Role of Glutamine in the Mediation of E-cadherin, p120-catenin and Inflammation in Ventilator-induced Lung Injury

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Abstract

Background: Ventilator-induced lung injury (VILI) is commonly associated with barrier dysfunction and inflammatory reaction. Glutamine could ameliorate VILI, but its role has not been fully elucidated. This study examined the relationship between inflammatory cytokines (interleukin [IL]–6, tumor necrosis factor [TNF]–α, and IL–10) and adherens junctions (E-cadherin, p120-catenin), which were ameliorated by glutamine in VILI, both in vitro and in vivo.

Methods: For the in vivo study, 30 healthy C57BL/6 mice weighing 25–30 g were randomly divided into five groups with random number table (n = 6 in each group): control (Group C); low tidal volume (Group L); low tidal volume + glutamine (Group L + G); high tidal volume (Group H); and high tidal volume + glutamine (Group H + G). Mice in all groups, except Group C, underwent mechanical ventilation for 4 h. For the in vitro study, mouse lung epithelial 12 (MLE-12) cells pretreated with glutamine underwent cyclic stretching at 20% for 4 h. Cell lysate and lung tissue were obtained to detect the junction proteins, inflammatory cytokines, and lung pathological changes by the Western blotting, cytokine assay, hematoxylin and eosin staining, and immunofluorescence.

Results: In vivo, compared with Group C, total cell counts (t = −28.182, P < 0.01), the percentage of neutrophils (t = −28.095, P < 0.01), IL-6 (t = −28.296, P < 0.01), and TNF-α (t = −19.812, P < 0.01) in bronchoalveolar lavage (BAL) fluid, lung injury scores (t = −6.708, P < 0.01), and the wet-to-dry ratio (t = −15.595, P < 0.01) were increased in Group H; IL-10 in BAL fluid (t = 9.093, P < 0.01) and the expression of E-cadherin (t = 10.044, P < 0.01) and p120-catenin (t = 13.218, P < 0.01) were decreased in Group H. Compared with Group H, total cell counts (t = 14.844, P < 0.01), the percentage of neutrophils (t = 18.077, P < 0.01), IL-6 (t = 18.007, P < 0.01), and TNF-α (t = 10.171, P < 0.01) in BAL fluid were decreased in Group H + G; IL-10 in BAL fluid (t = −7.531, P < 0.01) and the expression of E-cadherin (t = −14.814, P < 0.01) and p120-catenin (t = −9.114, P < 0.01) were increased in Group H + G. In vitro, compared with the nonstretching group, the levels of IL-6 (t = −21.111, P < 0.01) and TNF-α (t = −15.270, P < 0.01) were increased in the 20% cyclic stretching group; the levels of IL-10 (t = 5.450, P < 0.01) and the expression of E-cadherin (t = 17.736, P < 0.01) and p120-catenin (t = 16.136, P < 0.01) were decreased in the 20% cyclic stretching group. Compared with the stretching group, the levels of IL-6 (t = 11.818, P < 0.01) and TNF-α (t = 8.631, P < 0.01) decreased in the glutamine group; the levels of IL-10 (t = −3.203, P < 0.05) and the expression of E-cadherin (t = −13.567, P < 0.01) and p120-catenin (t = −10.013, P < 0.01) were increased in the glutamine group.

Conclusions: High tidal volume mechanical ventilation and 20% cyclic stretching could cause VILI. Glutamine regulates VILI by improving cytokines and increasing the adherens junctions, protein E-cadherin and p120-catenin, to enhance the epithelial barrier function.

Key words: Adherens Junctions; Glutamine; Inflammatory Cytokines; Ventilation-induced Lung Injury

Introduction

Ventilator-induced lung injury (VILI) results from injury to the barrier dysfunction and inflammatory reaction...
caused by mechanical ventilation irregularly in general anesthesia and intensive care units.\textsuperscript{[1−3]} It is characterized by increased alveolar permeability and pulmonary edema. Major underlying mechanisms have indicated that the injury includes volutrauma due to alveolar overdistension, atelectrauma due to ventilation at low lung volumes, and biotrauma with the release of mediators in the lung.\textsuperscript{[4]} However, there are limited methods to prevent VILI, protect the lung issue, and avoid the damage.

Glutamine is a conditional essential amino acid and plays an important role in energy source for cell proliferation.\textsuperscript{[5]} Recent studies showed that glutamine possessed immunomodulatory function, which could attenuate the release of tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-8 caused by oxidative stress and prevent lung injury in acute respiratory distress syndrome (ARDS).\textsuperscript{[6−8]} Glutamine could ameliorate a loss of epithelial barrier function and epithelial proliferation caused by total parenteral nutrition by upregulating E-cadherin and β-catenin expression.\textsuperscript{[9]} Pretreatment of glutamine could be useful for VILI, but the exact mechanism remains unknown.

