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Effects of Hydrocortisone on Activation of Inflammation *via* Interleukin-33 in Ventilator-Induced Lung Injury

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Abstract

Despite mechanical ventilation being a very important life-saving intervention, ventilator-induced lung injury (VILI) is related with inflammatory effects and causes high mortality. Our previous study demonstrated that the interleukin-33 (IL-33) cytokine pathway is a biomarker of VILI. The purpose of this study was to further explore the effects of hydrocortisone sodium succinate (HC) on pro-inflammatory IL-33 activation by VILI. The rats were intubated and received ventilation at 20 cmH_2O of inspiratory pressure (PC20) by a G5 ventilator for 4 h as a control group, and an intervention group received the same inspiratory pressure as well as treated with HC at 1 mg/kg at the third hour of ventilation (PC20+HC). The hemodynamic and respiratory data showed similar changes in the two groups that were exposed to VILI. The pathophysiological results showed that the HC treatment attenuated the VILI severity. Treatment of HC increased IL-33 expression in the bronchoalveolar lavage fluid. These results demonstrated that IL-33 is involved in VILI processing and HC treatment attenuated IL-33 involvement in inflammatory activation in VILI. In conclusion, IL-33 may play an important role in VILI.

Key Words: IL-33, ventilator, ventilator-induced lung injury

Introduction

Mechanical ventilation serves an essential life-saving purpose, which is to provide adequate gas exchange while reducing the bodily efforts required for breathing. The good predictive accuracy of the weaning index is subsequently very important strategy for ventilator weaning successful. Such as rapid shallow breathing index measurement seemed to have the best diagnostic accuracy of predicting weaning success (4). Despite these issues for challenge, negative side effects of mechanical ventilation are well known. One particularly undesirable side effect is ventilator-induced lung injury (VILI), which occurs with some frequencies, and may result in death (28, 30). VILI was first reported in 1744 in a case report published by John Fothergill (12), in which he discussed that mechanical forces generated by bellows for artificial ventilation, rather than by mouth-to-mouth resuscitation, could lead to injuries in patients. Based on these historical perspectives and the clinicalimplications indicating that mechanical ventilation can produce physiological and morphological alterations in the lungs, the term "ventilator-induced lung injury" (VILI) was introduced (3, 8, 11). Therefore, it is important to develop strategies to prevent VILI

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when ventilators are used.

The consequences of pulmonary damage in VILI were characterized pathologically by inflammatory cell infiltrates, hyaline membranes induced, increased vascular permeability and pulmonary edema (28, 30, 33). However, four mechanisms that most commonly lead to the occurrence of VILI and its consequences include excessive tidal volume (volutrauma), excessive airway pressure (barotrauma), cyclical opening and closing of small airways or lung units (atelectrauma), and subsequent generation of bio-mediators of activation and lung inflammatory injury (biotrauma) (5, 27, 28, 37).

Prior investigations of the biotrauma mechanism of VILI have resulted in very few effective treatments. Previous studies have demonstrated that biotrauma is strongly associated with the accumulation of proinflammatory cytokines, namely, IL-1β, TNF-α, IL-6 and IL-8 (7, 9, 15, 16, 26), dramatic in creases in the expression of which are seen in the lung lavage and serum levels. Conversely, it has been found that increased expression of the anti-inflammatory IL-10 cytokine can reduce lung injury and mortality in limiting pulmonary biotrauma of VILI (14, 37). Recently, interleukin-33 (IL-33), which is as an inflammasome-regulated and mechanically responsive cytokine, has become a subject of many studies (10, 18, 29). Our previous study also demonstrated that increased expression of the inflammatory IL-33 cytokine in lung tissues in a VILI rat model (38). Our results showed that the ST2L receptor of IL-33 was dramatically exhibited in membrane translocation, suggesting that this mechanism may cause lung injury in the VILI rat model. However, further investigation of VILI is required to determine whether any treatments can minimize the inflammatory response by altering the expression of IL-33.

