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Effect of early fluid resuscitation on the lung in a rat model of lipopolysaccharide-induced septic shock

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Abstract. – BACKGROUND: Many clinical trials have showed that early fluid resuscitation can improve the prognosis and reduce the mortality rate of patients with septic shock. However, some experiments suggest that abundant fluid may injure the lung and other tissues.

AIM: To evaluate the protective effect of early fluid resuscitation and simultaneous norepinephrine treatment on lung function by using the rat model of lipopolysaccharide (LPS)-induced septic shock.

MATERIALS AND METHODS: Male Wistar rats were randomly divided into four groups: normal control group, septic shock control group, early fluid resuscitation treatment group, early fluid resuscitation and simultaneous norepinephrine treatment group. Blood gas, lactate, fluid volume, and dose of norepinephrine were recorded. Pathological change was observed by hematoxylin and eosin staining and transmission electron microscopy. The activities of hydroxyl radicals, MDA, SOD and MPO were detected by spectrophotometry. The expression of IL-6, IL-8, and TNF-alpha were determined with ELISA kits.

RESULTS: LPS could induce rats to suffer from acute lung injury in early stage of septic shock. Early fluid resuscitation could guarantee effective circulating blood volume and tissue perfusion pressure, improve microcirculatory derangements, increase oxygen partial pressure and oxygenation index, but have the tendency to aggravate pulmonary edema. Simultaneous norepinephrine treatment in early stage could decrease the fluid volume, alleviate the degree of pulmonary edema, reduce the expression level of pro-inflammatory mediators in the serum and BALF, and increase the oxygenation index.

CONCLUSIONS: Early fluid resuscitation and simultaneous norepinephrine treatment may be a superior alternative to protect lung injury secondary to septic shock.

Key Words:
Septic shock, Early fluid resuscitation, Acute lung injury, Lipopolysaccharide, Lung.

Introduction

Septic shock has been paid close attention in the medical community because of its association with substantial mortality and multi-organ dysfunctions or failures. Though there're some developments on researching its pathogenesis and therapeutic approaches, the mortality rate hasn't been controlled effectively[1,2]. Early fluid resuscitation is an essential strategy to reverse oligoemia caused by septic shock[3,4] and treat the multiple organ dysfunction syndrome (MODS)[5]. Many trials have showed that early fluid resuscitation can significantly improve the prognosis of patients suffering from septic shock, save time for the followed treatments and adjustments of strategies, and reduce the costs of care and the mortality[6-9].

Acute lung injury (ALI), as the most common complicating disease with septic shock, has also attracted more attention from scholars since it may directly affect prognosis of patients with septic shock[10]. Management of negative fluid balance for ALI patients has been widely accepted in clinical trials, which could reduce the mortality rate and shorten the duration of mechanical ventilation significantly[11-15].

Though several studies have reported that early fluid resuscitation can improve the patient outcomes of septic shock complicated by ALI[16], some other studies show that excessive fluid infusion may lead to fluid trapped within the interstitial space and aggravate lung injuries[15,17]. How to manage fluid resuscitation for patients with septic shock and ALI remains a challenge for many clinical doctors.

The traditional fluid resuscitation strategy to treat septic shock is based on the principle of enlarging blood volume. If fluid resuscitation couldn't sustain effective perfusion pressure, vasoactive agents (such as norepinephrine) would

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be used. However, recent research has demonstrated that early simultaneous norepinephrine treatment could significantly reduce the fluid volume for reaching target blood pressure, and meanwhile, have some positive effects on peripheral circulation when ensuring center perfusion pressure[18]. However, there are no reports about its effects on lung functions. The objective of this study is to investigate the effects of two different early fluid resuscitation strategies on lung functions, using rat model with lipopolysaccharide (LPS)-induced septic shock complicated by ALI.

Materials and Methods

Healthy male Wistar rats (180 ~ 200 g) were provided by Department of Experimental Animals of Chinese Medical University. The HX300 animal ventilator, the HP portable ECG monitor, and the AVLOMNI blood gas analyzer were provided by AVL Company in Switzerland. Endotoxin (LPS, Sigma L-2880 From E. coli serotype 055: B5), hydroxyl radical, malondialdehyde (MDA), superoxide dismutase (SOD) and myeloperoxidase enzyme (MPO) activity kit were purchased from Jiancheng Bioengineering Institute (Nanjing, China). IL-6, IL-8, and TNF-alpha ELISA kit were purchased from Boster Biological Engineering Co. Ltd (Wuhan, China).

