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Protective effect of ulinastatin combined with dexmedetomidine on lung injury after cold ischemia-reperfusion in rats

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Abstract. – OBJECTIVE: We investigated the protective effect of ulinastatin combined with dexmedetomidine on lung injury after hepatic ischemia-reperfusion in rats.

MATERIALS AND METHODS: A total of 60 healthy and clean male Sprague Dawley (SD) rats were divided into the blank control group (group O), the model control group (group K), the ulinastatin and dexmedetomidine group (group F) according to random number table with 20 rats in each group.

RESULTS: The plasma concentrations of tumor necrosis factor-alpha *(TNF-α)*, interleukin-6 *(IL-6)*, interleukin-8 *(IL-8)* and malondialdehyde *(MDA)* at T_1, T_2 and T_3 time points in rats of the three groups were significantly higher than those of the T0 time point ($p<0.05$). The superoxide dismutase (SOD) activity in the plasma of rats of the three groups was significantly lower at T_1, T_2 and T_3 time point when compared with that of T0 ($p<0.05$). The concentrations of *TNF-α*, IL-6, IL-8 and MDA in group K at T_1, T_2 and T_3 moments were significantly higher than those of group O ($p<0.05$). However, the concentrations of IL-6, IL-8, *TNF-α* and MDA in group F at T_1, T_2, T_3 were significantly lower than those of group K ($p<0.05$). The activities of SOD in group K at T_1, T_2, T_3 were all significantly higher than those of group O ($p<0.05$). Meanwhile, the activities of SOD in group F at T_1, T_2, T_3 were significantly higher than those of group K ($p<0.05$).

CONCLUSIONS: Ulinastatin combined with dexmedetomidine can reduce the inflammatory response and inhibit lipid peroxidation, eventually alleviating acute lung injury after hepatic ischemia-reperfusion in rats.

Key Words

Ulinastatin, Dexmedetomidine, Lung injury, Cold ischemia-reperfusion, Rats.

Introduction

Liver transplantation has become an effective treatment for end-stage liver diseases caused by various etiologies. However, postoperative complications can affect the survival of grafts and even recipients. Acute lung injury (ALI) after liver transplantation is a serious complication affecting the short-term prognosis of patients with liver diseases. Meanwhile, ALI may cause acute respiratory distress syndrome (ARDS), even leading to death[1,2]. Cell metabolic disorders and structural damages are aggravated in the viscera when blood flow blocking is removed and reperfusion recovers. This damage further aggravates ischemic injury after blood reperfusion and is also known as ischemia-reperfusion injury. Therefore, the prevention and treatment of lung injury after liver transplantation is essential to improve the success rate of liver transplantation.

Ischemia-reperfusion injury makes the lung to become the most vulnerable distal organ. This may cause acute lung injury and related complications[3,4], even affecting the efficacy of surgical treatment and the long-term life quality of patients. Researches[5,6] have shown that oxidative stress and inflammation may be related to ischemia-reperfusion injury, although the underlying mechanism in lungs remains controversial. The occurrence of ALI after liver transplantation ranges from 34.2% to 77.8%. Moreover, ARDS is one of the main causes of deaths in liver transplant patients, whose incidence ranges from 7.8% to 23.6%. In addition, lung injury is one kind of diseases caused by various factors, such as surgical injury, sepsis, massive transfusion and reactive oxygen species[7,8]. However, the specific mechanism of lung injury remains unclear.

