Original Article

Effect of post recruitment maneuver ventilation by different tidal volume on lung vascular endothelial diastole function in rats with acute lung injury

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BACKGROUND: This study aimed to observe the effect of recruitment maneuver (RM) and post-RM ventilation at different tidal volume on lung vascular diastole endothelial function in rats with acute lung injury (ALI).

METHODS: A ALI rat model was produced by intravenous infusion of lipopolysaccharide (6 mg/kg). Twenty-five rats were randomly divided into five groups: control group ($n=5$), ALI group ($n=5$), low tidal volume group (LV group, VT 6 mL/kg, $n=5$), sustained inflation (SI) with low tidal volume group (SI+LV group, VT 6 mL/kg, $n=5$), and SI with moderate tidal volume group (SI+MV group, VT 12 mL/kg, $n=5$). RM was performed with SI, airway pressure 30 cmH₂O for 30 seconds, and positive end-expiratory pressure (PEEP) was set to 5 cmH₂O. Lung tissue was taken after 5 hours of mechanical ventilation. Mean arterial blood pressure (MAP) was monitored during the experiment. Endothelin-1 (ET-1), endothelial nitricoxide synthase (eNOS), Ach-induced endothelium-dependent relaxation response of isolated pulmonary artery rings were determined at 5 hours.

RESULTS: LPS increased ET-1 level, decreased the expression of eNOS in lung tissue, impaired the Ach-induced endothelium-dependent relaxation response in the pulmonary artery, without obvious effect on systemic hemodynamics. SI+LV significantly reduced LPS-induced elevation of ET-1 level, increased the expression of eNOS, significantly improved endothelial dysfunction, and improved the dysfunction of endothelium-dependent relaxation in the pulmonary artery.

CONCLUSIONS: RM with a high or low tidal volume ventilation could improve the lung vascular endothelial function of rats with acute lung injury, and RM with low tidal volume ventilation could lower significantly the injury of lung vascular endothelial diastole function in rats with acute lung injury.

KEY WORDS: Acute lung injury; Recruitment maneuver; Low tidal volume; Lung vascular endothelium; Pulmonary artery diastole

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INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are the common clinical syndromes of acute hypoxic respiratory insufficiency or failure. Pulmonary hypertension is one of the essential features of ALI/ARDS, with the clinical manifestations of continuous and irreformable hypoxemia and pulmonary artery pressure increase. It is associated with the severity

of the lung injury of ALI/ARDS patients as well as the independent risk factor affecting the prognosis of ALI/ARDS patient.^[1-4]

Mechanical ventilation is an important means to treat ALI/ARDS, but inappropriate mechanical ventilation may cause overexpansion of alveolus and over high airway plateau pressure. At present, it is still unclear whether the function of the pulmonary vessel

endothelium in ALI/ARDS patients can be improved by implementing different models of mechanical ventilation, and therefore the diastolic function mediated by endothelium can be improved and pulmonary artery pressure is lowered. Hence, it will be of great significance to study the effect of different models of mechanical ventilation on the function of the pulmonary vessel endothelium.

In this study, we used lipopolysaccharide (LPS) to produce ALI models in rats, and observed the changes of the pulmonary vessel endothelium based on the histopathological, immunological, molecular biological findings. We also observed the effect of recruitment maneuver (RM) and different tidal volumes after RM on the function of the pulmonary vessel endothelium in order to find the best model of mechanical ventilation for ARDS patients.

METHODS

Animals and instruments

Twenty-five Sprague-Dawley rats, 12 females and 13 males, weight 270 to 350 g, were provided by the Laboratory Animal Center, Nanjing General Hospital of the Nanjing Military Command of PLA (certification: SCXK (Military) 2007-0004).