Recently, many studies involving clinical trials have provided evidences that VILI can be avoided by using lung-protective ventilation, which is associated with lower serum cytokine and chemokine levels, less severe organ dysfunction, and lower mortality in patients with acute respiratory distress syndrome (10, 11, 32). Numerous studies have also shown that the administration of steroids could ameliorate lung injury in addition to possibly reducing systemic inflammation (13, 20, 31). Increases of serum IL-6, nitrite oxides and aspartate aminotransferase concentration were attenuated by treatment of intraperitoneal dexamethasone to high tidal volume (35 mL/kg) induced-VILI (24). In addition, HC had been found that could also inhibit the glucose transporter type 4 membrane translocation in the exercise training group and then blocked the inflammatory response of skeletal muscle (6).

Therefore, we were interested in conducting a study focused on the steroid being administered to improve VILI consequences, as well as on whether the mechanism of such improvement was *via* the IL-33 inflammatory pathway. However, the IL-33-mediated production of IL-8 by human lung cells was almost completely suppressed by corticosteroid treatment (37). Another recent study also found that corticosteroids attenuated IL-33-induced airway inflammation and accumulation of natural helper cells (17). The aim of the present study was to explore whether the HC suppressed IL-33 expression in the serum and blocked VILI-induced pathophysiology changes.

Materials and Methods

Experiment Protocol

The experimental protocols were approved by the Laboratory Animal Care Committee of Fu-Jen Catholic University. Adult male Wistar rats (weighing 220 ~ 300 g) were obtained from a provider of rats for animal experiments (BioLASCO CO., LTD, Taipei, Taiwan). As in the previous study, the intraperitoneal space of each rat was injected with a mixture of 20-40 mg/kg of Zoletil 50 (Vibac Laboratories, Carros, France) and 5-10 mg/kg of Rompun (Bayer, Leverkusen, Germany) to anesthetize the rat (35). Frequent checks were conducted to ensure that the animals were adequately anesthetized throughout the experiments. For each rat, a cannula with a 250-gauge catheter was inserted into the trachea and a G5 ventilator (Hamilton Medical AG, Switzerland) was applied and connected to an end tidal expiratory CO_2 monitor ($ETCO_2$; Hamilton Medical AG, Bonaduz, Switzerland). Rectal temperature monitoring and external heating were used to maintain a body temperature of 37.0 - 38.5°C. The left femoral artery was cannulated with a 50-gauge catheter to aspirate blood for blood gas analysis and arterial blood pressure monitoring. The blood pressure was measure through acquisition system instruments (MP 100) (Biopac System Inc., Santa Barbara, CA, USA) and recorded *via* computer (Chart 4 record system; Biopack Systems Inc.). Subsequently, intravenous injection of saline at 10 ml/kg/h *via* the left femoral vein with the 50-gauge catheter was conducted to avoid body fluid loss and to compensate for blood sampling. In addition, The animals in the latter group were ventilated at the pressure control mode setting in a G5 ventilator, including inspiratory pressure at 20 cmH_2O, a respiratory rate of 50 beat/min, an inspiratory time of 0.3 seconds, positive end-expiratory pressure (PEEP) at 0 cmH_2O, and a inspiratory oxygen fraction (FiO_2) of 21% for 30 min to maintain the $PaCO_2$ at 35-45 mmHg and PaO_2 at 90-100 mmHg, after which baseline data were collected.

Experimental Groups

The PC20 group in which the rats were subjected to mechanical ventilation with 20 cmH_2O of inspiratory pressure, and an intervention group (PC20+HC) in which the rats were subjected to hydrocortisone sodium succinate (HC) (Solu-Cortef; Upjohn Co., Kalamazoo, MI, USA) treatment of HC 1 mg/kg *via* IV after 2 h with 20 cmH_2O of inspiratory pressure during mechanical ventilation. The ventilator intervention was performed on each animal in both groups for a total of 4 h, during which data were collected.

