

## Original Article

# Protective effects of low tidal volume mechanical ventilation associated with WISP1 -TRL4 signaling pathway in a sepsis model of mice

Xi Jiang<sup>1,2</sup>, Shuqing Jin<sup>1</sup>, Xibing Ding<sup>1</sup>, Yao Tong<sup>1</sup>, Lingyu Wang<sup>1</sup>, Zhixia Chen<sup>1</sup>, Quan Li<sup>1,3</sup>

<sup>1</sup>Department of Anesthesiology, East Hospital, Tongji University School of Medicine, Shanghai, P.R. China;

<sup>2</sup>Department of Anesthesiology, Peking University Third Hospital, Beijing 100191, P.R. China; <sup>3</sup>Department of Anesthesiology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen 518116, P.R. China

Received June 26, 2015; Accepted May 19, 2016; Epub August 15, 2018; Published August 30, 2018

**Abstract:** Purpose: Sepsis is an independent risk factor of acute lung injury (ALI). The use of low tidal volume (LTV) is considered to contribute to decreasing ventilation induced lung injury (VILI) demonstrating protective effects. The purpose of our experiment is to study the time-course effect after low tidal volume mechanical ventilation and evaluate whether the protective effects of low tidal volume mechanical ventilation are associated with WISP1 -TRL4 signaling pathway in a sepsis model of mice. Materials and methods: Adult male wild type C57 mice were subject to mechanical ventilation with low tidal volume (6 ml/kg) and breathe rates 200/min. The cecal ligation puncture (CLP) model was applied to induce a polymicrobial abdominal infection. The mice were divided to 4 groups: 1) Control group; 2) LTV group; 3) CLP group; 4) CLP+LTV group. Lung tissues were harvested to detect the Evans blue album (EBA) permeability, wet/dry ratio, histological and morphometric analysis, as well as The WNT1 inducible signaling pathway protein (WISP1) and Toll-like receptor 4 (TLR4) protein and mRNA expression. Serum and bronchoalveolar lavage fluid (BALF) were obtained to analysis proinflammatory cytokines (interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$ ). Results: A significant increase in the EBA permeability, wet/dry ratio, IL-6 and TNF- $\alpha$  protein levels in serum and BALF was seen in CLP-treated mice compared with the control mice. The low tidal volume contributed to the decrease of lung injury from CLP-treated mice. The decrease was the most significant at the 4<sup>th</sup> hour while receded with time prolonged. The expression of WISP1 and TLR4 protein and mRNA were synergistically down-regulated when LTV mechanical ventilation was treated. Conclusion: After ventilation with LTV for 1 h, 2 h, 4 h, 8 h, 12 h the impairment for the lung of the sepsis mice are gradually improved while aggravate with time prolonged. At the 4<sup>th</sup> hour, the protective effect of low tidal volume to sepsis-induced lung injury is the most obvious. The latent mechanism that the low tidal volume ventilation contributes to the decrease lung injury in the sepsis-induced ALI model, which is partially associated with WISP1 -TLR4 signaling pathway.

**Keywords:** Low tidal volume, mechanical ventilation, sepsis, WISP1, TLR4

## Introduction

Sepsis was first described by Hippocrates in 400 BC, which is a common and severe condition with great mortality [1, 2]. Martin et al have reported that between 1979 and 2000, there was an annualized increase in the incidence of sepsis of 8.7 percent, from about 164,000 cases (82.7 per 100,000 population) to nearly 660,000 cases (240.4 per 100,000 population) in the United States. Although the in-hospital mortality rate due to sepsis fell from 27.8

percent to 17.9 percent in approximately 20 years, but the total number of deaths continued to increase [3]. Thus, sepsis is life-threatening and desperate situation that is of pivotal importance which needs improving therapy.

Literatures indicated that Sepsis is an independent risk factor of acute lung injury/acute respiratory distress syndrome (ALI/ARDS). When the septic focus is extrapulmonary, it constitutes 33% of indirect ALI/ARDS [5, 6, 13]. The intravenous infusion of Mesenteric lymph collected

from intraperitoneally infected rats to healthy rats showed significant IL-6, TNF- $\alpha$  and NF- $\kappa$ B as well as TLR4 expression contrary to control rats which demonstrated sufficient induction of acute lung injury [33]. Endotoxin can induce endothelial cell injury, which can increase permeability of endothelial cells and cytokine release, which is an important feature of both ALI/ARDS and sepsis [5, 14]. The Pathogenesis of ALI is explained by injury to both the vascular endothelium and alveolar epithelium [7, 20], characteristic of acute hypoxemic respiratory failure and bilateral pulmonary infiltrates that are not attributed to left atrial hypertension [8, 10]. CLP-induced sepsis caused MOF accompanying histopathological changes characteristic for acute lung injury. Such pathological changes of the lung result in the respiratory failure seem to be the most critical problem causing death of the patients [57]. Mechanical ventilation (MV) is an important supportive strategy for patients with ALI/ARDS and is essential for the management of many patients with sepsis-induced lung injury. However, MV can be deleterious to increases in vascular permeability leading to alveolar flooding, particularly at high tidal volumes, a phenomenon known as ventilator-induced lung injury (VILI). Injurious ventilation is considered to be "second hit" for indirect ALI/ARDS patients [5, 6, 10, 12, 14].

Substantial clinical and animal studies have demonstrated that the use of low tidal volumes can contribute to decreasing VILI. In the year 2000, the ARDS Network trial published on the New England Journal of Medicine indicated that the use of low tidal volumes of 6 ml/kg as compared to high tidal volumes of 12 ml/kg can decrease the mortality rate of ARDS in adults [4]. The NIH NHLBI ARDS Network also reported crude mortality was lower for patients with ALI who received lower tidal volume ventilation compared with those who received higher tidal volume ventilation, but this was not a statistically significant trend (test for trend  $P = 0.19$ ) [9]. As result, the protective effect of the ventilation strategy with low tidal volume is recognized to those ALI/ARDS patients. Except for critically severe condition, low tidal volume can be beneficial to surgery patients as well. In a meta-analysis including seventeen recent clinical trials among surgical and critically ill patients without lung injury, the protective ventilation group had a lower incidence of ALI (RR

0.27, 95% CI 0.12-0.59) and lung infection (RR 0.35, 95% CI 0.25-0.63); however, application of protective ventilation did not affect atelectasis (RR 0.76, 95% CI 0.33-1.37) or mortality (RR 1.03; 95% CI 0.67-1.58) compared with conventional ventilation [19]. The similar conclusion was demonstrated of beneficial effect of low tidal volume ventilation by Wolthuis et al [21] and Severgnini et al [22] team. In terms of animal studies, Chun Pan et al discovered in an indirect ALI rat model, compared with MV group (12 mL/kg), LV group (6 ml/kg) significantly reduced LPS-induced expression of ET-1 level ( $113.79 \pm 7.33$  pg/mL vs.  $152.52 \pm 12.75$  pg/mL,  $P < 0.05$ ) and TNF- $\alpha$  ( $3305.09 \pm 334.29$  pg/mL vs.  $4144.07 \pm 608.21$  pg/mL,  $P < 0.05$ ) [5]. And Jesu's Villar et al manifested that low tidal volume (6 ml/kg) plus 10 cm H<sub>2</sub>O PEEP could decrease gene expression and serum levels of cytokines in a sepsis-induced lung injury rat model [11]. However, the long-term mechanical ventilation leads to plenty of complications, for example, ventilator associated pneumonia and ventilator dependence et al The proper opportunity of extubation is not accepted universally. Additionally, the study of time-course effect of low tidal volume mechanical ventilation in sepsis-induced lung injury is rarely reported.

