, removal of foreign particles and dead cells, recruitment and activation of inflammatory cells, regulation of antibody production, and elimination of immune complexes. However, excessive or uncontrollable complement activation could lead to severe tissue damage. Thus, inhibition of excessive complement activation was considered to alleviate the lung injury caused by intestinal ischemia and reperfusion[6-8].

Cobra venom factor (CVF) is the stable anti-complement protein from cobra venom, and it is a structural and functional analog of complement component C3. CVF functionally resembles C3b, the activated form of C3[9]. Like C3b, CVF binds to factor B, which is subsequently cleaved by factor D to form the bimolecular complex CVF/Bb. CVF/Bb is a C3/C5 convertase that cleaves both complement components C3 and C5. Moreover, CVF/Bb cannot be easily inactivated by endogenous H and I factors. Thus, CVF/Bb can continuously activate C3 for a long time to deplete complement protein, achieving the desired anti-complement effect[10,11]. CVF treatment could cause a transient activation of complement system while pretreatment 24 h before the operation could lead to the complement depletion. The research of CVF application is mainly focus in the field of transplantation[12-17]. Complement activation plays a critical role in the pathological process of transplantation[18]. Lung injury induced by IIR is a common complication in the intestinal transplantation[19]. If we take action before the operation of transplantation, it would be beneficial to the patients. Therefore, the effect of CVF pretreatment on IIR induced lung injury was observed in this study and the possible mechanism was investigated.

Materials and Methods

Animals

Adult Sprague-Dawley rats (male, 200-220 g) were provided by the Experimental Animal Center of the Second Military Medical University (Shanghai, China). Rats were placed in the SPF (specific-pathogen-free) environment, with free access to food and water under a natural day/night cycle, and allowed to acclimate for a week before being used in studies. All procedures were performed in accordance with Guidelines of the Committee on Animals of the Second Military Medical University.

Establishment of Rat Model of Lung Injury Induced by IIR

The rat model of lung injury induced by IIR was established as described in our previous research[20]. Before introduction of anesthesia for the experiment, each rat was subjected to 12 h of fasting with no restriction of water access. The rats were anesthetized intraperitoneal (i.p.) with 10% (weight/volume) chloralhydrate (0.4 mL/100 g body weight) and placed in a supine position. All animals spontaneously breathed room air throughout the experiment. The tail vein was cannulated to administer lactated Ringer's solution as the maintenance fluid. The right carotid artery was also cannulated to monitor arterial blood pressure and obtain blood sample. Rectal temperature, mean arterial blood pressure (MAP), heart rate (HR) and respiratory frequency were continuously monitored. The superior mesenteric artery (SAM) was occluded by atraumatic microvascular clamp for 60 min followed by 4 h reperfusion. The ischemia was confirmed when the mesenteric pulsations ceased and the intestines became pale. Reperfusion was verified by the return of pulsation of SAM after clamp removal. Most rats required an additional bolus of 10% chloralhydrate (0.2 mL/100 g body weight, i.p.) during the period of reperfusion to ensure stable anesthesia.

Experimental Design

Rats were randomly divided into three groups (n = 10 each): (A) Sham-operated group, (B) Intestinal ischemia-reperfusion (IIR group), (C) IIR + CVF group. For CVF pretreatment, rats in group C were given CVF (50 μg/kg) via the tail vein 24 h before the operation[21,22]. As the controls, rats were given normal saline in groups A and B. CVF solution was freshly made by dissolving the CVF frozen powder in normal saline. CVF, purified from the chinese Cobra venom, was obtained commercially from the Ruiqing Biological Company (No. 070625). At the end point of reperfusion, all rats were sacrificed, and the blood draw and tissue were taken and analyzed.

Analysis of the Plasma Level of CH50

Plasma CH50 level was assessed by hemolytic assay as described previously[23]. Briefly, rabbit red blood cells were sensitized with goat anti-

serum to rabbit red blood cells and exposed at 30°C for 60 min to serial dilutions of serum samples in gelatin veronal buffer containing Ca^{2+}/Mg^{2+} (Sigma, St. Louis, MO, USA). Following a centrifugation step (2500 g, 5 min), absorbance of the supernatant fluid was measured at 405 nm, and concentration inducing 50% of hemolysis (CH-50) was determined.

Histological Analysis

Histological analysis was performed by hematoxylin-eosin (HE) staining. Briefly, a segment (~3 cm) of small intestine was removed from 10 cm proximal to the terminal ileum, and the specimens of the right lower lung were harvested. The excised tissues were washed by saline and fixed with 10% (volume/volume) formalin for 24 h. Then the fixed tissues were embedded in paraffin and cut at 4 μm sections. Finally, these sections were stained with HE and observed under light microscope. Two independent and blinded researchers performed the histological scoring. Briefly, Morphological lung injury was scored under the criteria as described previously: 0, normal tissue; 1, minimal inflammatory change; 2, no damage to the lung architecture; 3, thickening of the alveolar septae; 4, formation of nodules or areas of pneumonitis that distorted the normal architecture; and 5, total obliteration of the field[24]. Morphological damages of small intestine were assessed by Chiu et al histological injury scoring system of intestinal villi (0, normal mucosa; 1, slight-; 2, moderate-; 3, massive subepithelial detachments; 4, denudes villi; 5, ulceration)[25].