Animal preparation

The rats were intraperitoneally injected with pentobarbital sodium (50 mg/kg), and then fixed in a supine position. The trachea was open, and was connected with a small animal ventilator (type ASV0691-001, produced by US Harvard Company) for volume controlled ventilation (VCV), and the positive end expiratory pressure (PEEP) was 5 cmH₂O (1 cmH₂O=0.098 kPa). The respiratory rate was adjusted to keep the arterial partial pressure of carbon dioxide (PaCO₂) at 35 to 50 mmHg (1 mmHg=0.133 kPa), with FiO₂ 100%. The catheter femoral artery was used to monitor invasive arterial blood pressure, and the indwelling canal in the femoral vein was used for transfusion.

Establishment of models and animals' group

LPS (6 mg/kg, *E. coli* O111:B4, US Sigma Company) was injected into the vein of rats to produce ALI models, while rats in the control group were injected with the same dosage of sterilized saline.

The rats were randomly divided into five groups, five rats in each group: control group, ALI group, low tidal volume (VT) group (LV group, VT of 6 mL/kg),

sustained inflation (SI)+low VT group (SI+LV group, VT of 6 mL/kg), and SI+ conventional tidal volume group (SI+MV group, VT of 12 mL/kg). The recruitment maneuver was performed with 30 cmH₂O SI.

At the end of expiration, the ventilation by a breathing machine stopped, the air supply valve was closed, the exhalation valve was open, and the expiration end was set at 30 cmH₂O below a water-sealed bottle, and a three-way pipe was connected for expiration for 30 seconds. After that, we set the basic ventilation mode of PEEP 5 cmH₂O. RM in the SI group was performed one time at 1, 2, 3, and 4 hours, respectively.

At one hour after the model was successfully established, RM and mechanical ventilation were performed in different groups (except the control group and LPS group). After 5 hours, the rats were killed.

During the experiment, pentobarbital sodium (25 mg/kg) was intraperitoneally injected interruptedly, and pancuronium bromide (0.1 mg/kg) was intramuscularly injected to keep the rats in general anesthesia and muscular relaxation. The arterial blood pH (pHa) was maintained at 7.35-7.45; if pHa was <7.25, and the rats were intravenously injected with sodium bicarbonate.

Parameters

Blood pressure

The rats were connected with a transducer and monitored through a catheter inserted into the femoral artery. The mean arterial pressure (MAP) was recorded every hour.

Pathological changes in lung tissue and grade of lung injury

About 0.5 cm×0.5 cm tissue was taken from the right middle lung lobe. The biopsy specimen was stained with hematoxylin and eosin (HE) and fixed with neutral gum. Radiography was performed with a microscope, and the degree of lung injury was evaluated. Smith scoring was used to make semi-quantitative analysis on pneumonedema, inflammation and hemorrhage in pulmonary alveoli and interstitia, and formation of atelectasis graded from 0 to 4, respectively. Observation was made under the microscope at 10 high magnifications (×400) in each rat, and the mean value was calculated.^[5]

Lung water content

The lung wet/dry weight ratio was used to determine lung water content. After the rats were killed by blood-letting, the lower lobe of the right lung was taken and

covered immediately with water and blood using absorbent paper. The lower lobe (wet weight) was weighed and then put into a thermostatic oven at 70 °C for 72 hours to reach a constant weight. It was taken out and weighed again (dry weight). The ratio of two weights, i.e. W/D indicated pneumonema.^[6]

eNOS protein expression of lung tissue

The Envision two-step method^[7] was used for observation under a light microscope. Normal rabbit serum or PBS was used to replace the first antibody, and then absorption test and blank test were performed. Sections were observed under an optical microscope and photos were taken. Brown yellow granules showed positive staining of the target protein, and blue granules showed staining of hematoxylin double-stained cell nuclei. Image Pro plus 6.0 image analysis software was used to make a semi-quantitative analysis of the expression of target protein. Integrated optical density (IOD) of positive cells was determined at 5 magnifications (×400) for each section, and the mean value was calculated.