The WNT1 inducible signaling pathway protein (WISP1) is a cysteine-rich, secreted matricellular protein. Matricellular proteins are a subset of the extracellular matrix (ECM) proteins responsible for modulating cellular responses, such as cell growth, differentiation and survival, but do not exhibit structural function [23]. There is evidence that WNT signaling plays an important role in the inflammation in sepsis [34, 35]. Toll-like receptors (TLRs) detect host invasion by pathogens and constitute the key link between the innate and adaptive immune responses [11, 24], including TLR2 and TLR4. WISP1 appears to be a critical link between mechanical stretch and innate immunity in airway epithelium associated with lung diseases [31, 25, 26] and TLR4 appears to be important in ventilation induced lung injury (VILI) [11, 30]. According to Li's study, WISP1 as an endogenous signal acts through TLR4-signaling to increase alveolar-capillary permeability in VILI [31]. Nevertheless, activation of innate immunity through exogenous endotoxin has previ-

## The role of WISP1-TRL4 in the effects of mechanical ventilation in sepsis model

ously been shown to enhance VILI [27]. In aggregate, the purpose of this study is to investigate if the protective effect of low tidal volume ventilation works through WISP1-TRL4 signaling pathway under infectious circumstances, established upon a sepsis model of mice.

### Materials and methods

#### *Preparation of experimental animals*

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Tongji University. Adult male pathogen-free C57BL/6 mice (8-12 wks old) were obtained from the Laboratory Animal Research Center of Shanghai. All of them were housed standardized with 12:12 h dark light cycle with free access to water and food.

#### *Experiment design*

The mice were divided into four groups according to the protocol: (1) control: anesthetized, spontaneously breathing; (2) LTV groups: ventilated mice for 1 h, 2 h, 4 h, 8 h, 12 h with low tidal volume of 6 ml/kg plus zero positive end-expiratory pressure; (3) CLP groups: The cecal ligation and puncture (CLP) model was constructed to induce a systemic inflammatory response from a polymicrobial abdominal infection. After 24 hours, The CLP group mice were subject to continuously spontaneous breathing 1 h, 2 h, 4 h, 8 h, 12 h and (4) CLP+LTV groups: 24 hours after CLP operation, CLP mice were ventilated for 1 h, 2 h, 4 h, 8 h, 12 h respectively with low tidal volume.

#### *Cecal ligation and puncture*

Cecal ligation and puncture was reported by Wichterman et al [28] and was optimized with following minor modifications [29]. CLP, unlike surrogates like endotoxin injection, recreates sepsis progression most similarly to humans, with comparable hemodynamic and inflammatory profiles [55]. CLP-induced sepsis led to pathological changes of the lung result in the respiratory failure, the most critical problem causing death of the patients. So we performed CLP operation to mice to establish sepsis model. The mice were anesthetized by i.p. administration of 100 mg/kg ketamine and 10 mg/kg xylazine. After the abdominal fur was shaved, the midline laparotomy was conducted.

Before the cecum was exposed and ligated at half the distance between the distal pole and the base of the cecum, the feces in ileocecal valve were squeezed to the blind end, which was able to maintain bowel continuity and prevent infection transmission. At the mesentery and in the antimesenteric direction, puncture was implemented by a 21 G needle subsequently. The cecum was then returned to the peritoneal cavity and the operation for closing abdomen with a 4-0 sterile synthetic absorbable suture as well as peritoneum. The CLP model was then built after closing abdominal cavity. The mice were freed back to food and water in 24 h.

#### *VILI model*

In order to create ventilator induced lung injury (VILI) model, a time-cycled, volume-limited rodent ventilator was used (Model Inspira, Harvard Apparatus, South Natick, MA, USA). Five mice per group were subject to mechanical ventilation with the settings: tidal volume of 6 ml/kg, rate 200 breaths/minute and zero cm H<sub>2</sub>O PEEP, fraction of inspired oxygen: 0.21. Firstly, mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine, and were placed supine fixed on a stiff board. Anesthesia was maintained by supplementing with one-third of the initial dose of anesthetic agents regularly at approximately every 1 h during the experimental period. Followed by tracheostomy with a 18 G catheter inserted into the trachea, the mice were ventilated by connecting the ventilator with catheter. Body temperature was maintained at 30°C with a thermal insulation blanket (ALC-HTP, Alcott Biotech CO.LTD, Shanghai, China).

#### *Measurement of alveolar-capillary permeability*

Alveolar-capillary permeability was determined by using the Evans blue albumin (EBA) colorimetric assay. Evans blue (0.5% EB, Sigma-Aldrich, St Louis, MO, USA) was dissolved in Ca<sup>2+</sup>/Mg<sup>2+</sup>-free phosphate-buffered saline (PBS; Sigma-Aldrich), and conjugated to albumin (4% EBA). To evaluate alveolar-capillary permeability, EBA (25 mg/kg body weight) was injected via the internal jugular vein 1 h before lung harvesting and mice sacrificed. Blood samples were obtained from the right heart, and the pulmonary vasculature was subse-

## The role of WISP1 -TRL4 in the effects of mechanical ventilation in sepsis model

quently infused with 1 mL PBS. Then the right lung was isolated, weighed and stored in liquid nitrogen until these samples were used for EBA analysis. After the lung tissue was ground into a fine powder in 2 mL PBS, and incubated with an additional 1 ml/100 mg of formamide (Sigma-Aldrich) (18 h; 60°C), formamide extracts were centrifuged (15,000 g × 30 min; 4°C), and the centrifuged supernatants were collected to quantify lung EBA content by using a wavelength (620 nm) spectrophotometric method. Pulmonary EBA absorbance at 620 nm was measured. The EBA permeability index was calculated by dividing pulmonary EBA absorbance at 620 nm/g of lung tissue by plasma EBA absorbance at 620 nm [30, 31].

### *Measurement of lung wet-to-dry weight ratio*

The wet-to-dry weight (W/D) ratio of the lung was assessed to evaluate the severity of pulmonary edema. The left lung was removed at the end of experiment period. And it was weighed immediately. Torrefied in a drying oven at 65°C for 48 hrs, the lung was then reweighed and the W/D ratio was calculated.

### *Measurement of TNF- $\alpha$ and IL-6 cytokine in serum and BALF*

At the termination of each experiment, all animals were euthanized and blood samples were exsanguinated via the right heart with a heparinized 1 ml needle tubing. The supernatant after centrifugation (5,000 r × 15 min; 4°C) was extracted for subsequent analysis. 1 ml saline filled in needle tubing was perfused into the lung through the catheter to collect Bronchoalveolar lavage fluid (BALF). These data illuminate protein leak from the circulation into the broncho-alveolar space. Serum and BALF samples collected from each of the groups were separated and stored at -80°C prior to analysis. IL-6 and TNF- $\alpha$  levels were measured by using highly sensitive enzyme-linked immunosorbent assay (ELISA) kits that specifically contraposed the analysis of cytokines in murine samples (R&D Systems, Minneapolis, MN, USA).

### *Histological and morphometric analysis*

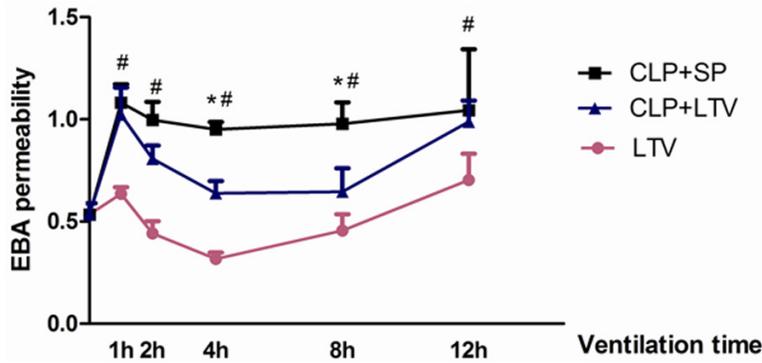
Right lung lobes were promptly fixed in 4% paraformaldehyde in PBS overnight at 4°C for fur-

ther analysis. Sections were examined by routine H&E staining. Edema, alveolar and interstitial inflammation, alveolar and interstitial hemorrhage, atelectasis, and hyaline membrane formation were each scored on a 0 to 4 point scale [32]: no injury = score of 0; injury in 25% of the field = score of 1; injury in 50% of the field = score of 2; injury in 75% of the field = score of 3; and injury throughout the field = score of 4. Five high-powered (400 ×) microscopic fields from each slide were analyzed. The sums of tissue slides were averaged to evaluate the severity of lung injury.