In this study, we used models of VILI in vivo and in vitro to explore the protective effect mechanisms of glutamine in VILI, which could be a novel manner of prevention and treatment in VILI.

**Methods**

**Cell culture, treatment with glutamine, and cyclic stretching**

Mouse alveolar epithelial (MLE-12) cells were purchased from the American Type Culture Collection (Manassas, VA, USA). MLE-12 cells were plated at a density of $5 \times 10^4$ cells/ml on culture dishes or collagen I-coated flexible bottom BioFlex plates (Flexcell International, McKeesport, PA, USA) in Dulbecco’s Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (Gibco, Grand Island, NY, USA) with 10% fetal bovine sera (Gibco) and 100 U/ml penicillin and 100 µg/ml streptomycin, maintained at 37°C in an atmosphere of 5% CO$_2$ in air, and pH 7.4 for 48 h. MLE-12 monolayers were serum-deprived for 2 h before experiments. For the study, glutamine (Cayman Chemical, Michigan, USA) (4 mmol/L) was added to the plate of confluent MLE-12 cells 60 min before cyclic stretching.\textsuperscript{[10,11]}

MLE-12 cells on collagen-coated flexible bottom BioFlex plates were exposed to cyclic stretching using a FX-5000T Flexercell Tension Plus system (Flexcell International) equipped with a 25-mm BioFlex loading station. After a 48 h culture, cell monolayers were mounted onto the Flexercell system with a cyclic stretching pattern of a frequency 0.5 Hz for 30 cycles/min and a stretch-to-relaxation relation of 1:1.\textsuperscript{[10,11]} Cyclic stretching was conducted at 20% of the change in the basement membrane surface area applied in a cyclic manner. These surface area changes correspond to 80% of total lung capacity.\textsuperscript{[12,13]} The cyclic stretching time was 4 h at 37°C in a humidified incubator containing 5% CO$_2$. A computer controlled all processes. Nonstretched cells were used as controls.

**Animals and grouping**

Thirty healthy C57BL/6 mice weighing 25–30 g were purchased from the Laboratory Animal Center of Shandong University. All animal procedures were reviewed and approved by the Laboratory Animal Ethics Committee of Shandong University. Mice, which were housed in a specific pathogen-free conditions and used in experiments at 8–9 weeks of age, were randomly divided into five groups (n = 6 in each group): control (Group C); low tidal volume (Group L); low tidal volume + glutamine (Group L + G); high tidal volume (Group H); and high tidal volume + glutamine (Group H + G).

**Experimental protocol**

Mice in Group C were not treated with anything. Mice in the other four groups were treated with mechanical ventilation for 4 h using an ALC-V8 animal ventilator (Shanghai Alcott Biotech Co., Shanghai, China). Mice were treated with a tidal volume of 7 ml/kg, a respiratory rate of 120 times/min, and positive end-expiratory pressure (PEEP) of 5 cm H$_2$O in Group L and Group L + G, and a tidal volume of 20 ml/kg, a respiratory rate of 40 times/min, and PEEP of 0 cm H$_2$O in Group H and Group H + G. The common ventilation parameters were set as follows: I/E ratio of 1:2, and a fraction of inspired oxygen of 21%. Mice in Group L + G and Group H + G were pretreated with glutamine (0.75 g/kg at an intravenous (IV) bolus) for 30 min before anesthesia.

Animals were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg) and ketamine (80 mg/kg). Anesthesia was maintained by infusion of pentobarbital at 15 mg/kg every 30 min through the tail vein. Muscle relaxation was maintained with pancuronium (2 mg·kg$^{-1}$·h$^{-1}$).\textsuperscript{[14]} Vital signs of mice were monitored with Mouse Ox pulse oximetry system (Starr Life Sciences Inc., USA).

**Tissue processing**

After 4 h of mechanical ventilation, mice were sacrificed, and the lung injury score was recorded. Acute lung injury (ALI) was scored according to the following four items: alveolar congestion, hemorrhage, infiltration or aggregation of neutrophils in the airspace or the vessel wall, and thickness of the alveolar wall/hyaline membrane formation. Each item was graded according to a 5-point scale: (0) minimal (little) damage; (1) mild damage; (2) moderate damage; (3) severe damage; and (4) maximal damage.\textsuperscript{[15]} Total score was the four items summed. The lungs were removed; the right lung upper lobe was quickly frozen in liquid nitrogen, which was used for the western blotting, and the remnant right lung tissue was fixed in 4% paraformaldehyde for 48–72 h for hematoxylin and eosin (H and E) staining. The left lung was lavaged to collect the bronchoalveolar lavage (BAL) fluid for the cytokine detection, and then used to calculate the pulmonary wet-to-dry (W/D) ratio to quantify the magnitude of pulmonary edema. After measuring the wet lung weight,
tissues were incubated in a 70°C incubator for 72 h to gain the dry weight.