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Ulinastatin is a broad-spectrum proteolytic enzyme inhibitor, which has been demonstrated to reduce inflammatory responses and protect organ function[9,10]. Various clinical and animal works[11,12] have proven that ulinastatin can effectively reduce multiple links of hepatic ischemia-reperfusion injury, including postoperative complications, systemic inflammation, the activation of protease-activated receptors-related inflammatory responses and tissue two-hit. Dexmedetomidine (DEX) is a new type of highly selective alpha 2-receptor agonist, which has a dose-dependent sedative, analgesic, anxiolytic and inhibitory effect on sympathetic function. Meanwhile, DEX can inhibit the release of pro-inflammatory cytokines and suppress oxidative stress, thereby reducing the systemic inflammatory responses caused by surgical trauma and playing a protective role in lungs. Clinical studies have also shown that DEX has a superior anti-inflammatory action that can enhance the function and anti-apoptosis of macrophages. It also exhibits no influence in the tendency of neutrophil phagocytosis and the production of superoxide anion. Meanwhile, it can maintain the activity of natural killer cells and surgery after general anesthesia, reduce inflammation caused by endotoxin limit, as well as the levels of tumor necrosis factor alpha (*TNF-α*) and interleukin-6 (*IL-6*)[13,14]. Thus, the aim of the present study was to evaluate the pretreatment effect of ulinastatin combined with dexmedetomidine on hepatic ischemia-reperfusion-induced lung injury in rats, and to explore the underlying mechanism.

Materials and Methods

Experimental Animals

A total of 60 healthy male Sprague Dawley (SD) rats weighing 200 ± 25 g were provided by the Experimental Animal Center of Military Medical Science Academy of the PLA. During the experiment, the disposition of animals was strictly in accordance with the guidance of the treatment of experimental animals issued by the Ministry of Science and Technology in 2014. This study was approved by the Animal Ethics Committee of Tianjin First Center Hospital Animal Center.

Study Design

After one week of adaptive feeding, all the rats were divided into the blank control group (group O), the model control group (group K), and the ulinastatin and dexmedetomidine group (group F) by the random number table. All rats were fasted for 10-12 h with free access to water. Rats in group O were not treated, while rats in group K were intraperitoneally injected with 10% chloral hydrate solution (3 ml/kg) for anesthesia. After abdominal hair removal and iodophor disinfection, sterile operation was performed and the abdominal cavity was opened along the abdominal midline. The liver cold ischemia-reperfusion injury model was constructed according to a previous report[15].

After rat liver exposure, the falciform ligament suprahepatic inferior vena cava was isolated above the level of free right renal vein inferior vena cava. Portal and setline was isolated, and the hepatic artery was isolated above the hepatoduodenal ligament. From the portal vein into the liver, the insulin needle was injected into 1 ml heparin, and then insulin was extracted from normal saline. The needle was compressed with a sterile cotton ball. After sufficient hemostasis, the vessels were clamped in the portal vein, the superior and inferior vena cava, and the hepatic artery in sequence. Meanwhile, the inferior vena cava was clamped from the upper vena cava of the right renal vein to make the whole liver free of blood flow. The needles were inserted through the portal vein, and 4°C lactated Ringer's solution was injected to maintain the drop speed of 6-8 mL/min perfusion of liver. The opening of the inferior vena cava perfusion fluid outflow was incised, and inferior vena cava blood flow and perfusion were timely suctioned. The perfusion process was performed on ice to maintain the low temperature of the liver, and cold perfusion time was 20 min. During the infusion, the liver became pale and cold. To open the portal vein, hepatic artery, hepatic vein and inferior vena cava and to restore the hepatic blood flow, perfusion was stopped with 8-0, no damage vascular sutures for the inferior vena cava outflow. The liver was from pale white to bright red, and warm saline was used for peritoneal lavage finally. The operation was performed in warming blanket, and rats' rectal temperature was maintained no less than 37°C.

Rats in group F were anesthetized, and rats in group K were operated. After subcutaneously incision, 30*104 IU ulinastatin was intravenously infused, and was repeated every 4 h. At the same time, dexmedetomidine intravenously infused at a temporal speed of 4 ug/(kg h). Finally, the abdomen was closed by the same method after reperfusion.

Table I. The concentrations of TNF-α, IL-6, IL-8, MDA and SOD in the three groups of rats at different time points (n=20, $\bar{x}\pm s$).