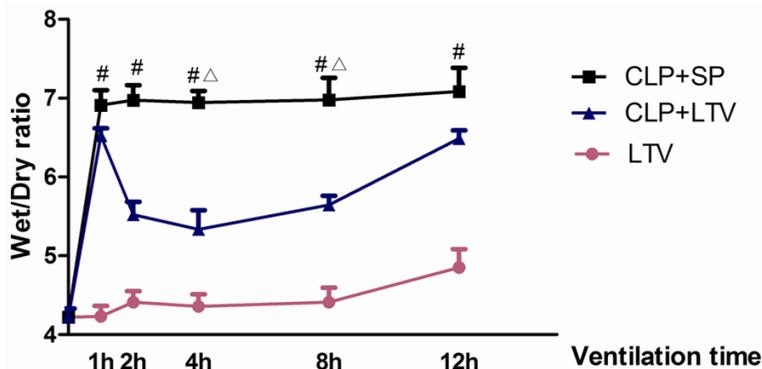
### *Western blot analysis*

According to the previous study [31], western blotting for WISP1 and mTLR4 was performed to analyze the protein expression. Proteins were extracted from frozen lung tissues and pyrolysed in radio-immunoprecipitation lysis buffer, which included Radio Immunoprecipitation Assay (RIPA), protease inhibitors (Roche, Mannheim, Germany) and phenylmethylsulfonyl fluoride (PMSF). The homogenates were centrifuged for 30 min at 12,000 rpm in 4°C. Supernatants of the tissues were collected, and protein concentrations were subsequently determined by standard BCA assay. Equivalent amounts of 50  $\mu$ g protein were separated by gel electrophoresis by using a 10% SDS-polyacrylamide electrophoresis gel after mixed with 6 × sodium dodecyl sulfate (SDS) loading buffer. Then dispersive proteins were transferred to a nitrocellulose membrane. Next, the membrane was blocked with 5% nonfat milk (WISP1) or 5% bovine serum albumin (BSA) (TLR4) for 1 h at room temperature. After that, nitrocellulose membranes were incubated overnight at 4°C with either rabbit polyclonal primary antibody against WISP1 (ab178547; Abcam, Hong Kong, China), rabbit polyclonal primary antibody against TLR4 (ab47093; Abcam, Hong Kong, China) or mouse monoclonal antibody against  $\beta$ -actin (ab8226; Abcam, Hong Kong, China). On the second day, the membranes were washed in TBST three times. Then the membranes were incubated with secondary antibody (926-32221 IRDye 680 anti-rabbit secondary antibody; Licor Biosciences, Lincoln, NE, USA) for 1 h at 37°C, followed by being rewashed in TBST three more times. Eventually, the membranes were scanned by an Odyssey image analysis system (Licor Bio-

## The role of WISP1-TRL4 in the effects of mechanical ventilation in sepsis model



**Figure 1.** EBA (*Evans blue albumin*) permeability of isolated mice lungs. CLP+SP = CLP mice with spontaneous breathing. CLP+LTV = CLP mice with low tidal volume mechanical ventilation. LTV = healthy mice with low tidal volume mechanical ventilation. \* $P < 0.05$  vs. CLP+LTV, # $P < 0.001$  vs. control. Graphed values represent the means  $\pm$  SD ( $n = 5$  animals per group).



**Figure 2.** Wet/Dry ratio of isolated mice lungs. CLP+SP = CLP mice with spontaneous breathing. CLP+LTV = CLP mice with low tidal volume mechanical ventilation. LTV = healthy mice with low tidal volume mechanical ventilation.  $\Delta P < 0.001$  vs. CLP+LTV, # $P < 0.001$  vs. control. Graphed values represent the means  $\pm$  SD ( $n = 5$  animals per group).

sciences). Western blots were quantitated by using Quantity One software (Bio-Rad, Foster City, CA, USA) and normalized to  $\beta$ -actin signal.

### Reverse transcription PCR (RT-PCR)

The mRNA expression levels of WISP1 and TLR4 were detected by Reverse transcription PCR (RT-PCR). Total RNA was prepared from lung tissues by using the TRIzol reagent (Sigma-Aldrich). Total RNA was then converted into cDNA by using a PrimeScript RT reagent kit (TaKaRa Bio Inc. Shiga, Japan). Primers for WISP1 amplification (137 bp) were: position 280 forward 5'-CAGCACCCTAGAGGAAACGA-3', position 394 reverse 5'-CTGGGCACATATCTTACAGCATT-3'. Primers for TLR4 amplification (126 bp) were: position 62 forward 5'-CACTGTTCTTCTCCTGCCTGAC-3', position 187 re-

verse 5'-TGGTTGAAGGAATGTCATC-3'. Primers for  $\beta$ -actin amplification (154 bp) were: position 163 forward 5'-GGCTGTATTCCCCTCCATCG-3', position 295 reverse 5'-CCAGTTGGTAACAATGCCATGT-3'. Integrated with 2  $\mu$ L of the chromogenic agent SYBR green, the product obtained from reverse transcription was amplified by the Premix Taq Version 2.0 assay (TaKaRa Bio Inc.). A 2% agarose gel was used to separate the total mRNA. The expression of mRNA was subjected to being detected by Image Lab software (Bio-Rad). And it was normalized to the  $\beta$ -actin signal as well.

### Statistical analysis

The statistical analysis method used in the current study for each analysis was presented as the mean  $\pm$  SD by using a one-way analysis of variance (ANOVA) and post hoc testing was performed with Bonferroni correction of the t-test. The individual studies performed throughout this work represent at least five independent studies. Power

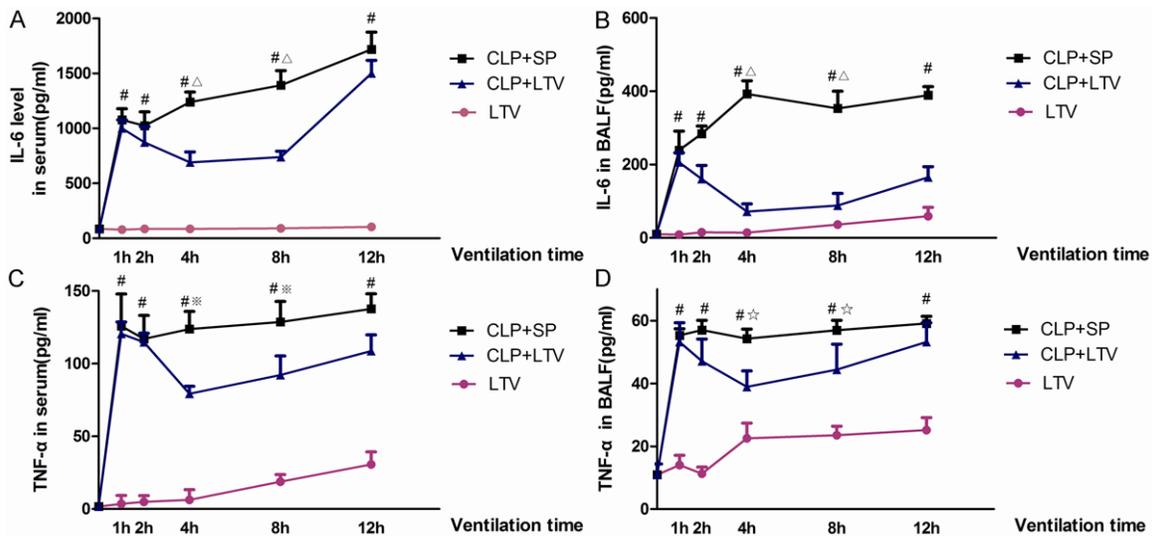
analyses were performed by using a Type I error probability of 0.05, with a power of 0.9, to determine the sample size necessary to reject the null hypothesis. All statistical analyses were carried out by using the GraphPad Prism 5.0 program.