**Bronchoalveolar lavage fluid**

The neck and chest of the mice were dissected, the trachea and right lung lobe were ligated. The lungs were washed with phosphate buffer saline (PBS) (0.3 ml) three times through the puncture needle, which was inserted into the upper of the trachea. The recovered lavage fluid was centrifuged at 1500 × *g* for 10 min at 4°C. The liquid supernatant was used for the detection of cytokines.

**Immunofluorescence and histological analysis**

For the in vitro study, after cyclic stretching, the plates with the cyclic stretching cells were washed with cold PBS, and the cells were fixed in 4% formaldehyde (10 min) and incubated in 1% bovine serum albumin (Solarbio, Beijing, China) for 1 h. The cells were then incubated with rabbit anti-E-cadherin polyclonal antibody overnight at 4°C. After washing with cold PBS, the cells were incubated for 1 h at 37°C with the secondary antibody (green) goat anti-rabbit IgG (H + L). 4’,6-diamidino-2-phenylindole (Solarbio) was used to stain cell nuclei (blue) for 3 min. Then, fluorescence microscopy was used to observe the protein changes due to cyclic stretching.

For the in vivo study, lung tissues blocked by embedding in paraffin were sectioned (5 mm thick) and stained with H and E according to the protocol, which was well established. Hematoxylin was applied for 5 min, and eosin was applied for 2 min. Then, the lung histology changes were observed through light microscopy.

**Cytokine assays**

*In vivo*, levels of TNF-α, IL-6, and IL-10 in BAL fluid samples (*n* = 6/group) were detected using enzyme-linked immunosorbent assay (ELISA) kits (Dakewe, Beijing, China) according to the manufacturer’s instructions. *In vitro*, after the experiments, cell culture media were collected and centrifuged at 1000 × *g* for 3 min, and the liquid supernatant was frozen at −80°C. The cytokines were quantified using the ELISA kits. The mRNA level of cytokines was measured using real-time polymerase chain reaction (PCR) according to the manufacturer’s instructions. Each sample was tested in triplicate. Total RNA was isolated with TRIzol (Invitrogen, Paisley, UK). cDNA was synthesized with TaKaRa PrimeScript™ reagent kit (TAKARA, Kusatsu, Japan). PCR reactions were performed with SYBR Premix Ex Taq II (Tli RNaseH Plus) (TAKARA). The threshold amplification cycle number was determined for each reaction within the linear phase of the amplification plot, and relative gene expression was determined using the 2^−ΔΔCt method. The values were normalized against the housekeeping gene GAPDH.

**Western blotting**

For the in vivo study, tissue fragments were lysed in radioimmunoprecipitation assay buffer supplemented with a cocktail of protease inhibitors. For the in vitro study, for the preparation of total cell extracts, monolayer cultures were washed in cold PBS and lysed in the appropriate amount of radioimmunoprecipitation assay (Beyotime, Shanghai, China) buffer supplemented with the protease inhibitor phenylmethylsulfonylfluoride (Beyotime). The lysate was collected, and the protein concentration was determined using a bichinchoninic acid protein assay kit. Equal amounts of protein were denatured and separated on 10% SDS-PAGE gels and then transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA) for electrophoresis at 100 V for 1 h. After blocking with skim milk (5%), the appropriate primary antibodies (Abcam, Cambridge, MA, USA) were added and incubated overnight at 4°C. The horseradish peroxidase-conjugated secondary antibody (Abcam) was added to the 37°C shaking incubator for 1 h. After sequential washing of membranes in T-PBS to remove excess secondary antibody, signals were detected by chemiluminescence using the ECL system. Relative band densities of the various proteins were measured from scanned films using ImageJ Software (National Institutes of Health, Bethesda, MD, USA).^{16}

**Statistical analysis**

Representative experiments from at least three independent experiments are shown. Statistical analysis was performed using the SPSS 19.0 statistics package (IBM, Armonk, NY, USA). All data are expressed as the mean ± standard deviation. Statistical differences were assessed using the Student’s *t*-test. *P* < 0.05 was considered statistically significant.

**Results**

**Acute lung injury caused by high tidal volume mechanical ventilation in mice**

Mice treated with high tidal volume mechanical ventilation for 4 h could lead to ALI. Compared with Group C, total cell counts [Figure 1a] and the percentage of neutrophils [Figure 1b] in BAL fluid were higher in Group H (total cell counts, *t* = −28.182, *P* < 0.01; percentage of neutrophils, *t* = −28.095, *P* < 0.01) and were not significantly changed in Group L (total cell counts, *t* = −2.175, *P* = 0.055; percentage of neutrophils, *t* = −1.927, *P* = 0.083). After treatments, compared with Group C, lung injury scores and the W/D ratio [Table 1] were measured using the procedures mentioned above, were increased in Group H (lung injury scores, *t* = −6.708, *P* < 0.01; ratio of wet/dry, *t* = −15.595, *P* < 0.01), and were not significantly changed in Group L (lung injury scores, *t* = −1.746, *P* = 0.124). Compared with Group C and Group L, H and E staining showed that alveolar congestion, infiltration or aggregation of neutrophils in the airspace or the vessel wall, and thickening of the alveolar wall were caused in Group H, and H and E staining in Group L was not significantly changed [Figure 2].