Measurement of Physiological Parameters

For each rat, the arterial blood pressure was continuously recorded in terms of systemic mean arterial blood pressure (MABP), while the heart rate (HR) was continuously monitored through the arterial blood pressure monitoring system; Chart 4 software was used to correct the MABP and HR per hour. Analysis of arterial blood gas (ABG) was also conducted, including measurements of arterial oxygen tension (PaO_2), carbon dioxide tension ($PaCO_2$), acidity (pH) and O_2 saturation (SaO_2). The ABG was collected from the left femoral artery at each hour. A portable blood gas analyzer (i-STAT, Princeton, NJ, USA) was used to perform measurements of the blood samples.

Pathophysiological Analysis of Lung Injury

Hematoxylin and eosin staining of right middle lung lobe tissues was used to determine histopathological changes due to lung injury, and representative photographs of whole lungs were also taken for both experimental groups. Levels of lung injury in each field were assigned a lung injury score based on microscopic investigation. Consistent with a previous study, each score was calculated according to (A) the infiltration or aggregation of neutrophils in the airspace or vessel wall and (B) the thickness of the alveolar wall. Each assessment yielded a grade of 0, 1, 2 or 3, for no, mild, moderate or severe injury, respectively (38). The two scores were then summed to determine the overall score for the severity of lung injury. Furthermore, quantification of proteins in bronchoalveolar lavage fluid (BALF) was also conducted to determine lung injury levels after 4 h of VILI. After sacrifice of a given rat, the lungs were removed and tied on the right side. The BALF samples were then obtained through the processing of lavage by 5 mL of PBS fluid, as in a previous study (34). The cell pellets and supernatants of BALF were separated by centrifugation (900 Xg, 10 min) with a centrifuge. A bicinchoninic acid (BCA) protein assay kit (Thermo scientific, Rockford, IL, USA) was then used to determine the protein concentration in the BALF samples. The supernatants of BALF were stored in a refrigerator for ELISA assays.

Measurement of Cytokine Levels

IL-33 levels were measured using a standardized sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D system, Minneapolis, MN, USA). The absorbance was read at 450 nm using an Epoch™ ELISA reader (BioTek Instruments, Winooski, Vermont, USA).

Statistical Analysis

All the numeric data presented are mean ± SD. Statistical analyses were performed using SPSS (version 13.01S; Beijing Stats Data Mining Co. Ltd., Beijing, P.R.C). Statistical comparisons between groups were performed using ANOVA, the Bonferroni *post hoc* test and Student's *t*-test. Differences were regarded as significant at values of $P < 0.05$.

Results

Effects on Respiratory Parameters of Rats with VILI

Rats were subjected to high inspiratory pressure ventilation through the use of an adult ventilator, which simulated the development of VILI in a clinical situation. In the pressure control mode of the mechanical ventilation, hyperventilation was maintained *via* a peak airway pressure of 20 cmH_2O and a tidal volume of around 10 mL, in which real time of airway pressure and tidal volume were recorded by the ventilator waveform monitor (Fig. 1A). However, the hyperventilation consequence of VILI was demonstrated *via* the hypocapnia expression. The trend in the $ETCO_2$, PCO_2 and PaO_2 changes of these two groups were not significantly different (Fig. 1B, C and D), although the levels of $ETCO_2$ and PCO_2 were decreased and PaO_2 was increased from the baseline to the 4 h time point of both groups. The respiratory parameters exhibited no significant differences between the PC20 and PC20+HC groups during the fours of experiment.