## Results

### *Evans blue albumin permeability*

The EBA permeability reflects the damage degree of pulmonary capillary. When compared with control group, the EBA permeability of CLP-treated mice was increased significantly (CLP+SP 4 h vs. Control,  $0.95 \pm 0.04$  vs.  $0.53 \pm 0.06$ ,  $P < 0.001$ ). The low tidal volume contributed to the decrease of alveolar-capillary permeability in lung tissue from CLP-treated mice

## The role of WISP1-TRL4 in the effects of mechanical ventilation in sepsis model



**Figure 3.** IL-6 and TNF- $\alpha$  protein levels in serum and BALF. CLP+SP = CLP mice with spontaneous breathing. CLP+LTV = CLP mice with low tidal volume mechanical ventilation. LTV = healthy mice with low tidal volume mechanical ventilation. Graphed values represent the means  $\pm$  SD ( $n = 5$  animals per group).  $\Delta P < 0.001$  vs. CLP+LTV,  $\text{※} P < 0.01$  vs. CLP+LTV,  $\text{☆} P < 0.05$  vs. CLP+LTV,  $\text{\#} P < 0.001$  vs. control.

(CLP+SP 4 h vs. CLP+LTV 4 h,  $0.95 \pm 0.04$  vs.  $0.64 \pm 0.06$ ,  $P < 0.05$ ). And the decrease was more obvious with the ventilation time extending (CLP+LTV 1 h vs. CLP+LTV 4 h,  $1.02 \pm 0.13$  vs.  $0.64 \pm 0.06$ ,  $P < 0.01$ ). The decrease was the most significant at the 4<sup>th</sup> hour while receded with time prolonged. No statistical differences were evident between the LTV 4 h groups compared with control group ( $P > 0.05$ ) (Figure 1). And the same is true of the CLP+SP groups ( $P > 0.05$ ) (Figure 1).

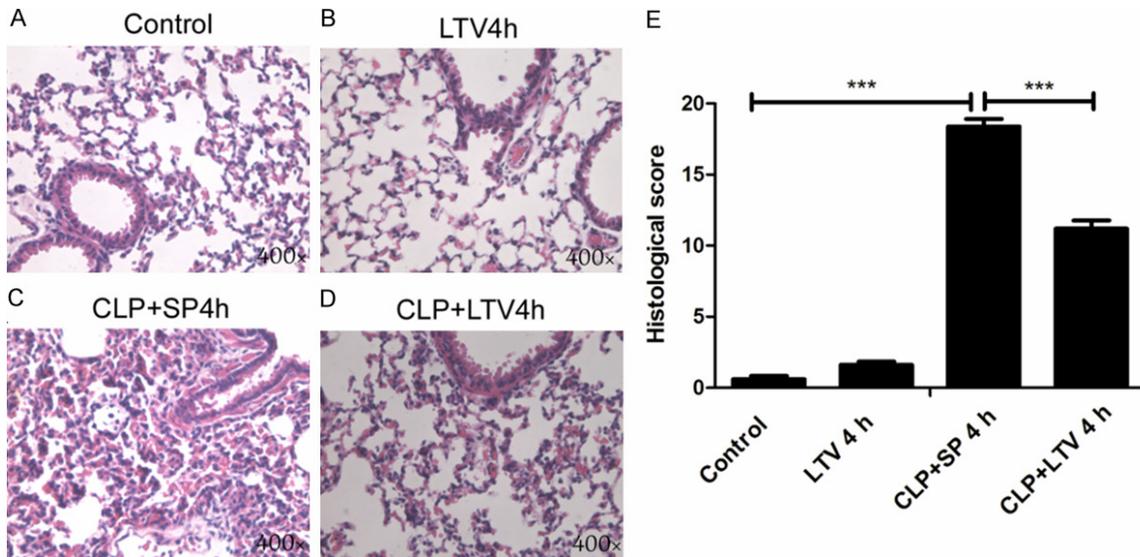
### W/D ratio of lung tissue

The wet-to-dry ratio represents the severity of pulmonary edema. When compared with control group, the wet-to-dry ratio of CLP-treated mice had a markedly increase (CLP+SP 4 h vs. Control,  $6.94 \pm 0.15$  vs.  $4.22 \pm 0.11$ ,  $P < 0.001$ ). The low tidal volume contributed to the decrease of wet-to-dry ratio in lung tissue from CLP-treated mice (CLP+SP 4 h vs. CLP+LTV 4 h,  $6.94 \pm 0.15$  vs.  $5.65 \pm 0.11$ ,  $P < 0.05$ ). And the decrease was more apparently observed with the ventilation time extending (CLP+LTV 1 h vs. CLP+LTV 4 h,  $6.52 \pm 0.09$  vs.  $5.65 \pm 0.11$ ,  $P < 0.01$ ). The decrease was the most significant at the 4<sup>th</sup> hour while receded with time prolonged. No statistical differences were evident between the LTV 4 h groups compared with control group ( $P > 0.05$ ) (Figure 1A). No statistical differenc-

es were evident between the CLP+SP groups ( $P > 0.05$ ) (Figure 2).

### IL-6 and TNF- $\alpha$ protein levels in serum and BALF

Serum and BALF samples were collected from mice in all the experiment groups. A significant increase in both IL-6 and TNF- $\alpha$  protein levels were seen in CLP-treated mice compared with the control mice (CLP+SP 4 h vs. Control: Serum IL-6,  $1239.55 \pm 92.04$  vs.  $86.30 \pm 24.83$ ,  $P < 0.001$  (Figure 3A); BALF IL-6,  $392.04 \pm 36.04$  vs.  $10.4790 \pm 4.32$ ,  $P < 0.001$  (Figure 3B); Serum TNF- $\alpha$ ,  $123.85 \pm 12.04$  vs.  $1.74 \pm 0.32$ ,  $P < 0.001$  (Figure 3C); BALF TNF- $\alpha$ ,  $54.27 \pm 3.04$  vs.  $10.95 \pm 3.44$ ,  $P < 0.001$  (Figure 3D). The low tidal volume contributed to the decrease of proinflammatory cytokines in serum and BALF from CLP-treated mice (CLP+SP 4 h vs. CLP+LTV 4 h: Serum IL-6,  $1239.55 \pm 92.04$  vs.  $691.57 \pm 95.04$ ,  $P < 0.001$  (Figure 3A); BALF IL-6  $392.04 \pm 36.04$  vs.  $71.74 \pm 21.04$ ,  $P < 0.001$  (Figure 3B); Serum TNF- $\alpha$ ,  $123.85 \pm 12.04$  vs.  $79.34 \pm 5.04$ ,  $P < 0.01$  (Figure 3C); BALF TNF- $\alpha$ ,  $54.27 \pm 3.04$  vs.  $38.96 \pm 5.04$ ,  $P < 0.05$  (Figure 3D). In the first 4 hours, the decrease of IL-6 and TNF- $\alpha$  were more obviously observed in the CLP groups proportional to the time (CLP+LTV 1 h vs. CLP+LTV 4 h: Serum IL-6,  $1000.44 \pm 76.55$  vs.  $691.57 \pm 95.04$ ,  $P <$



**Figure 4.** Histopathological and morphometric analysis of lung tissues. A-D. H&E staining of lung tissue samples of A. Control group; B. CLP+SP = CLP mice with spontaneous breathing; C. CLP+LTV = CLP mice with low tidal volume mechanical ventilation; D. LTV = healthy mice with low tidal volume mechanical ventilation. E. Histologic scores of lung injury in the groups. Graphs values represent the means  $\pm$  SD. Five microscopic fields from each slide were analyzed per group. \*\*\* $P < 0.001$ . All images were acquired using 400  $\times$  magnification.