**Cytokines and adherens junctions, E-cadherin and p120-catenin, change caused by high tidal volume mechanical ventilation in mice**

After mechanical ventilation, level of cytokines in BAL fluid was measured using ELISA. Compared with Group C,
the level of IL-6 [Figure 3a] and TNF-α [Figure 3b] in BAL fluid was increased in Group H (IL-6, t = −28.296, P < 0.01; TNF-α, t = −19.812, P < 0.01) and was not significantly changed in Group L (IL-6, t = −1.910, P = 0.085; TNF-α, t = −2.027, P = 0.070). While the level of IL-10 [Figure 3c] in BAL fluid was reduced in Group H (t = 9.093, P < 0.01), it was not significantly changed in Group L (t = 1.509, P = 0.162). The barrier function proteins also changed after the mechanical ventilation in mice. Compared with Group C [Figure 4], the expression of E-cadherin and p120-catenin was decreased in Group H (E-cadherin, t = 10.044, P < 0.01; p120-catenin, t = 13.218, P < 0.01) and was not significantly changed in Group L (E-cadherin, t = 1.156, P = 0.312; p120-catenin, t = 1.298, P = 0.264).

Glutamine can alleviate inflammatory and barrier function caused by high tidal volume mechanical ventilation in mice

Glutamine (0.75 g/kg at an intravenous bolus) was pretreated for 30 min before mechanical ventilation in Group L + G and Group H + G. We found that glutamine could alleviate the lung injury caused by high tidal volume mechanical ventilation. Compared with Group H, total cell counts [Figure 1a], the percentage of neutrophils [Figure 1b], and the levels of IL-6 [Figure 3a] and TNF-α [Figure 3b] in BAL fluid decreased in Group H + G (total cell counts, t = 14.844,
with Group C; p120-catenin expressions; (b) E-cadherin and p120-catenin relative intensity in mice were normalized to GAPDH expression. (a) Representative Western blotting of E-cadherin and p120-catenin levels by the western blotting. Compared with the nonstretching group, the levels of IL-6 [Figure 5a and 5b] and TNF-α [Figure 5c and 5d] were increased in the 20% cyclic stretching group (IL-6, \( t = -10.171, P < 0.01 \); TNF-α, \( t = 10.171, P < 0.01 \)). From the H and E staining results, compared with Group H [Figure 2], the degree of lung injury was alleviated in Group H + G. At the same time, glutamine could enhance the barrier function by regulating the expression of adherens junctions E-cadherin and p120-catenin. Compared with Group H [Figure 4], the expression of E-cadherin and p120-catenin increased in Group H + G (E-cadherin, \( t = -14.814, P < 0.01 \); p120-catenin, \( t = -9.114, P < 0.01 \)).

**Twenty percent cyclic stretching mediated the downregulation of E-cadherin and p120-catenin and the level of interleukin-6, tumor necrosis factor-α and upregulation of interleukin-10**

MLE-12 cells were randomly divided into three groups as follows: a sham group, with nonstretching; a stretching group, with 20% cyclic stretching for 4 h; and a glutamine group, pretreated with glutamine for 60 min before 20% cyclic stretching. The protein level of cytokines was detected by ELISA: Enzyme-linked immunosorbent assay. Compared with the nonstretching group, the levels of IL-10 [Figure 5c and 5d] were increased in the 20% cyclic stretching group (IL-10, \( t = -7.531, P < 0.01 \); TNF-α, \( t = -13.567, P < 0.01 \); p120-catenin, \( t = 16.136, P < 0.01 \)). With immunofluorescence, we observed that the distribution of E-cadherin was more limited under microscope in the stretching groups than that in the nonstretching group [Figure 7].

**Glutamine can rescue the changing of cytokines and loss of E-cadherin and p120-catenin caused by 20% cyclic stretching**

Glutamine (4 mmol/L) was pretreated for 60 min before 20% cyclic stretching. Compared with the stretching group, the levels of IL-6 [Figure 5a and 5b] and TNF-α [Figure 5c and 5d] decreased in the glutamine group (IL-6, \( t = 11.818, P < 0.01 \); TNF-α, \( t = 8.631, P < 0.01 \)). Compared with the stretching group, the levels of IL-10 [Figure 5e and 5f] and the expression of E-cadherin and p120-catenin [Figure 6] were increased in the glutamine group (IL-10, \( t = 18.077, P < 0.01 \); E-cadherin, \( t = 17.736, P < 0.01 \); p120-catenin, \( t = 16.136, P < 0.01 \)). With immunofluorescence, we found that the distribution of E-cadherin was better in the glutamine group than that in the stretching group [Figure 7].