Groups	Moments	TNF-α (ug/L)	IL-6 (ng/L)	IL-8 (ng/L)	MDA (umol/L)	SOD (u/ml)
Group O	T_0	33.56±2.96	69.13±3.69	15.87±1.26	3.72±0.31	92.31±2.43
	T1	34.47±1.32	65.24±1.52	17.63±1.24	4.05±0.29	90.43±1.94
	T2	32.34±1.61	71.53±1.78	15.47±1.12	3.36±0.75	92.35±1.36
	T3	33.17±1.57	68.83±1.82	16.07±1.59	3.94±0.56	91.26±2.05
Group K	T0	32.86±1.81	71.42±3.72	15.13±2.01	3.81±0.59	91.47±2.18
	T1	134.47±7.32#	89.74±4.52#	59.86±3.24#	4.35±0.69#	75.43±2.01#
	T2	182.54±7.61#	101.83±4.72#	83.47±3.12#	9.36±0.75#	68.59±1.36#
	T3	155.27±6.57#	117.53±4.82#	92.07±2.59#	8.94±0.56#	57.42±2.05#
Group F	T0	33.16±3.12	68.89±2.50	16.72±2.31	3.62±0.42	93.45±2.13
	T1	116.07±5.63*	78.13±2.61*	50.26±2.31*	4.09±0.74*	82.65±1.86*
	T2	147.05±5.09*	90.64±3.87*	74.51±2.04*	8.07±0.55*	68.53±1.34*
	T3	141.26±6.21*	97.53±3.10*	78.64±2.83*	7.32±0.61*	62.93±1.66*

Note: In group O rats each time between $p>0.05$; group K rats, compared with group T0, #$p<0.05$; group F rats, compared with group T0 ; *$p<0.05$; compared with group O; #$p<0.05$; compared with group K, *$p<0.05$.

Sample Collection and Determination

A total of 3 mL venous plasma samples were collected in all rats. Radioimmunoassay was used to detect the concentration of interleukin 6 (*IL-6*), interleukin 8 (*IL-8*), tumor necrosis factor alpha (*TNF-α*) and malondialdehyde thiobarbituric acid (MDA) at 4 time points, including before surgery (T_0), 30 min after anhepatic phase (T_1), 2 h after liver transplantation (T_2) and end of operation (T_3). Meanwhile, the activity of superoxide dismutase (SOD) was determined by the xanthine oxidase method, and the standard kit was provided by Nanjing Jiancheng Biological Company. At the time point of T2, 0.5 cm*0.5 cm lung tissue was taken from the left middle lung of all rats. The tissue samples were fixed with 10% Formaldehyde Solution, embedded in paraffin, and cut into 3-4 mm slices. Hematoxylin-eosin (HE) staining was performed for section staining, and pathological results of the lung tissues were observed under a 200 optical microscope. Paraffin sections from 3 specimens were selected for measuring the protein expression of TNF-α and IL-6 (kit purchased from Beijing Zhongshan Bioengineering Company).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM, Armonk, NY, USA) were used for all statistical analysis. Data were presented as mean ± standard deviation ($\bar{x}\pm s$). *t*-test was used to compare the difference between two groups. Classified variables were analyzed by using x^2-test. One-way ANOVA was applied to compare the differences among multiple groups, followed by the Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Determination of TNF-α, IL-6, IL-8, MDA and SOD

The levels of *TNF-α*, *IL-6*, *IL-8*, *MDA* and SOD were not statistically different at different time points, moreover, no significant difference was observed among the three groups at T_0 ($p>0.05$). The concentrations of *TNF-α*, *IL-6* and *IL-8* at T_3, and the level of MDA at T_1, T_2 in group K were significantly higher than those of group O ($p<0.05$). The concentrations of *TNF-α*, *IL-6*, *IL-8* at T_3 and the level of MDA at T_1, T_2 in group F were significantly lower than those of group K and group O ($p<0.05$). The activities of SOD at T_1, T_2 and T_3 in group K were significantly higher than those of group O ($p<0.05$). Meanwhile, the activities of SOD at T_1, T_2 and T_3 in group F were significantly higher than those of group K ($p<0.05$) (Table I).