Experimental Groups and Animal Model

Rats were randomly divided into four groups: normal control group (group A, n = 15), septic shock control group (group B, n = 15), early fluid resuscitation treatment group (group C, n = 15), early fluid resuscitation and simultaneous norepinephrine treatment group (group D, n = 15). The rats were anaesthetized by intraperitoneal administration of chloral hydrate (10%, 300 mg/kg). They were tracheotomized and mechanically ventilated with HX300 animal ventilator, with a respiratory rate of 100 breath/min, and a tidal volume of 10 ml/kg. For monitoring heart rate and mean arterial pressure (MAP), the pressure sensors and monitors were connected after punctuation of left common carotid artery. The femoral vein was cannulated to administer the medicine and fluid infusion. The maintenance of body temperature was completed by electric heaters. After the rats had been in stable state for half an hour, 0.2 ml of arterial blood was collected to analyze their blood gas, which was regarded as base values, and then 0.2ml saline was injected to supplement blood volume. Models of septic shock were established by intravenous injection of 15 mg/kg LPS. For control group A, the same amount saline was used instead of LPS. The traditional fluid resuscitation strategy in group C was that saline (10 ml/kg/15 min) was injected firstly; 30 min later, norepinephrine (0.5-6 μg/kg per min) was then administered according to the level of MAP. The program in group D was saline injection (10 ml/kg per 15 min) and simultaneous norepinephrine administration (0.5-6 μg/kg per min). Vital signs, fluid volume and dose of norepinephrine in each group were also recorded.

Specimen Collection

After establishment of the rat models for 2 hours, the rats were sacrificed, and 1 ml blood was collected for analysis of blood gas. After centrifugation of other 4 ml of blood at 3000 g for 10 min, the supernatant was collected and stored at –20°C for detection of the expression of inflammatory mediators. For electron microscopy, the lobe of right lung was cut and divided into several sections, with the size of 0.1 cm × 0.1 cm × 0.1 cm, then fixed in 2.5% glutaraldehyde. The wet weight of the lung (W) and the dry weight (D) were recorded and their ratio was calculated. One of the sections was fixed in 4% poly-formaldehyde at 4°C for hematoxylin-eosin (HE) staining; and another one was reserved at –70°C for analysis of oxidation index. To detect the expression of inflammatory mediators, we needed to ligate the right hilum of lung, lavage the left lung three times with 2 ml saline, and then collect the supernatant liquid after centrifugation at 1500 g for 4 min of initial bronchoalveolar lavage fluid (BALF, 3.0-3.6 ml).

Determination Method

For observation of the pathological changes with light microscopy, we immersed the lung tissue in 4% polyoxymethylene overnight, dehydrated and embedded it conventionally, cut it into 5 μm slices, and stained them with HE. Some lung tissues were fixed with 2.5% glutaraldehyde, dehydrated, embedded with epoxy resin, and made into ultrathin sections for analysis of the changes of organelles by transmission electron microscopy (JEM-2000EX, JEOL, Japan). After centrifugation of the lung tissue homogenates at 12000 g for 20 min, we collected the supernatant and determined the activities of hydroxyl radicals, MDA, SOD and MPO by

Table I. Comparison of blood gas and fluid volume (mean ± SD).