Hemodynamic Changes in Rats with VILI

The MABP in rats with VILI was dramatically reduced at the 0-h time point of PC 20 cmH_2O, and continuously decrease until the 4 h time point (Fig. 2A). The mean HR in the VILI experiment was initially decreased at 0 h and 1 h of PC 20 cmH_2O,

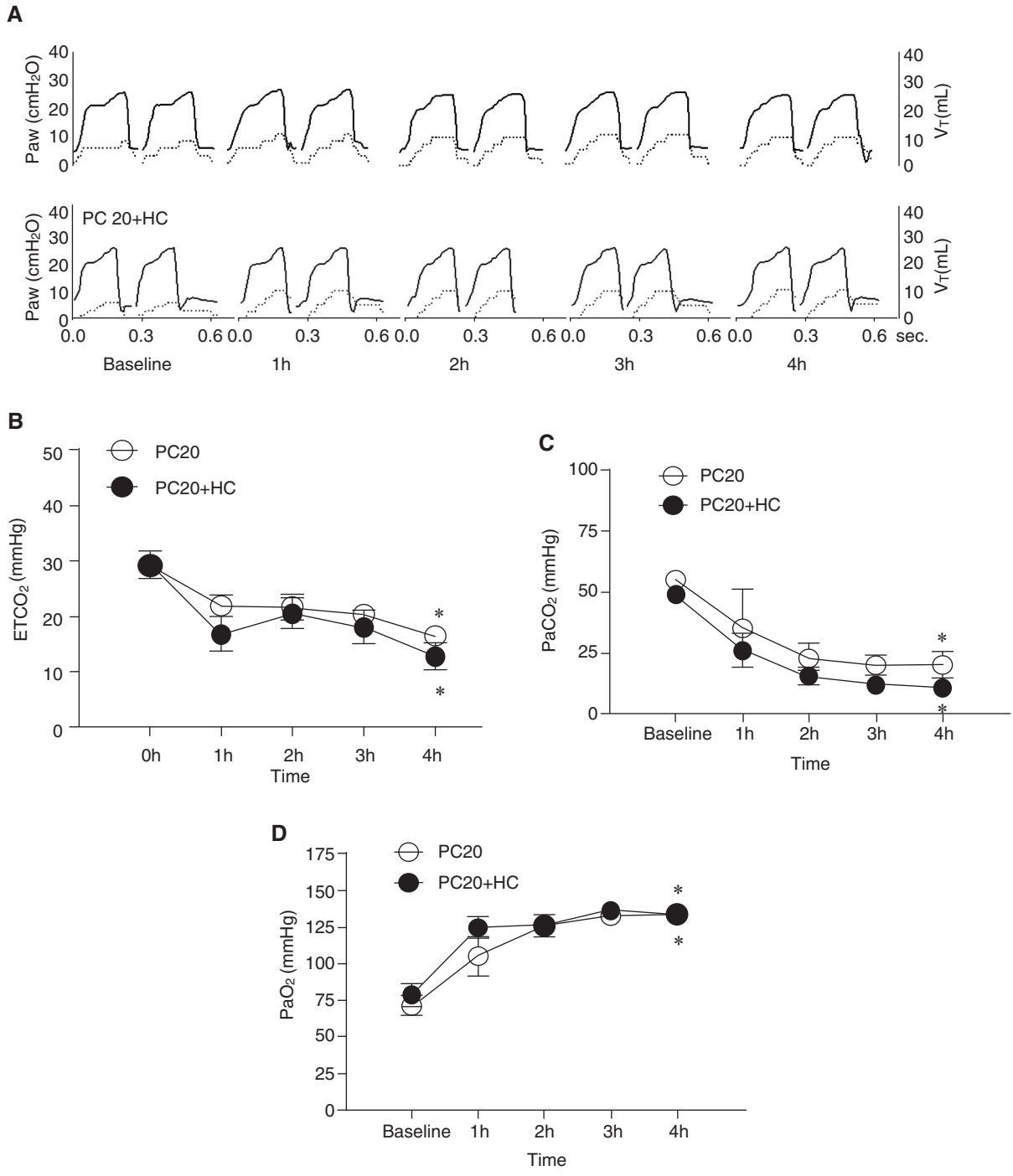

Fig 1. Effects on respiratory parameters of rats with VILI. (A) High inspiratory pressure from mechanical ventilation was used to cause severe hyperventilation with high airway pressure (paw; solid line) and tidal volume ($V_{T;}$ dot ted line) by ventilator waveform recording in the rat model of VILI. (B) $ETCO_2$ trends were recorded by a sidestream CO_2 sampling monitor. Both trends in (C) hypocapnia in $PaCO_2$ and (D) PaO_2 were analyzed by the arterial blood gas technique at each respective time point. $^*P < 0.05$ compared with the baseline. The symbols are presented in terms of mean ± standard error. HC: Hydrocortisone. (n = 5).

but it was subsequently increased until the 4 h time point (Fig. 2B). However, the MABP and HR measurements showed the same trends in both groups. The hemodynamic measurements for both groups also indicated that the animals were effectively subjected to VILI.