Morphological Changes and Immunohistochemistry Results

HE staining results showed that there was no edema and widening of alveolar septa as well as no bleeding or exudation in the alveolar space in the rats of group O. However, the lung tissues in group K was in disorder. Meanwhile, widened

lung interstitium, hemorrhage, congestion, the occurrence of alveolar edema and massive neutrophil infiltration were observed. These pathological changes reached peak at T_2 and were then gradually improved. The lung injury in group F was significantly lower than that of group K (Figure 1). The expression of *TNF-α* and *IL-6* in lung tissues was detected by SABC, and the positive expression was brownish yellow. Both *TNF-α* and *IL-6* were expressed in alveoli cells, and the expression levels of these two molecules in group K and F were higher than those of group O. Moreover, the levels of *TNF-α* and *IL-6* in group K were higher than group F (Figure 2 and 3).

Discussion

Lung injury caused by ischemia-reperfusion is a continuous process that affects the success rate of liver transplantation and the post-operative survival rate of patients. It is suggested that the production of massive oxygen free radicals is the main outcome of lung injury induced by hepatic ischemia-reperfusion[16,17]. Moreover, the imbalance of oxidation and the antioxidant system leads to the increase of lipid peroxidation and massive reactive oxygen species production. Moreover, it also plays a cytotoxic role in destroying the structure of lung tissues, eventually resulting in pulmonary edema and lung dysfunction[18]. As one of the metabolites of lipid peroxidation, MDA plays an important role in understanding the extent injury of tissue cells. Similarly, SOD reflects the ability of organisms to indirectly scavenge oxygen free radicals. Therefore, transcription of the inflammatory factors activation is an important factor leading to ischemic reperfusion injury. The release of pro-inflammatory cytokines, such as the activation and migration of *TNF-α*, *IL-6*,