The concentration of endothelin-1 in lung tissue determined by radioimmunoassay (RIA)

Totally 100 mg left lung tissue was taken from each rat, added with 1 mL physiological saline, homogenized with an electric homogenizer for 15 minutes, and centrifuged at 3000 r/min at 4 °C. The supernatant was taken for test. Before the test, the dilutability of the sample was confirmed and 3-5 samples served as small samples. They were diluted to 1: 1, 1: 5, 1: 20. Radioimmunoassay was performed to detect the concentration of ET-1 according to the instructions of the kit produced by Yanbian North Biological Technology Co., Ltd. The automatic γ counter produced by USTC Chuangxin Co., Ltd, Zonkia Branch was used to calculate the result.

The diastolic function of the pulmonary artery

After the rats were killed by blood-letting, the chest was immediately open to remove the heart and lung and put them in Krebs-Henseleit (K-H liquid) buffer at 37 °C (home-made: NaCl 118.30 mmol/L, KCl 4.70 mmol/L, CaCl₂ 2.50 mmol/L, KH₂PO₄ 1.20 mmol/L, MgSO₄ 1.20 mmol/L, NaHCO₃ 25 mmol/L, and glucose 11.10 mmol/L), and then the gas mixed with 95 °C O₂ and 5 °C CO₂ was blew into it. The blood on the surface was washed, the pulmonary arteries were separated, the connective tissues on the surface of the arteries were removed, and finally

2 to 3 pulmonary arterial rings with a length of 2 mm were produced. The rings were hung with two stainless steel wires in the bath filled with K-H buffer, and then the same mixed gas CO₂ was blown in at 37 °C. One end of the ring was fixed at the glass hook in the bath, and the other was connected with a tension transducer, which recorded the changes of ring tension through biological signal collection and the disposal system. The basic tension of the pulmonary arterial ring was 1 g. One hour after balancing, phenylephrine was added at a final concentration of 10⁻⁵ mol/L to determine the activity of the sample. After steady contraction of the vessel, acetylcholine (Ach)^[8] was added in time sequence with the accumulative final concentration varying from 10⁻⁹ mol/L to 10⁻⁴ mol/L. Sodium nitroprusside (SNP)^[9] was added in time sequence with the accumulative concentration varying from 10⁻⁹ mol/L to 10⁻⁴ mol/L in another vessel. After 5 minutes at each concentration, the change of tension of vessels was recorded to draw the accumulative concentration-diastolic response curve. The percentage of maximum diastolic response was taken as a longitudinal coordinate, and the concentration of a drug as a horizontal axis. The diastolic percentage = (maximum contractile tension-diastole tension) / (maximum contractile tension-rest tension) × 100%.

TNF- α in lung tissue determined by ELISA

Totally 100 mg of left lung tissue was taken, added with physiological saline, homogenized with an electric homogenizer for 10 minutes, and centrifuged at 4 °C and 3000 r/min. The supernatant was taken and stored under -70 °C. The cytokine concentration was determined according to the instructions of the kit produced by US R&D Company.

Statistical analysis

Data were expressed as mean value $\pm$ standard deviation. Software STATA9.0 was used to conduct statistical analysis. Homogeneity of variance was determined before comparing the means of samples by one-way analysis of variance. Student-Newman-Keuls procedure was used to compare the means of several samples. A *P* value less than 0.05 was considered statistically significant.

RESULTS

General condition

During the experiment, the blood pressure fluctuated in the normal range, and no obvious change was

observed between the groups and inter-groups ($P>0.05$, Table 1).

Pathological findings of lung tissue

Under a light microscope, no obvious pulmonary interstitial and intra-alveolar hemorrhage, edema or inflammation was observed in the CON group. The alveolar space was dry, and the alveolar septum was even, with part of pulmonary alveoli dilated. In the ALI group, obvious pulmonary interstitial and intra-alveolar hemorrhage and drainage were observed with a lot of pulmonary interstitial inflammatory cells. The alveolar septum was broadened, focal alveoli were collapsed, and pulmonary atelectasis was seen. In the LV group, pulmonary interstitial inflammatory cells were infiltrated or congestive, and part of pulmonary alveoli were bleeding, seeping or collapsed. In the SI+LV group, the lung tissue was congestive, the alveolar septum was broadened slightly, and the neutrophil granulocytes

were infiltrated. In the SI+MV group, pulmonary interstitial inflammatory cells were infiltrated or obviously congestive; part of the pulmonary alveoli were over-dilated, bleeding, seeping, or collapsed (Figure 1).