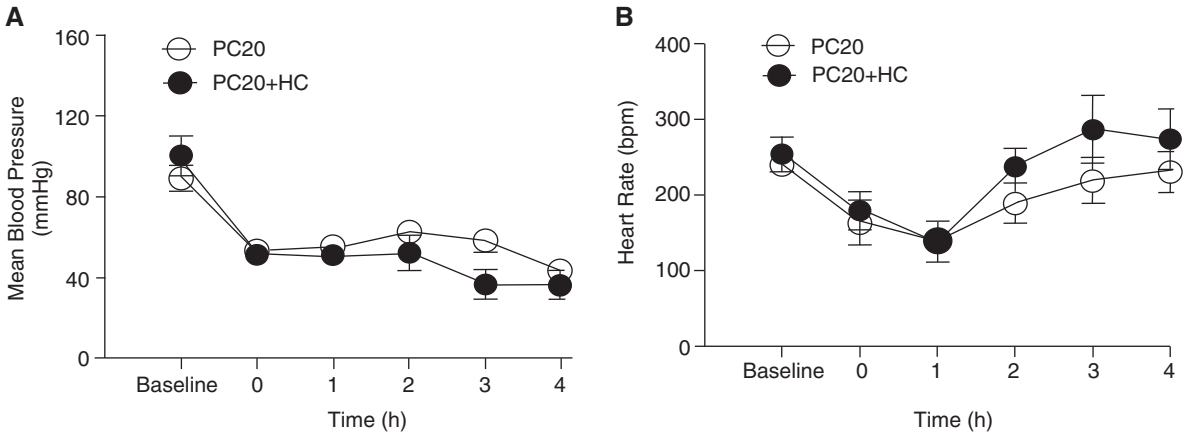

Fig 2. Hemodynamic changes in rats showing manifestations of VILI. (A) A gradually decreased trend in mean arterial blood pressure. (B) Heart rate presented the trend gradually the decrease in the baseline to 1 h and then compensatory increase until 4 h. The symbols of each respective time point are presented in terms of mean ± standard error. HC: Hydrocortisone. (n = 5).

Hydrocortisone Contribution to the Attenuation of VILI Severity

The appearance of whole lung for each rat in both groups was first checked. There is no lung tissue damage in the control rats that were intubated but were not ventilated with high pressure (Fig. 3A). Lung injury lesions were indicated by the red coloration in the PC20 rats, whereas the red coloration in the lungs of the PC20+HC rats significantly decreased (Fig. 3, B and C). H&E staining of the alveoli to determine histopathological damage also showed that there was no sign of injury (Fig. 3D). But, H&E staining indicated infiltration or aggregation of neutrophils in the airspace and hyaline membrane, and thicker vessel wall in the PC20 rats (Fig. 3E), and HC treatment attenuated the ventilator-induced lung tissue damage in the PC20+HC rats (Fig. 3F). In addition, the mean lung injury score was also significantly decreased in the PC20+HC rats compared with the PC20 rats (Fig. 3G). HC also significantly decreased VILI-induced protein secretion into BALF (Fig. 3H). The lung compliance measurements for both groups, however, were not significantly different (Fig. 3I). The above pathophysiological assay results demonstrated that HC attenuated the harmful effects of VILI.

