原著論文

Ventilation-induced lung injury increases lung angiotensin II in rats

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Abstract

We hypothesized that lung injury and inflammation in ventilation-induced lung injury (VILI) are related to angiotensin (Ang) II. Adult male Sprague-Dawley rats were randomized to receive two ventilation strategies for 2 hours: 1) the high-volume zero PEEP group (HVZP) was ventilated with a high tidal volume (40 mL/kg) and zero positive end expiratory pressure (PEEP); 2) the low-volume with PEEP group (LVP) was ventilated with a low tidal volume (8 mL/kg) and PEEP (5 cmH_2O). Another group which did not receive ventilation served as the control. Total protein in bronchoalveolar lavage fluid (BALF) was significantly higher in HVZP group than in the control and LVP groups. Rats treated with HVZP ventilation had a significantly higher lung injury score than did the control and LVP groups. BALF macrophage inflammatory protein-2 (MIP-2) and lung Ang II were significantly higher in HVZP and LVP groups when compared with the control group. Lung Ang II correlated positively with MIP-2 in BALF in all study rats. These results indicate that local angiotensin system is involved in the pathogenesis of VILI and suggest that blockade of Ang II might have potential therapeutic implications in alleviating VILI.

Key words: angiotensin; bronchoalveolar lavage; macrophage inflammatory protein-2
Running title: Ventilation-induced lung injury and lung angiotensin II

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Introduction

Mechanical ventilation has been used to support acutely ill patients for several decades. Despite the life-saving potential of this support, it has several potential disadvantages and complications.[1] Mechanical ventilation with high tidal volumes causes hemorrhage and edema in lungs and activates inflammatory pathways. This process is referred to as ventilation-induced lung injury (VILI).[2,3] Research revealed a broader range of VILI, physiologically and histopathologically indistinguishable from acute lung injury. The spectrum of VILI includes disruption of endothelial and epithelial cells, increases in endothelial and epithelial permeability, and increases in pulmonary inflammatory mediators.[2,3]

Angiotensin (Ang) II can be generated locally in the lung tissue and may have autocrine and paracrine actions at the cellular level.[4] Ang II is also known to affect tissue neutrophil accumulation and to stimulate the release of a neutrophil chemoattractant from parenchymal cells.[5] However, little is known about the role of Ang II in VILI in vivo. We hypothesized that lung injury, lavage protein and cytokine contents, and interactions of these injurious processes in VILI are related to Ang II.

Materials and methods

Animal preparation

This experimental protocol was approved by the Institutional Animal Use Committee at Taipei Medical University and was performed with adult male Sprague Dawley rats weighing between 250 g and 300 g. Rats were maintained on a 12-h light-dark cycle with free access to food and water. The rats were anesthetized intraperitoneally with pentobarbital (50 mg/kg, Abbott, North Chicago, IL, USA). A tracheostomy was performed, and a 14-gauge plastic canula was inserted into the trachea. The animals were then ventilated with a volume-cycled ventilator (Small Animal Ventilator, Model SAR-830/AP; CWE Inc., Ardmore, PA, USA) for 2 h.

Rats were randomized to receive one of two ventilatory strategies: 1) the high-volume zero PEEP group (HVZP, n=8) was ventilated with a high tidal volume (V_T) and zero positive end expiratory pressure (PEEP) (V_T 40 mL/kg, RR 25 breaths/min, PEEP 0 cmH_2O, and FiO_2 0.21); 2) the low-volume with PEEP group (LVP, n=7) was ventilated with low V_T and PEEP (V_T 8 mL/kg, RR 40 breaths/min, PEEP 5 cmH_2O, and FiO_2 0.21). Another group which did not receive ventilation served as the control (n=7).

Pressure-volume curve measurement

Animals were deeply anesthetized with an intraperitoneal injection of pentobarbital (100 mg/kg) at 2 h after mechanical ventilation. The rats were exsanguinated via the abdominal aorta, and the tracheostomy was clamped for 3 min to allow absorption atelectasis to occur. A static deflation pressure-volume curve was obtained by inflating the lung with air to 25 cm H_2O pressure for 1 min and recording the maximal lung volume. Then the pressure was progressively decreased and held for 30 s at 20, 15, 10, 5, and 0 cm H_2O with the lung volume measured at each pressure. The measured volumes at all pressures were recorded, corrected for the compression volume of the system, and expressed as mL/kg

body weight.[6]

Bronchoalveolar lavage

After the static pressure-volume curve measurements had been performed, the chest was opened and the lung was removed intact from the animal with the tracheostomy tube in place and lavaged with 7 ml of 0.9% saline at 4°C for three times. Aliquots of the bronchoalveolar lavage fluid (BALF) from each animal were used to measure the total protein content with bovine serum albumin as the standard and macrophage inflammatory protein-2 (MIP-2) using an ELISA kit (R&D Systems, Minneapolis, MN, USA); values were expressed as mg/kg body weight and pg/mL, respectively.