Estimation of Pulmonary Edema and Pulmonary Microvascular Permeability

For the pulmonary microvascular permeability assay, the left jugular vein was isolated and cannulated. Evans blue dye solution (30 mg/kg) was injected into the left jugular vein 30 min before the animal was killed. At the end of the reperfusion period, the animals were killed by cutting the ascending aorta. The hilus of the left lung was clamped and resected for assessment of pulmonary edema. After lungs were removed from the thoracic cavity, the left lung was weighed, and then placed in a drying oven at 80°C for 48 h. After this drying procedure, the specimen was reweighed and the lung wet-to-dry weight (W/D) ratio was calculated by dividing the wet weight by the dry weight. The right lung was used to estimate pulmonary microvascular permeability by using the Evans blue dye method described previously[26]. At the end of reperfusion, the right lung was perfused via the pulmonary artery for 2 min with saline (37°C) at 0.04 mL/g body weight per min using an infusion pump to eliminate residual blood and Evans blue dye from the pulmonary bed. Then the lung was weighed, placed in an oven, and heated at 90°C for 16 h. The dried tissues were incubated in 2 mL of formamide at 37°C for 24 h. The concentration of Evans blue dye extracted from the lungs was measured by spectrophotometry at 620 nm and expressed as micrograms of dye per milligram wet lung weight.

Analysis of the Activity of Diamine Oxidase (DAO)

The activity of DAO in plasma was analyzed using the commercial DAO Fluorometric Detection Kit according to the manufacturer's instruction (Genmed Scientifics, Shanghai, China).

Analysis of Malonaldehyde (MDA) and Myeloperoxidase (MPO) Activity

Pulmonary MDA content and MPO activity were determined with chemical method described as the manufacturer's instructions (Nanjing Jiancheng Biochemistry Co., Nanjing, China). Lung tissue (100 mg, wet wt) was homogenized in 2 mL of 10 mM phosphate buffer (pH 7.4). After centrifugation at 12,000 g for 20 min, the MDA content and MPO activity in the supernatant were measured using the corresponding kits. MDA content was analyzed by the thiobarbituric acid (TBA) spectrometric assay. Protein content in the sample was determined by Commassie blue assay. Concentrations were expressed as nmol MDA per mg of protein. MPO activity was determined by using a spectrophotometric reaction. The change in absorbance was measured at 490 nm. MPO activity was expressed as U/g wet tissue.

Cytokine Measurement

The lung tissues were homogenized in cold PBS (phosphate buffered saline) and then centrifuged at 3000 g for 15 min at 4°C. interleukin (IL)-8 concentration was measured in the supernatant using the ELISA kit from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The protein concentrations of the samples were determined by Coomassie Blue assay kit and the IL-8 concentration was normalized with the protein concentration.

Immunohistochemical Analysis of ICAM-1

ICAM-1 (Intercellular Adhesion Molecule-1) in the lung tissue was stained immunohistochemically. Briefly, the formalin-fixed lung tissue were embedded in paraffin and cut into four μm think sections. The paraffin sections were deparaffinized and blocked with 1% BSA (bovine serum albumin) in PBS containing 0.5% Triton X-100 for 30 min at room temperature. Then, the sections were incubated overnight at 4°C with polyclonal rabbit anti-rat ICAM-1 antibody (R&D Systems, Minneapolis, MN, USA). After washing, sections were incubated with biotinylated sheep anti-rabbit antibody (R&D) for 30 min and then streptavidin-peroxidase for 15 min before addition of 3,3'-diaminobenzidine (DAB). Finally, tissue sections were counterstained in hematoxylin.

RNA Isolation and Real-time PCR

Total RNA was extracted from the lung tissues with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and quality of RNA was assessed spectrophotometrically at wavelengths of 260 and 280 nm. The first-strand complementary DNA (cDNA) was generated using the Reverse Transcription System kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Real time PCR was performed in a SYBR Green PCR Master mix (Applied Biosystems Inc., San Mateo, CA, USA) using 7500 Sequence Detection System (MJ Research, Saint bruno, Quebec, Canada) with the following protocol: 95°C (5 min), 40 cycles for 95°C (15 s), 60°C (32 s). The following primers were used: ICAM-1 with *TGTCGGTGCTCAGGTATC* (forward primer) and *AGTGGTCTGCTGTCTTCC* (reverse primer); GAPDH with *AGAACATCATCCCTGCATCC* (forward primer) and *TGGATACATTGGGGGTAGGA* (reverse primer). The relative expression of genes was calculated and expressed as $2^{-\Delta\Delta CT}$. The GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was used as an endogenous control.

Statistical Analysis

Results were expressed as means ± SD and analyzed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

Analysis of Variance (ANOVA) was used for the multiple comparisons among all groups followed by post-hoc tests using Fisher's LSD (least significant difference) method. For comparing the difference of CH50, Student *t*-test was used. Historical injury scores were expressed as the median-interquartile range and comparisons among groups were performed using nonparametric Kruskal-Wallis analysis. When overall differences were identified, Mann-Whitney U test and Bonferroni correction were utilized to analyze comparisons between two groups. *p* value of less then 0.05 was considered statistically significant.