Hydrocortisone Elevated IL-33 Expression in the BALF Detection

The expression of IL-33 in the collected BALF was significantly decreased in the PC20 group compared with the control group. The IL-33 level was decreased in BALF after VILI. However, the IL-33 levels in BALF was more significantly increased in the PC20+HC group than the PC20 group (Fig. 4A). The serum levels were not significantly different in each time point, as well as in the experimental (Fig. 4B). Therefore, IL-33 cytokine was demonstrated to be involved in the effect of lung protection in rats receiving HC treatment after VILI. The results, thus, demonstrated that the lung injury attenuation after HC administration was probably associated with the effects of IL-33 in the animal VILI model.

Discussion

In our previous study, IL-33 was shown to cause lung injury in VILI *via* its receptor ST2-produced membrane translocation (38). The aim of the present study was to determine whether steroid would attenuate the inflammatory response of IL-33 and thus contribute to decrease lung injury in VILI. We found that when a rat was administered HC after 2 h of VILI ventilation, the severity of lung injury was attenuated and the IL-33 level in BALF was significantly increased in the PC20+HC group compared with the PC20 group. These findings might suggest that the steroid treatment improved lung injury in VILI *via* its effects on the mechanism of IL-33 inflammation.

IL-33 is as a member of the IL-1 family of cytokines (2, 23). IL-33 has been revealed to have various functional roles; for example, IL-33 is involved in the mechanism of asthma attacks, with higher IL-33 levels being found in endobronchial biopsies of human asthmatic subjects, primarily in bronchial epithelial cells (25, 26). In contrast, it has also been shown in a murine model that intraperitoneal treatment with an anti-IL-33 antibody could result in the