Measurements of Ang II levels in lung tissue

Lung tissue was homogenized in lysis buffer and the supernatant solution was used for the measurements of Ang II levels with enzyme-linked immunosorbent assay kit (SPI-BIO, Massy Cedes, France). Protein content was measured by the Lowry method.[7]

Histological examination

Immediately after the bronchoalveolar lavage was finished, right lung was embedded in paraffin, stained with hematoxylin and eosin. Acute lung injury was scored according to the following four items: 1) alveolar congestion, 2) hemorrhage, 3) infiltration of neutrophils in the airspace or the vessel wall, and 4) thickness of the alveolar wall/hyaline membrane formation.[8] Each item was graded according to a five-point scale: 0, minimal (little) damage; 1, mild damage; 2, moderate damage; 3, severe damage; and 4, maximal damage.

Statistical analysis

The lung injury score data are given as the median (range), whereas other data are presented as the means ± SEM. Statistically significant differences were analyzed by ANOVA followed by Scheffe's post hoc analysis. Differences were considered significant at $p<0.05$.

Results

Pressure-volume curves

With regards to the deflation pressure-volume curves, control animals and rats treated with LVP had comparable pressure-volume curves. Treatment with HVZP ventilation exhibited lower lung volumes than those in the control and LVP groups, although the differences were not statistically significant (Fig. 1)

Lavage protein and MIP-2

Total protein content recovered from BALF was significantly higher in rats ventilated with the HVZP protocol (17.9±2.4 mg/kg) than in control animals (6.4±1.7 mg/kg) and in animals ventilated with the LVP protocol (7.0±1.2 mg/kg) ($p<0.01$; Fig. 2A). MIP-2 in BALF increased after mechanical ventilation, and the concentration was significantly higher in rats ventilated with the HVZP and LVP ventilation when compared with the control group ($p<0.01$; Fig. 2B).

Lung Ang II concentration

Rats ventilated with HVZP and LVP ventilation had significantly higher lung Ang II concentrations than did the control rats ($p<0.05$; Fig. 3). Lung Ang II levels correlated positively

with MIP-2 in BALF ($r=0.802$, $p<0.001$).

Histology

After 2 h of ventilation, rats treated with HVZP ventilation had a significantly higher lung injury score than did the control and LVP groups ($p<0.001$; Table 1). These findings were consistent with the changes of alveolar damage found in acute lung injury.

Discussion

Our in vivo model showed that mechanical ventilation at a high tidal volume decreased lung volume and increased total protein and MIP-2 contents in the BALF. These phenomena are consistent with the alterations of VILI. However, the exact mechanisms mediating the deleterious effects of mechanical ventilation remain unclear. The main finding of this study is that the development of VILI was associated with an increase of Ang II levels in lung tissues. These data indicate that high-volume ventilation may injure the lungs and suggest that the local tissue angiotensin system may play a role in VILI.

Pulmonary edema is a prominent feature of VILI in small-animal models.[2] The high protein content of the edema fluid suggests that it is due to increased permeability and implicates changes in both the epithelial and microvascular endothelial barriers. In this study, we found that 2 h of injurious mechanical ventilation (40 mL/kg V_T) led to severe pulmonary edema as assessed by the high concentrations of protein in BALF compared with that found in rats ventilated with 8 mL/kg V_T. Bronchoalveolar lavage levels of cytokines have been shown to be key mediators of injurious ventilation.[9,10] MIP-2 is a rodent homologue of human IL-8, and both are important mediators of neutrophil recruitment and activation. We found increased MIP-2 in BALF after mechanical ventilation, and the highest level was seen in the HVZP group. These findings are consistent with the observations of Ricard et al., who found high BALF protein concentrations and similar BALF MIP-2 concentrations in intact rats ventilated with a high volume than in animals ventilated with a low volume.[11] These results suggest that the mechanism of pulmonary edema formation may differ from the mechanisms of cytokine release and that cytokines might not be necessary for initiation of pulmonary edema.

The renin-angiotensin system plays an important role in the regulation of blood pressure, fluid, and electrolyte homeostasis.[12] Ang II is released from its precursor angiotensinogen by enzymatic processing with renin and then by angiotensin converting enzyme. Although angiotensinogen is mainly synthesized in the liver and secreted into the circulating blood, angiotensin formation has also been shown to occur in diverse tissues other than the liver.[13] Ang II may immediately stimulate bovine and human endothelial cells to release neutrophil chemoattractant substances and influence neutrophil accumulation.[5] Our study found increased lung tissue Ang II and BALF MIP-2 levels in the ventilated groups, and there was a linear relationship between lung tissue Ang II and BALF MIP-2 levels ($r=0.802$, $p<0.001$). These data imply that Ang II may be involved in the BALF MIP-2 production and then neutrophil infiltration into alveolar spaces in VILI.

