

Effects of Mechanical Ventilation on Lung Grafts during Cold Ischemia Phase

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ABSTRACT

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Background: The preservation of donor lungs during cold ischemia phase plays an important role in improving the function of lung grafts after transplantation. Donor lungs were conventionally preserved with a method of static inflation during ischemia phase, but how mechanical ventilation would affect lungs during this stage was unknown. This study was undertaken to investigate the effects of mechanical ventilation on donor lungs during cold ischemia phase.

Methods: Isolated lungs from 45 rats were randomized into control (CON), mechanical ventilation without positive end-expiratory pressure (PEEP) (MVNP) and mechanical ventilation with PEEP (MVP) groups. During the cold ischemia phase, lungs in the CON group were preserved with a conventional method of static inflation, while lungs in the MVNP and MVP groups were mechanically ventilated with tidal volume (VT) of 7 ml/kg without PEEP and VT of 7 ml/kg with 3 cm H₂O PEEP, respectively. After 2 hours, bronchoalveolar lavage fluid (BALF) collected from lungs was subsequently analyzed for surfactant, inflammatory cytokines, and total protein; lung tissues were collected for analysis of morphology and the wet-to-dry (W/D) weight ratio; peak airway pressure was recorded before and after lung preservation to reflect the change of lung compliance.

Results: Compared to CON group, the levels of surfactant protein B (SP-B), surfactant protein C (SP-C), total surfactant, and active large aggregates (LA) in MVP group raised significantly ($P < 0.05$); meanwhile, W/D ratio, the lavage concentrations of interleukin (IL)-6, and tumor necrosis factor (TNF)- α in the MVNP group were higher than those in the CON group ($P < 0.05$). In contrast with CON group, lung injury scores were higher in both MVNP and MVP groups ($P < 0.05$); the level of peak airway pressure in the MVNP group was dramatically higher than that in the CON and MVP groups ($P < 0.05$), while there were no differences between CON and MVP groups.

Conclusions: Mechanical ventilation during cold ischemia phase with low VT and PEEP promoted the level of lung surfactant, but aggravated lung injuries with no effects observed on lung compliance.

Lung transplantation has been well-established as an effective therapy for patients suffering from any form of benign end-stage lung disease. According to previous reports, the number of lung transplant operations is increasing dramatically since the mid of 1990s (1). Although it had been developed a lot during the past two decades, this form of therapy was still hampered by persistent obstacles, such as primary graft dysfunction, which was responsible for 28.8% of deaths within the first 30 days after lung transplantation according to the Registry by the International Society for Heart and Lung Transplantation (2). Better methods for lung preservation during perioperative period are necessary to reduce the incidence of primary graft dysfunction, because those processes play an important role in reducing the incidence of primary graft dysfunction and improving the function of donor lungs. Conventional preservation of donor lungs is centered on cold static preservation and inflation of oxygen (3). Cold storage is aimed at slowing cell metabolism, reducing oxygen and other substrate consume to prevent organ deterioration ultimately. While the purpose of inflating the lungs with oxygen is to allow ongoing energy-efficient aerobic metabolism, and the benefit of this strategy lies in maintaining the integrity of the alveolocapillary barrier (4, 5). A previous study in terms of donor lungs preservation during warm ischemia indicated that post-mortem room air-inflation was as good as ventilation in prolonging warm ischemia tolerance, and that prevention of alveolar collapse in warm ischemia stage appeared to be critically important in protecting the lung grafts from reperfusion injury independent of continuous oxygen supply (6). However, what effects mechanical ventilation might have on pulmonary grafts during cold ischemia phase is unknown.