0.01 (**Figure 3A**); BALF IL-6,  $206.31 \pm 26.13$  vs.  $71.74 \pm 21.04$ ,  $P < 0.01$  (**Figure 3B**); Serum TNF- $\alpha$ ,  $120.61 \pm 8.13$  vs.  $79.34 \pm 5.04$ ,  $P < 0.01$  (**Figure 3C**); BALF TNF- $\alpha$ ,  $53.22 \pm 6.13$  vs.  $38.96 \pm 5.04$ ,  $P < 0.05$  (**Figure 3D**). The decrease was the most significant at the 4<sup>th</sup> hour while receded with time prolonged. No statistical differences were evident between the LTV groups compared with control group ( $P > 0.05$ ).

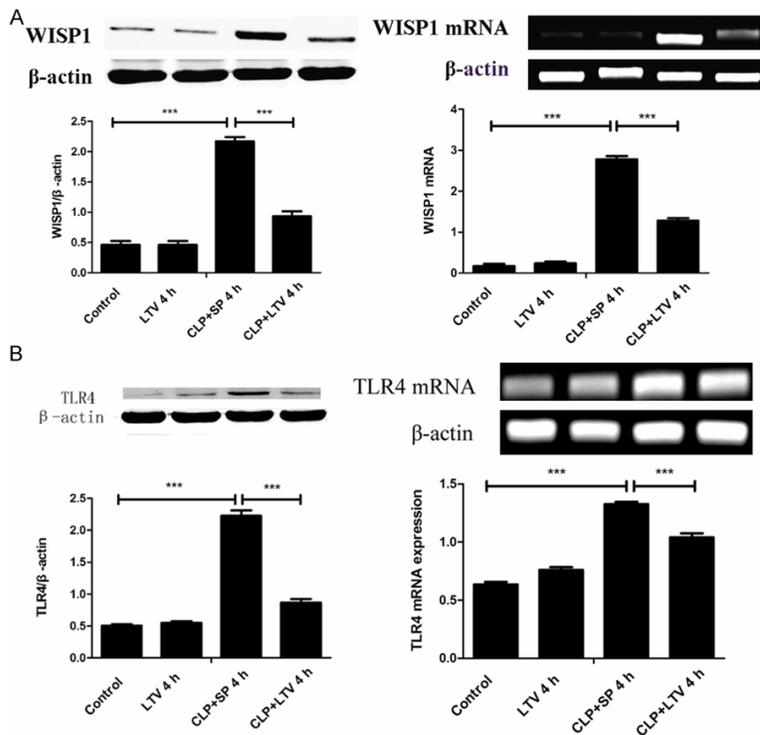
#### Histopathological examination of lung tissues

No abnormal histological alterations of the lung samples were observed in the control group and LTV 4 h group (**Figure 4A, 4B**). In the CLP-treated lungs, however, diffuse interstitial edema, hemorrhage and inflammatory cell infiltration were apparent (**Figure 4C**). When the CLP mice were introduced into following LTV mechanical ventilation, histopathology of lung samples isolated from these mice improved (**Figure 4D**). Consistent with these histological findings, the scoring after CLP treatment was significantly higher compared with controls (CLP+SP vs. control,  $18.40 \pm 1.14$  vs.  $0.6 \pm 0.55$ ,  $P < 0.001$ ) (**Figure 4E**) based on the histological scoring scheme that we utilized to analyze the severity of lung injury. The low tidal volume contributed to the decrease on pathology of lung injury from CLP-treated mice

(CLP+SP vs. CLP+LTV,  $18.40 \pm 1.14$  vs.  $11.2 \pm 1.30$ ,  $P < 0.001$ ) (**Figure 4E**).

#### Protein and mRNA expression of WISP1 and TLR4

WISP1 and TLR4 protein expressions were detected by western blot and mRNA expressions were detected by RT-PCR. Both the protein expression and mRNA expression of WISP1 and TLR4 were evidently increased seen in CLP-treated mice compared with the control mice (CLP+SP 4 h vs. Control: WISP1 protein,  $2.17 \pm 0.12$  vs.  $0.46 \pm 0.11$ ,  $P < 0.001$ ; WISP1 mRNA,  $2.78 \pm 0.14$  vs.  $0.17 \pm 0.09$ ,  $P < 0.001$ ; TLR4 protein,  $2.23 \pm 0.15$  vs.  $0.50 \pm 0.04$ ,  $P < 0.001$ ; TLR4 mRNA,  $1.33 \pm 0.03$  vs.  $0.64 \pm 0.04$ ,  $P < 0.001$ ) (**Figure 5A, 5B**). The low tidal volume contributes to the synergy decrease of the expression of WISP1 and TLR4 proteins expression and mRNA expressions from CLP-treated mice (CLP+SP 4 h vs. CLP+LTV 4 h: WISP1 protein,  $2.17 \pm 0.12$  vs.  $0.93 \pm 0.15$ ,  $P < 0.001$ ; WISP1 mRNA,  $2.78 \pm 0.14$  vs.  $1.28 \pm 0.11$ ,  $P < 0.001$ ; TLR4 protein,  $2.23 \pm 0.15$  vs.  $0.87 \pm 0.10$ ,  $P < 0.001$ ; TLR4 mRNA,  $1.33 \pm 0.03$  vs.  $1.04 \pm 0.06$ ,  $P < 0.001$ ) (**Figure 5A, 5B**). No statistical differences were evident between LTV 4 h groups compared with control groups.



**Figure 5.** Protective effects of low tidal volume mechanical ventilation for sepsis-induced acute lung injury model on WISP1 and TLR4 protein and mRNA expression levels. A. Representative blots and bar graph showing mean densitometry values of WISP1 protein and mRNA expression levels in mice lungs (n = 5 animals per group). B. Representative blots and bar graph showing mean densitometry values of TLR4 protein and mRNA expression levels in mice lungs (n = 5 animals per group). Graphed values represent the means ± SD. \*\*\*P < 0.001.

## Discussion

Our study demonstrated that low tidal volume ventilation contributes to the protective effects in sepsis-induced lung injury. The protective effects were the most significant at the 4<sup>th</sup> hour while receded with time prolonged. To our knowledge, this is the first report to claim the time-dependent protective effects of low tidal volume ventilation in the two-hit model via WISP1 -TLR4 signaling pathways. Our main findings include the following: 1) The low tidal volume ventilation doesn't induce lung injury in healthy lungs. 2) The sepsis after 24 h causes significant lung injury. 3) After ventilation with LTV for 1 h, 2 h, 4 h, the impairment for the lung of the sepsis mice got gradually improved, but deteriorated with time prolonged. 4) The latent mechanism that the low tidal volume ventilation contributes to the decrease lung injury in the two-hit model is associated with WISP1 -TLR4 signaling pathways.