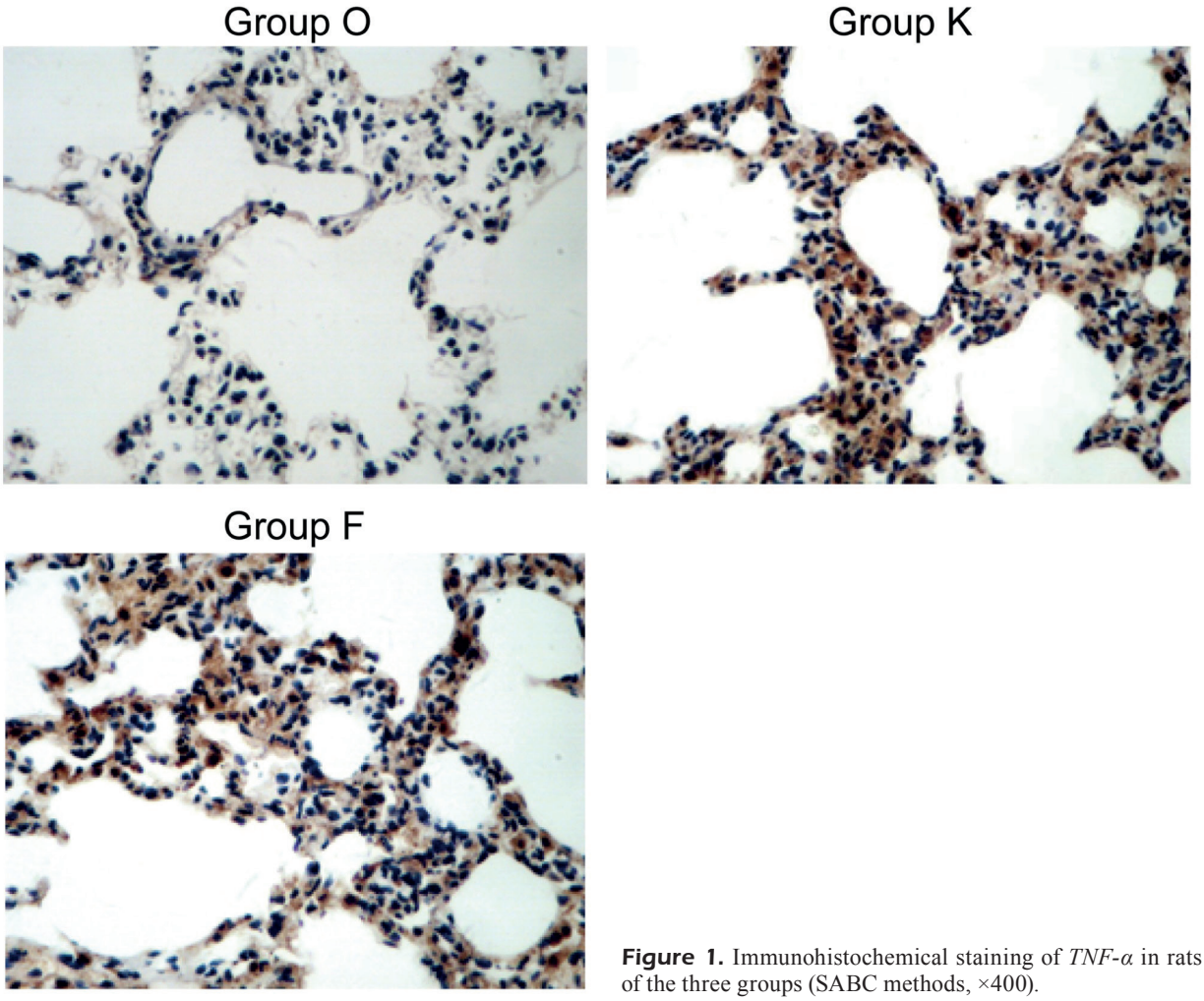

Figure 1. Immunohistochemical staining of *TNF-α* in rats of the three groups (SABC methods, ×400).