Mechanical ventilation is an essential supportive treatment for many conditions, but it may also directly cause lung damage through multiple mechanisms (7, 8). Low tidal volume (VT, 6 to 8 ml per kilogram of predicted body weight) was demonstrated to be an important part of lung-protective ventilation strategy (9). Also, positive end-expiratory pressure (PEEP) plays important roles in avoiding lung collapse during expiration. Low VT with 3 cm H₂O was taken as rela-

tively non-injurious ventilation strategy (10). Previous studies concerning the effects of mechanical ventilation on lung surfactant showed contradictory data (10, 11). Pulmonary surfactant is a complex mixture of phospholipid and protein, and the main function of surfactant is to reduce surface tension at the alveolar surface sequentially conferring stability to the lung (12). The biophysical activity is accomplished by one of the two major subfractions of surfactant, the large-aggregate (LA) (13). The other subfraction of surfactant, the inactive small-aggregate (SA) is generated from LA during ventilation. Surfactant protein B (SP-B) and surfactant protein C (SP-C) are hydrophobic surfactant-associated proteins. We could utilize the changes of LA, SA, SP-B, and SP-C to reflect the effects of different preservation methods on surfactant system.

Taken together, we explored the effects of mechanical ventilation on donor lungs with low VT during the period of cold ischemia in terms of pulmonary surfactant, lung morphology, wet-to-dry (W/D) weight ratio, lung compliance and the concentrations of inflammatory cytokines in the lungs.

MATERIALS AND METHODS

Animals and Ethics

All procedures in the present study were approved by the Institutional Animal Care and Use Committee of Harbin Medical University. Adult male Sprague-Dawley (SD) rats weighing 280-320 g were purchased from the Animal Experiment Center of The Second Hospital of Harbin Medical University.

Animal Preparation

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), intubated through a tracheostomy. Heparinization was accomplished by injecting heparin (150 U) into the caudal vein. Following a midline sternotomy, 20 ml 4°C low-potassium dextran (LPD) solution was used to flush the lungs by an antegrade route through annulation of the pulmonary artery, the left atrium was used for outflow. In this process, we ventilated the lungs with VT of 7 ml/kg and at a rate of 50 breaths/minute to allow homogeneous distribution of the perfus-

ate. Then, the heart-lung block was carefully excised as previously reported (10). The isolated lungs from 45 SD rats were randomized into control (CON), mechanical ventilation without PEEP (MVNP) and mechanical ventilation with PEEP (MVP) groups. Lung grafts from CON group maintained statically inflated by ligating the airway in the end of inspiration during the period of cold ischemia. While pulmonary grafts from MVNP and MVP groups were mechanical ventilated with low VT of 7 ml/kg without PEEP and the same VT with PEEP of 3 cm H₂O respectively and at a same rate of 50 breaths/minute. During 2 hours of cold ischemia phase, all lungs were stored in the ice-water mixture of LPD solution, the tube of air inlet end in the MVNP and MVP groups was submerged in ice-water to cool the gas. We placed a probe into the bronchus to record the temperature in the process of storage. The gas mixture used for inflation and ventilation consists of 40% O₂ and 60% N₂.

Lavage Procedure

After 2 hours, five of the isolated rat lungs in each group were subjected to a whole lung lavage with sterile saline as previously described (14). Briefly, we filled the lungs with 10 ml of saline until they appeared fully distended, and then withdrew and reinfused the saline for two additional times. This procedure was repeated three times with fresh saline for a total of three washes. The volume of total lavage was recorded and then the total lavage was spun at 150 × g for 10 minutes. The 150 × g supernatant was collected. A 3 ml aliquot of this supernatant (total surfactant) was aliquoted and frozen at -20°C for analysis of total surfactant. The remaining 150 × g supernatant was used to separate the LA from the SA by centrifugating at 40,000 × g for 15 minutes (15). Then we aliquoted the supernatant containing the SA surfactant and froze it at -20°C. The pellet of the 40,000 × g spin (the LA fraction) was resuspended with 2 ml of saline and was also frozen at -20°C for further analysis.

In addition, the left sides of the other ten lungs in each group were filled with 3 ml of saline, after which the saline was withdrawn and reinfused for another two times. The process was also repeated three times with sterile saline. The total lavage volume was recorded and spun

at 1,500 × g for 10 minutes at 4°C. We aliquoted and froze the collected supernatant at -20°C for further analysis of SP-B and SP-C, as well as total protein (TP) and inflammatory cytokines in bronchoalveolar lavage fluid (BALF).