**Discussion**

In this study, we have shown that high tidal volume mechanical ventilation and 20% pathological cyclic mechanical stretch could cause VILI in mice and MLE-12 cells. We have also found that glutamine pretreatment could alleviate the injury degree of VILI by upregulating the expression of E-cadherin...
and p120-catenin, the level of IL-10, and downregulating levels of IL-6 and TNF-α in mice and MLE-12 cells.

Mechanical ventilation is an essential respiratory support in intensive care units and clinical anesthesia; if not used correctly, it can cause serious lung injury, including increasing permeability, barrier dysfunction, and pulmonary edema.\textsuperscript{17-20} The worsened damage could lead to ARDS.\textsuperscript{21}

In this study, we simulated VILI as the reference indicated...
in vivo and in vitro.\textsuperscript{22,23} Taken together, we found that high tidal volume mechanical ventilation could cause serious lung injury by lung injury scores measurement and HE staining.

The mechanisms of VILI are intricate, and studies have shown that they are related to inflammation and barrier function.\textsuperscript{24,25} There are limited ways to prevent and treat VILI; glutamine pretreatment may be a novel way to alleviate the degree of VILI, but it requires further study. Glutamine is an essential amino acid that could attenuate ALI caused by acid aspiration by regulating the cytokines IL-1\(\beta\), IL-6, IL-10, and TNF-\(\alpha\).\textsuperscript{26} The inflammation mechanisms of glutamine might be attenuation of inflammatory cell infiltration into the lung and the biorauma reduced from the cytokine responses. TNF-\(\alpha\) might play an important role leading to pulmonary inflammation mediated by neutrophil recruitment.\textsuperscript{27} Studies showed that the level of TNF-\(\alpha\) was associated with the incidence and severity of ARDS.\textsuperscript{28} Similarly, we found that the level of TNF-\(\alpha\) increased following VILI, and glutamine could attenuate the level to reduce the neutrophil infiltration.

Glutamine could regulate the immune function and reduce the inflammation reaction by regulating cytokines.\textsuperscript{7} The cytokine IL-6 is an important pro-inflammatory factor and was found to be involved in ALI together with other cytokines, TNF-\(\alpha\) and IL-1\(\beta\), in the innate immune response.\textsuperscript{29,30} The level of IL-6 could increase in VILI.\textsuperscript{31} Consistently, we found that mechanical ventilation could increase the level of IL-6. The cytokine IL-10 is an anti-inflammatory factor that could inhibit proinflammatory cytokines, such as IL-1\(\beta\), IL-6, and TNF-\(\alpha\).\textsuperscript{32} Anti-inflammatory factors and pro-inflammatory factors are dynamically balanced in our body.\textsuperscript{33} When the body confronts injury and infection, the dynamic balance is destroyed, and the anti-inflammatory factors decrease as the other factors increase. In our study, we found that mechanical ventilation could destroy the dynamic immune balance to decrease the level of IL-10 and increase the levels of IL-6 and TNF-\(\alpha\). Glutamine pretreatment could restore the balance by upregulating the level of IL-10 and downregulating the levels of IL-6 and TNF-\(\alpha\).

VILI could cause barrier dysfunction to regulate the adherens junctions and tight junctions.\textsuperscript{10,31,32,34} We previously reported that high tidal volume mechanical ventilation could destroy the barrier function by activating c-Src kinase to downregulate the tight junction occludin.\textsuperscript{22} There are not many studies on whether adherens junctions take part in VILI, and thus, this requires further study. In this study, we assumed E-cadherin and p120-catenin participated in VILI and found that glutamine could attenuate the VILI through upregulating the adherens junctions of E-cadherin and p120-catenin.

In vivo, we used whole lung tissue to study the VILI, while in vitro, we used mouse lung epithelial (MLE-12) cells. Both epithelial and endothelial cells were important in the lung tissue; the mechanisms of endothelial cells in VILI have been thoroughly studied, but the mechanisms of epithelial cells are not well known. We previously reported that the epithelial cells might take part in VILI and be important to protect the function barrier.\textsuperscript{22} Epithelial cells are known to be involved in alveolar barrier function, and the excessive expansion and collapse of cells could damage the integrity of the alveolar membrane.\textsuperscript{23} In this study, we focused on the role epithelial cells play in barrier function in vitro. The current method of stretching alveolar epithelial cells using a stretch machine to simulate lung expansion and contraction is well-recognized.\textsuperscript{135} Frequency and maximum amplitude of stretching has been reported to change cell permeability and barrier function.\textsuperscript{36} Experiments have confirmed that a stretch area expanded by more than 20–30% is defined as pathological stretch stimulation.\textsuperscript{131} In this study, we used 20% cyclic stretching to simulate VILI.

