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Resolvin D1 attenuates ventilator-induced lung injury by reducing HMGB1 release in a HO-1-dependent pathway☆

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ABSTRACT

Mechanical ventilation (MV) is a major support for patients with severe clinical disease, surgery and anesthesia. However, complications of mechanical ventilation especially ventilator-induced lung injury(VILI) can make the course and prognosis worse. Resolvin D1(RvD1) is a class of endogenous polyunsaturated fatty acid derivative, which has protective effects on various pulmonary inflammatory diseases. However, the mechanism of RvD1 in the process of VILI has not been fully elucidated. Our study found that RvD1 does have a protective effect on VILI including inhibiting inflammatory responses, reducing tissue damage and improving pulmonary function. The protective effect of RvD1 is positively related to its dose. Our research suggested RvD1 plays a role that increases the expression of heme oxygenase-1 (HO-1) and decreases the expression of the high mobility group chromosomal protein B1 (HMGB-1) in VILI. HO-1 can exert the protective effect of organism through multiple mechanisms such as anti-inflammatory, anti-oxidation, anti-apoptosis, etc. HMGB1 is a potent inflammatory response factor in the body, which can aggravate the inflammatory response of the body. Our study demonstrated that RvD1 can ameliorate lung inflammation and reduce pathological changes in lung tissue in a model of lung injury induced by mechanical ventilation. The protective role of RvD1 is closely linked to the increased expression of HO-1 and the decreased expression of HMGB1. Moreover, we found that RvD1 can increase the expression of Nrf2 and inhibit the expression of NF-κB. We found the specific inhibitor of HO-1, ZnPP, can significantly inhibit the protective role of RvD1 in VILI. When HO-1 is inhibited, pathological damage and inflammatory reaction in the lungs are considerably aggravated, and pulmonary function is significantly weakened. In addition, the expression of HMGB1 is drastically increased. This indicates that the HO-1-HMGB1 pathway plays an important role in the protective effect of RvD1 on mechanical ventilation lung injury.

1. Introduction

Mechanical ventilation is an essential supportive approach for the treatment of respiratory insufficiency by artificial ventilation using a ventilator. Its main functions include increasing alveolar ventilation [1], reducing respiratory work and improving oxygenation. Timely and effective mechanical ventilation is critical for the body to survive respiratory failure caused by various severe illnesses and can created conditions for the treatment of the diseases. However, complications often occur during mechanical ventilation therapy, the most common of which is heterogeneous lung parenchymal injury represented by ventilator-induced lung injury (VILI) [2,3]. How to treat VILI in different lung diseases and even healthy lungs during mechanical ventilation is an urgent problem to be solved. In recent years, research on the pathogenesis of VILI has made a progress [4,5]. In the process of VILI, the inflammatory response activated by the damage of alveolar epithelial and capillary endothelial plays an important role. It is estimated that its pathogenesis mainly includes barotrauma, volume injury, collapse

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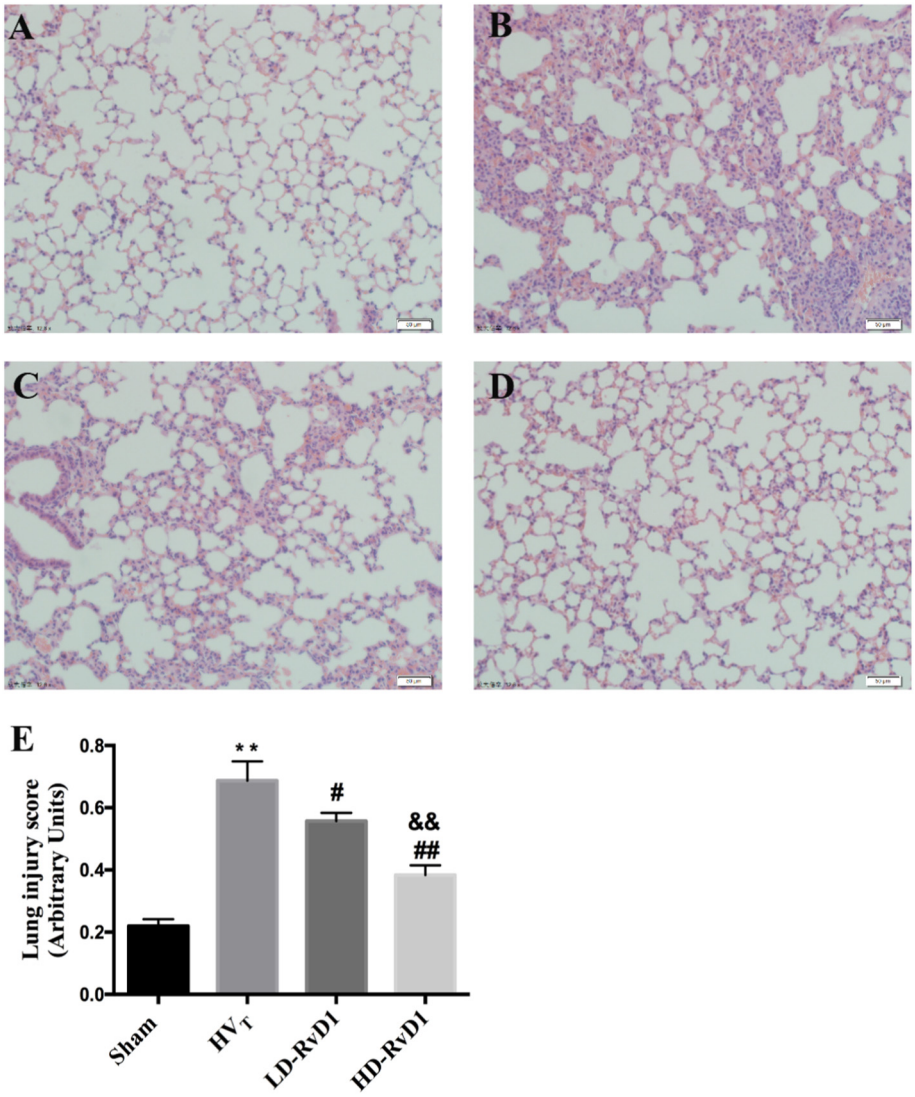

