

# Maresin 1 Attenuates Ventilator-induced Lung Injury by Reducing Oxidative Stress through the Nrf2/HO-1 and NF- $\kappa$ B Pathways

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## Research Article

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# Abstract

Mechanical ventilation could support the lives of patients with respiratory failure in a variety of ways, including maintaining airway patency and improving oxygenation, etc. However, mechanical ventilation itself could lead to lung damage, which is called mechanical ventilation-related lung injury (VILI). The incidence of VILI is high and the prognosis is poor, so clarifying the mechanism of VILI and seeking effective preventive and therapeutic measures are the urgent medical problem to be resolved. By constructing the VILI model, we studied the effect of Maresin1 on VILI and explored its possible mechanism at the animal level. We tested the related indicators of lung injury, the oxidative stress response, and the inflammatory response. The results indicated that Maresin1 could inhibit the oxidative stress response and excessive inflammation, thus ameliorating lung injury in VILI. We also detected the expression levels of the principal oxidative stress pathway Nrf2 / HO-1 and the key inflammatory transcription factor NF- $\kappa$ B. We used the HO-1 inhibitor ZnPP to further confirm our conclusion. Our results suggested for the first time that Maresin1 could promote the activation of the Nrf2 / HO-1 pathway and significantly suppress the expression of NF- $\kappa$ B to exert a positive role in VILI.

## 1. Introduction

Mechanical ventilation is a vital means of life support for the patients who cannot rely on their spontaneous breathing function to meet the need of their normal life activities[1], such as the patients who are under general anesthesia and suffering from respiratory failure. However, while mechanical ventilation provides respiratory support and improves the oxygenation function of the body, mechanical ventilation itself could also cause or aggravate the damage of lung tissue, known as ventilator-induced lung injury (VILI). VILI could even evolve into acute respiratory distress syndrome (ARDS) and endanger the patient's life[2]. Though the protective lung ventilation strategies have made definite progress, VILI still poses challenges for the clinical practice[3]. Therefore, the search for new and effective treatments for VILI is an important problem to be solved urgently[4].

The excessive stretch stimulation of large tidal volume mechanical ventilation on the alveolar wall may cause obvious pathological damage including the alveolar-capillary exudation, the neutrophil infiltration, the alveolar hemorrhage, and the alveolar wall thickening, etc.[5] Many studies suggested that in addition to the mechanical damage to VILI, the oxidative stress and the excessive inflammatory response could also significantly increase the severity of VILI[6]. Therefore, controlling the excessive oxidative stress response and the inflammatory responses provide targets for the prevention and treatment of VILI.

Nuclear factor erythroid 2 related factor2 (Nrf2) could promote the transcription of heme oxygenase-1 (HO-1), the indirect metabolite of which has a strong antioxidant capacity, so Nrf-2 / HO-1 plays an important role in cell adaptation and antioxidant stress[7]. HO-1 is the rate-limiting enzyme in the heme metabolism pathway in the human body. As a protective protein, HO-1 has numerous biological activities such as anti-oxidation, anti-inflammatory, anti-tracheal epithelial cell

proliferation, anti-apoptosis, and regulation of microcirculation. HO-1 could negatively regulate the inflammatory response and the oxidative stress response in acute lung injury[8].

NF- $\kappa$ B, as a target for many inflammatory diseases, is regulated by multiple signals. ROS, a vital production of oxidative stress, is considered to be one of the important regulators of NF- $\kappa$ B activation and plays an important role in the occurrence and development of inflammation[9]. ROS is necessary for the objects to play a role in eliminating bacteria, but overexpressed ROS could induce inflammation and results in tissue damage. Studies have shown that the activation of Nrf2 / HO-1 could inhibit the production of ROS induced by lipopolysaccharides (LPS) and reduce the expression of inflammatory mediators to play a protective role in improving lung injury[10, 11].

Specialized pro-resolving mediators(SPM) such as maresins and resolvins, which are derived from polyunsaturated fatty acids, play an important role in controlling inflammation and oxidative stress[12, 13]. As a novel SPM[14], the effect of Maresin1 on improving lung injury through multiple mechanisms has been confirmed[15, 16]. There was also a recent investigation shown that MaR1 could inhibit the oxidative stress in lung IRI[17]. However, whether MaR1 could inhibit the oxidative stress and the inflammation response to exert a protective role in VILI has not been reported.