Results

CVF Pretreatment Resulted in Complement Depletion

CVF is a structural and functional analog of complement C3 isolated from Cobra venom. To verify CVF pretreatment induced complement depletion, plasma complement activities were determined in Saline or CVF pretreated rats both before IIR and 4 h after IIR by the CH50 assay. As shown in Table I, CH50 was significantly reduced 4 h after IIR, as compared with that in pre-IIR (71.59 ± 9.41 vs. 103.26 ± 14.15, $p < 0.01$). However, when rats had been injected with purified CVF i.v. 24 h before, the plasma complement activity decreased significantly as compared with that of rats with saline pretreatment (10.03 ± 2.54 vs. 103.26 ± 14.15, $p < 0.01$). In the meantime, there is no significant difference of CH50 levels between pre-IIR and 4 h after IIR in the CVF pretreated group (Table I).

CVF Pretreatment Prevented the Damage of Intestinal and Lung

In the rats of IIR groups, multiple erosions and bleeding were observed in the small intestine. Notably, pretreatment with CVF at a dose of 50 μg/kg protected the small intestine from erosions and bleeding. As shown in Figure 1, although the ileum of sham-operated rats exhibited normal mucosal architecture with intact villi, IIR result-

Table I. The plasma levels of CH50 in saline or CVF pretreated IIR rats.

Groups	Saline (U/ml)	CVF (U/ml)
Pre-IIR	103.26 ± 14.15	10.03 ± 2.54**
IIR-4h	71.59 ± 9.41##	9.84 ± 2.15

Data are expressed as mean ± SD (n = 10). **$p < 0.01$ vs. saline group; ##$p < 0.01$ vs. Pre-IIR.

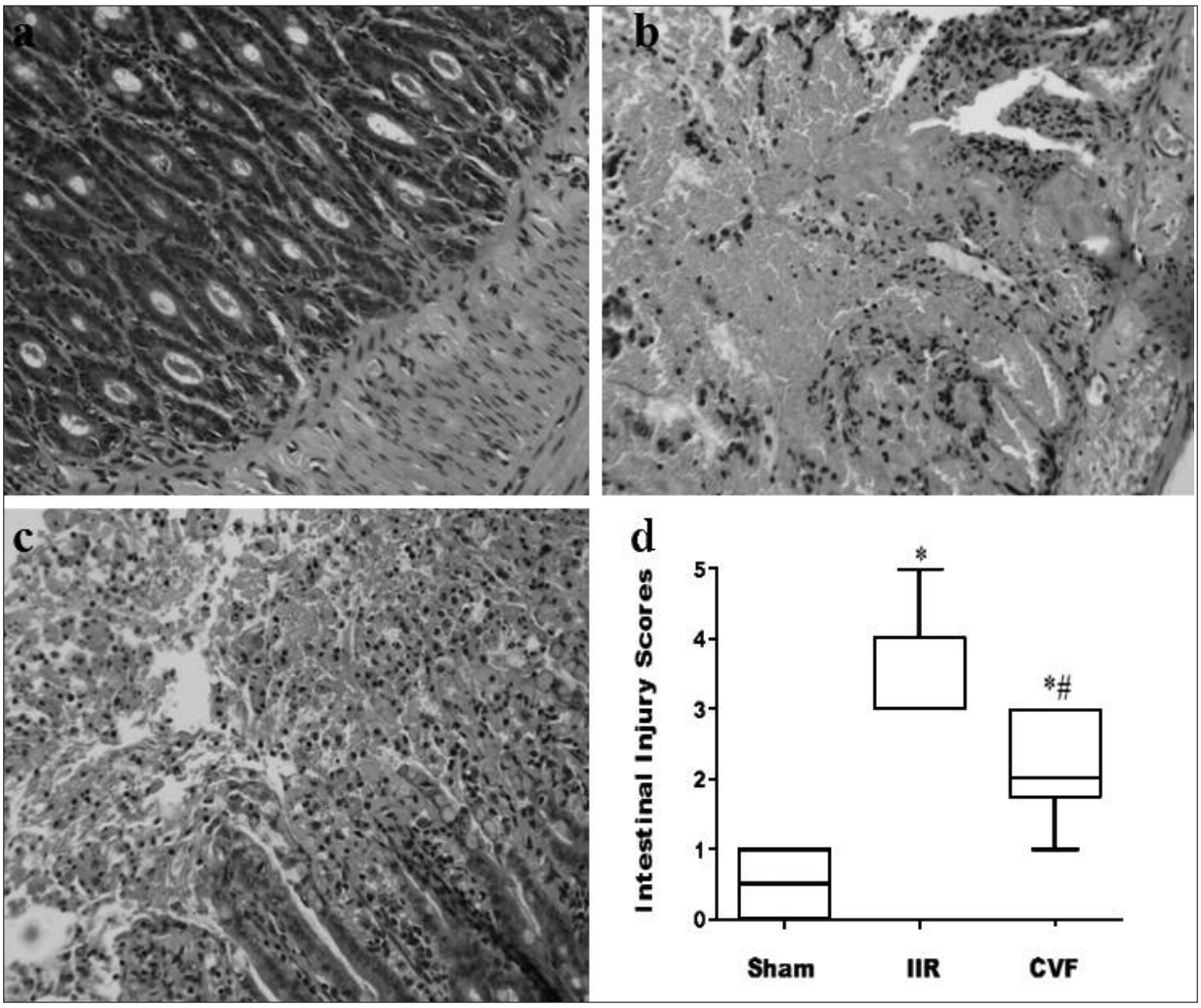

Figure 1. Histological observation of intestinal injury in the sham-operated IIR and IIR+ CVF groups. Intestinal tissue sections were stained with hematoxylin-eosin. Photomicrographs were taken at 200 × magnification. One representative image of the intestinal microscopic photograph in the sham-operated ***(a)*** IIR ***(b)*** and IIR+ CVF groups ***(c)*** in three independent experiments is shown. The histological injury score performed by two independent and blinded researchers is shown ***(d)***. In the box plots, median, the interquartile range, and minimum-maximum values were shown (n = 10). $^{*}p < 0.01$ vs. sham-operated group; $^{\#}p < 0.01$ vs. IIR group.