Pulmonary Surfactant Analysis

We determined the amount of total surfactant, SA, and LA fractions in all groups via phospholipid-phosphorus measurements. Aliquots of the different samples were extracted by the method as described by Bligh and Dyer (16). Subsequent measurements of phospholipid phosphorus in each of the lipid extracts were determined by means of Duck-Chong (17). The amounts of surfactant in the total lung lavage were calculated and expressed as milligrams phospholipid per kilogram body weight.

SP-B and SP-C in BALF were analyzed using specific enzyme-linked immunosorbent assay (ELISA) kits (Shanghai BlueGene Biotech, Shanghai, China) for rats according to the manufacturers' instructions.

Total Protein and Inflammatory Cytokines Analysis

The concentrations of TP in BALF were determined using a bicinchoninic acid (BCA) assay kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's protocol. Levels of inflammatory cytokines in BALF, including tumor necrosis factor (TNF)- α and interleukin (IL)-6 were quantified using specific ELISA kits (Shanghai BlueGene Biotech, Shanghai, China) for rats.

Wet-to-Dry Weight Ratio

The right upper lobes of the lung grafts, which were desiccated at 70°C for 72 hours after being weighed, were used to measure the W/D ratio via dividing wet by the dry lung weight.

Histologic Examination and Scoring

The isolated inferior lobes in the right lungs were separated and immediately submerged in 4% formalin. The lung tissues from each animal were sectioned sagittally and stained with hematoxylin and eosin for light microscopy. All sections were evaluated by a pathologist who was blind to the study. Lung injury degree was grad-

ed from 0 (normal) to 4 (severe) based on following categories adapted from previous study (18): 1) neutrophil infiltration; 2) interstitial edema; 3) airway epithelial cell damage; 4) alveoli collapse; and 5) alveolar overdistension. A total histologic lung injury scores were calculated by summing the 5 scores.

Lung Compliance Analysis

We recorded the peak airway pressure using a pressure transducer after the lung grafts were excised and at the end of cold ischemia to reflect the change of lung compliance through different management during the period of cold ischemia.

Statistical Analysis

Data were expressed as means \pm standard deviation (SD). Differences in the measured variables between groups were determined by a one-way analysis of variance (ANOVA). Specially, the data of peak airway pressure were determined by analysis of covariance. The significant difference level was set at $P < 0.05$.

RESULTS

There were no significant differences in animal weight, volumes of lavage collected from the left lungs and the whole lungs, as well as the temperature inside the lung bronchus ($P > 0.05$).

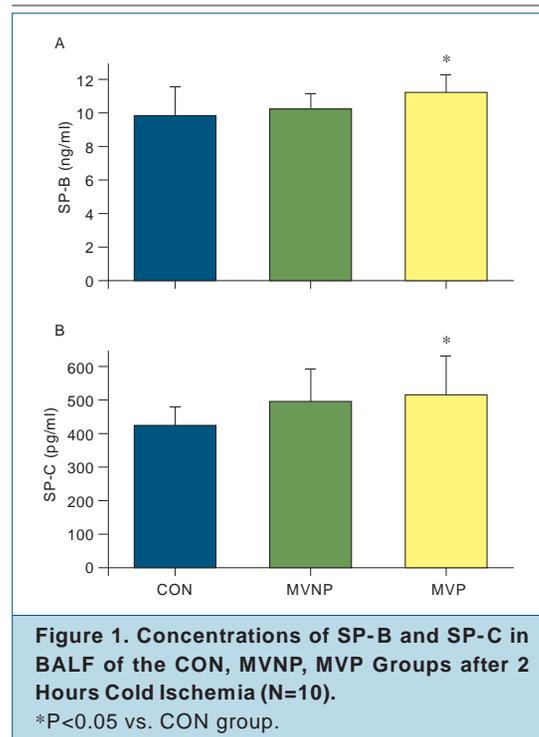
Lung Surfactant in BALF

As shown in figure 1, compared with CON group, the concentrations of SP-B and SP-C in BALF of MVP group were significantly higher ($P < 0.05$), the values in the MVNP group were also higher than CON group, but there were no statistical differences.