E-cadherin plays an important role in maintaining the integrity of epithelial cells,\textsuperscript{37} p120-catenin, which regulated the cell-cell adhesion by interaction of E-cadherin and p120-catenin, could not only increase the E-cadherin internalization from the cell surface but also decrease its recycling back to the cell surface.\textsuperscript{38} p120-catenin binding E-cadherin could prevent the endocytosis and degradation of E-cadherin.\textsuperscript{11} The loss of p120-catenin and E-cadherin may weaken the adhesive forces between cells and barrier dysfunction. In our study, we found that E-cadherin and p120-catenin expression decreased following both 20% cyclic stretching and high tidal volume.
mechanical ventilation. Glutamine has been proven to improve the barrier function resulting in a redistribution of intracellular β-catenin and the loss of E-cadherin.[9] In this study, glutamine had beneficial effect to alleviate VILI by increasing the expression of E-cadherin and p120-catenin to enhance the barrier function.

The current in vivo and in vitro studies using high mechanical ventilation and cyclic stretching showed that glutamine could alleviate the degree of VILI by regulating the inflammatory factors together with barrier function proteins. Immunofluorescence examination showed that glutamine improved the distribution of E-cadherin with cyclic stretching. Results from HE staining, lung injury score and W/D ratio in mice showed that glutamine could ameliorate pulmonary edema and alleviate alveolar hemorrhage, inflammatory cell infiltration and destroy pulmonary architecture.

In conclusions, this study provides evidence for the novel way of glutamine to protect against VILI. Glutamine ameliorated VILI by improving the cytokines and increasing the adherens junction protein E-cadherin and p120-catenin to enhance the epithelial barrier. The exact mechanisms of glutamine in VILI require further study and the protective effects of glutamine pretreatment for lung injury also require further clinical studies. This approach would be a novel manner to prevent and treat with VILI.

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Conflicts of interest
The authors declare that they have no competing interests.