Fig. 1. Pathological changes and lung injury scores in mouse lung tissue. Representative hematoxylin-eosin staining pictures of lung tissue (magnification 200×). Sham group (panel A), HVT group (panel B), LD-RVD1 group (panel C), HD-RVD1 group (panel D), Lung injury score (panel E). Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\#P < 0.05$ versus HVT group; && $P < 0.01$ versus the HD-RvD1 group.

injury and biological injury. There are also studies showing that small tidal volume protective ventilation strategy can reduce the incidence of mechanical ventilation complications and achieve better clinical results [6], but in fact, based on the original lung disease, mechanical ventilation often inevitably leads to the damage of the remaining normal lung tissue, namely VILI.

Pathophysiological changes in VILI include inflammatory factors release and neutrophil recruitment, increased vascular permeability, etc. These changes further lead to pulmonary edema, decreased lung compliance, abscess of alveolar surfactant, and oxygenation disorders. Different mechanical stresses interact with lung tissue during mechanical ventilation, resulting in damage to lung structures such as type I and type II alveolar epithelial cells, endothelial cells, macrophages, small airways, cell junctions, and cytoskeleton structures [7]. During this process, alveolar epithelial and capillary endothelial damage caused by various factors that activates the inflammatory response [8,9] and affects the progression and prognosis of VILI. Therefore, prevention and reduction of the inflammatory response during mechanical ventilation are of great significance for VILI.

Studies by Serhan et al. found that some endogenous lipid mediators play a crucial role in promoting regression of inflammation [10–12]. These specialized pro-resolving mediators (SPMs) are mainly derived from polyunsaturated fatty acids (PUFA) represented by omega-6 fatty acids and omega-3 fatty acids [13]. The former one is mainly represented by peanuts (arachidonic acid) (Eicosatetraenoic acid, C20: 4n-6); the latter one mainly includes Eicosapentaenoic acid (EPA) (C20: 5n-3) and Twenty-two carbon six Docosahexaenoic acid (DHA) (C22: 6n-3). Among them, arachidonic acid is derived from lipoxins, and EPA and DHA are derived from Resolvins, Protectins and Maresins.

So far, the research on the role and mechanism of resolvins has been extensively and deeply studied [14–17]. Many studies have shown that resolvins have protective effects on various pulmonary inflammatory diseases, such as ALI, asthma, and cystic fibrosis, bacterial pneumonia, chronic obstructive pulmonary disease, etc. [18–21]. In addition, some studies have confirmed that RvD1 anti-inflammatory action is through the activation of peroxisome proliferator-activated receptor gamma (PPAR-y) to inhibit nuclear factor-KB (NF-κB) [22]. NF-κB is a key transcription factor that regulates the expression of numerous inflammatory genes, which usually presents in the cytoplasm in an inactive state. Inappropriate activation of NF-κB is closely linked to cancer, inflammation and autoimmune diseases, septic shock, viral infection, etc. Previous studies have shown that NF-κB is related to the expression of HO-1 [23]. It has been reported that ω polyunsaturated fatty acids are natural ligands for PPAR-y [24] and RvD1 can protect

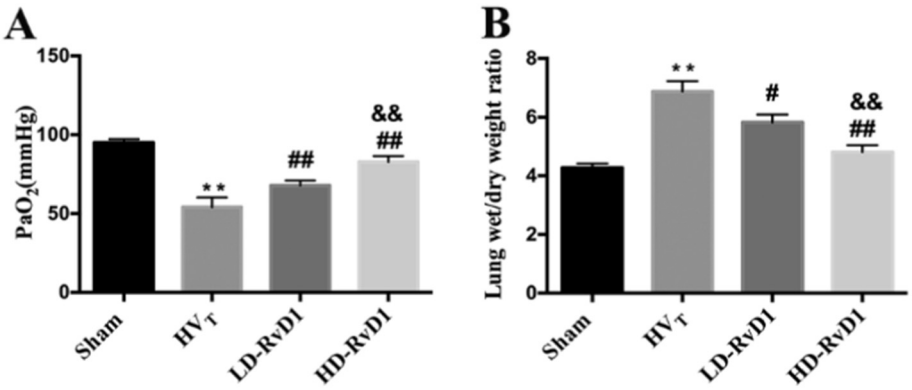

Fig. 2. RVD1 ameliorated pulmonary function in VILI mice. PaO2 (panel A), lung wet-to-dry ratio (panel B), the protein concentration in alveolar lavage fluid (panel C), the lung MPO activity (panel D). Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\#P < 0.05$ versus HVT group; && $P < 0.01$ versus the HD-RvD1 group.