ed in a large loss of epithelial crypt area, infiltration of a large number of neutrophils throughout the mucosa, erosion, and mucosal bleeding. In contrast, the pretreatment with CVF resulted in much smaller erosions and infiltration of fewer neutrophils. The scoring result is shown in Figure 1d. In summary, the macroscopic and microscopic lesions in the rat intestine were all significantly alleviated by the pretreatment with CVF.

As DAO (diamine oxidase) is an enzyme with high activity in the mature upper villus cells of rat intestinal mucosa, the plasma DAO levels were detected to reflect the mucosal injury as a marker of the integrity of the intestinal mucosa. Figure 2 shows that the levels of plasma DAO activity in IIR rats increased significantly compared with that in sham-operated rats (13.95 ± 1.62 vs. 8.72 ± 1.68, $p < 0.01$). However, CVF pretreatment significantly reduced the level of plasma DAO activity in rats with IIR (11.01 ± 1.43 vs. 13.95 ± 1.62, $p < 0.01$).

For the lung injury induced by IIR, representative HE-stained sections of the lung tissues from sham-operated, IIR, and IIR + CVF rats are presented in Figure 3 a-c. The lung tissues of rats in the IIR group showed severe edema, alveolar hemorrhage, inflammatory cell infiltration and destructed pulmonary architecture (Figure 3b),

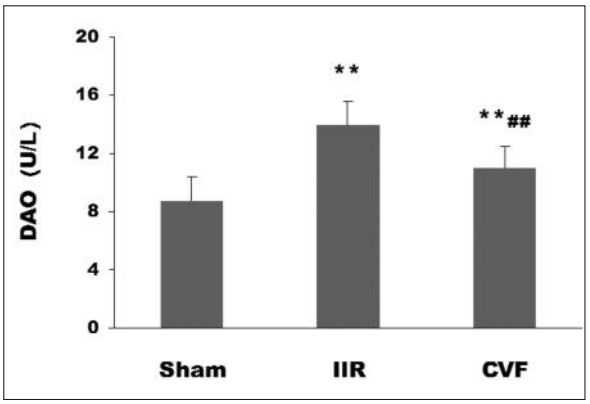

Figure 2. DAO activity levels in the sham-operated, IIR and IIR+ CVF groups. Data are expressed as mean ± SD (n = 10). $^{**}p < 0.01$ vs. sham-operated group; $^{\#\#}p < 0.01$ vs. IIR group.

while the pulmonary architecture was rarely destructed with mild edema and alveolar hemorrhage in the IIR+ CVF group (Figure 3c). The scoring result is shown in Figure 3d.

In addition, lung W/D ratio determination and Evans Blue dye assay was performed to assess the pulmonary edema and pulmonary microvascular permeability respectively. Figure 4 shows that in IIR rats lung W/D ratio increased significantly compared with that in sham-operated rats (5.72 ± 0.72 vs. 4.11 ± 0.34, $p < 0.01$). However, CVF pretreatment significantly reduced lung W/D ratio in rats with IIR (4.93 ± 0.41 vs. 5.72±0.72, $p < 0.01$). Similarly, the Evans Blue dye assay showed that IIR induced a significant pulmonary microvascular permeability increase