Figure 2 showed the amounts of total surfactant, the levels of LA and SA from different experimental groups. There was significantly more total surfactant in the lavage from MVP group when compared with CON group ($P < 0.05$). Analysis of the LA revealed similar difference as total surfactant. No differences were found with SA among the three groups.

W/D Ratio and TNF- α , IL-6, TP Levels in BALF

W/D ratio of lung tissue in the MVNP group was higher than that in CON group ($P < 0.05$).



The concentrations of TNF- α and IL-6 in BALF of MVNP group were significantly higher when compared with CON and MVP groups ($P < 0.05$). No differences were observed in the level of TP among groups (Figure 3).

Lung Histopathologic Changes

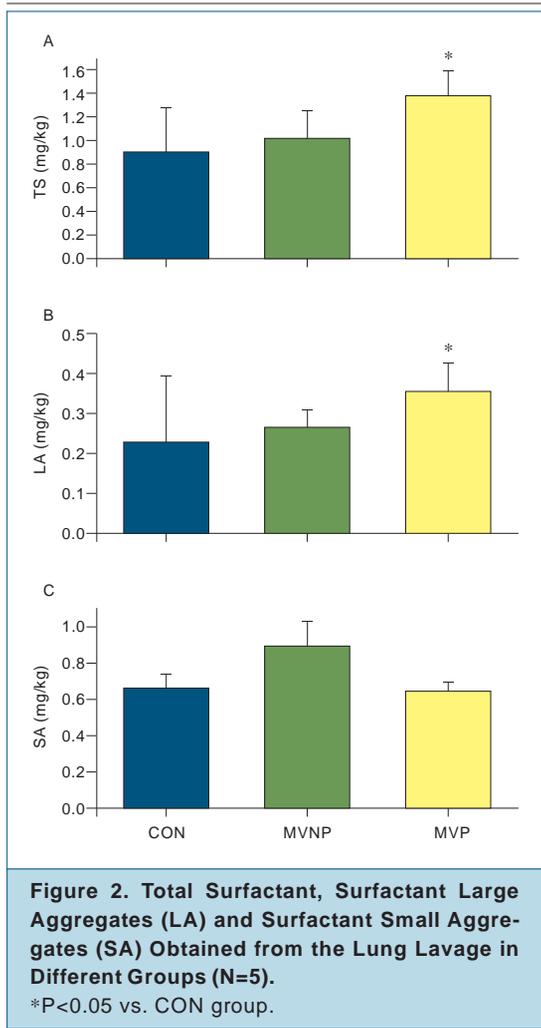
Light microscope images showed that tissues of three groups existed varying degrees of lung injury involving the scoring items. Lung injuries in MVNP and MVP groups were more serious than those in CON group with significant differences according to the lung injury scores ($P < 0.05$) (Figure 4).

Peak Airway Pressure

We compared the adjusted peak airway pressure (after cold storage for 2 hours) by balancing initial peak airway pressure (before cold storage) to reflect lung compliance. As figure 5 showed, peak airway pressure of MVNP group was dramatically higher than both CON and MVP groups ($P < 0.05$), but there was no difference between CON and MVP groups.