Our previous studies have shown that Resolvin D1 could inhibit the expression of HMGB-1 via the Nrf2 / HO-1 pathway and thus play a positive role in VILI[18]. However, whether Maresin1 could reduce the oxidative stress response and the inflammatory response through the Nrf2 / HO-1 pathway and NF- $\kappa$ B have not been confirmed. Therefore, in this experiment, we chose the VILI model to verify whether Maresin1 could inhibit the oxidative stress response and the inflammatory response in VILI and whether its related effects are exerted through the Nrf2 / HO-1 and the NF- $\kappa$ B pathways.

## **2. Materials And Methods**

### **2.1 Animals**

The 6-8 weeks old, weighing 20-22 g, SPF male C57BL/6 mice were purchased from the Experimental Animal Center of Wuhan University. The mice were randomly divided into 3 per cage and with free access to food and water. All the mice were housed in the animal experiment center of the SPF environment with the humidity of 60–65%, the light-dark cycles 12/12h, and the temperature of 22-24°C. All the mice were acclimatized for one week before the start of the experiment. The experiment was performed in accordance with the Chinese Animal Research Guidelines and was approved by the Laboratory Animal Management Committee of Tongji Medical College of Huazhong University of Science and Technology.

### **2.2 Experimental design**

The construction method of the VILI model is the same as that described in our previous literature[18]. All experiments were repeated no less than three times.

The mice were first randomly divided into the following 4 groups (n=7): (1)The sham operation group (the Sham group): tracheal intubation was performed, but no mechanical ventilation was performed. 200  $\mu$ l of saline was injected into the tail vein 30 minutes before the tracheal intubation (2) The maresin1(Cayman Chemical, Ann Arbor, MI, USA) intervention control group—the MaR1 group: tracheal intubation was performed, but no mechanical ventilation was performed. Tail vein injection of Maresin1 1 ng(200  $\mu$ l) 30 minutes before the tracheal intubation; (3) The large tidal volume mechanical ventilation group (the HVT group): tidal volume 40 ml/kg; 200  $\mu$ l of saline was injected into the tail vein 30 minutes before the tracheal intubation. (4) the Maresin1 intervention group—the MaR1 + HVT group): tidal volume 40 ml/kg, tail vein injection of Maresin1 1 ng(200  $\mu$ l) 30 minutes before the beginning of the tracheal intubation. After that, we used Znpp, the specific HO-1 inhibitor. The mice were randomly divided into the following six groups(n=7). (1) The Sham group(2) The MaR1 group (3) The ZnPP intervention control group—the Znpp group: tracheal intubation was performed, but no mechanical ventilation was performed. The HO-1 antagonist ZnPP (10 mg/kg) was intraperitoneally injected and 200  $\mu$ l of saline was injected into the tail vein 30 minutes before the tracheal intubation. (4) The HVT group (5) The MaR1 + HVT group (6) The HO-1 antagonist zinc protoporphyrin + Maresin1 group (the ZnPP + Maresin1 group): ZnPP (10 mg/kg) was intraperitoneally injected 30 minutes before the start of the tracheal intubation and the rest of the treatment was consistent with the MaR1 + HVT group. The mice in the sham group, the MaR1 group, the HVT group, and the MaR1 + HVT group were intraperitoneally injected additional saline(10 mg/kg) 30 minutes before starting the experiment. The respiratory rate of each group of mechanical ventilation is 80 times/min, the mechanical ventilation time is 4h, FiO<sub>2</sub>=21%, I:E=1:2, PEEP=0 mmHg.

## 2.3 Histological analysis of lung tissues

The right upper lobe of the lung tissue was harvested for the pathological analysis. The lung tissue was made into slices about approximated 4  $\mu$ m thick after being fixed in 4% paraformaldehyde for 24h. The slices were stained with the hematoxylin and the eosin. Then the sections were observed under the ordinary optical microscope. The pathological injury score of the lung tissue was performed by using the lung tissue injury scoring criteria published by the American Thoracic Society[19].