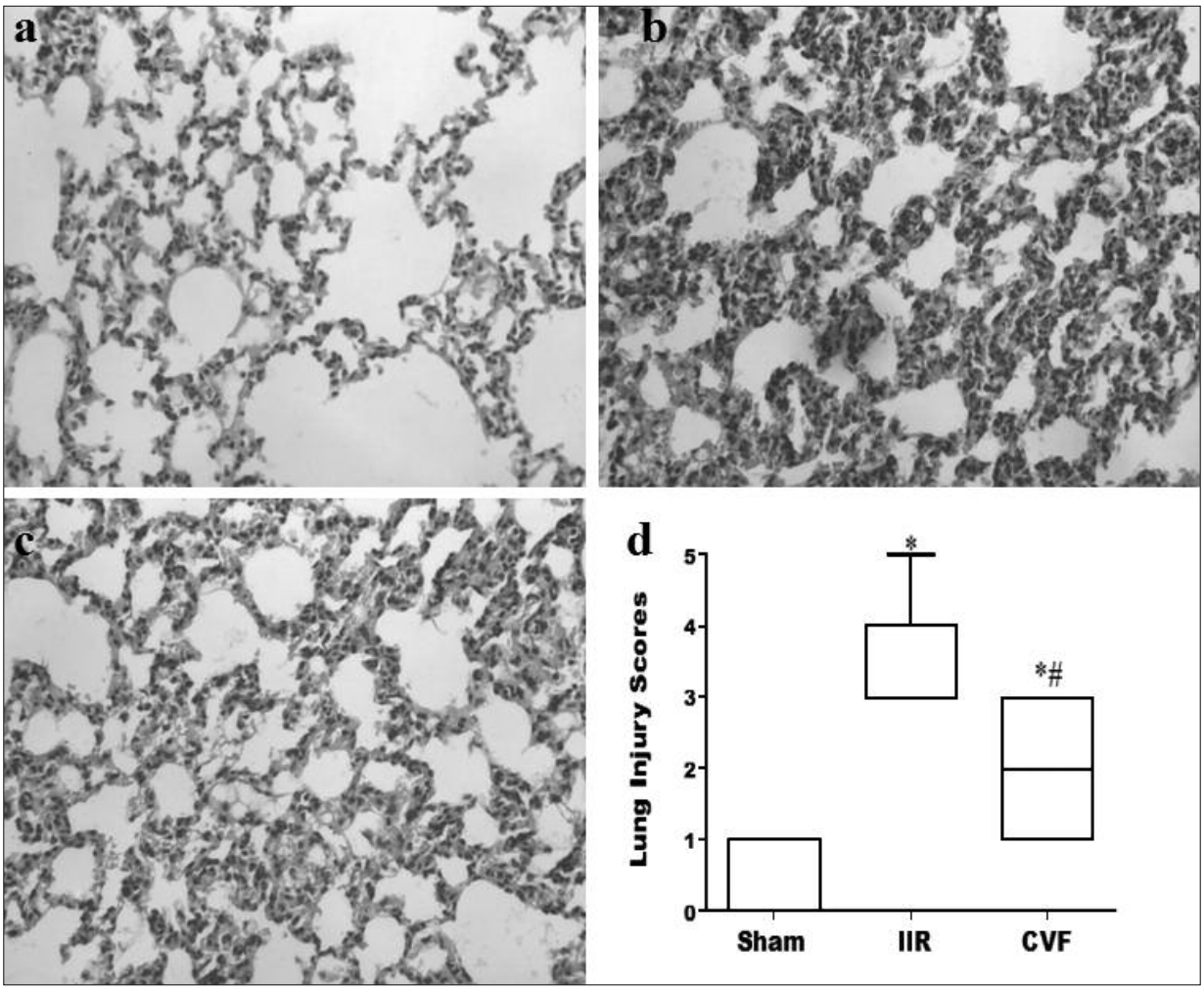

Figure 3. Histological observation of lung injury in the sham-operated, IIR and IIR+ CVF groups. Lung tissue sections were stained with hematoxylin-eosin. Photomicrographs were taken at 400 × magnification. One representative image of the lung microscopic photograph in the sham-operated ***(a)*** IIR ***(b)*** and IIR+ CVF groups ***(c)*** in three independent experiments is shown. The histological injury score performed by two independent and blinded researchers is shown ***(d)***. In the box plots, median, the interquartile range, and minimum-maximum values were shown (n = 10). $^{*}p < 0.01$ vs. sham-operated group; $^{\#}p < 0.01$ vs. IIR group.

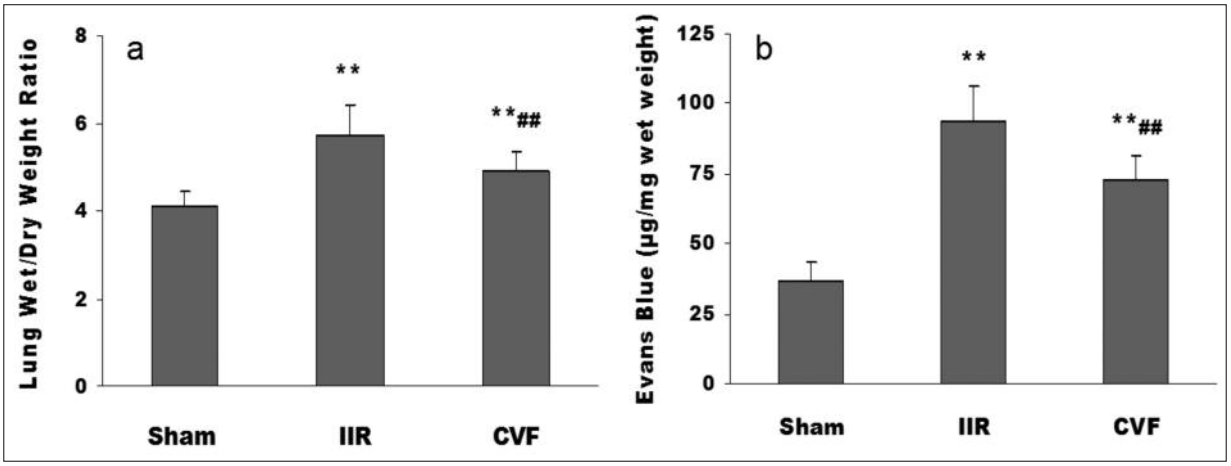

Figure 4. Lung wet/dry weight ratio and pulmonary Evans Blue permeability assay in the sham-operated, IIR and IIR+ CVF groups. Data are expressed as mean ± SD (n = 10). $^{**}p < 0.01$ vs. sham-operated group; $^{\#\#}p < 0.01$ vs. IIR group.

as compared with that of sham animals (93.59 ± 12.52 vs. 37.01 ± 6.44, $p < 0.01$) and the CVF pretreatment markedly prevented the pulmonary microvascular permeability elevation (72.85 ± 8.39 vs. 93.59 ± 12.52, $p < 0.01$) (Figure 4b).