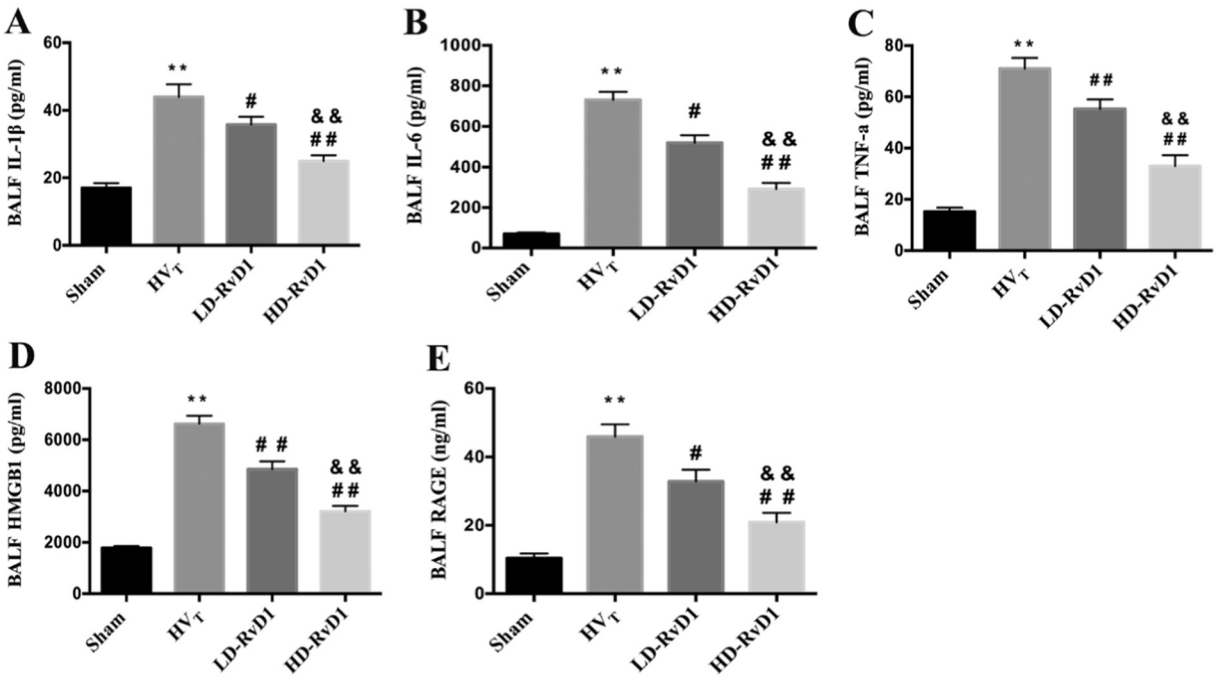

Fig. 3. Concentrations of IL-6, TNF-a, IL-1β, HMGB1 and RAGE in BALF. BALF IL-1β (panel A), BALF IL-6 (panel B), BALF TNF-a (panel C), BALF HMGB1 (panel D). BALF RAGE (panel E). Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\#P < 0.05$ versus HVT group; &&$P < 0.01$ versus the HD- RvD1 group.

against lung injury in multiple animal models by activating PPAR-y [19,25–28]. In recent years, some studies have also confirmed that PPAR-y activation is accompanied by an increase in the expression of heme oxygenase-1 (HO-1) which can protect the cardiovascular system from oxidative stress and inflammatory damage as well as lung injury [29–31]. HO-1 is involved in the pathophysiological processes of various diseases and can play a protective role in organ tissues through multiple mechanisms such as anti-inflammatory, anti-oxidation and anti-apoptosis [32]. The metabolites of HO-1 in animal tissues and cells also have anti-inflammatory effects [33]. The high mobility group chromosomal protein B1 (HMGB1) and advanced glycation end product receptor (RAGE) are potent inflammatory response factors that can