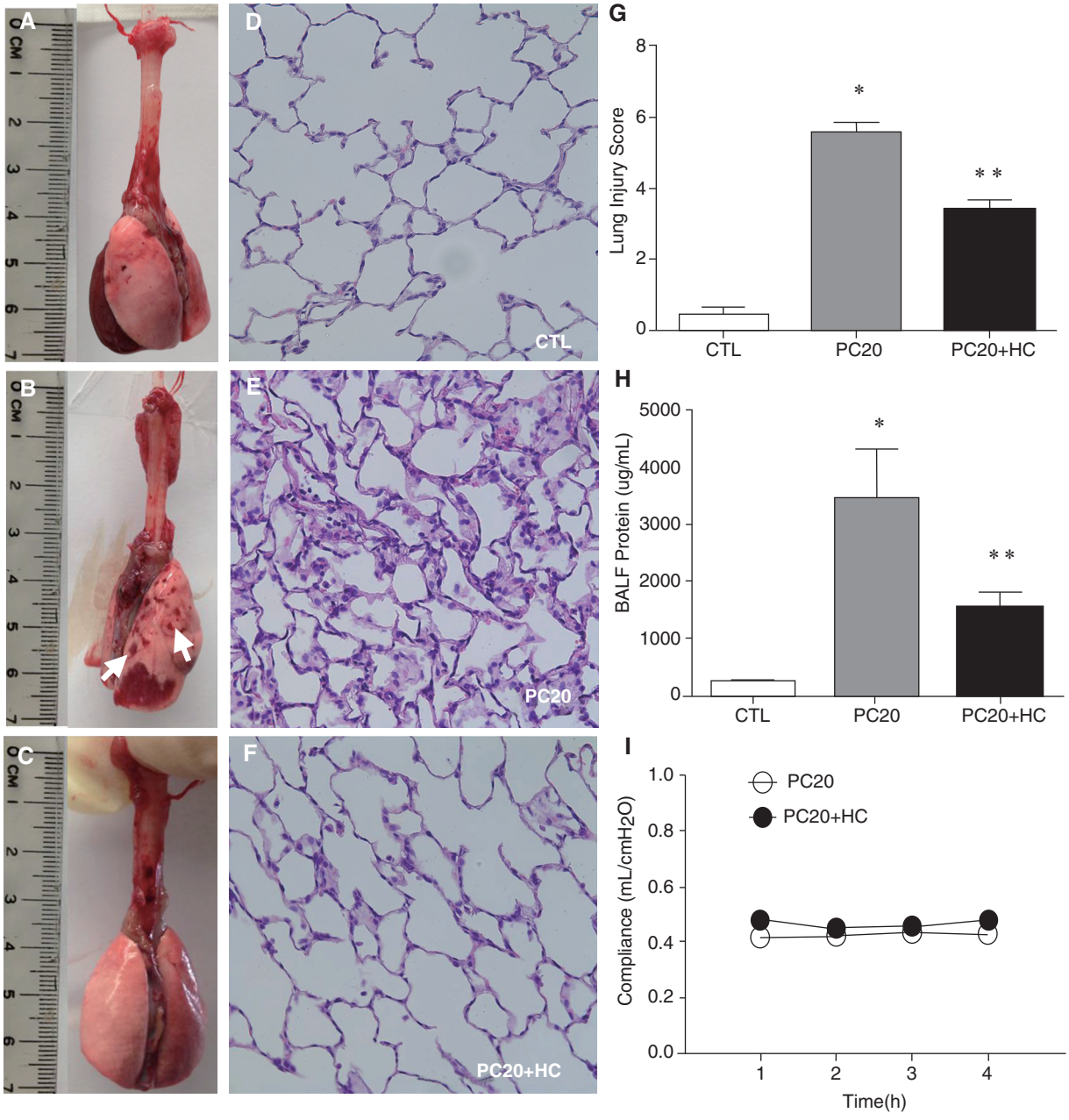

Fig. 3. Pathophysiological changes caused by VILI in rats. Lung injury in rats due to VILI was attenuated after administration of HC. (A) Photograph of a whole lung of control rat. (B) Photograph of whole lung of PC20 rat; the red coloration (arrow) indicates the presence of lung injury lesion. (C) Photograph of a whole lung of PC20+HC rat. (D-F) H&E staining images demonstrated pathohistological alveolar injury, the expression of which was dramatically attenuated in a PC20+HC rat (F). The following three results of figures were significantly lower in the PC20+HC group than the PC20 group, including: (G) Calculation of lung injury score, (H) Protein assay in BALF, (I) Lung compliance as measured by ventilator monitor. The bars or symbols at each respective time point are represented in terms of mean ± standard error. HC: Hydrocortisone (n = 5). BALF: Bronchoalveolar lavage fluid. *$P < 0.05$ compared with PC20 group.

inhibition of allergen-induced lung eosinophilic inflammation and mucus hypersecretion (21). In a pneumonia-related study, the researchers indicated that IL-33 expression was clearly induced in influenza A virus-infected murine lungs (19). It was also shown that IL-33 played a role in lung protection. A recent study by Luzina and colleagues (22) has implicated that full-length IL-33 acts as a regular culprit in pulmonary inflammation and fibrosis. In contrast to the effect of mature IL-33, full-length IL-33 remains predom-