CVF Pretreatment Alleviated the Elevation of MDA and MPO in the Lung Tissues

Previous studies have demonstrated that lipid peroxidation plays a critical role in ischemia and reperfusion injury[27]. So we detect the level of MDA (malondialdehyde) in lung tissues, which is an important product of membrane lipid peroxidation. In this study, IIR resulted in a markedly elevation of MDA in lung tissues (2.53 ± 0.33 vs. 1.15 ± 0.16, $p < 0.01$) and CVF pretreatment decreased the MDA level significantly (1.75 ± 0.32 vs. 2.53 ± 0.33, $p < 0.01$) (Figure 5a).

MPO (myeloperoxidase) is a lysosomal protein stored in azurophilic granules of the neutrophils and it is expressed most abundantly in the neutrophils, so we determine MPO activity in lung tissue to assess the neutrophil infiltration. As showed in Figure 5b, the MPO activity in the lung tissue was significantly elevated in IIR injury (2.05 ± 0.36 vs. 0.40 ± 0.06, $p < 0.01$). However, CVF pretreatment markedly reduced the elevation of MPO activity induced by IIR (1.50 ± 0.22 vs. 2.05 ± 0.36, $p < 0.01$).

CVF Pretreatment Reduced the Expression of IL-8 and ICAM-1 in Lung Tissues

Figure 6 showed that the IL-8 concentration in IIR group was significantly increased by 3.25-fold compared with that sham-operated controls

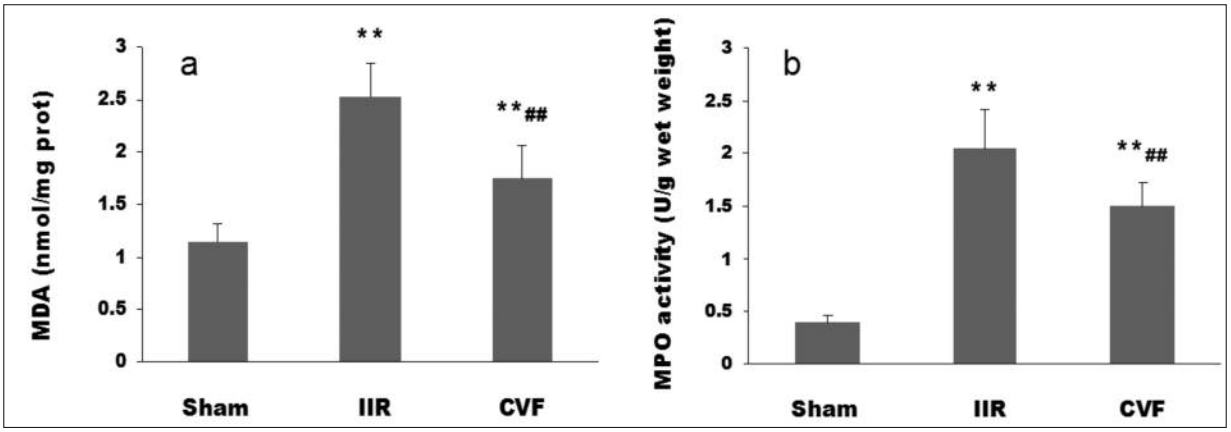

Figure 5. Pulmonary MDA level and MPO activity in the sham-operated, IIR and IIR+ CVF groups. Data are expressed as mean ± SD (n = 10). $^{**}p < 0.01$ vs. sham-operated group; $^{\#\#}p < 0.01$ vs. IIR group.

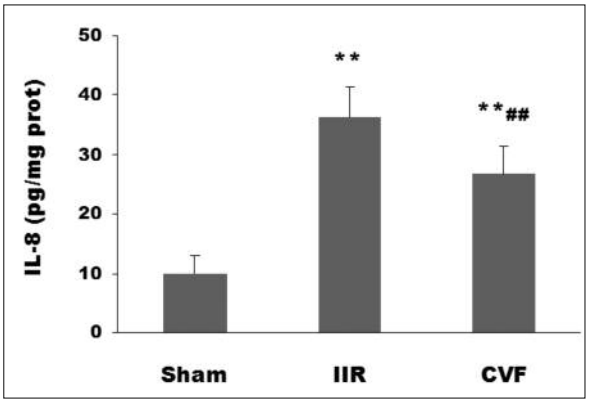

Figure 6. Pulmonary IL-8 level in the sham-operated, IIR and IIR+ CVF groups. Data are expressed as mean ± SD (n = 10). $^{**}p < 0.01$ vs. sham-operated group; $^{\#\#}p < 0.01$ vs. IIR group.

(36.48 ± 4.87 vs. 9.80 ± 3.07, $p < 0.01$). After the pretreatment with CVF, the levels of IL-8 in lung tissues were significantly reduced (26.74 ± 4.51 vs. 36.48 ± 4.87, $p < 0.01$).