The severity of pathological injury of lung tissue was higher in the ALI group than in the CON group (15.18 ± 0.45 vs. 4.39 ± 0.62 , $P<0.05$). The severity of the injury in the LV group (13.13 ± 0.50), SI+LV group (10.61 ± 0.76), SI+MV group (13.58 ± 0.50) was lower than in the ALI group ($P<0.05$). The severity of the injury in the LV group and SI+MV group was higher than in the SI+LV group ($P<0.05$, Table 2).

Content of lung water

The W/D of the lung in the ALI group was higher than that in the CON group (6.86 ± 0.50 vs. 4.15 ± 0.20 , $P<0.05$). The W/D in the LV group and SI+MV group was increased more obviously than that in the SI+LV group

Table 1. Effect of different tidal volumes after RM on MAP in ALI rats

Groups	0 h	1 h	2 h	3 h	4 h	5 h	F	P
CON	114.50±12.30	115.60±7.00	114.50±2.90	117.10±6.10	112.50±7.70	110.90±7.20	0.41	0.84
ALI	114.60±6.50	118.10±4.70	109.10±11.20	100.70±16.00	109.50±10.10	105.60±12.90	1.33	0.29
LV	106.50±12.30	111.50±6.30	105.80±11.80	108.50±16.30	106.50±9.50	111.50±15.10	0.42	0.83
SI+LV	103.00±5.70	114.30±12.40	106.30±15.30	113.70±11.50	111.30±9.30	109.20±14.70	0.33	0.89
SI+MV	109.40±2.70	110.50±11.10	107.80±9.20	111.60±7.70	108.50±10.30	107.90±7.3	0.18	0.97
F	1.67	0.613	0.51	1.30	0.31	0.20	—	—
P	0.20	0.66	0.73	0.30	0.87	0.93

Figure 1. Pathological findings in lung tissue of ALI rats (HE, original magnification $\times 200$). A: CON group; B: ALI group; C: LV group; D: SI+MV group; E: SI+LV group.

(5.18 ± 0.16 vs. 6.19 ± 0.33 vs. 6.69 ± 0.23 , $P < 0.05$). The W/D was also higher in the SI+MV group than in the LV group (6.19 ± 0.33 vs. 6.69 ± 0.23 , $P < 0.05$) (Table 2).

eNOS protein expression

In the CON group, a large number of eNOS protein expressions was seen, whereas the expressions were less in the ALI group than in the CON group. The eNOS protein expressions in the lung tissues of the SI+LV group, SI+MV group, and LV group were not significantly different, but more than those in the ALI group (Figure 2).

The IOD value for eNOS protein expression in the lung tissue in the ALI group was 6762.65 ± 3221.63 , relatively lower than that in the CON group (17544.04 ± 2675.42 , $P < 0.05$). The IOD value in the LV group (9339.53 ± 3366.40), SI+LV group (12663.83 ± 1348.93),

and SI+MV group (9208.12 ± 2773.68) was higher than that in the ALI group ($P < 0.05$). There was no statistical difference between the SI+LV group and SI+MV group ($P > 0.05$, Table 3).