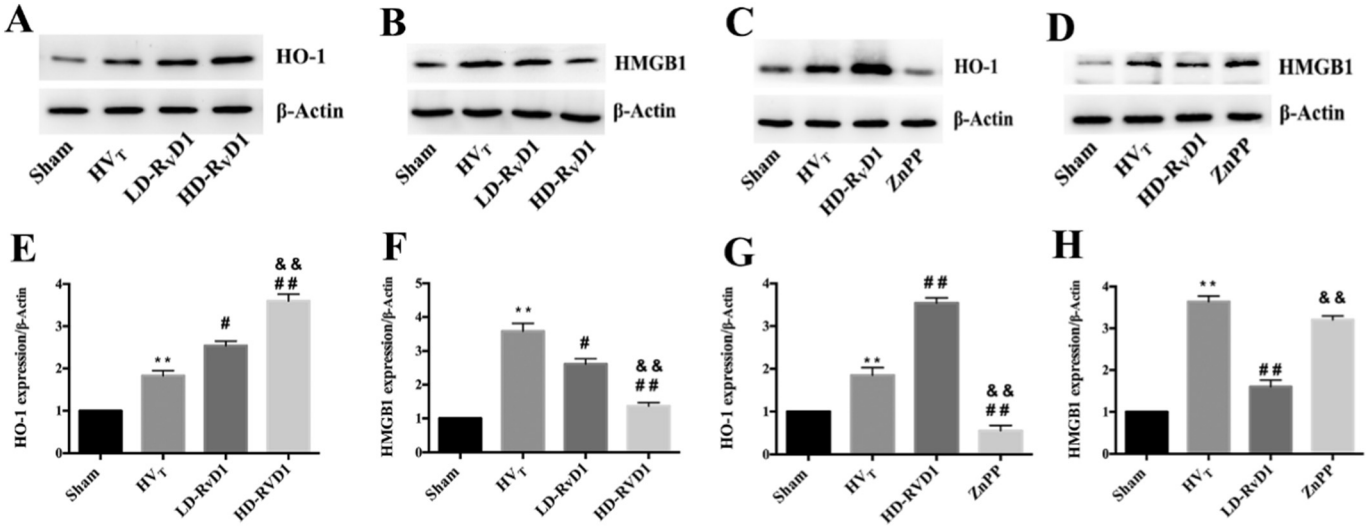

Fig. 4. Expression of HO-1 and HMGB-1 in lung tissue of mice in each group. HO-1 expression in the Sham group, HVT group, LD-RVD1 group and HD-RVD1 group (panel A). HMGB1 expression in the Sham group, HVT group, LD-RVD1 group and HD-RVD1 group (panel B). HO-1 expression in the Sham group, HVT group, HD-RVD1 group and ZnPP + RvD1 group (panel C). HO-1 expression in the Sham group, HVT group, HD-RVD1 group and ZnPP + RvD1 group (panel D). panels E, F, G, and H are statistical charts corresponding to panels A, B, C, and D, respectively. Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\#P < 0.05$ versus HVT group; $\&\&P < 0.01$ versus the HD-RvD1 group.

participate in and aggravate the occurrence and progression of a series of inflammatory reactions in the body. Nuclear factor E2-related factor 2(Nrf2) is a key factor in cellular oxidative stress and previous studies have shown that the expression of HO-1 is also regulated by Nrf2 [34,35].

Based on the above discussion, we hypothesized that RvD1 has a protective effect on mice with VILI. In addition, the up-regulation of HO-1 expression and the down-regulation of HMGB-1 play a key role in the protective role of RvD1. At the same time, we verified if the expression of NF-κB and Nrf2 may also be affected by RvD1 in VILI.

2. Materials and methods

2.1. Animals

SPF male C57BL/6 mice, 6–8 weeks old, weighing 20–25 g, were purchased from Experimental Animal Center of Wuhan University. During the experiment, the animals were kept in the animal experiment center of SPF environment (12/12 h of the light/dark cycle, 22–24 °C) with free access to food and water. The experiment was carried out according to the Chinese Animal Research Guidelines and approved by the Laboratory Animal Management Committee of Tongji Medical College of Huazhong University of Science and Technology.

2.2. Experimental design

Mice were weighed and then anesthetized with ketamine (120 mg/kg, intraperitoneally) and xylazine (8 mg/kg, intraperitoneally). One-fourth of the initial dose of anesthetic drugs was supplied about every 1 h to maintain anesthesia during the experimental period. Te mice were fixed in a supine position on a heating blanket and were subjected to tracheotomy. A 20G vein catheter was inserted for mechanical ventilation (Model 863 Ventilator; Harvard, USA). Mice in all groups, except the sham group, had 40 ml/kg ventilation and 80 breaths/min for 4 h as described previously [36] and underwent mechanical ventilation for 0 positive end-expiratory pressure.

C57BL/6 were first randomly divided into 4 groups($n = 8$): (1) sham operation group (Sham group), all surgical operations were performed, and spontaneous breathing was retained; (2) Large tidal volume mechanical ventilation group (HVT group): tidal volume 40 ml/kg;(3) low dose RvD1 intervention group (LD RvD1 group): tidal volume 40 ml/kg, intraperitoneal injection of RvD1 100 ng at the beginning of mechanical ventilation; (4) high dose RvD1 intervention group (HD RvD1 group): tidal volume 40 ml/kg, intraperitoneal injection of RvD1 500 ng at the beginning of mechanical ventilation; After that, we used HO-1 inhibitors. The experiments were divided into the following four groups.(1) Sham group (2) HVT group (3) HD RvD1 group (4) HO-1 antagonist zinc protopophyrin + RvD1 group (ZnPP + RvD1 group): The HO-1 antagonist ZnPP (10 mg/kg) was intraperitoneally injected 30 min before the start of mechanical ventilation and the rest of the treatment was consistent with the HD RvD1 group.

2.3. Histological analysis

The lung tissue of the right upper lobe was taken and fixed in 4% paraformaldehyde for 24 h. Sections were made approximated 4 μm thick and observed under the ordinary optical microscope when stained with hematoxylin-eosin. Lung injury scores were based on the lung tissue injury scoring criteria published by the American Thoracic Society in 2011.