	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	Lactate (mmol/L)	Fluid volume (ml)	Usage of noradrenaline (μg/kg/min)
Group A	7.38 ± 0.12	93.9 ± 2.23	38.65 ± 1.78	1.50 ± 0.34	0	0
Group B	6.89 ± 0.13*	50.19 ± 3.78*	34.54 ± 1.89	6.98 ± 2.45*	0	0
Group C	7.21 ± 0.15$^{\Delta}$	62.34 ± 2.46$^{\Delta}$	35.09 ± 2.01	4.37 ± 1.36$^{\Delta}$	12 ± 3$^{\Delta}$	3.5 ± 1.8$^{\Delta}$
Group D	7.35 ± 0.19$^{\#}$	86.75 ± 2.13$^{\#}$	37.46 ± 1.48	3.12 ± 1.32$^{\#}$	6 ± 2$^{\#}$	2.3 ± 1.2$^{\#}$

*The differences were significant while comparing group B with group A. $^{\Delta}$The differences were significant, compared group C with group B. $^{\#}$There were significant differences while comparing group D with group C.

spectrophotometry (Shimadzu UV-2401 PC spectrophotometer, Tokyo, Japan). We unfreezed BALF and serum on ice, and after centrifugation at 1000 g for 15 min, detected the expression levels of IL-6, IL-8, and TNF-α with ELISA kit (Boster Biological Engineering Co. Ltd., Wuhan, China).

Statistical Analysis

All data were shown as mean ± SD and analyzed by SPSS13.0 (SPSS Inc., Chicago, IL, USA). The differences between the two groups were investigated by the *t* test at the 5% significant level.

Results

Fluid Volume and Blood Gas Analysis

There were no significant differences in the basal blood pressure, heart rate and blood gas among the four groups, while MAP declined 15 min later after being injected with LPS. Compared with group A, pH and oxygen partial pressure in group B decreased significantly, while lactate increased notably ($p < 0.05$). In comparison with group B, pH and oxygen partial pressure of group C both rose, accompanied by evident decline of lactate ($p < 0.05$). pH and oxygen partial pressure of group D were much higher than that in group C, and lactate further decreased ($p < 0.05$). Furthermore, fluid volume and dose of norepinephrine in group D were a lot less than that in group C ($p < 0.05$). There was no significant differences of carbon dioxide pressure values among the groups ($p > 0.05$) (Table I).

W/D Ratio in Lung Tissue

Compared with group A, the W/D ratio of group B increased significantly, which meant aggravated pulmonary edema, followed by group C that was much severer. However, there were no significant differences between B and C. Early norepinephrine treatment significantly decreased W/D ratio compared with group C (see Table II).

Activities of Hydroxyl Radicals, MDA, MPO and SOD in Lung Tissue

As shown in Table II, activities of hydroxyl radicals, MDA, and MPO of group B were significantly higher than those of group A, while activity of SOD was clearly lower than that in group A. While comparing group B with C, there were no evident differences. In comparison with group C, only the activity of MPO in group D decreased significantly.

Table II. Comparisons of W/D and indicators of Oxidation – reduction in lung tissue.

	W/D	Inhibition of hydroxyl radical (U/mgprot)	MDA (nmol/mgprot)	MPO (U/dprot)	SOD (U/mgprot)
Group A	3.85 ± 0.34	87.52 ± 3.45	2.98 ± 0.64	1.69 ± 0.14	365.78 ± 10.26
Group B	6.89 ± 0.23*	12.89 ± 1.52*	10.17 ± 1.12*	6.79 ± 1.02*	250.32 ± 8.56
Group C	6.96 ± 0.25	13.14 ± 2.24	9.56 ± 1.37	6.02 ± 1.16	262.98 ± 5.47
Group D	5.12 ± 0.33$^{\#}$	13.78 ± 2.18	9.49 ± 1.27	3.08 ± 0.33$^{\#}$	268.75 ± 6.56

*The differences were significant while comparing group B with group A. $^{\#}$There were significant differences compared group D with group C.

HE Staining in Lung Tissue

Observing lung tissue sections stained by HE under light microscope indicated that the alveolar structure of group A was integrated, and there were no significant congestion, neutrophil invasion and interstitial edema (Figure 1A); in group B, alveolar structure was disordered, the integrity of the alveolar wall was destructed, accompanied by thickened alveolar wall and edema; massive neutrophil accumulated in alveolar space; alveolar capillary congestion and exudation happened; part of the alveolus pulmonis collapsed; and there was some hemorrhage in alveolar space (Figure 1B); compared with group B, accumulation of neutrophil of group C reduced significantly, alveolar capillary leakage was not severer yet whereas there was no significant improvement on alveolar edema (Figure 1C); as for group D, neutrophil infiltration in alveolar space reduced clearly compared with group B, congestion and edema of alveolar wall were alleviated significantly compared with group C (Figure 1D).