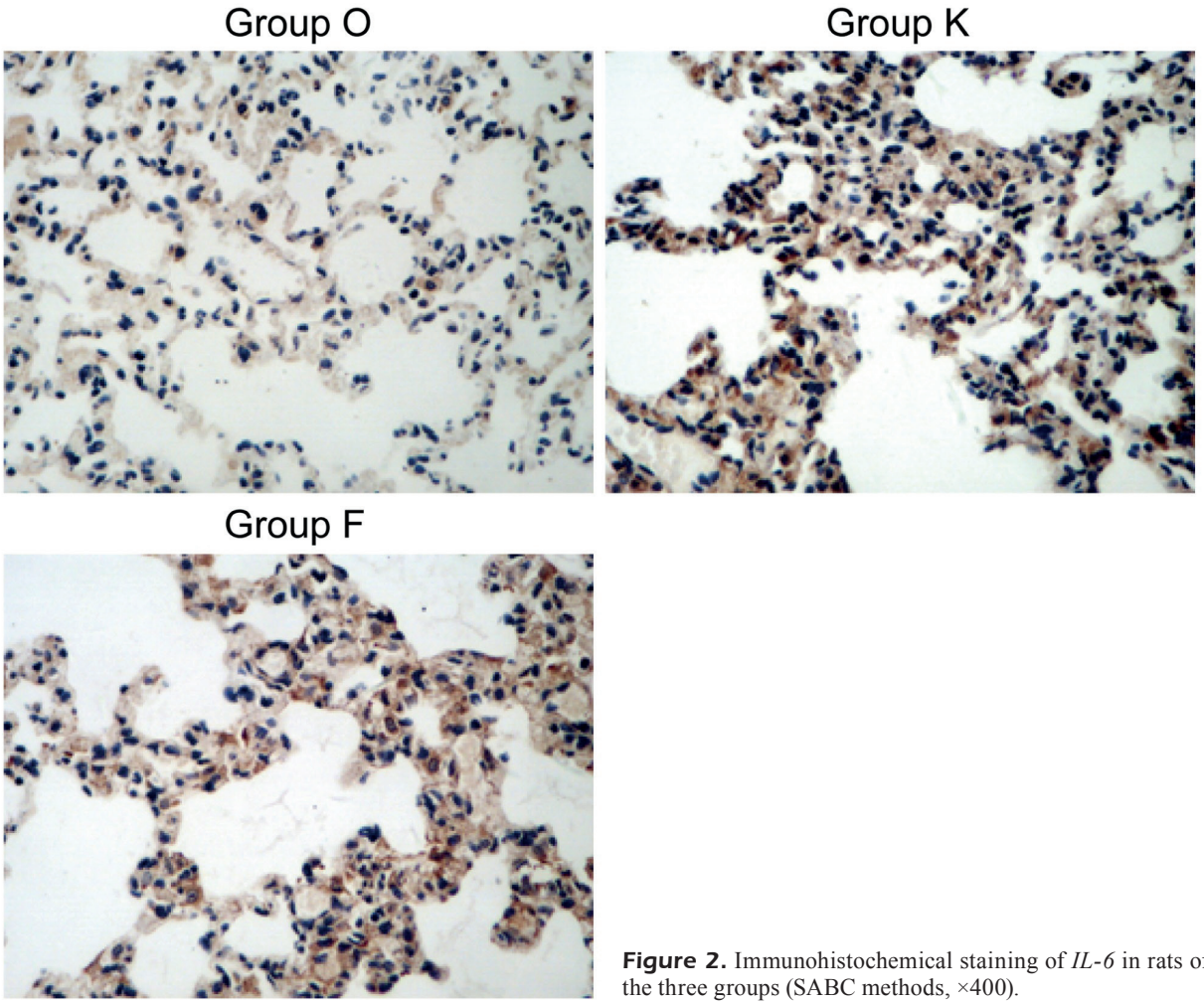

Figure 2. Immunohistochemical staining of *IL-6* in rats of the three groups (SABC methods, ×400).

IL-8, can induce neutrophils and cause systemic inflammatory damage of various organs[19-21].

In the present study, the hepatic ischemia-reperfusion model was replicated in SD rats, and rats' liver was subjected to warm ischemia, transient cold perfusion and reperfusion. The inferior vena cava and portal vein blood was blocked, the intestinal system of liver was congested and swollen, and the liver ischemia-reperfusion process was simulated. This method was similar with the clinical pathology during the perioperative period of hepatic ischemia-reperfusion, and was suitable for observing early remote organ damage induced by hepatic ischemia-reperfusion[22-24].

Our results showed that the concentrations of *TNF-α*, *IL-6*, *IL-8* and *MDA* in the serum of the ulinastatin and dexmedetomidine group at T_1, T_2, T_3 were significantly lower than those of the model group. Meanwhile, the activity of SOD was significantly higher than that of the model group. MDA in the ulinastatin combined with dexmedetomidine group decreased significantly when compared with the model control group. Conversely, SOD was significantly increased compared with the model control group, suggesting that ulinastatin combined with dexmedetomidine could reduce ischemia-reperfusion and lipid lung tissues caused by oxygen free radical peroxidation reperfusion injury. Meanwhile, consistent results were obtained from the morphological changes and immunohistochemistry. Therefore, these data implied that ulinastatin combined with dexmedetomidine had a good therapeutic effect on lung injury induced by hepatic ischemia-reperfusion. Various pathological changes were observed after orthotropic liver transplantation, and the lesions changed the most significantly at two hours after new liver stage.