2.4. Lung wet-to-dry weight ratio

After cleaning the surface with absorbent paper, the right middle lung was weighed and placed in an oven until constant weight. Calculate the W/D ratio to assess the severity of pulmonary edema.

2.5. Measurement of PaO2

At the end of the experiment, arterial blood (heparin anticoagulant) of the abdominal aorta was extracted, and the level of PaO2 in the blood was measured using a blood gas analyzer.

2.6. Bronchoalveolar lavage analysis

Bronchoalveolar lavage was performed using three aliquots of 0.5 ml phosphate-buffered saline (PBS). After centrifugated at 12000 rpm at 4 °C for 15 min, the supernatant was collected and the concentration of TNF-a, IL-6, IL-1β, HMGB1 and RAGE in BALF was detected strictly according to the instructions of the ELISA kit. The

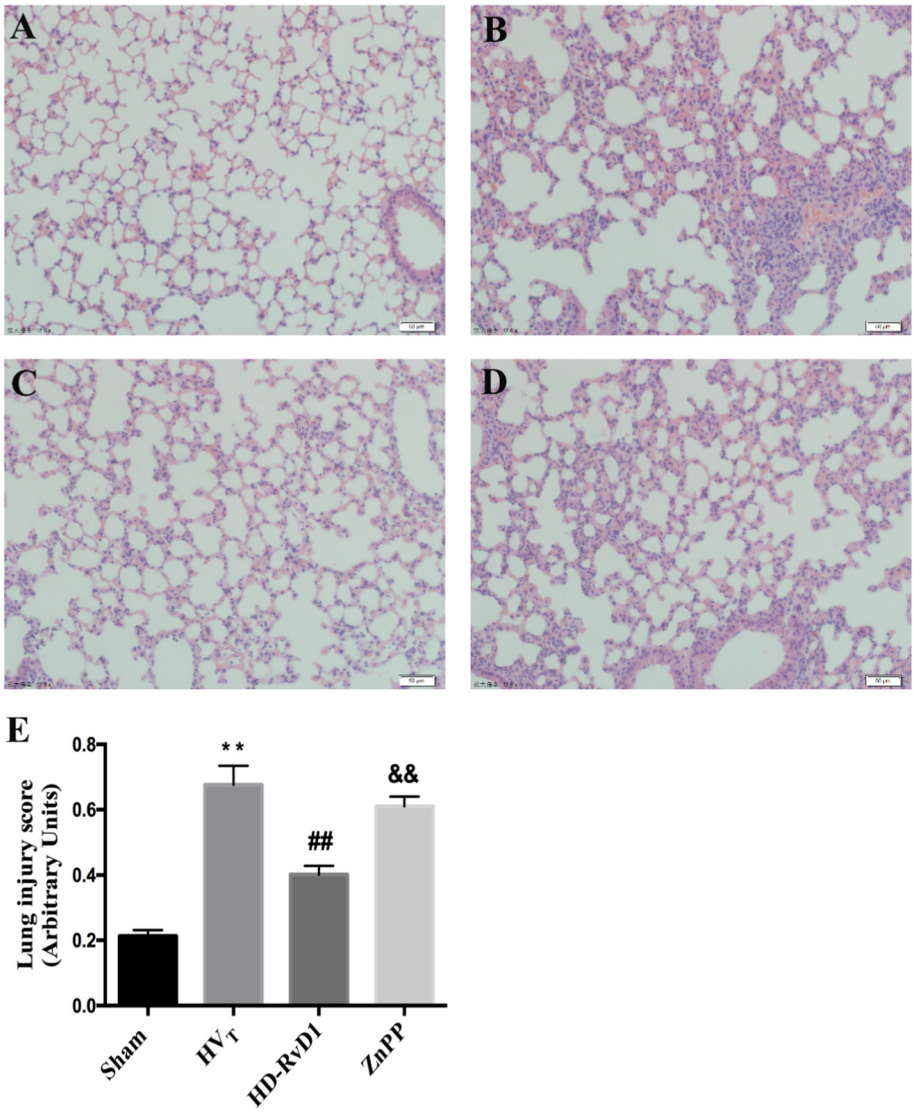

Fig. 5. Pathological changes and lung injury scores in mouse lung tissue. Representative hematoxylin-eosin staining pictures of lung tissue (magnification 200×). Sham group (panel A), HVT group (panel B), HD-RVD1 group (panel C), ZnPP + RvD1 group (panel D), Lung injury score (panel E). Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\#P < 0.05$ versus HVT group; $\&\&P < 0.01$ versus the HD-RvD1 group.