TNF- α content

The TNF- α content in the lung tissue of the ALI group increased more significantly than that of the CON group (2280.18 ± 290.05 ng/L ($P < 0.05$). The TNF- α content in the LV group (3370.75 ± 314.17 ng/L, SI+LV group (2374.53 ± 410.60 ng/L, and SI+MV group (3468.86 ± 659.25) ng/L was lower than that in the ALI group (4831.13 ± 514.88) ng/L ($P < 0.05$). Moreover, the TNF- α content in the LV group and SI+MV group increased more significantly than in the SI+LV group ($P < 0.05$); but no statistical difference was observed in the LV group and SI+MV group ($P > 0.05$, Table 3).

Table 2. Effect of RM and different tidal volumes on severity of damage of lung tissue and W/D of ALI rats (mean $\pm$ SD)

Groups	n	Grade of damage of lung tissue	Pneumon-edema (W/D)
CON	5	4.39 ± 0.62	4.15 ± 0.20
ALI	5	$15.18 \pm 0.45^*$	$6.86 \pm 0.50^*$
LV	5	$13.13 \pm 0.50^{*#}$	$6.19 \pm 0.33^{*#}$
SI+LV	5	$10.61 \pm 0.76^{*#}$	$5.18 \pm 0.16^{*#}$
SI+MV	5	$13.58 \pm 0.50^{*#\Delta}$	$6.69 \pm 0.23^{*\Delta}$
F		266.38	92.9
P		0.00	0.00

Compared with the CON group, $^*P < 0.05$; compared with the ALI group, $^#P < 0.05$; compared with the SI+LV group, $^\Delta P < 0.05$

Table 3. Changes of eNOS IOD value, TNF- α and ET-1 content of ALI rats

Groups	n	IOD value	TNF- α (ng/L)	ET-1 (pg/mL)
CON	5	17544.04 ± 2675.42	2280.18 ± 290.05	90.32 ± 12.94
ALI	5	$6762.65 \pm 3221.63^*$	$4831.13 \pm 514.88^*$	$178.92 \pm 16.01^*$
LV	5	$9339.53 \pm 3366.40^*$	$3370.75 \pm 314.17^{*#\Delta}$	$152.35 \pm 8.21^{*\Delta}$
SI+LV	5	$12663.83 \pm 1348.93^{*#}$	$2374.53 \pm 410.60^{*#}$	$109.18 \pm 15.62^{*#}$
SI+MV	5	$9208.12 \pm 2773.68^*$	$3468.86 \pm 659.25^{*#\Delta}$	$158.79 \pm 30.40^{*\Delta}$
F		11.31	25.38	20.40
P		0.00	0.00	0.00

Compared with the CON group, $^*P < 0.05$; compared with the ALI group, $^#P < 0.05$; compared with the SI+LV group, $^\Delta P < 0.05$

Figure 2. Expression of eNOS of the lung of ALI rats (IHC, original magnification $\times 400$). A: CON group; B: ALI group; C: LV group; D: SI+MV group; E: SI+LV group.

Endothelin-1 (ET-1) content in lung tissues

ET-1 content in the lung tissue in the ALI group was higher than that in the CON group (178.92 ± 16.01 pg/mL vs. 90.32 ± 12.94 pg/mL, $P < 0.05$). The ET-1 content in the LV group and SI+MV group was higher than that in the SI+LV group (109.18 ± 15.62) pg/mL ($P < 0.05$), whereas statistical difference was observed between the LV group and SI+MV group ($P < 0.05$, Table 3).

Diastolic function of isolated pulmonary arterial rings Isolated pulmonary arterial ring's diastolic responses to Ach at different concentrations

With the action of Ach at concentrations varied from 10^{-9} mol/L to 10^{-4} mol/L, the pre-contracted vascular rings appeared with concentration-dependent vasodilatation in each group. With the action of Ach at different concentrations, the diastolic percentages in each group were different. The endothelium-dependent diastolic function of the ALI group in contrast to the CON group declined remarkably by the action of Ach at different concentrations ($P < 0.05$). The endothelium-dependent diastolic function of the ALI group and SI+MV group compared with the SI+LV group declined by the action of Ach at different concentrations ($P < 0.05$). Though there was no statistical difference between the LV group and SI+LV group, the Ach-mediated diastolic function in the LV group tended to decline ($P > 0.05$, Figure 3).