The Ultrastructure of Lung Tissue (Type II Alveolar Epithelial Cells) by Electron Microscopy

Nucleus shape of group A was normal roughly, and there were many mitochondrion appeared in the cytoplasm and lamellar structure in osmiophilic body (Figure 2A); in group B, the nucleus shape was irregular, a lot of heterochromatin condensed and marginated, mitochondrial cristae decreased, outer membrane destructed, lamellar structure in osmiophilic body reduced, and nuclear membrane became dropsical (Figure 2B); cell condition of group C was a little similar to group B but had some improvements (Figure 2C); in group D, there was visible microvilli in the surface of alveolar epithelial cells, the cell nucleus also had irregular shape, and there were also nuclear heterochromatin condensed. However, the phenotypes were not severer than those in group C, and the number of mitochondria increased significantly (Figure 2D).

Expression of Inflammatory Mediators in BALF

Compared with group A, expression levels of IL-6, IL-8 and TNF- in BALF increased significantly in group B; level of pro-inflammatory mediators in group C was significantly lower than that in group B, but significantly higher than group D (Table III and Figure 3A, B, C).

Expression of Inflammatory Mediators in Serum

Compared with group A, expression levels of IL-6, IL-8 and TNF-α in serum increased signifi-

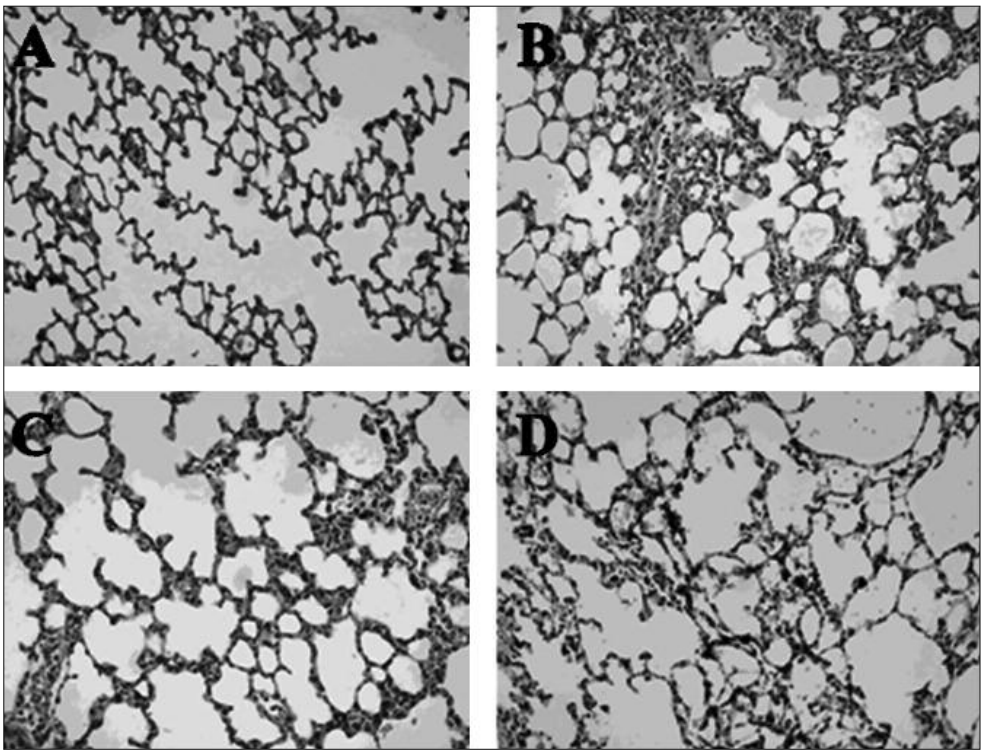

Figure 1. HE staining of the four groups (× 200). ***A,*** Normal control group. ***B,*** Septic shock control group. ***C,*** Early fluid resuscitation treatment group. ***D,*** Early fluid resuscitation and simultaneous norepinephrine treatment group.