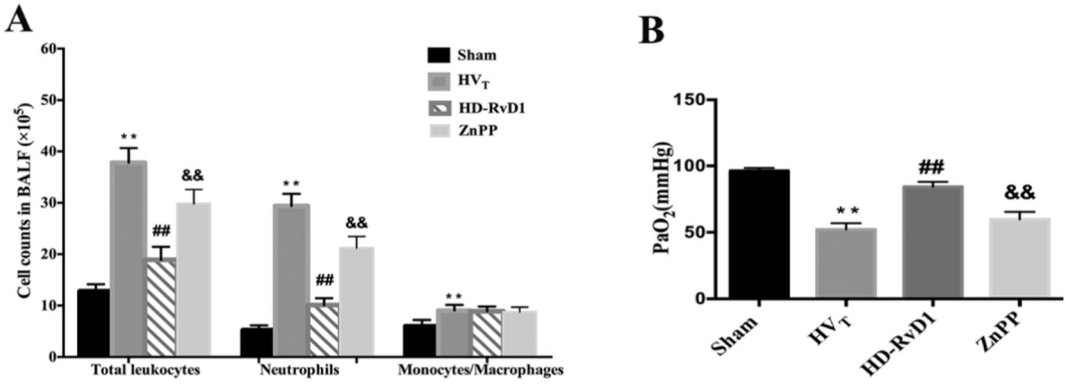

Fig. 6. PaO2 and the cell sorting count of alveolar lavage fluid in each group. Total number of cells, neutrophils and macrophages in alveolar lavage (panel A). The PaO2 in each group (panel B). Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\&\&P < 0.01$ versus the HD-RvD1 group.

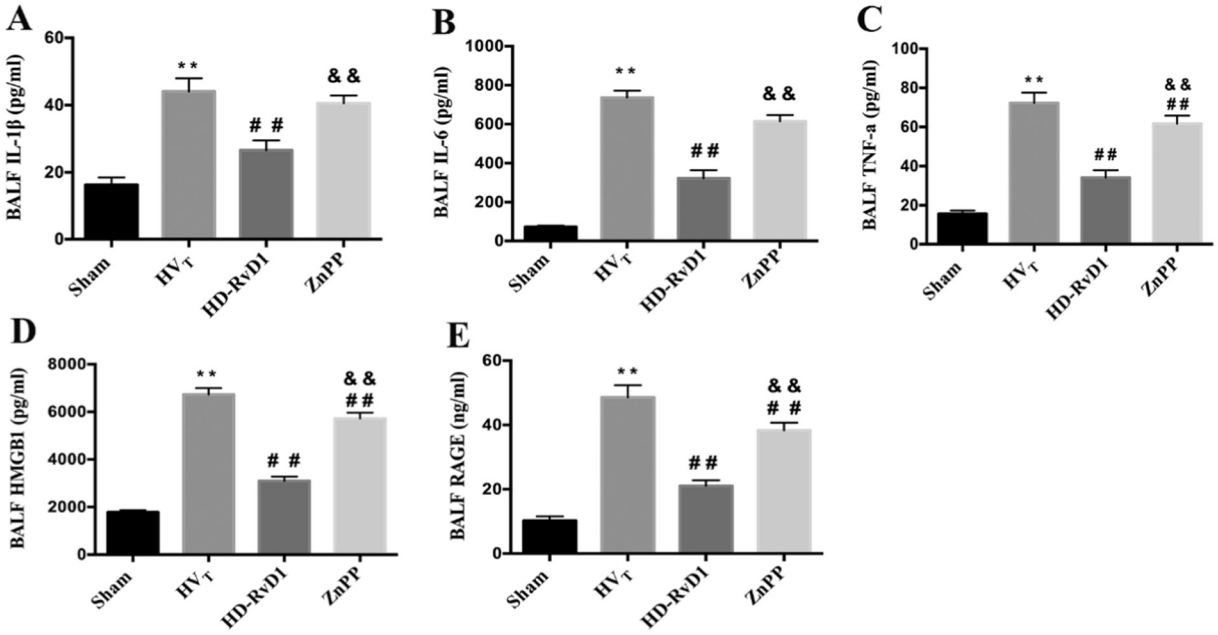

Fig. 7. Concentrations of IL-6, TNF-a, IL-1β, HMGB1 and RAGE in BALF. BALF IL-1β (panel A), BALF IL-6 (panel B), BALF TNF-a (panel C), BALF HMGB1 (panel D). BALF RAGE (panel E). Data are presented as means ± SEM, $n = 8$. $^{**}P < 0.01$ versus sham group; $\#\#P < 0.01$ versus HVT group; $\#P < 0.05$ versus HVT group; $\&\&P < 0.01$ versus the HD-RvD1 group.

protein concentration of BALF was measured according to the instructions of the Piece BCA Protein Assay kit.

2.7. Cell sorting count in alveolar lavage fluid

The BALF was centrifuged at 1500 r/min in a centrifuge at 4 °C for 10 min. The resulting pellet was resuspended in 1 ml of PBS, then a small amount of resuspended BALF was titrated on a cell counting plate and counted under a light microscope. In addition, a suitable amount of the suspension was applied to a glass slide (800 r/min) by a cytospin smear. 300 cells were counted under a light microscope after Wright staining, polymorphonuclear granulocytes and monocytes were counted respectively. The total number is calculated by determining the number of cells of each type.

2.8. Protein extraction and western blot

The total protein of lung tissue was isolated with the protein extraction kit (Nanjing KeyGen Biotech Co., Ltd.) according to the manufacturer's protocol. Protein concentrations were measured with the BCA Protein Assay kit. The samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) in 10% polyacrylamide gels. Immunoblotting was performed with Rabbit anti-HO-1 antibody (Abcam, USA), anti-HMGB-1 antibody, anti-NF-κB antibody(CST, USA) and anti-Nrf2 antibody (Abcam, USA),then using HRP-labeled-goat anti-rabbit antibody. Image J was utilized to quantify.