Isolated pulmonary arterial ring's diastolic responses to SNP at different concentrations

By the action of SNP at concentrations varied from 10^{-9} mol/L to 10^{-4} mol/L, the pre-contracted vascular rings in each group appeared with concentration-dependent vasodilatation. But no statistical difference was observed

in the endothelium-independent diastolic functions and diastolic percentages between the two groups by the action of SNP at different concentrations ($P > 0.05$, Figure 4).

DISCUSSION

The integrity of endothelium is a necessary condition for the existence of endothelium-dependent pulmonary vessels' diastolic function. During ALI, the integrity of endothelium is damaged, and then the endothelium-dependent relaxation^[6] is selectively injured. Endothelium-mediated pulmonary vessels' unbalance of contraction and relaxation appears as an increase of vessels' contractibility and pulmonary vascular resistance, which cause pulmonary hypertension. Pulmonary hypertension can increase the afterload of the right ventricle and blood shunts in the lung, which will aggravate the interstitial pulmonary edema^[10] of distal vessels. The constant existence of pulmonary hypertension is an independent risk factor^[11] in ALI/ARDS patients with poor prognosis.

Mechanical ventilation is one of the important means to treat ALI/ARDS, but ALI/ARDS patients have many complications, such as pulmonary edema, loss of type II alveolar epithelium, uneven distribution of gas caused by alveoli collapse, different compliances, and hyperventilation for part of alveoli. Hence, during the treatment of positive pressure ventilation, especially under the ventilation of large tidal volume, pulmonary artery pressure increases continuously and then lung injury aggravates.

Therefore, combination of RM with low tidal volume (6 mL/kg), lung opening with the controlled plateau pressure (below 30 cmH₂O), and protective ventilation is suggested to treat ALI/ARDS patients. However, there is no report on the effect of RM and small tidal volume

Figure 3. Concentration-effect curve of endothelium-dependent diastolic function induced by Ach. Compared to the CON group, the endothelium-dependent diastolic function of the ALI group declined obviously by the action of Ach at different concentrations ($P < 0.05$). Compared to the SI+LV group, the endothelium-dependent diastolic function of the ALI group and SI+MV group reduced by the action of Ach at different concentrations ($P < 0.05$).

Figure 4. The concentration-effect curve of the endothelium-independent diastolic function induced by SNP. There was no obvious difference in endothelium-independent diastolic functions between the two groups under the action of SNP at different concentrations, and the difference of diastolic percentages was not statistically significant ($P > 0.05$).

after RM on ALI/ARDS patient's pulmonary vascular endothelium-dependent diastolic function, and the mechanism is still unknown. In our study, RM and small tidal volume after RM improved the pulmonary vascular endothelium-mediated diastolic function. The increase in secretion of down-regulated inflammatory factor and secretion of up-regulated endothelium-derived relaxing factor was suggested to improve the RM's function on endothelial cells.

Inflammatory factor is one of the causes of pulmonary arterial endothelial cell injury and increase of pulmonary artery pressure. Our previous study^[11] revealed that the secretion of inflammatory factors may lead to damage the permeability of lung vascular endothelial cells. Moreover, in the *in vitro* culture of human pulmonary artery smooth muscle cells, interleukin (IL)-1 β , bradykinin (BK), and transforming growth factor (TGF)- β 1 could affect the function of prostacyclin receptor and damage the diastolic function of the pulmonary artery. Smooth muscle cells of the pulmonary artery show a strong capability of migration and proliferation^[12,13] to the inflammatory factors. In the present study, ALI increased the release of inflammatory factors, lung water, injury of lung tissue, and reduction of diastolic pressure of the pulmonary artery. While combination of RM with small tidal volume mechanical ventilation reduced the release of inflammatory factors, and alleviated the endothelial cell injury and the increase of pulmonary artery pressure.