## 2.5 Lung wet-to-dry weight ratio

The middle lobe of the right lung tissue from each group was collected to calculate the lung wet-to-dry weight ratio. After cleaning the surface of the fresh lung tissue with absorbent paper, weigh the initial weight of the lung tissue. Then put the lung tissue into the oven until the weight of the lung tissue becomes a constant weight. The weight of the lung tissue was weighed again. Calculate the lung wet-to-dry weight ratio and assess the extent of the lung tissue edema.

## 2.6 Analysis of arterial blood gas

At the end of the experiment, the blood (heparin anticoagulant) of the left carotid artery was collected and the arterial partial pressure of oxygen was immediately measured by a blood gas analyzer (Radiometer, Copenhagen, Denmark).

## 2.6 Measurement of the markers of the oxidative stress in lung tissues

According to the manufacturer's instructions, the malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), myeloperoxidase (MPO) and 15-F2t-isoprostane were detected by their corresponding commercial assay kits (Jiancheng Bioengineering Insti-

tute, Nanjing, China). The activities of these indicators were assessed by a spectrophotometer

## 2.7 Bronchial alveolar lavage fluid analysis

After the ligation of the right lung, 0.5 mL phosphate buffer saline (PBS) was utilized to lavage the left lung to obtain the BALF. The lavage process was repeated three times, and the recovery rate of more than 80% was considered effective.

The number of cells in the BALF was counted by a haematocytometer.

The protein concentration of the BALF was measured by using the BCA Protein Assay kit (Thermo Fisher Scientific).

According to the manufacturer's instructions, the corresponding murine ELISA kits (Dakewe Bioengineering Co., Ltd., Shenzhen, China) were used to evaluate the levels of the related inflammatory mediators (IL-6, TNF- $\alpha$ , IL- $\beta$ ) in the BALF.

## 2.10 Western blotting analysis

According to the manufacturer's protocol, the protein of the lung tissue was extracted by using the protein extraction reagent kit (KeyGEN BioTECH, Nanjing, China) and the nucleoprotein was extracted using the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, China). The protein concentration was measured with the BCA Protein Assay kit (Thermo Fisher Scientific). The western blotting analysis was performed as mentioned in our previous research[20]. The membrane was first incubated with the primary antibody: Nrf2 (Cell Signaling Technology, Danvers, MA, USA), HO-1 (Cell Signaling Technology, Danvers, MA, USA), NF- $\kappa$ B p65 (Cell Signaling Technology, Danvers, MA, USA), Histone 3 (Cell Signaling Technology, Danvers, MA, USA), GAPDH (Antgene, China) at 4°C overnight. Then the membrane was incubated with

the corresponding secondary antibody (Cell Signaling Technology, Danvers, MA, USA). Finally, the enhanced chemiluminescence reagent (Beyotime Institute of Biotechnology, Shanghai, China) and the UVP 160 imaging system (Upland, CA, USA) was used for exposure imaging. The grayscale value of the images was analyzed using the Image J software.

## 2.11 Statistical Analysis.

The whole data were expressed as means±SEM. The processes of the statistical analysis process and the mapping were performed by the Graphpad Prism 6.0 software. One-way analysis of variance (ANOVA) was used for comparison among groups, and the differences between groups were compared using Newman-Keuls.  $P < 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1 Maresin1 attenuated ventilator-induced lung injury and significantly improves pulmonary function.

The pathological changes of the lung tissue were assessed by HE staining (Figure 1A-D). The content of the lung injury in the sham group and the MaR1 group didn't show a significant difference, which indicated Maresin1 itself does not have obvious damage to the lung tissue. Excessive mechanical ventilation could cause evident lung injury and Maresin1 could attenuate the histological lung injury. The pathological injury score (Figure 1E) was in line with the results observed by He staining.