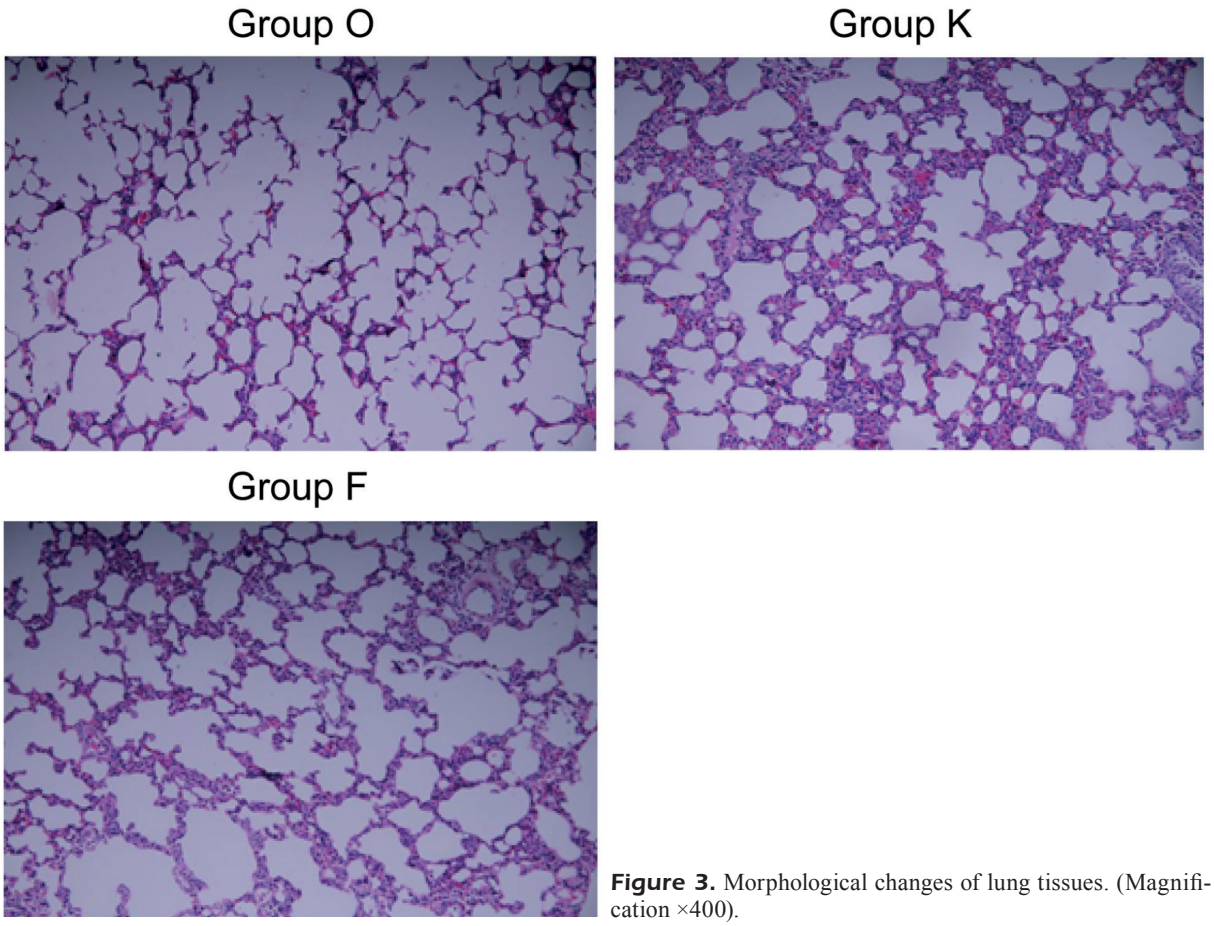

Figure 3. Morphological changes of lung tissues. (Magnification ×400).

Conclusions

We showed that ulinastatin combined with dexmedetomidine can reduce inflammatory responses and inhibit lipid peroxidation, as well as alleviate acute lung injury after hepatic ischemia-reperfusion injury in rats.

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Conflict of Interests:

The authors declared no conflict of interest.

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