References
1. Ngiam N, Kavanagh BP. Ventilator-induced lung injury: The role of gene activation. Curr Opin Crit Care 2012;18:16-22. doi: 10.1097/MCC.0b013e32834e7d00.
2. Liu D, Geng Z, Zhai W, Wang H, Chen Y, Liang J, et al. 15-deoxy-Delta12,14-prostaglandin J2 ameliorates endothoxin-induced acute lung injury in rats. Chin Med J 2014;127:815-20. doi: 10.3760/cma.j.issn.0366-6999.20131079.
3. Li H, Wu Z, Feng D, Gong J, Yao C, Wang Y, et al. BML-111, a lipoxin receptor agonist, attenuates ventilator-induced lung injury in rats. Shock 2014;41:311-6. doi: 10.1097/SHK.0000000000000104.
4. Kneyber MC, Zhang H, Slutsky AS. Ventilator-induced lung injury. Similarity and differences between children and adults. Am J Respir Crit Care Med 2014;190:258-65. doi: 10.1164/rccm.201401-0168CP.
5. Ahmad S, White CW, Chang LY, Schneider BK, Allen CB. Glutamine protects mitochondrial structure and function in oxygen toxicity. Am J Physiol Lung Cell Mol Physiol 2001;280:L779-91. doi: 10.1152/ajplung.2001.280.L779.
6. Coëffier M, Miralles-Barrachina O, Le Pessot F, Lalaude O, Daveau M, Lavoine A, et al. Influence of glutamine on cytokine production by human gut in vitro. Cytokine 2001;13:148-54. doi: 10.1006/cyto.2000.0813.
7. Singleton KD, Serkova N, Beecky VE, Wischmeyer PE. Glutamine attenuates lung injury and improves survival after sepsis: Role of enhanced heat shock protein expression. Crit Care Med 2005;33:1206-13. doi: 10.1097/01.CCM.0000166357.10996.8A.
8. Zhang Y, Zhou L, Zhou Y, Dião C, Han L, Yijie N, et al. Glutamine ameliorates mucosal damage caused by immune responses to duck plague virus. Vaccine 2017;35:8267-72. doi: 10.1016/j.vaccine.2017.06.042.
9. Feng Y, Sun X, Yang H, Teitelbaum DH. Dissociation of E-cadherin and beta-catenin in a mouse model of total parenteral nutrition: A mechanism for the loss of epithelial cell proliferation and villus atrophy. J Physiol 2009;587(Pt 3):641-54. doi: 10.1113/jphysiol.2008.162719.
10. Dai CY, Dai GF, Sun Y, Wang YL. Loss of p120 catenin aggravates alveolar edema of ventilation induced lung injury. Chin Med J 2013;126:2918-22. doi: 10.3760/cma.j.issn.0366-6999.20122343.
11. Wang Y, Minshall RD, Schwartz DE, Hu G. Cyclic stretch induces alveolar epithelial barrier dysfunction via calpain-mediated degradation of p120-catenin. Am J Physiol Lung Cell Mol Physiol 2011;301:L197-206. doi: 10.1152/ajplung.00048.2011.
12. Kaufman CD, Geiger RC, Dean DA. Electroporation- and mechanical ventilation-mediated gene transfer to the lung. Gene Ther 2010;17:1098-104. doi: 10.1038/gt.2010.57.
13. Geiger RC, Kaufman CD, Lam AP, Budinger GR, Dean DA. Tubulin acetylation and histone deacetylase 6 activity in the lung under cyclic load. Am J Respir Cell Mol Biol 2009;40:76-82. doi: 10.1165/rcmb.2007-0307OC.
14. Ding N, Wang F, Xiao H, Xu L, She S. Mechanical ventilation enhances HMGB1 expression in an LPS-induced lung injury model. PLoS One 2013;8:e74633. doi: 10.1371/journal.pone.0074633.
15. Huang CS, Kawamura T, Lee S, Tochigi N, Shigemura N, Buchholz BM, et al. Hydrogen inhalation ameliorates ventilator-induced lung injury. Crit Care 2010;14:R234. doi: 10.1186/cc8939.
16. Gu C, Liu M, Zhao T, Zhai L, Wang Y. Recombinant human annexin A5 can repair the disrupted cardiomyocyte adherens junctions in endotoxemia. Shock 2015;44:83-9. doi: 10.1097/SHK.0000000000000370.
17. Pinhu L, Whitehead T, Evans T, Griffiths M. Ventilator-associated lung injury. Lancet 2003;361:332-40. doi: 10.1016/S0140-6736(03)12329-X.
18. Slutsky AS, Ranieri VM. Ventilator-induced lung injury. N Engl J Med 2013;369:2126-36. doi: 10.1056/nejmci1400293.
19. dos Santos CC, Slutsky AS. The contribution of biophysical lung injury to the development of biotrauma. Annu Rev Physiol 2006;68:585-618. doi: 10.1146/annurev.physiol.68.072304.113443.
20. Randolph AG, Meert KL, O’Neil ME, Hanson JH, Luckett PM, Arnold JH, et al. The feasibility of conducting clinical trials in infants and children with acute respiratory failure. Am J Respir Crit Care Med 2003;167:1334-40. doi: 10.1164/rcrm.2002011‑175NOC.
21. Amato MB, Barbas CS, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi-Filho G, et al. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. N Engl J Med 1998;338:347-54. doi: 10.1056/NEJM199802193380602.
22. Zhao T, Liu M, Gu C, Wang X, Wang Y. Activation of e-src tyrosine kinase mediated the degradation of occludin in ventilator-induced lung injury. Respir Res 2014;15:158. doi: 10.1186/s12931-014-0158-2.
23. Villar J, Cabrera NE, Valladares F, Casula M, Flores C, Blanch L, et al. Activation of the Wnt/β-catenin signaling pathway by mechanical ventilation is associated with ventilator-induced pulmonary fibrosis in healthy lungs. PLoS One 2011;6:e23914. doi: 10.1371/journal.pone.0023914.
24. Wang S, Shi P, Wang Y. TRPA1 ion channels in vagal afferent nerves contribute to ventilator-induced lung injury in a rat model. Gen Physiol Biophys 2013;32:389-94. doi: 10.4149/gpb_2013045.
25. Dipaolo BC, Davidovich N, Kazanietz MG, Margulies SS. Rac1 pathway mediates stretch response in pulmonary alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 2013;305:L141-53. doi: 10.1152/ajplung.00298.2012.
26. Lai CC, Liu WL, Chen CM. Glutamine attenuates acute lung injury caused by acid aspiration. Nutrients 2014;6:3101-16. doi: 10.3390/nu6083101.
27. Strieter RM, Kunkel SL, Keane MP, Standiford TJ. Chemokines in lung injury: Thomas A. Neff lecture. Chest 1999;116:103S-10S. doi: 10.1378/chest.116.suppl.1.103S.

28. Larrick JW, Kunkel SL. The role of tumor necrosis factor and interleukin 1 in the immunoinflammatory response. Pharm Res 1988;5:129-39. doi: 10.1007/A1015904721223.

29. Ito K, Mizutani A, Kira S, Mori M, Iwasaka H, Noguchi T, et al. Effect of ulinastatin, a human urinary trypsin inhibitor, on the oleic acid-induced acute lung injury in rats via the inhibition of activated leukocytes. Injury 2005;36:387-94. doi: 10.1016/j.injury.2004.06.018.

30. Ware LB. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Semin Respir Crit Care Med 2006;27:337-49. doi: 10.1055/s-2006-948288.

31. Yildiz C, Palaniyar N, Otulakowski G, Khan MA, Post M, Kuebler WM, et al. Mechanical ventilation induces neutrophil extracellular trap formation. Anesthesiology 2015;122:864-75. doi: 10.1097/ALN.0000000000000605.

32. Scumpia PO, Moldawer LL. Biology of interleukin-10 and its regulatory roles in sepsis syndromes. Crit Care Med 2005;33:S468-71. doi: 10.1097/01.CCM.0000186268.53799.67.