DISCUSSION

Our study adopted ventilation strategies of low



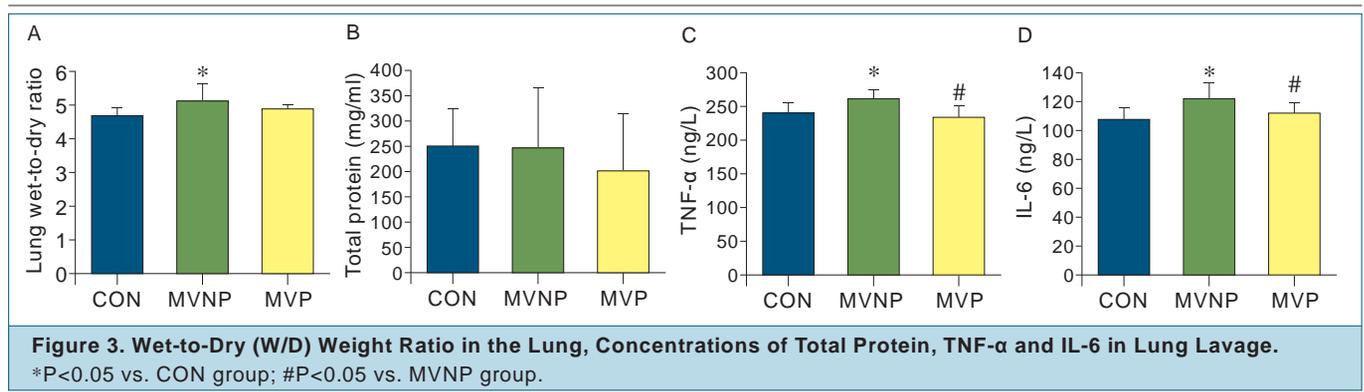
VT with or without PEEP to explore the effects of mechanical ventilation on isolated lungs in terms of the secretion of lung surfactant, donor lungs injury and the change of lung compliance during cold ischemia phase by comparing with conventional storage method of static inflation. The isolated lung models were chosen specially to address the lung-specific effects of ventilation without the influence of other systemic factors as reported by previous studies (19, 20).

In our study, we found that total surfactant, surface tension reducing-related surfactant sub-fractions (LA) and proteins (SP- B and SP- C) were significantly higher in lungs ventilated with low VT and PEEP compared with those preserved with conventional inflation. W/D ratio, which reflected the degree of pulmonary edema was higher with significant differences in lungs ventilated without PEEP when compared with

lung grafts both statically inflated and ventilated with PEEP. The comparisons of inflammatory cytokines TNF- α and IL-6 revealed the same results as W/D ratio. There were no significant differences in concentrations of TP in BALF of three groups. Lung injury scores of lungs ventilated with or without PEEP during cold storage were both higher than statically inflated lungs. The comparison of peak airway pressure showed that the compliance of lungs ventilated without PEEP was worse than lungs inflated or ventilated with PEEP, while there was no difference between the latter two situations.

A previous study using a conventional non-injury ventilation strategy to ventilate healthy rat lungs for 24 hours reflected that there was a significantly higher amount of total surfactant recovered from the ventilated animals compared with the nonventilated animals, and this increase was due to a significant increase in the LA subfraction in the ventilated groups, while no differences were found in the comparison of SAs (21). The result was similar to the changes observed in our study. Compared with conventional static inflation, increases of total surfactant and LA in lavages from lung grafts ventilated with low VT and PEEP were likely connected to stretch-related increases in surfactant secretion (22). The regionally alveolar atelectasis due to the lung injury induced by mechanical ventilation without PEEP may decrease the secretion of lung surfactant, as a result, the levels of total surfactant and LA in lavage from lungs ventilated with low VT without PEEP were not significantly higher than those from lungs conventionally statically inflated. The changes of surfactant were also reflected in the concentrations of hydrophobic SP-B and SP-C, which presented the same comparison results as total surfactant and LA.

Furthermore, it has been proved that serum protein infiltrated into the alveolar can inhibit the surface tension reducing activity of LA, and contribute to the decrease of lung compliance (23, 24). Lungs flushed with saline of the vasculature before ventilation had higher lung compliance compared with non- flushed lungs. This was associated with lower protein concentrations and improved surfactant activity (25). We perfused the lung grafts with 4°C LPD solution