The detection results of the arterial partial pressure of oxygen (Figure 1F), the W/D ratio (Figure 1G), and the protein concentration in BALF (Figure 1H) were consistent with the results of the pathological changes. Maresin1 itself had no significant effect on the partial arterial oxygen pressure, the W/D ratio, and the protein concentration in BALF. The PaO<sub>2</sub> of the HVT group was significantly lower than the sham group ( $P < 0.01$ ) while the W/D ratio and the protein concentration in BALF were significantly higher than the normal mice ( $P < 0.01$ ), indicating that excessive mechanical ventilation could contribute to the lung injury. Compared with the HVT group, the PaO<sub>2</sub> in the MaR1 + HVT group was significantly increased ( $P < 0.01$ ), while the W/D ratio and the protein were significantly reduced ( $P < 0.01$ ), suggesting that Maresin1 could reduce lung injury and improve lung oxygenation function.

### 3.2 Maresin inhibited the excessive inflammatory response in VILI

The excessive inflammation is the main cause that leads to the occurrence of the VILI. Our results (Figure 2) showed that the cell count in BALF (Figure 2A), the MPO activity (Figure 2B) and the levels of pro-inflammatory factors (Figure 2C-E) in the HVT group were significantly increased compared to the normal

mice( $P < 0.01$ ), while Maresin1 significantly reduced the levels of pro-inflammatory factors, the cell count, and the MPO activity. These results suggested that excessive mechanical ventilation could cause a significant inflammatory response that leads to lung injury and Maresin1 had a significant role in anti-inflammatory.

### **3.3 Maresin1 inhibited the oxidative stress response in VILI**

Oxidative stress plays an indispensable role in the initiation and progression of the VILI. As shown in Figure 3A–F, compared with the sham group, the HVT group showed low expression levels in GSH-PX and CAT but a higher level of the ROS, MDA, SOD, and 15-F2t-isoprostane( $P < 0.01$ ). These tests revealed that excessive oxidative stress was indeed involved in VILI. Compared with the HVT group, the GSH-PX and CAT levels of the MaR1 + HVT group were higher( $P < 0.01$ ), and the ROS, MDA, SOD, and 15-F2t-isoprostane levels were lower( $P < 0.01$ ), clearing that Maresin could significantly inhibit the oxidative stress response.

### **3.4 Maresin1 promote the activation of the Nrf2/HO-1 pathway and inhibited the NF- $\kappa$ B.**

The level of the protein expression level of the Nrf2, Histone 3, HO-1, GAPDH, and NF- $\kappa$ B p65 protein in the lung tissues were analyzed by Western blotting to clarify the possible mechanism by which Maresin1 ameliorates the VILI. The results (Figure4 A-D) suggested that Maresin1 could significantly increase the protein expression level of HO-1 and the level of Nrf2 in the nucleus, at the same time, the expression of NF- $\kappa$ B p65 could be significantly inhibited by Maresin1. Furthermore, the inhibitor of HO-1, ZnPP, could significantly inhibit the effect of Maresin1(Figure4 E-H) on Nrf2/HO-1 and NF- $\kappa$ B p65. ZnPP reduced the expression of HO-1 and Nrf2 and increased the expression of NF- $\kappa$ B.

### **3.5 ZnPP inhibited the protective effect of Maresin1 on lung tissue in VILI**

As shown in Figure 5, there were no obvious pathological changes that occurred in the sham group, the MaR1 group, and the ZnPP group, indicating that Maresin1 and ZnPP do not cause lung injury by themselves. Pathological sections of lung tissue in the HVT group showed noticeable damage to lung tissue compared with the sham group( $P < 0.01$ ). The pathological changes in the lung caused by HVT became not obvious after the treatment of Maresin1. However, the histological changes in the lungs of the ZnPP + Maresin1 group were obviously more severe than those in the MaR1 + HVT group( $P < 0.01$ ). The lung injury score accorded with the pathohistological change. These results indicated that Maresin1 decreased the pathological damage of lung tissue caused by mechanical ventilation in part by increasing the expression of HO-1

## 3.6 ZnPP weakened the inhibition effect of Maresin1 on the oxidative stress and inflammatory response.

As shown in Figure 6, the MPO, the IL-1 $\beta$ , the TNF- $\alpha$ , the ROS, the MDA, and the 15-F2t-isoprostane in the HVT group were significantly higher than those in the sham group ( $P < 0.01$ ). Maresin1 could inhibit the expression of the factors referred to above ( $P < 0.01$ ). When the expression of HO-1 was inhibited by ZnPP, the MPO, the IL-1 $\beta$ , the TNF- $\alpha$ , the ROS, the MDA, and the 15-F2t-isoprostane showed a significant increase compared with the MaR1 + HVT group ( $P < 0.01$ ). These phenomena indicated that ZnPP could weaken the inhibitory effect of Maresin1 on the inflammation response and the oxidative stress response.