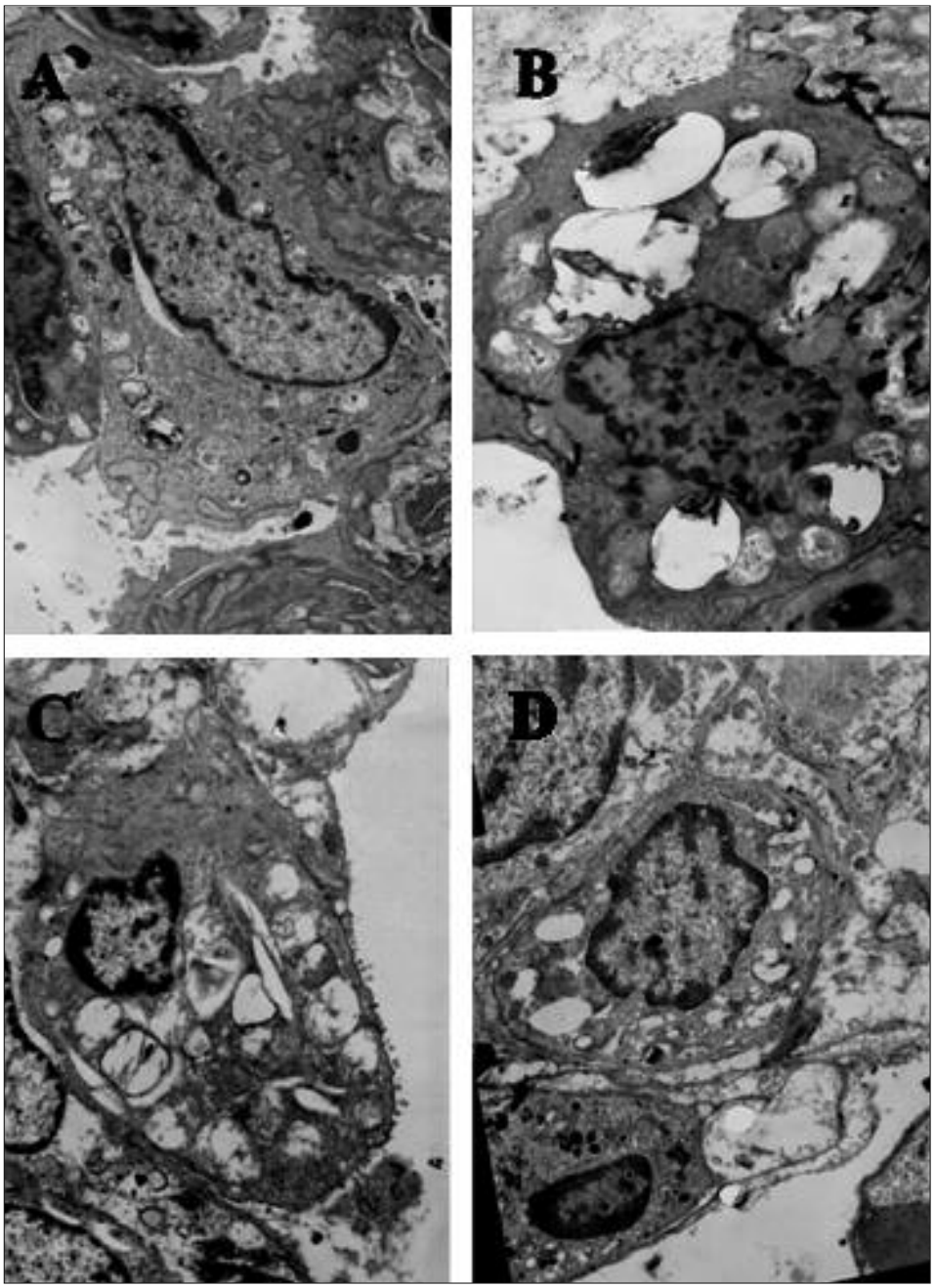

Figure 2. Type II alveolar epithelial cells of the four groups (×6000). ***A,*** Normal control group. ***B,*** Septic shock control group. ***C,*** Early fluid resuscitation group. ***D,*** Early fluid resuscitation and simultaneous norepinephrine treatment group.