Similar results were obtained in ICAM-1 expression. As shown in Figure 7a, ICAM-1 mRNA expression was significantly increased in the IIR group compared with that in the sham-operated group (3.89 ± 0.95 vs. 1 ± 0.28, $p < 0.01$). Pretreatment with CVF remarkably attenuated the elevation of ICAM-1 in the IIR group (2.05 ± 0.43 vs. 3.89 ± 0.95, $p < 0.01$). Consistent to the reduced ICAM-1 mRNA expression, ICAM-1 protein was demonstrated to be reduced by CVF pretreatment in the immunohistochemical analysis (Figure 7b-d).

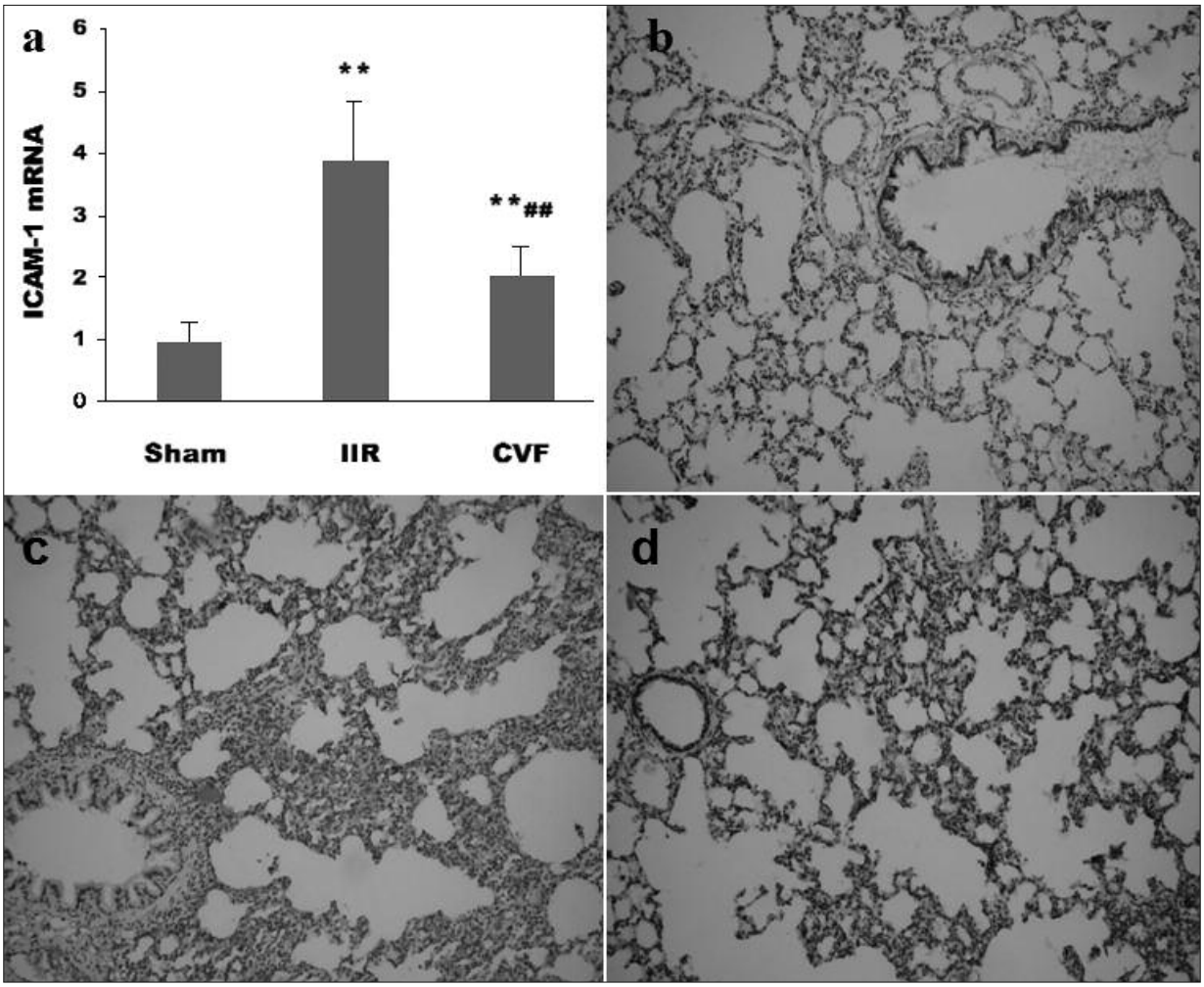

Figure 7. Pulmonary ICAM level in the sham-operated IIR and IIR+ CVF groups. ***(a)*** Real-time PCR analysis of ICAM-1 mRNA level in the sham-operated IIR and IIR+ CVF groups. Data are expressed as mean ± SD (n = 10). $^{**}p < 0.01$ vs. sham-operated group; $^{\#\#}p < 0.01$ vs. IIR group. ***(b-d)*** Immunohistochemical analysis of ICAM-1 expression in sham-operated ***(b)***, IIR ***(c)*** and IIR+ CVF (d) groups. Photomicrographs were taken at 400 × magnification. One representative image of three independent experiments is shown.

Discussion

IIR is a common physiological and pathological process in clinic and it plays an important role in the pathological development of strangulated intestinal obstruction, bowel transplantation, burn, severe infection, cardiopulmonary insufficiency and hemorrhagic shock[28,29]. The consequences of IIR included the following three aspects: (1) Intestinal mucosal barrier injury. This injury can induce acute or chronic functional change of mucosal absorption, leading to undernutrition and high mortality. (2) Bacterial translocation. The enteric bacteria pass through the damaged mucosal barrier to the lymph nodes, liver, spleen and even whole body, resulting in SIRS (Systemic Inflammatory Response Syndrome). (3) Remote organ damage (especially ARDS-Acute Respiratory Distress Syndrome) caused by the release of a large number of inflammatory mediators[30].