## 4. Discussion

As a common clinical treatment method, mechanical ventilation was widely used in clinical critical illness such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). However, the current reports suggested that the prevalence of VILI in surgical patients with normal lung function is between 6.2% and 24% [21, 22]. For patients with pulmonary dysfunction, the incidence of VILI is higher. Various known pathophysiological factors including inappropriate inflammatory response, excessive destruction of airway barrier, alveolar edema, cell apoptosis, etc. could contribute to the occurrence of the VILI, but the precise pathological mechanism of VILI has not yet been elucidated.

Although the research on the prevention and treatment of VILI has made certain progress, such as small tidal volume ventilation, the application of related drugs and biological agents [23, 24], the incidence of VILI remains remarkable and exploring the exact and effective treatment for VILI are still medical problems to be solved urgently.

There were studies have shown that Maresin 1 could ameliorate the lung ischemia/reperfusion injury by suppressing the oxidative stress via activating the Nrf-2-mediated HO-1 signaling pathway [17, 25]. However, there is no research indicating whether Maresin1 could mitigate lung injury by suppressing the oxidative stress in VILI. So we constructed the VILI model to clarify the role of the Maresin1 exerts in VILI and explore and the possible mechanism and treatment methods of VILI. Our previous research showed that Maresin1 could inhibit the excessive inflammation caused by sepsis in mice [20, 26] in a dose-dependent manner, the 1ng dose of Maresin1 can significantly protect mice and is a very suitable dose choice. There was also related study shown that 1ng of Maresin1 can effectively inhibit the oxidative stress response in lung tissue [17]. What's more, we chose two doses of Maresin1 of 0.1ng and 1ng for pre-experiment. The pathological results of the lung showed that the protective effect of 1 ng of Maresin1 on VILI was more stable and effective, so we chose 1 ng of Maresin for research.

We first verified the protective effect of Maresin1 in VILI. The results of the pathological section showed that Maresin1 could significantly ameliorate the extent of the pathological damage of lung tissue in VILI. Then we used the number of the cells in BALF to reflect the overall inflammation level, the protein concentration in BALF to assess the vascular permeability, and the changes in lung tissue W / D ratio to

measure the severity of the pulmonary edema. The large exudation of capillary fluid and protein is the main cause of pulmonary edema[27]. Our results showed that Maresin1 could mitigate the pathological damage of lung tissue, reduce the permeability of microvascular, and extenuate the degree of pulmonary edema in VILI. In addition, Maresin1 could dramatically improve lung oxygenation function.

Considering the important role of inflammatory response in VILI, we tested the relevant inflammatory indicators. The inflammatory mediators closely related to VILI are mainly MPO, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, etc[28]. MPO is a specific marker of PMN[19]. Our results showed that mice could develop severe inflammation after hyperventilation. The VILI mice showed a huge increase in related inflammatory indicators, such as cell counts in BALF and the levels of related inflammatory factors (eg IL-1 $\beta$ , IL-6, TNF- $\alpha$ ). Maresin1 could markedly inhibit the inflammation response.

As we mentioned earlier, oxidative stress and inflammation are closely related, both played a vital role in the start and development of VILI. So we also explored the oxidative stress response in VILI. When oxidative stress occurs, the imbalance between oxidation and antioxidants of the body could be reflected by the measurement of the levels of a number of related indicators. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) could prevent lung injury from ROS exposure[29]. MDA could reflect the degree of lipid peroxidation in the body, 15 F 2t isoprostane is a highly specific and sensitive indicator of lipid peroxidation products in the body. Therefore, we tested the above indicators in lung tissue, and the results showed that mechanical ventilation could significantly worsen the oxidative stress response, and Maresin1 could inhibit the oxidative stress response.