Table III. Inflammatory mediators expression in BALF of all the groups (means ± SD).

	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)
Group A	35.88 ± 3.12	24.29 ± 4.23	19.65 ± 1.78
Group B	446.89 ± 37.24*	411.19 ± 32.78*	158.54 ± 16.89*
Group C	387.79 ± 31.58$^{\Delta}$	347.34 ± 29.37$^{\Delta}$	104.09 ± 9.18$^{\Delta}$
Group D	158.32 ± 14.25$^{\#}$	132.75 ± 11.43$^{\#}$	64.46 ± 4.48$^{\#}$

*The differences were significant while comparing group B with group A. $^{\Delta}$The differences were significant, compared group C with group B. $^{\#}$There were significant differences while comparing group D with group C.

Table IV. Expression of inflammatory mediators in serum (means ± SD).

	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)
Group A	50.38 ± 3.24	35.93 ± 4.37	25.32 ± 2.18
Group B	478.33 ± 30.78*	458.19 ± 31.32*	167.41 ± 14.47*
Group C	394.21 ± 28.15$^{\Delta}$	395.34 ± 28.46$^{\Delta}$	121.05 ± 8.01$^{\Delta}$
Group D	167.35 ± 14.22$^{\#}$	143.42 ± 12.13$^{\#}$	47.46 ± 3.35$^{\#}$

*The differences were significant while comparing group B with group A. $^{\Delta}$The differences were significant, compared group C with group B. $^{\#}$There were significant differences while comparing group D with group C.

cantly in group B; level of pro-inflammatory mediators in group C was significantly lower than that in group B, but significantly higher than group D (Table IV and Figure 3D, E, F).

Expression Ratio of Inflammatory Mediators in BALF and Serum

In all the groups, the expression levels of IL-6, IL-8 and TNF-α in serum were higher than those