2.9. Statistical analysis

All results are expressed as means ± standard errors of the means (SEM) and analyzed using SPSS 18.0. One-way analysis of variance (ANOVA) was used for comparison between groups and the least significant difference (LSD) method was used for comparison between the two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. RvD1 attenuated ventilator-induced lung pathological injury

As shown in Fig. 1A, no significant change in the tissue of the sham operation group. Conversely, samples from HVT animals was obviously damaged, suggesting a significant improvement in inflammatory cell infiltration, alveolar interstitial edema, protein fragments filled with airspace and transparent membranes(Fig. 1B). RvD1 attenuated VILI in a dose-dependent manner: the histologic damage was significantly lower in the group receiving high dosage of RvD1 than in the one receiving low dosage of RvD1 (Fig. 1C). The lung injury scores were in accordance with the pathological change of lung tissues (Fig. 1E). This indicates that RvD1 has a protective effect on mechanical ventilation-induced lung injury, and the protective effect is associated with to the dose.

3.2. RvD1 significantly improves pulmonary function

PaO2 is an essential indicator of pulmonary function, and lung wet-to-dry ratio is an indicator of the degree of pulmonary edema. Alveolar lavage protein and MPO are important indicators for assessing lung inflammation. As shown in Fig. 2, The PaO_2 value of the sham operation group was normal, while the PaO2 value of the HV_T group was significantly lower than that of the sham group. As we expected,LD-RvD1 enhanced the level of PaO2, and this effect was dose-dependent (Fig. 2A). As for the lung dry-to-wet ratio (Fig. 2B), protein concentration in alveolar lavage fluid (Fig. 2C), and MPO (Fig. 2D) showed the opposite trend that mechanical ventilation significantly increased the above-mentioned indicators and RvD1 significantly improved the situation. The effect of high doses of RvD1 was substantially stronger than that of low doses. This indicates that RvD1 can improve pulmonary function in lung injury caused by mechanical ventilation and is positively correlated with dose.

3.3. RvD1 ameliorated concentrations of cytokines in alveolar lavage fluid

As shown in Fig. 3. The concentrations of TNF-a, IL-1β, IL-6,

HMGB1 and RAGE of BALF in the HV_T group were highly upregulated than those in Sham group explains that mechanical ventilation does lead to increased lung inflammation. The increase in the cytokines levels was significantly reduced in the LV-RvD1 group, and even lower in the HD-RvD1 group. It was shown that RvD1 can inhibit the production of pro-inflammatory cytokines, thereby alleviating lung inflammation. RvD1 inhibited the inflammatory response in a dose-dependent manner.

3.4. RvD1 increased expression of HO-1 and inhibited the production of HMGB-1 in lung tissue

As shown in Fig. 4, compared with the sham operation group, the production of HO-1 was increased in the HV_T group. Compared with the HV_T group, the expression of HO-1 in the LD-RvD1 group was significantly up-regulated and the up-regulation is more pronounced in the HD-RvD1 group. This indicated that the inflammatory response itself can promote the expression of HO-1 to a certain extent, while RvD1 promoted the expression of HO-1 in a dose-dependent manner. As for HMGB-1, the HMGB-1 value of HV_T group was significantly higher than that of sham operation group. LD-RvD1 could mitigate the expression of HMGB-1 and the inhibitory effect of HD-RvD1 was more obvious. The role of RvD1 in promoting HO-1 expression and inhibiting HMGB1 expression can be inhibited by the HO-1 specific inhibitor ZnPP, indicating that HMGB1 contribute to the downstream component of the HO-1 pathway.

3.5. ZnPP reversed the effect of RvD1 on lung tissue pathological injury

As shown in Fig. 5, no significant change in the tissue of the sham operation group. Pathological sections of lung tissue in HV_T group showed obvious damage to lung tissue compared with the sham group. The pathological changes of the lung caused by HV_T ventilation were not obvious when treated with high dose RvD1. However, the histological changes in the lungs of the ZnPP + RvD1 group were similar to those in the HV_T group. Lung injury scores accord with the pathohistological change. This indicated that RvD1 decreased the pathological damage of lung tissue caused by mechanical ventilation in part by increasing the expression of HO-1.

3.6. ZnPP inhibited the effect of RvD1 improves pulmonary function

As shown in Fig. 6, compared with the sham group, the PaO2 of HVT group was significantly decreased and the cell count of alveolar lavage fluid was considerably increased. The PaO2 of HD-RVD1 group was significantly higher than HVT group and the cell count of the alveolar lavage fluid was significantly lower than that of the HVT group. ZnPP inhibited RvD1 with increasing oxygen partial pressure and reducing the number of lymphocytes and neutrophils in alveolar lavage fluid suggesting that RvD1's role in improving pulmonary function is closely related to elevated HO-1 expression.

3.7. ZnPP escalated the expression of inflammatory cytokines in alveolar lavage fluid

As shown in Fig. 7. The concentrations of TNF-a, IL-1β, IL-6, HIGB1 and RAGE of BALF in the HVT group were significantly higher than those in the Sham group ($P < 0.05$). HD-RvD1 can inhibit the expression of the above inflammatory factors and this inhibition can be markedly attenuated by ZnPP. This indicates that the role of RvD1 reduces pulmonary inflammation is closely related to the expression of HO-1. At the same time, it was also shown that HMGB1 and Rage are downstream signals of HO-1, when HO-1 expression is inhibited, the expression of both HMGB1 and Rage are significantly increased.