The reduced diastolic pressure of pulmonary vessels is associated with the secretion of relaxing factors and shrinking factors from endothelium, which is another cause of pulmonary hypertension. eNOS exists in vascular endothelial cells, and acts on substrate to produce NO, while NO will disperse into vascular smooth muscle to play a role in relaxing the vessels.^[14] When mechanical ventilation with different tidal volumes was given to eNOS transgenic mice and normal mice, the mice with eNOS over expression under the condition of large tidal volume (20 mL/kg) showed less leukocyte infiltration and blood plasma, lower lung water content in pulmonary alveoli, and less TNF- α s in alveolus lavage fluid than normal mice.^[15]

ET-1 often causes pulmonary vessels to contract through mechanical tension, cytokines, and low oxygen incited secretion. The intravenous infusion of ET-1 can increase pulmonary artery pressure,^[16] and alleviate the contraction of pulmonary arterial vessels^[17] after blocking endothelins from combining with receptor. Similarly, the concentration of ET-1 in circulation in ALI patients is positively correlated to right arterial pressure

(RAP), pulmonary arterial systolic pressure (PASP), mean pulmonary artery pressure (MPAP), and pulmonary vascular resistance index (PVRI). The increase of ET-1 concentration causes the contraction of pulmonary vessels in ALI patients.^[18] In our study, although there was no statistical difference in the expression of eNOS in lung tissues between the SI+LV group and SI+MV group under the ventilation with different tidal volumes, the expression of ET-1 in the SI+LV group was much lower. This indicated that combination of RM with a small tidal volume can reduce the secretion of shrinking factors, but increase the secretion of relaxing factors.

A large amount of pulmonary alveoli collapsed in ALI/ARDS, thus the lung volume reduced remarkably. RM can make the collapsed alveoli re-expand, reduce the mechanical stimulus caused by shear force to endothelial cells, and then reduce the secretion of cytokines and ease the inflammatory reaction in the lung. Borges et al^[19] reported that due to the heterogeneity of ALI lung parenchyma, even a mechanical ventilation at small tidal volume was given to the patient, over expansion of alveoli still existed at the end of respiration. A study^[20] using CT proved that low oxygen in early ARDS was negatively correlated with alveoli collapse, and that RM could make the collapsed alveoli re-expand to improve oxygenation. In the present study, RM lowered the endothelium-mediated diastolic pressure of pulmonary vessels, and reduced ET-1 and the secretion of inflammatory mediators, thus alleviating the damage of the endothelium.

Ach acts on the M2 receptor of pulmonary vessel endothelium, and then activates the Gi protein coupled with M2 receptor to release the endogenous NO and relax the endothelium. When the pulmonary vessel endothelium is injured, the diastolic response of the pulmonary artery to Ach is reduced. As SNP can replenish NO exogenously,^[9] the pulmonary artery still has diastolic response to SNP when pulmonary vessel endothelium is injured. It has been proved that the combination of RM with different tidal volumes has different responses to Ach-mediated endothelium-dependent pulmonary artery diastolic pressure. Compared with the ALI group and SI+MV group, the endothelium-dependent diastolic pressure in the SI+MV group was improved. Although there was no significant difference between the LV group and SI+LV group, the Ach-mediated diastolic pressure tended to decline. There was no significant difference in the groups with response to SNP, which further proved that combination of RM with mechanical ventilation by a small tidal volume can prevent the decline endothelium-mediated diastolic pressure.

In conclusion, the combination of RM with a high tidal or low tidal volume mechanical ventilation can improve the diastolic pressure of pulmonary vessel endothelium in ALI rats. The combination of RM with a small tidal volume can reduce the injury to endothelial cells, increase the expression of eNOS protein, down-regulate the inflammatory reactions in the lung and the secretion of ET-1, and improve endothelial cell mediated pulmonary vessel diastolic pressure, and pulmonary artery pressure of ALI/ARDS patients. Also it is important to get better prognosis.

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