Low tidal volume has long been advocated to be an important manipulation in managing ARDS patients [15, 16, 36] and normal lungs in surgery patients [17, 18], but the mechanisms involved are not well clarified. Recently, several studies proposed a consensus that the specific meaning of protective ventilation strategy represents 1) physiologic low VT values (6 to 8 ml per kilogram of predicted body weight). 2) moderate to high levels of PEEP of 6 to 8 cm of water and 3) recruitment maneuvers [18, 19].

Our findings that LTV decreases the permeability of alveolar-capillary of lung, the pulmonary edema level and cytokines (IL-6 and TNF-α) in serum and BALF in CLP mice suggest that low-VT strategy is less injurious in sepsis-induced ALI. A number of mechanisms are postulated as the following: 1) Lower VT is used to prevent overdistension and might be more lung

protective [4, 15, 49]. 2) Protective MV can modify gene expression of important components of the extracellular matrix and accelerate remodeling and repair of damaged lung tissue [51]. Nevertheless, ventilation with too low tidal volumes might increase lung injury by causing increased dead space ventilation, intrapulmonary shunting, hypoxia and hypercapnia [5, 40, 47, 48]. The increased respiratory rate leads to obvious respiratory acidosis in clinical ALI patients. However, Hans Fuchs indicated that oxygenation and lung protection were maintained at extremely low tidal volumes in association with very severe hypercapnia in a ARDS rabbit model [47]. And other studies also pointed out that hypercapnic acidosis itself induced by administration of carbon dioxide to the inspired air (so called therapeutic hypercapnia) protects the lung [52, 53]. We also found that after 4 hours of LTV mechanical ventilation, the protective effect of LTV receded. It is implied

that extubation in the early stage in clinical application is the better choice to avoid complications of long-term mechanical ventilation. This is consistent with Maes K et al's conclusion of 12 hours of controlled mechanical ventilation with tidal volume of 0.5 mL/100 g in rats with intraperitoneal injection of lipopolysaccharide augmented diaphragm interleukin-6 levels [56].

WISP1, secreted matricellular protein belongs to the CCN protein family, is expressed in epithelial and mesenchymal cells in several organs that have been associated with cell-cell adhesion, AII-to-ATI cell transition, proliferation, and epithelial-mesenchymal transition (EMT) and ECM deposition. If dysregulated, WISP1 may contribute to the initiation and progression of several lung diseases-particularly, lung cancer and fibrosis [37, 41]. The CCN proteins support adhesion and regulate migration, proliferation, survival and apoptosis of several cell types involved in inflammatory or fibrotic processes. Also, they could contribute to the recruitment of immune cells or angiogenesis, crucial processes involved in inflammatory response, whose expression is regulated by cytokines reciprocally [38]. Activation of Wnt/ $\beta$ -catenin signaling during lung injury, type 2 epithelial cells (AT2) promoted survival, migration, and differentiation toward an AT1-like phenotype. As consequence, the reduction of surface tension is decreased and the lung compliance is lost [44]. In Ding et al study, in vitro the LPS-treated RAW 264.7 cells were observed that WISP1 mRNA expression displays a time- and dose-effect relationship. And in vivo, WISP1 expressions in lung were found increased significantly, peaking at 24 h after CLP by using a cecal ligation and puncture model to simulate clinical sepsis patients which is consistent with our study [39]. Jesu's Villar et al demonstrated that Protein levels of WNT5A,  $\beta$ -catenin, and MMP7 in the lungs increased in animals with sepsis-induced ALI, protective MV (6 ml/kg) down-regulated the WNT/ $\beta$ -catenin signaling pathway and promoted lung recovery, which caters to the concept that WNT/ $\beta$ -catenin signaling pathway is modulated early during sepsis and ventilator-induced lung injury [50]. Konigshoff et al examined increased expression of the WNT target gene, WISP1, in hyperplastic AII cells in experimental lung fibrosis [42]. In general, these findings suggest that the

secreted WISP1 protein plays a critical role in the pathogenesis of lung fibrosis, inflammation and sepsis-induced ALI. Similar to our study, WISP1 can be a potential therapeutic target protein associated with acute lung injury in sepsis patients, and also could be the protective mechanism target of low tidal ventilation.

Michiel Vaneker et al manifested that MV induced-inflammation seemed at least partially TLR4 dependent, and cytokines (e.g., keratinocyte-derived chemokine and IL-6) significantly decreased in the plasma and lung tissues in TLR4 KO mice compared with the ventilated WT mice [43]. Jesu's Villar stated that MV with low VT plus PEEP attenuated sepsis-associated TLR-4 activation [11]. In Li's study, HTV-induced WISP1 coimmunoprecipitated with glycosylated TLR4 in sensitive A/J lung homogenates, which indicated that WISP1 acts as an adjuvant adaptor molecule that contributes to VILI in mice, most likely by modulating and/or amplifying TLR4-mediated cellular functions [31]. Our experiments also show that after LTV mechanical ventilation in CLP mice, both WISP1 and TLR4 expression in lung tissue were down-regulated.

The present study has several limitations. First, the limited facilities didn't endow us the ability to observe invasively or noninvasively the hemodynamic of sepsis-treated ventilation mice, but Chun's experiment revealed that LTV (6 ml/kg) induced the reduction of oxygen in blood, but it was comparable during the 5 h ventilation. And the values of PaCO<sub>2</sub> were comparable at baseline [5]. We think that our data could have important clinical implications of sepsis-induced ALI. Second, we didn't access PEEP over low tidal volume ventilation because of limited rodent ventilator settings. The use of low VT ventilation without PEEP (or very low levels of PEEP) promotes loss of lung aeration and atelectasis formation [45], as well as associated with recruitment and de-recruitment of unstable lung units referring to atelectrauma [19, 45, 46]. Further studies are needed to examine the effect of PEEP as well as recruitment maneuvers of complete protective ventilation strategy. Third, we didn't fully confirm that the WISP1 -TLR4 signaling pathway is involved in the experiment design. However, Ding et al have reported that the EBA permeability was decreased by WISP1 antibody ( $P <$

0.01) intratracheal instillation directly in CLP mice. White blood cell number also decreased, and total protein concentration in BALF and lung wet-to-dry ratio as well [39]. Li also reported that intratracheal instillation of recombinant mouse WISP1 protein increased HTV-induced EBA permeability in resistant CBA/J mice [31]. In addition, TLR4 is a common-known receptor to react proinflammatory effects in innate immune system [30, 54], but TLR4 KO mice should be used to further examine the mechanism in future experiments.

In summary, our study confirms that the low tidal volume ventilation is not detrimental to healthy lungs and has protective effects to sepsis-induced lung injury. Administration of ventilation with LTV for CLP mice ameliorates the permeability of alveolar-capillary, the pulmonary edema level and cytokines (IL-6 and TNF- $\alpha$ ) in serum and BALF increase as well as the histopathologic change with the time prolonged, which indicates that the impairment for the lung of the sepsis mice is gradually improved. According to our experiment result, the impairment is the least at the 4<sup>th</sup> hour. The latent mechanism of the protective effects of low VT is associated with WISP1 and TLR4 for synergetic downregulation of both molecules. Our study recommends that the proper extubation opportunity of a sepsis patients depending on ventilator is on the early stage. The clinical application of low tidal volume ventilation in ICUs and operative rooms, aiming at decreasing lung injury for patients, may have been reported. Nevertheless, our experiments suggest a novel role and further mechanisms of the application. Modulation of WISP1-TRL4 pathway may represent a promising therapeutic target for attenuating or preventing the pathological consequences of sepsis-associated ALI.