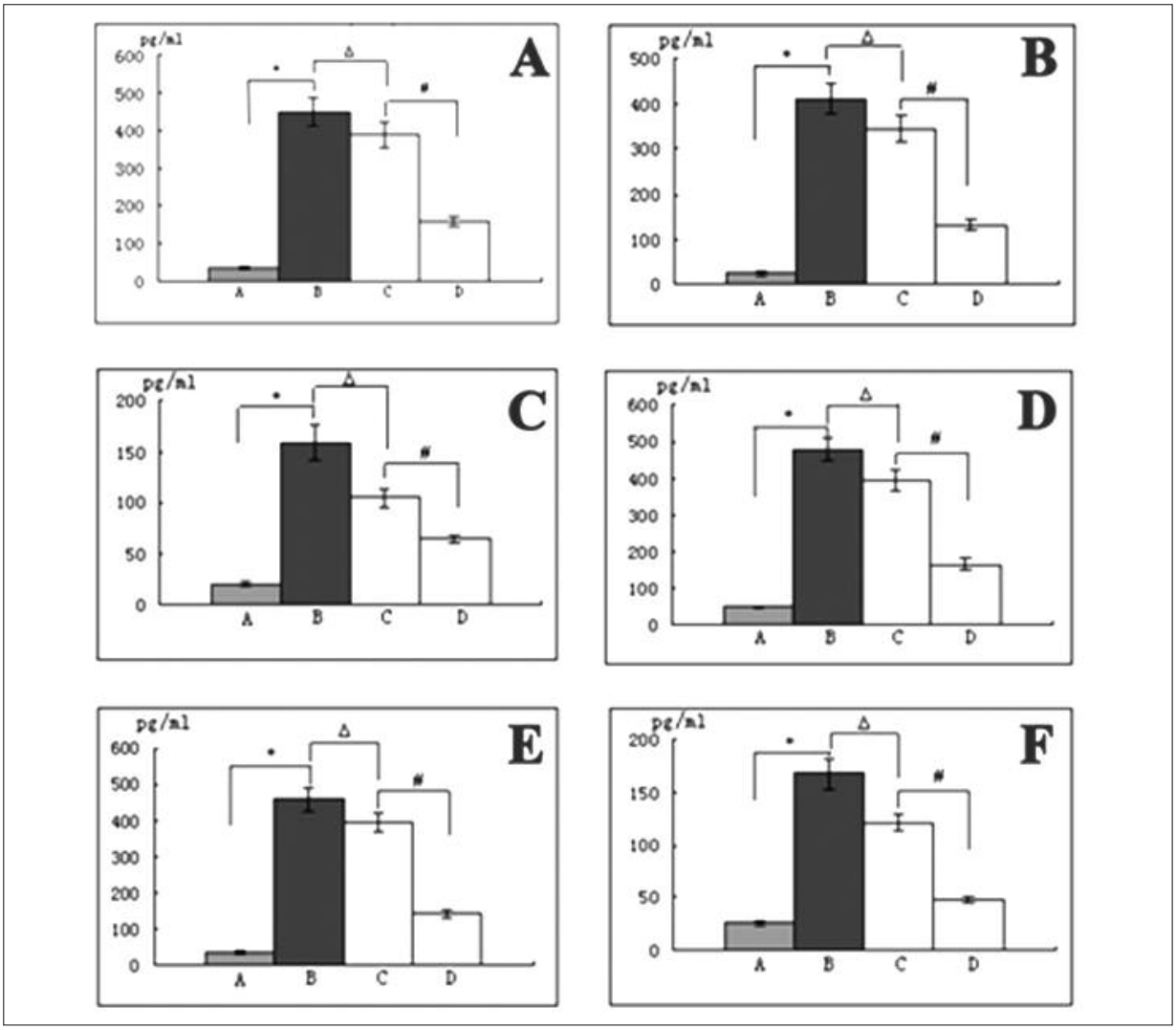

Figure 3. Changes in expression levels of IL-6, IL-8, or TNF-α in BALF or serum. ***A,*** IL-6 in BALF. ***B,*** IL-8 in BALF. ***C,*** TNF-α in BALF. ***D,*** IL-6 in serum. ***E,*** IL-8 in serum. ***F,*** TNF-α in serum.

in BALF; compared with group A, ratio of inflammatory mediators in BALF and serum of the other three groups increased significantly; there were no significant differences among group B, C and D (Table V).

Discussion

LPS is the main component of cell wall of Gram-negative bacteria, the main factor to cause infectious diseases. LPS could progress to septicemia and further lead to systemic inflammatory response syndrome and even a most common and severest disease, ALI. Physiological characteristics of the lung make it as the first target organ to be involved and attacked[19]. Our results showed that rats could behave hypotensive shock rapidly after being given LPS intravenously, accompanied by ALI which result in significantly reduced oxygenation index in rats. According to HE staining of lung tissue and the observation by electron microscopy, there were inflammatory cell infiltration in lung, pulmonary edema caused by the increase of capillary permeability, structural damage of the type II alveolar epithelial cells, and reduction of lamellar structure in osmiophilic body which could impact on the release of pulmonary surfactant and thus affect lung function.

Microcirculation obstacle is the central link in septic shock, and tissue hypoxia is the basic problem of the shock. Therefore, to improve the perfusion of organs and tissues and reverse tissue ischemia and hypoxia is the key to recover the shock. Early fluid resuscitation is an important and necessary way to reverse the inadequate effective circulating blood, caused by septic shock, and prevent MODS. The concept of early-goal directed therapy (EGDT)[6] confirms the important role of adequate early fluid resuscitation of septic shock, and gives specific targets for early fluid resuscitation, which become an important part of international guidelines to treat shock[20]. In addition, a number of experiments have proved that early fluid resuscitation can significantly reduce the mortality of severe infection and septic shock patients[21].