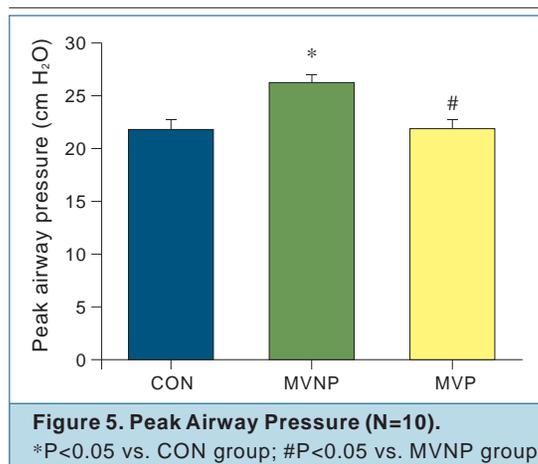
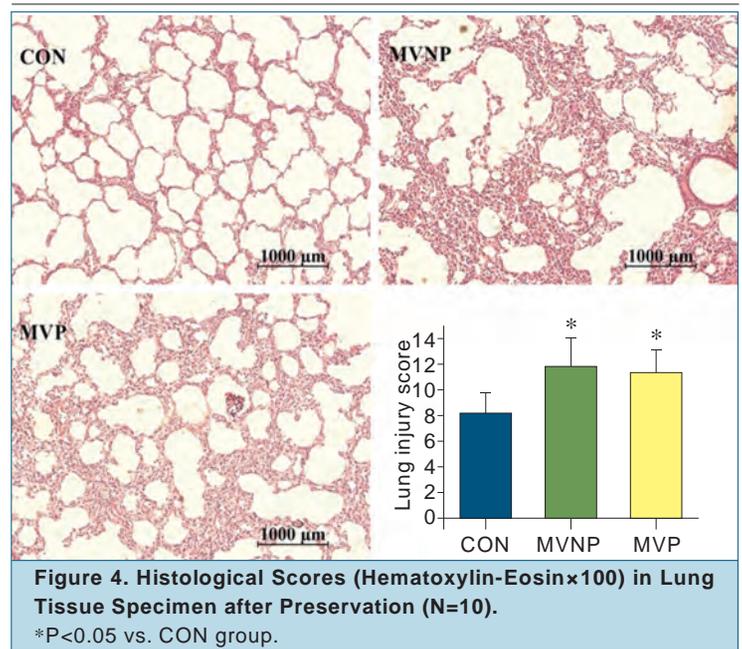


before pulmonary cold preservation in all groups of our study, so the protein was mostly flushed out from the pulmonary vascular. As a result, there were no significant differences in concentrations of TP in BALF of three groups, which could help us eliminate the interfere roles of TP in the surface tension reducing activity of surfactant.

Mechanical ventilation has been demonstrated to induce release of cytokines into the air-space (19). Furthermore, cytokines have been reported to inhibit surfactant protein A (SP-A), SP-B, SP-C synthesis by type II cells (26). Given the information, we could explain the changes of cytokines and surfactant in lungs ventilated without PEEP. Also, we could deduce that PEEP might alleviate the change of cytokines, subsequently increase the levels of active surfactant and surfactant proteins. Furthermore, the advantage of ventilation with PEEP was reflected in decreased W/D ratio and peak airway pressure when compared with lungs ventilated without PEEP.

The repeated distension and collapse of the lung contributes to more serious lung injury caused by mechanical ventilation. Formation of edema might be caused by stretch-induced pulmonary capillary leak (21).

We reflected the change of lung compliance via comparison of the peak airway pressure after 2 hours preservation, rather than determining the surface tension reducing activity of LA and lung compliance. Results showed that peak airway pressures of lungs ventilated without PEEP were higher than those of conventionally statically inflated lungs and lungs ventilated with PEEP. Moreover, although lungs ventilated with low VT and PEEP presented higher level of LA, they



didn't reveal significantly lower peak airway pressure compared with lungs statically inflated. Given these results, we could deduce that lung compliance might be associated with not only the lev-

el of lung surfactant but also the degree of lung injury. The difference between lungs ventilated with and without PEEP might be owed to the difference existed in the levels of active LA and hydrophobic SP-B and SP-C. Moreover, the higher levels of TNF- α and IL-6 in lungs ventilated without PEEP might further impair the surface tension reducing function of surfactant.

The limitation of the study was that we just explored the effects of mechanical ventilation on isolated lung grafts during cold ischemia stage without evaluation of lung function and in-

jury after reperfusion. Further studies are required to investigate how mechanical ventilation during cold ischemia phase will affect lung grafts after lung transplantation.

Overall, this study demonstrated that mechanical ventilation during cold ischemia phase with low VT and PEEP promoted the level of lung surfactant, but aggravated lung injuries with no effects observed on lung compliance.

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