In conclusion, these results show that Ang II is involved in the pathogenesis of VILI and

Fig. 1. *Average deflation pressure-volume curves in the control, HVZP, and LVP groups. Control and LVP groups had comparable pressure-volume curves. HVZP group exhibited lower lung volumes than those in the control and LVP groups, although the differences were not statistically significant*

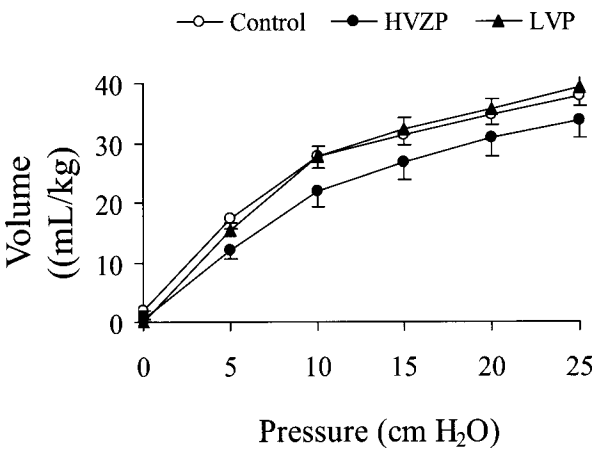


Fig. 2. *Total protein contents and MIP-2 in BALF.*
*(A) Total protein content recovered from BALF was significantly higher in the HVZP group than in the control and LVP groups (**p<0.01).*

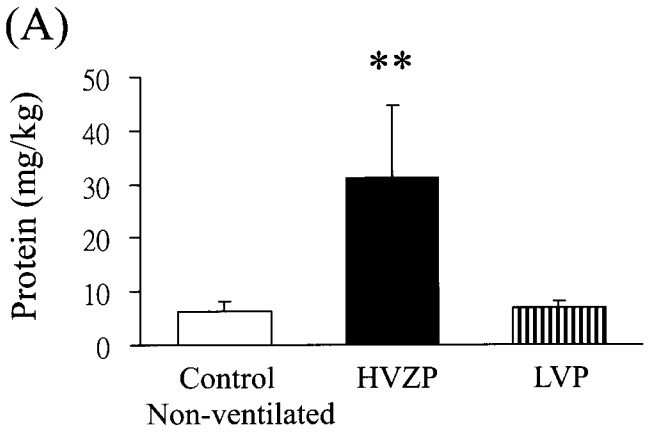
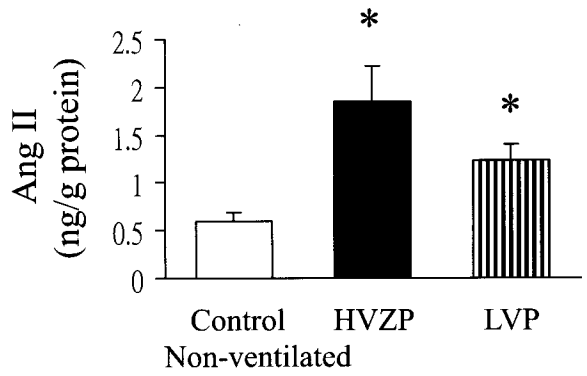


Fig. 3. *Lung Ang II levels. Rats treated with HVZP and LVP ventilation had significantly higher lung Ang II concentrations than did the control rats (*p<0.05).*

*(B) MIP-2 concentrations in BALF were significantly highein the HVZP and LVP groups when compared with the control group (**p<0.01).*



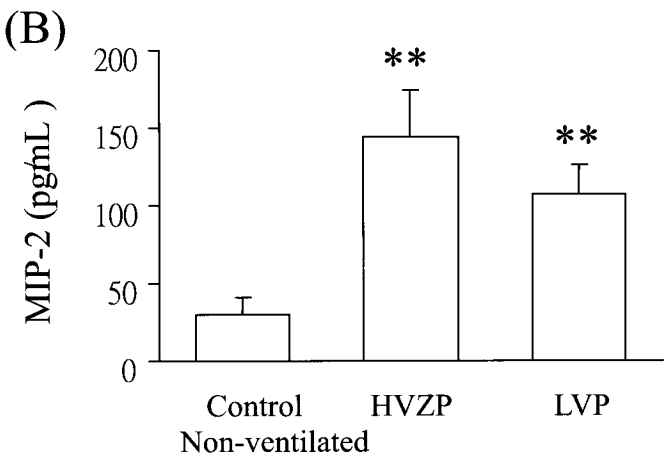


Table 1 *Lung injury scores (median [rang])*

Treatment	n	Alveolar congestion	Hemorrhage	Neutrophil infiltration	Alveolar wall thickness	Lung injury score
Control	7	0 (0-1)	1 (0-2)	1 (1-2)	0 (0-1)	2 (1-3)***
HVZP	8	3 (0-3)	3 (0-3)	3 (3-3)	0 (0-1)	9 (4-9)
LVP	7	2 (1-2)	2 (1-2)	2 (2-2)	0 (0-0)	6 (4-6)**

Values are expressed as the median (range).
***p<0.001 versus HVZP and LVP groups. **p<0.01 versus HVZP group.

suggest that blockade of Ang II might have potential therapeutic implications in alleviating VILI.

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