33. Vlahos R, Bozinovski S, Jones JE, Powell J, Gras J, Lilja A, et al. Differential protease, innate immunity, and NF-kappaB induction profiles during lung inflammation induced by subchronic cigarette smoke exposure in mice. Am J Physiol Lung Cell Mol Physiol 2006;290:1931-45. doi: 10.1152/ajplung.00201.2005.

34. Liu M, Gu C, Wang Y. Upregulation of the tight junction protein occludin: Effects on ventilation-induced lung injury and mechanisms of action. BMC Pulm Med 2014;14:94. doi: 10.1186/1471-2466-14-94.

35. Zhao T, Zhao H, Li G, Zheng S, Liu M, et al. Role of the PKCa-c-Src tyrosine kinase pathway in the mediation of p120-catenin degradation in ventilator-induced lung injury. Respirology 2016;21(8):1404-1410. doi: 10.1111/resp.12858.

36. Cohen TS, Cavanaugh KJ, Margulies SS. Frequency and peak stretch magnitude affect alveolar epithelial permeability. Eur Respir J 2008;32:854-61. doi: 10.1183/09031936.00141007.

37. Reynolds AB. P120-catenin: Past and present. Biochim Biophys Acta 2007;1773:2-7. doi: 10.1016/j.bbamcr.2006.09.019.

38. Miyashita Y, Ozawa M. Increased internalization of p120-uncoupled E-cadherin and a requirement for a dileucine motif in the cytoplasmic domain for endocytosis of the protein. J Biol Chem 2007;282:11540-8. doi: 10.1074/jbc.M608351200.
摘要

背景：机械通气相关肺损伤与屏障功能障碍和炎症反应有关。谷氨酰胺能够改善机械通气造成的肺损伤，但是其作用机制尚不十分清楚。本研究通过体内实验和体外实验两方面，探讨谷氨酰胺改善机械通气造成肺损伤的机制。

方法：30只体重为25~30g的健康C57BL/6小鼠，利用随机数字表法随机分为5组，每组6只小鼠：对照组（C组）、小潮气量组（L组）、小潮气量+谷氨酰胺组（L+G组）、大潮气量组（H组）、大潮气量+谷氨酰胺组（H+G组）。C组小鼠自主呼吸，其余四组小鼠进行机械通气4小时。肺泡上皮细胞（MLE-12）和谷氨酰胺预处理过的MLE-12细胞均以20%的牵张幅度进行机械牵张4小时。收集细胞和肺组织，用免疫印迹法、细胞因子测定、苏木精-伊红（HE）染色和免疫荧光来检测黏连蛋白（E-cadherin、p120-catenin）、炎症细胞因子（IL-6、TNF-α、IL-10）和肺病理变化。

结果：体内实验，与C组比较，H组肺泡灌洗液中的细胞总数（t=-28.182, P<0.01）、中性粒细胞（t=-28.095, P<0.01）、IL-6（t=-28.296, P<0.01）、TNF-α（t=-19.812, P<0.01）、肺损伤评分（t=-6.708, P<0.01）和湿干比（t=-15.595, P<0.01）均增加，肺泡灌洗液中IL-10（t=9.093, P<0.01）、E-cadherin（t=10.044, P<0.01）和p120-catenin（t=13.218, P<0.01）表达均下降。与H组比较，H+G组的肺泡灌洗液中的细胞总数（t=14.814, P<0.01）、中性粒细胞（t=18.077, P<0.01）、肺泡灌洗液中IL-6（t=18.007, P<0.01）和TNF-α（t=10.171, P<0.01）表达均降低，而肺泡灌洗液中IL-10（t=-7.531, P<0.01）、E-cadherin（t=-14.814, P<0.01）、p120-catenin（t=-9.114, P<0.01）表达均增加。体外实验，与对照组比较，机械牵张组IL-6（t=-21.111, P<0.01）和TNF-α（t=-15.270, P<0.01）表达增加，IL-10（t=-5.450, P<0.01）和E-cadherin（t=17.736, P<0.01）表达增加，p120-catenin（t=16.136, P<0.01）表达下降。与机械牵张组比较，谷氨酰胺预处理组的IL-6（t=11.818, P<0.01）、TNF-α（t=8.631, P<0.01）表达下降，IL-10（t=-3.203, P<0.05）、E-cadherin（t=-13.567, P<0.01）、p120-catenin（t=-10.013, P<0.01）表达增加。

结论：大潮气量机械通气和幅度为20%的机械牵张可能导致机械通气相关肺损伤。谷氨酰胺通过改善细胞因子和增加黏附连接蛋白E-cadherin和p120-catenin来改善机械通气肺损伤，增强屏障功能。

谷氨酰胺在机械通气肺损伤中调节E-钙黏素、p120连环蛋白及炎症的作用