### Acknowledgements

We are appreciative of the financial support from the National Natural Science Foundation [81270135] and the Shanghai Education Committee Key Project [13ZZ024]. We also acknowledge Shuya Mei, Zhenzhen Shao, Xiang Zhao, Mengzhu Li, Lingling Zhang, Xiaoyin Niu, Ting Guo for their valuable discussion and technical assistance.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Quan Li, Department of Anesthesiology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen 518116, China. Tel: +86 13816-262446; E-mail: liquantongji@163.com

### References

- [1] Ratzinger F, Eichbichler K, Schuardt M, Tsirkindou I, Mitteregger D, Haslacher H, Perkmann T, Schmetterer KG, Doffner G, Burgmann H. Sepsis in standard care: patients' characteristics, effectiveness of antimicrobial therapy and patient outcome—a cohort study. *Infection* 2015; 43: 345-52.
- [2] Kang JW, Kim SJ, Cho HI, Lee SM. DAMPs activating innate immune responses in sepsis. *Ageing Res Rev* 2015; 24: 54-65.
- [3] Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546-54.
- [4] Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000; 342: 1301-8.
- [5] Pan C, Wang J, Liu W, Liu L, Jing L, Yang Y, Qiu H. Low tidal volume protects pulmonary vasomotor function from “second-hit” injury in acute lung injury rats. *Respir Res* 2012; 13: 77.
- [6] Yehya N, Xin Y, Oquendo Y, Cereda M, Rizi RR, Margulies SS. Cecal ligation and puncture accelerates development of ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2015; 308: L443-51.
- [7] Johnson ER, Matthay MA. Acute lung injury: epidemiology, pathogenesis, and treatment. *J Aerosol Med Pulm Drug Deliv* 2010; 23: 243-52.
- [8] Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD. Incidence and Outcomes of Acute Lung Injury. *N Engl J Med* 2005; 353: 1685-93.
- [9] Erickson SE, Martin GS, Davis JL, Matthay MA, Eisner MD; NIH NHLBI ARDS Network. Recent trends in acute lung injury mortality: 1996-2005\*. *Crit Care Med* 2009; 37: 1574-9.
- [10] Chiumello D, Cressoni M. Respirator management of sepsis-related respiratory failure. *Curr Infect Dis Rep* 2009; 11: 365-71.

## The role of WISP1 -TRL4 in the effects of mechanical ventilation in sepsis model

- [11] Villar J, Cabrera N, Casula M, Flores C, Valladares F, Muros M, Blanch L, Slutsky AS, Kacmarek RM. Mechanical ventilation modulates Toll-like receptor signaling pathway in a sepsis-induced lung injury model. *Intensive Care Med* 2010; 36: 1049-57.
- [12] Walker MG, Yao LJ, Patterson EK, Joseph MG, Cepinskas G, Veldhuizen RA, Lewis JF, Yamashita CM. The Effect of Tidal Volume on Systemic Inflammation in Acid-Induced Lung Injury. *Respiration* 2011; 81: 333-42.
- [13] Hudson LD, Milberg JA, Anardi D, Maunder RJ. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995; 151: 293-301.
- [14] Finigan JH, Boueiz A, Wilkinson E, Damico R, Skirball J, Pae HH, Damarla M, Hasan E, Pearse DB, Reddy SP, Grigoryev DN, Cheadle C, Esmon CT, Garcia JG, Hassoun PM. Activated protein C protects against ventilator-induced pulmonary capillary leak. *Am J Physiol Lung Cell Mol Physiol* 2009; 296: L1002-11.
- [15] Needham DM, Yang T, Dinglas VD, Mendez-Tellez PA, Shanholtz C, Sevransky JE, Brower RG, Pronovost PJ, Colantuoni E. Timing of low tidal volume ventilation and intensive care unit mortality in acute respiratory distress syndrome. A prospective cohort study. *Am J Respir Crit Care Med* 2015; 191: 177-85.
- [16] Natalini G, Minelli C, Rosano A, Ferretti P, Militano CR, De Feo C, Bernardini A. Cardiac index and oxygen delivery during low and high tidal volume ventilation strategies in patients with acuterespiratory distress syndrome: a crossover randomized clinical trial. *Crit Care* 2013; 17: R146.
- [17] Severgnini P, Selmo G, Lanza C, Chiesa A, Frigerio A, Bacuzzi A, Dionigi G, Novario R, Gregoretti C, de Abreu MG, Schultz MJ, Jaber S, Futier E, Chiaranda M, Pelosi P. Protective mechanical ventilation during general anesthesia for open abdominal surgery improves postoperative pulmonary function. *Anesthesiology* 2013; 118: 1307-21.
- [18] Futier E, Constantin JM, Paugam-Burtz C, Pascal J, Eurin M, Neuschwander A, Marret E, Beaussier M, Gutton C, Lefrant JY, Allaouchiche B, Verzilli D, Leone M, De Jong A, Bazin JE, Pereira B, Jaber S; IMPROVE Study Group. A trial of intraoperative low-tidal-volume ventilation in abdominal surgery. *N Engl J Med* 2013; 369: 428-37.
- [19] Sutherasan Y, Vargas M, Pelosi P. Protective mechanical ventilation in the non-injured lung: review and meta-analysis. *Crit Care* 2014; 18: 211.
- [20] Villar J, Blanco J, Zhang H, Slutsky AS. Ventilator-induced lung injury and sepsis: two sides of the same coin? *Minerva Anestesiol* 2011; 77: 647-53.
- [21] Wolthuis EK, Choi G, Dessing MC, Bresser P, Lutter R, Dzoljic M, van der Poll T, Vroom MB, Hollmann M, Schultz MJ. Mechanical ventilation with lower tidal volumes and positive end-expiratory pressure prevents pulmonary inflammation in patients without preexisting lung injury. *Anesthesiology* 2008; 108: 46-54.
- [22] Severgnini P, Selmo G, Lanza C, Chiesa A, Frigerio A, Bacuzzi A, Dionigi G, Novario R, Gregoretti C, de Abreu MG, Schultz MJ, Jaber S, Futier E, Chiaranda M, Pelosi P. Protective mechanical ventilation during general anesthesia for open abdominal surgery improves postoperative pulmonary function. *Anesthesiology* 2013; 118: 1307-21.
- [23] Chen CC, Lau LF. Functions and mechanisms of action of CCN matricellular proteins. *Int J Biochem Cell Biol* 2009; 41: 771-83.
- [24] Poltorak A, Ricciardi-Castagnoli P, Citterio S, Beutler B. Physical contact between lipopolysaccharide and Toll-like receptor 4 revealed by genetic complementation. *Proc Natl Acad Sci U S A* 2000; 97: 2163-7.
- [25] Yang M, Zhao X, Liu Y, Tian Y, Ran X, Jiang Y. A role for WNT1-inducible signaling protein-1 in airway remodeling in a rat asthma model. *Int Immunopharmacol* 2013; 17: 350-7.
- [26] Heise RL, Stober V, Cheluvharaju C, Hollingsworth JW, Garantziotis S. Mechanical stretch induces epithelial-mesenchymal transition in alveolar epithelia via hyaluronan activation of innate immunity. *J Biol Chem* 2011; 286: 17435-44.
- [27] Martin TR, Frevert CW. Innate immunity in the lungs. *Proc Am Thorac Soc* 2005; 2: 403-11.
- [28] Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock-A review of laboratory models and a proposal. *J Surg Res* 1980; 29: 189-201.
- [29] Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nat Protoc* 2009; 4: 31-36.
- [30] Li H, Su X, Yan X, Wasserloos K, Chao W, Kaynar AM, Liu ZQ, Leikauf GD, Pitt BR, Zhang LM. Toll-like receptor 4-myeloid differentiation factor 88 signaling contributes to ventilator-induced lung injury in mice. *Anesthesiology* 2010; 113: 619-29.
- [31] Li HH, Li Q, Liu P, Liu Y, Li J, Wasserloos K, Chao W, You M, Oury TD, Chhinder S, Hackam DJ, Billiar TR, Leikauf GD, Pitt BR, Zhang LM. WNT1-inducible signaling pathway protein 1 contributes to ventilator-induced lung injury. *Am J Respir Cell Mol Biol* 2012; 47: 528-35.
- [32] Smith KM, Mrozek JD, Simonton SC, Bing DR, Meyers PA, Connett JE, Mammel MC. Prolonged