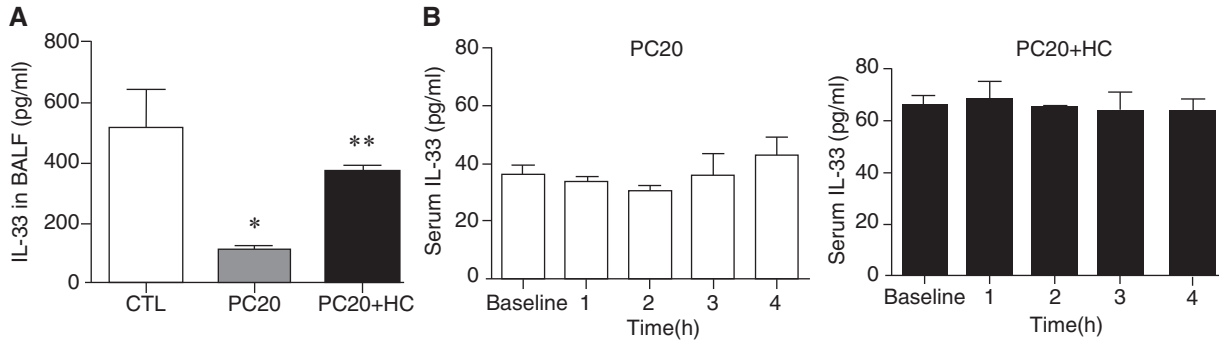

Fig 4. IL-33 expression of rats receiving HC treatment after VILI. (A) The expression level of IL-33 in BALF was significantly increased in the PC20+HC group compared with the PC20 group. (B) Analysis of serum IL-33 expression levels indicated no significant changes. Mean ± standard error are presented in each bar. HC: Hydrocortisone. (n = 5). *$P < 0.05$ compared with the control group and **$P < 0.05$ compared with the PC20 group.

inantly intracellular and regulates the expression of IL-6, heat shock protein-70 and other factors (22). The above evidences indicate that IL-33 in the lung regulates events between the proinflammatory and anti-inflammatory mechanisms as well as profibrotic and antifibrotic responses, which all serve to protect the lung (9, 23). However, our previous study also indicated that the IL-33/ST2 pathway was a new therapy target in VILI (38). The expression of IL-33 in lung tissues was predominantly increased by mechanically responsive lung injury, and was decreased in BALF detection. On the other hand, receptor of ST2L (ligand) was highly accumulated and sST2 (soluble) was decreased in the cell membrane fraction of lung tissues in rats with VILI. Therefore, this consequence indicated that a great amount ST2L receptor was translocated to the cell membrane to bind with IL-33, and that this also caused downstream activation and upregulation of NF-κB. Here, we also revealed that IL-33 expression was upregulated in BALF detection in VILI after HC treatment, and that the treatment attenuated lung injury. Furthermore, IL-33 moderated the inflammatory response such that IL-6 levels were also decreased. Future studies may further explore VILI mechanism and therapy in the ARDS pathophysiology, and focus on the effects of IL-33 cytokine on lung injuries and its mechanism.

The physiological parameters of VILI in animal models are always measured. The respiratory parameters including airway pressure and tidal volume were measured to determine VILI. The design of the VILI model in this study was also similar with that of our previous study, in which a clinical G5 ventilator was used to develop VILI in rats, an experimental situation similar with the clinical ventilator used in acute respiratory failure. Thus, the hyperinflation of VILI was similar to those of previous studies (33, 34). The pressure control mode in the ventilator was performed that included airway pressure kept at 20 cmH_2O and the tidal volume was approximately 25 mL/kg in rats. However, findings of this study that the blood pressure was decreased and the heart rate was increased were similar to those of previous studies, which indicated that the hyperinflation caused an increased intrathoracic pressure leading to hemodynamic deterioration (5, 7). The effects of VILI on the PC20 and PC20+HC groups were shown to be essentially the same, with no significant differences. Gas exchange measurements in this study revealed normal oxygenation and hypocapnea. However, HC treatment did not appear to affect gas exchange in the PC20+HC group as compared with the PC20 group. This is consistent with previous animal studies regarding gas exchange in the context of VILI (35, 38). In addition, VILI should also be diagnosed from photographs of pulmonary pathologies, including infiltration, air leaks, thickness of the alveolar wall and the rich protein aggregation of neutrophils or inflammatory mediators in the alveolar airspace or vessel wall (27, 33). Also similar to our previous studies (1, 27, 36), our findings from H&E staining images and lung injury scores all consistently indicated that lung injury was clearly induced during the process of high-pressure ventilation from the G5 ventilator, but that rats receiving HC were protected to some extent from lung injury in VILI.

In conclusion, it is very important that high pressure or hyperinflation should be avoided when using mechanical ventilation. Nonetheless, ventilator strategies for avoiding VILI are still being developed. The exploration of physiological mechanisms of VILI should also provide potential contributions to treatment. Through BALF detection, our study demonstrated that IL-33 cytokine is involved in VILI processing; however, the administration of hydrocortisone drug could attenuate the effects of IL-33 cytokine on inflamma-

tory activation in VILI.

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