The traditional fluid resuscitation strategy is based on the principle of enlarging blood volume. Vasoactive agents are used only if fluid resuscitation couldn't sustain effective perfusion pressure. Such approach can reduce the adverse effects to microcirculation of peripheral vascular contraction caused by vasoactive drugs. However, in septic shock, the body is more likely to exhibit hypotension[22] because of significantly decreased vascular tone resulting from reduced catecholamines secretion, and the application of sedative drugs during mechanical ventilation. This status is difficult to be corrected by simply fluid resuscitation[23]. vascular tone decreased significantly due to the reduce of secretion volume of catecholamines, complicated by the application of sedative drugs during mechanical ventilation. Norepinephrine could not only guarantee the effective center perfusion pressure through interaction with its receptor, but also the blood supply of vital organs[24,25], which is currently recognized as first- line vasopressor to treat septic shock[20]. Nacira et al have shown that the joint application of fluid resuscitation and norepinephrine can not cause microcirculatory derangements, but improve the mesenteric blood supply significantly[18]. Similarly, our results showed that two different strategies of early fluid resuscitation could both improve the effective circulating blood volume and tissue perfusion pressure. However, compared with traditional fluid strategy, application of norepinephrine could further reduce the blood lactate value. In view of the fact that the increase of blood lactate value is one specific indicator to reflect microcirculation disturbance[26], we concluded that use of norepinephrine in early stage improves the microcirculation disturbance, possibly because of the increase of angiotasis, reduction of the effective circulating blood volume leaking into the tissue space, faster velocity of the effective perfusion blood flow, decrease of blood stasis in the microcirculation and acceleration of anaerobic metabolite.

Septic shock could further progress to ALI or ARDS, because the endotoxin can activate neutrophils, monocytes/macrophages, lymphocytes, and endothelial cells, induce a large number of cytokines to release and aggregate in the lungs and damage the lung. Our results that the significant increase of IL-6, IL-8, TNF-α in BALF of control group with septic shock and the raised ratio of pre-inflammatory mediators in BALF and serum, suggested that lung would easily be damaged, and the inflammatory mediators could aggravate the lung injury by gradually producing the waterfall-like cascade reaction, inducing other endogenous inflammatory mediators and activating of the coagulation system and complement system. The inflammatory mediators play a key role in the occurrence and development of

ALI[27], and to reduce the level of pro-inflammatory mediators can decrease the degree of acute lung injury and improve lung function.

Our findings also suggested that the joint application of norepinephrine in early stage and fluid resuscitation could further decrease the expression level of pro-inflammatory mediators in the serum and BALF, reduce neutrophils invasion in the lung tissue, and improve the degree of lung injury, compared with traditional strategy. This may be because norepinephrine could decrease levels of IL-8 and TNF-α in serum[28], reduce the fluid volume and further the degree of pulmonary edema, lessen the damage of capillary endothelial cells, thereby reducing the inflammatory mediators on the whole. However, use of norepinephrine in early stage had no effect on the ratio of pro-inflammatory mediators in BALF and serum, probably because norepinephrine could alleviate the excessive release of pro-inflammatory mediators in lung tissue by improving the whole body's circulatory disturbance, but not the local lung tissue.

Furthermore, in this study, levels of hydroxyl radicals, MDA and MPO of group with septic shock, on behalf of the degree of oxidative damage, increased significantly, whereas level of SOD, on behalf of ability of reduction, decreased, compared with normal control group; use of norepinephrine in early stage only induced the expression level of MPO. The reason may be that septic shock belongs to hyperdynamic shock (arterio-venous shunt opens, and perfusion of true capillary tissue decreases) when the tissue is simultaneously in the state of relative hypoxia. Although the microenvironment has been improved, excessive fluid perfusion and use of vasoactive drug may lead to an ischemia-reperfusion status, in which large number of oxygen free radicals appear and thereby increase the oxidative damage. Therefore, to choose the appropriate antioxidant may further improve the prognosis of septic shock and ALI.

Conclusions

LPS could induce rats to have acute lung injury in early stage of septic shock. Early fluid resuscitation could guarantee effective circulating blood volume and tissue perfusion pressure, improve microcirculatory derangements, increase oxygen partial pressure and oxygenation index, but has the tendency to aggravate pulmonary edema yet. Compared with the traditional fluid resuscitation program, application of norepinephrine in early stage could further decrease the fluid volume used to resuscitate, alleviate the degree of pulmonary edema, reduce the expression level of pro-inflammatory mediators in the serum and BALF, and increase the oxygenation index. Thereby, early norepinephrine treatment and simultaneous fluid resuscitation may have a superior protective effect on lung injury.

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