## The role of WISP1-TRL4 in the effects of mechanical ventilation in sepsis model

- partial liquid ventilation using conventional and high-frequency ventilatory techniques: gas exchange and lung pathology in an animal model of respiratory distress syndrome. *Crit Care Med* 1997; 25: 1888-97.
- [33] Zhang YM, Zhang SK, Cui NQ. Intravenous infusion of mesenteric lymph from severe intraperitoneal infection rats causes lung injury in healthy rats. *World J Gastroenterol* 2014; 20: 4771-7.
- [34] George SJ. Wnt pathway: a new role in regulation of inflammation. *Arterioscler Thromb Vasc Biol* 2008; 28: 400-2.
- [35] Murahovschi V, Pivovarova O, Ilkavets I, Dmitrieva RM, Döcke S, Keyhani-Nejad F, Gögebakan Ö, Osterhoff M, Kemper M, Hornemann S, Markova M, Klötting N, Stockmann M, Weickert MO, Lamounier-Zepter V, Neuhaus P, Konradi A, Dooley S, von Loeffelholz C, Blüher M, Pfeiffer AF, Rudovich N. WISP1 is a novel adipokine linked to inflammation in obesity. *Diabetes* 2015; 64: 856-66.
- [36] Oh DK, Lee MG, Choi EY, Lim J, Lee HK, Kim SC, Lim CM, Koh Y, Hong SB; Korean Society of Critical Care Medicine H1N1 collaborative. Low-tidal volume mechanical ventilation in patients with acute respiratory distress syndrome caused by pandemic influenza A/H1N1 infection. *J Crit Care* 2013; 28: 358-64.
- [37] Berschneider B, Königshoff M. WNT1 inducible signaling pathway protein 1 (WISP1): A novel mediator linking development and disease. *Int J Biochem Cell Biol* 2011; 43: 306-9.
- [38] Kular L, Pakradouni J, Kitabgi P, Laurent M, Martinerie C. The CCN family: A new class of inflammation modulators? *Biochimie* 2011; 93: 377-88.
- [39] Ding X, Wang X, Zhao X, Jin S, Tong Y, Ren H, Chen Z, Li Q. RGD Peptides Protects Against Acute Lung Injury in Septic Mice Through Wisp1-Integrin  $\beta 6$  Pathway Inhibition. *Shock* 2015; 43: 352-60.
- [40] Oura T, Rozanski EA, Buckley G, Bedenice D. Low tidal volume ventilation in healthy dogs. *J Vet Emerg Crit Care (San Antonio)* 2012; 22: 368-71.
- [41] Königshoff M, Eickelberg O. WNT Signaling in Lung Disease: A Failure or a Regeneration Signal? *Am J Respir Cell Mol Biol* 2010; 42: 21-31.
- [42] Königshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A, Rose F, Fink L, Seeger W, Schaefer L, Günther A, Eickelberg O. WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. *J Clin Invest* 2009; 119: 772-87.
- [43] Vaneker M, Joosten LA, Heunks LM, Snijdelaar DG, Halbertsma FJ, van Egmond J, Netea MG, van der Hoeven JG, Scheffer GJ. Low-tidal-volume Mechanical Ventilation Induces a Toll-like Receptor 4-dependent Inflammatory Response in Healthy Mice. *Anesthesiology* 2008; 109: 465-72.
- [44] Flozak AS, Lam AP, Russell S, Jain M, Peled ON, Sheppard KA, Beri R, Mutlu GM, Budinger GR, Gottardi CJ. Beta-catenin/T-cell factor signaling is activated during lung injury and promotes the survival and migration of alveolar epithelial cells. *J Biol Chem* 2010; 285: 3157-67.
- [45] Futier E, Godet T, Millot A, Constantin JM, Jaber S. Mechanical ventilation in abdominal surgery. *Ann Fr Anesth Reanim* 2014; 33: 472-5.
- [46] Pinhu L, Whitehead T, Evans T, Griffiths M. Ventilator-associated lung injury. *Lancet* 2003; 361: 332-40.
- [47] Fuchs H, Mendler MR, Scharnbeck D, Ebsen M, Hummler HD. Very Low Tidal Volume Ventilation with Associated Hypercapnia - Effects on Lung Injury in a Model for Acute Respiratory Distress Syndrome. *PLoS One* 2011; 6: e23816.
- [48] Terragni PP, Del Sorbo L, Mascia L, Urbino R, Martin EL, Birocco A, Faggiano C, Quintel M, Gattinoni L, Ranieri VM. Tidal Volume Lower than 6 ml/kg Enhances Lung Protection Role of Extracorporeal Carbon Dioxide Removal. *Anesthesiology* 2009; 111: 826-35.
- [49] Jaber S, Coisel Y, Chanques G, Futier E, Constantin JM, Michelet P, Beaussier M, Lefrant JY, Allaouchiche B, Capdevila X, Marret E. A multicentre observational study of intraoperative ventilatory management during general anaesthesia: tidal volumes and relation to body weight. *Anaesthesia* 2012; 67: 999-1008.
- [50] Villar J, Cabrera NE, Casula M, Valladares F, Flores C, López-Aguilar J, Blanch L, Zhang H, Kacmarek RM, Slutsky AS. WNT/ $\beta$ -catenin signaling is modulated by mechanical ventilation in an experimental model of acute lung injury. *Intensive Care Med* 2011; 37: 1201-9.
- [51] Pelosi P, Rocco PR. Effects of mechanical ventilation on the extracellular matrix. *Intensive Care Med* 2008; 34: 631-9.
- [52] Laffey JG, Honan D, Hopkins N, Hyvelin JM, Boylan JF, McLoughlin P. Hypercapnic acidosis attenuates endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 2004; 169: 46-56.
- [53] Sinclair SE, Kregenow DA, Lamm WJ, Starr IR, Chi EY, Hlastala MP. Hypercapnic acidosis is protective in an in vivo model of ventilator-induced lung injury. *Am J Respir Crit Care Med* 2002; 166: 403-8.
- [54] Kuipers MT, van der Poll T, Schultz MJ, Wieland CW. Bench-to bedside review: Damage-asso-

## The role of WISP1 -TRL4 in the effects of mechanical ventilation in sepsis model

- ciated molecular patterns in the onset of ventilator-induced lung injury. *Crit Care* 2011; 15: 235.
- [55] DeJager L, Pinheiro I, Dejonckheere E, Libert C. Cecal ligation and puncture: the gold standard model for polymicrobial sepsis? *Trends Microbiol* 2011; 19: 198-208.
- [56] Maes K, Stamiris A, Thomas D, Cielen N, Smuder A, Powers SK, Leite FS, Hermans G, Decramer M, Hussain SN, Gayan-Ramirez G. Effects of controlled mechanical ventilation on sepsis-induced diaphragm dysfunction in rats. *Crit Care Med* 2014; 42: e772-82.
- [57] Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL; International Surviving Sepsis Campaign Guidelines Committee; American Association of Critical-Care Nurses; American College of Chest Physicians; American College of Emergency Physicians; Canadian Critical Care Society; European Society of Clinical Microbiology and Infectious Diseases; European Society of Intensive Care Medicine; European Respiratory Society; International Sepsis Forum; Japanese Association for Acute Medicine; Japanese Society of Intensive Care Medicine; Society of Critical Care Medicine; Society of Hospital Medicine; Surgical Infection Society; World Federation of Societies of Intensive and Critical Care Medicine. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008; 36: 296-327.