Lung injury is one of the most usual remote organ damage induced by IIR. Recently, more and more evidences indicate that the excessive complement activation is closely related to the occurrence and development of the lung injury caused by IIR. C3a and C5a, which are produced in complement activation, can also promote phagocytic cells to release granzyme, prostaglandins, and TNF-α, IL-1 , IL-6 and other proinflammatory cytokines, stimulate the release of lysosomal enzymes and promote mast cells to release vasoactive substances such as histamine, resulting in small vasodilatation and increased vascular permeability, which are common symptoms of pulmonary edema[31].

C3 is an important inherent component of the complement system, and it is located in the converging point of the three activation pathways of complement, and plays a pivotal role in the activation process of complement system[32]. CVF is a structural and functional homolog to the activated form of C3, which causes exhaustive activation of the alternative pathway resulting in depletion of complement components in mammalian serum.

In this study, we found that after IIR, the plasma CH50 level significantly reduced, indicating that the complement activation occurs at an early stage. In contrast, when animals were pretreated with CVF, the CH50 level was significantly lowered either before or after IIR, which showed complement depletion. Consequently, CVF pretreatment significantly protected the damage of both intestinal mucosal and lung tissue of rats subjected to IIR injury. This also revealed that complement activation was involved in the pathogenesis of intestinal damage after IIR. These results are consistent with the results of previous researches. Ikai M et al[33] found that complement play a critical role in mediating shock induced by intestinal ischemia, and CVF treatment could significantly improve the symptoms of shock. Fruchterman et al[34] also demonstrated that the complement activation mediated the intestinal epithelial cell dysfunction and intestinal injury in hemorrhagic shock, and the use of complement inhibitors could significantly reduce the intestinal damage.

In addition, we found that CVF pretreatment significantly prevented the MPO and MDA elevation. MPO is the marker enzyme of PMN (polymorphonuclear cells), and the elevated activity of MPO in tissues indicates the increase of PMN infiltration. MDA is one of the major products of membrane lipid peroxidation, and its content can reflect the level of lipid peroxidation. Large amounts of oxygen free radicals activated by PMN and the lipid peroxidation caused by ROS (Reactive Oxygen Species) are the pathological basis of lung injury after IR[35]. IIR resulted in a significant increase of MPO and MDA in the lung tissues. When CVF was administrated preoperatively, the MPO and MDA elevation was lowered. The result indicates PMN activation might be properly reduced by CVF pretreatment.

Furthermore, two type of molecules play critical roles in the process that white blood cells move out of blood vessels and reach the local tissue in inflammatory response: one is the adhesion molecules expressing in the endothelial cell surface, the other is chemokines in the local tissues[36]. So we further observed the effect of CVF pretreatment on the ICAM-1 and IL-8 expression. ICAM-1 (Intercellular Adhesion Molecule-1, CD54) is an endothelial- and leukocyte-associated transmembrane protein long known for its importance in stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration. The combination of the adhesive complex and ICAM-1 can induce the firm adhesion of PMN and endothelial cells and result in PMN migration[37,38]. ICAM-1 is lowly expressed under physiological conditions, and its expression is significantly increased in the inflammatory tissues[39]. In our results, both RT-PCR and immunohistochemical analysis demonstrates that ICAM-1 increase significantly in IIR animals, suggesting that the upregulation of ICAM-1 is associated with lung injury.

IL-8 is a chemokine whose primary function is the induction of chemotaxis in its target cells (e.g., neutrophil granulocytes). It serves as a chemical signal that attracts neutrophils at the site of inflammation, and, therefore, is also known as neutrophil chemotactic factor[40].

Our results showed that, CVF pretreatment reduced both ICAM-1 and IL-8 in lung tissues of IIR rats, which may be responsible for the reduced neutrophil infiltration and oxidative damage.

Here, we found that complement activation may play an important role in the pathological process of lung injury induced by IIR. When intestinal transplantation operation performed, the remote lung injury often occurs as the ischemia-reperfusion happens. If we take some preventive action before the operation of transplantation, it would be beneficial to the patients. This study showed that complement depletion by CVF pretreatment might be an option to the transplanted patients. However, the use of CVF might cause complications as well. Firstly, the function of immune system might be decreased and the infection might happen when complement depletion by CVF. Secondly, CVF, purified from cobra venom, would be an exogenous antigen for patients. Thirdly, CVF treatment affects widely and cannot target to the specific injured organ. So much more work needs to be done if we want to use CVF as a preventive method in clinical condition.

Conclusions

CVF pretreatment could alleviate the acute lung injury induced by IIR. The mechanism might include, at least in part, the inhibition of oxidant generation, filtration of neutrophils, ICAM-1 expression and IL-8 release. Collectively, CVF might have a great potential for preventing the severe clinical outcomes of IIR injured patients.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 30901411), Young Researchers Fund of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (No. 098YJ01) and Research and Innovation Fund of Shanghai Municipal Education Commission (No. 09YZ89).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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