4. Discussion

At present , mechanical ventilation is one of the most important clinical treatments, but the ventilator-related lung injury caused by it also poses challenges for clinical and basic research. As mentioned earlier, the occurrence of VILI involves multiple interactive pathophysiological mechanisms and inflammatory response. Except the application of small tidal volume mechanical ventilation, almost no effective clinical strategy has been developed to reduce the progression of VILI.

The existing research results suggested that the control and treatment of VILI through drug intervention to regulate pulmonary inflammation may become a new strategy for VILI. Endogenous lipid mediators can not only control the inflammatory response, but also promote inflammation cancellation. These lipid mediators can exert therapeutic effects on diverse inflammatory diseases by reducing the accumulation of inflammatory cells in local tissues and the expression of inflammatory factors. Although current research on RvD1 remains primarily in the cellular and animal stages, it provides a new direction for the treatment of acute lung injury including VILI [37].

Our study showed that in the VILI animal model of the experimental mice, the administration of RvD1 can significantly reduce the inflammatory response, ameliorate the pathological changes of lung tissue and reduce the expression of inflammatory factors. At the same time, RvD1 can significantly improve pulmonary function.

HO-1 plays an important protective role in many diseases, which is closely related to its anti-inflammatory and anti-oxidative stress effects. As mentioned early, it has been demonstrated that in the cardiovascular system, PPAR-y activation can induce up-regulation of HO-1 expression and thus play a protective role. Therefore, in the VILI model, whether the protective effect of RvD1 on lung injury is mediated by HO-1 is worthy of further investigation. In this study, administration of RvD1 attenuated lung injury and the application of the HO-1 specific inhibitor ZnPP eliminated the protective effect of RvD1 on lung. The results indicated that the protective effect of RvD1 on lung injury is at least partially mediated by HO-1. Interestingly, we found mechanical ventilation can increase the total number of cells and neutrophils in alveolar lavage fluid, but the increase in macrophage number is not obvious, at the same time, there was no statistically significant difference in the number of macrophages between ZnPP + RvD1 group and other groups. We speculate that this phenomenon may be related to the short time of our model, the macrophages inherent in lung tissue have not been polarized and the number of macrophages that migrate to the lungs through the peripheral circulation is also limited. Moreover, macrophages can exert anti-inflammatory effects by scavenging other inflammatory cells. The inflammatory response of the HVT group is enhanced, so that the anti-inflammatory response is also correspondingly improved.

NF-κB signaling pathway plays an important role in inflammation-related diseases including VILI. Nrf2 is a key regulator of cellular antioxidant system, which can regulate the expression of antioxidase [38] to maintain cell oxidation/antioxidant balance and protect cells. As mentioned early, there have been studies shown that Nrf2 [39] and NF-κB [40] are closely related to the expression of HO-1, so we verified the regulation of RvD1 on Nrf2 and NF-κB in VILI. Our studies (The results are shown in the supplementary materials.) showed that RvD1 inhibits the expression of NF-κB and improves the expression of Nrf2 in VILI in a dose-dependent manner. But unfortunately, we have not further explored the exact mechanism of RvD1's regulation of Nrf2 and NF-κB.

HMGB1 is an evolutionarily rich and highly conserved DNA binding protein. HMGB1 is derived from the passive release of necrotic cells or is actively secreted by the inflammatory cells into the extracellular environment during cellular stress to regulate innate and adaptive immunity. HMGB1 stimulates the immune response by binding to and interacting with its primary receptor, RAGE. Studies have shown that in acute lung injury, the content of HMGB1 is significantly increased and its elevation is closely related to clinical mortality [41], at the same

time, the expression of the RAGE is also significantly increased. The combination of the two can activate a series of signaling pathways including NF-KB, MAPK and PI3K etc. This leads to the production and release of inflammatory factors. Our study further confirmed that in the mouse VILI model, the content of HMGB1 and RAGE in alveolar lavage fluid was significantly increased accompanied by neutrophil exudation. The production of inflammatory factors IL-6, IL-1β and TNF-a was also significantly increased.

In recent years, activation of PPAR-y has been shown to reduce HMGB1 production and thereby inhibit inflammatory responses and reduce lung injury [42], however, the precise mechanism remains unclear. The results of this study showed that in the VILI model, RvD1 markedly inhibited HMGB1 production, RAGE expression, neutrophil infiltration and inflammatory factor release. As mentioned above, the activation of PPAR-y results in an increase in the expression of HO-1, so we further verified the relationship of HO-1, HMGB1 and rage. The results showed that ZnPP, the specific inhibitor of HO-1, can significantly inhibit the expression of HMGB1and RAGE. This suggests that RvD1 can protect the mouse VILI model through the HMGB1-RAGE signaling pathway and this effect is closely linked to HO-1.

5. Conclusion

In summary, RvD1 can decrease lung injury during mechanical ventilation and can protect the mouse VILI model through HMGB1-RAGE signaling pathway, which is related to the up-regulation of HO-1 expression.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105825.

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