We next studied the classical pathway of oxidative stress Nrf2 / HO-1 and the important inflammation response transcription factor NF- $\kappa$ B. Under normal physiological conditions, Nrf2 combined with Keap1 and localized in the cytoplasm. When the oxidative stress response occurs, Nrf2 dissociates from the conjugate, translocates into the nucleus, and induces HO-1 transcription, thereby exerting antioxidant effects[30]. Uncontrolled oxidative stress response could also contribute to the activation of the NF- $\kappa$ B[31]. Under normal physiological conditions, the p65 subunit of NF- $\kappa$ B in the cytoplasm combines with I $\kappa$ B $\alpha$  to form the NF- $\kappa$ B - I $\kappa$ B $\alpha$  complex[32]. When cells are stimulated by oxidative stress, I $\kappa$ B $\alpha$  is phosphorylated and bound to ubiquitin to degrade. The degradation of I $\kappa$ B $\alpha$  results in that NF- $\kappa$ B is activated to bind to specific DNA sequences in the nucleus, thereby participating in the process of the inflammation responses[33]. So we examined the protein expression of HO-1 and the expression of Nrf2 and NF- $\kappa$ B in the nucleus after the Maresin1 treatment. The results showed that Maresin1 could promote the Nrf2 to translocate into the nucleus and significantly inhibit the translocation of NF- $\kappa$ B. To confirm our findings, we further used the HO-1 antagonist ZnPP. After the treatment of ZnPP, the expression levels of HO-1 and Nrf2 were drastically reduced, while the expression of NF- $\kappa$ B was significantly increased, indicating that Maresin1 could indeed inhibit excessive oxidative stress to improve lung injury in VILI by activating Nrf2 /HO-1 pathway.

To further verify that Maresin1 does exert antioxidant and anti-inflammatory effects through the Nrf2 / HO-1 pathway and NF- $\kappa$ B, we continued to use ZnPP to validate our hypothesis. As expected, when ZnPP

was used to inhibit the expression of HO-1, the degree of pathological damage in the lung tissue was significantly increased, and the blood oxygen partial pressure was considerably reduced. At the same time, we re-examined the relevant indicators of the oxidative stress response and the inflammation response. The results showed that when HO-1 was inhibited, the oxidative stress response and the inflammation response were significantly worsened. All these results indicated that Maresin1 significantly inhibited the production of pro-inflammatory factors via the suppression of the NF- $\kappa$ B and exerted the antioxidative effects via promoting the activation of the HO-1/Nrf2 pathway.

## 5. Conclusion

Our study showed that Maresin1 could reduce the oxidative stress response and the inflammation response through the Nrf-2 / HO-1 and the NF- $\kappa$ B signaling pathway, thereby significantly improving VILI.

## Abbreviations

Acute lung injury (ALI); Acute respiratory distress syndrome (ARDS) ; Catalase (CAT); Glutathione peroxidase (GSH-PX);The transcription of heme oxygenase-1 (HO-1); Nuclear factor erythroid 2 related factor2 (Nrf2) ;Superoxide dismutase (SOD);Specialized pro-resolving mediators(SPM) Lipopolysaccharides (LPS); Mechanical ventilation-related lung injury (VILI);

## Declarations

## Ethics approval and consent to participate

The experiment was performed in accordance with the Chinese Animal Research Guidelines and was approved by the Laboratory Animal Management Committee of Tongji Medical College of Huazhong University of Science and Technology.

## Consent for publication

All authors knew and agreed to publish.

## Availability of data and material

Data and materials can be obtained by contacting the corresponding author.

## Competing interests

The authors declare no competing financial interests.

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## Authors' contributions:

F.W. and G.P. wrote this article and performed the experiments. M.C. and J.W. established the animal models and collected samples. G.P. and F.W. analysed the data. J.W. and M.C. prepared the figures. H.X., S.Y. and D.Z. designed the experiments and revised the article.

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Not applicable

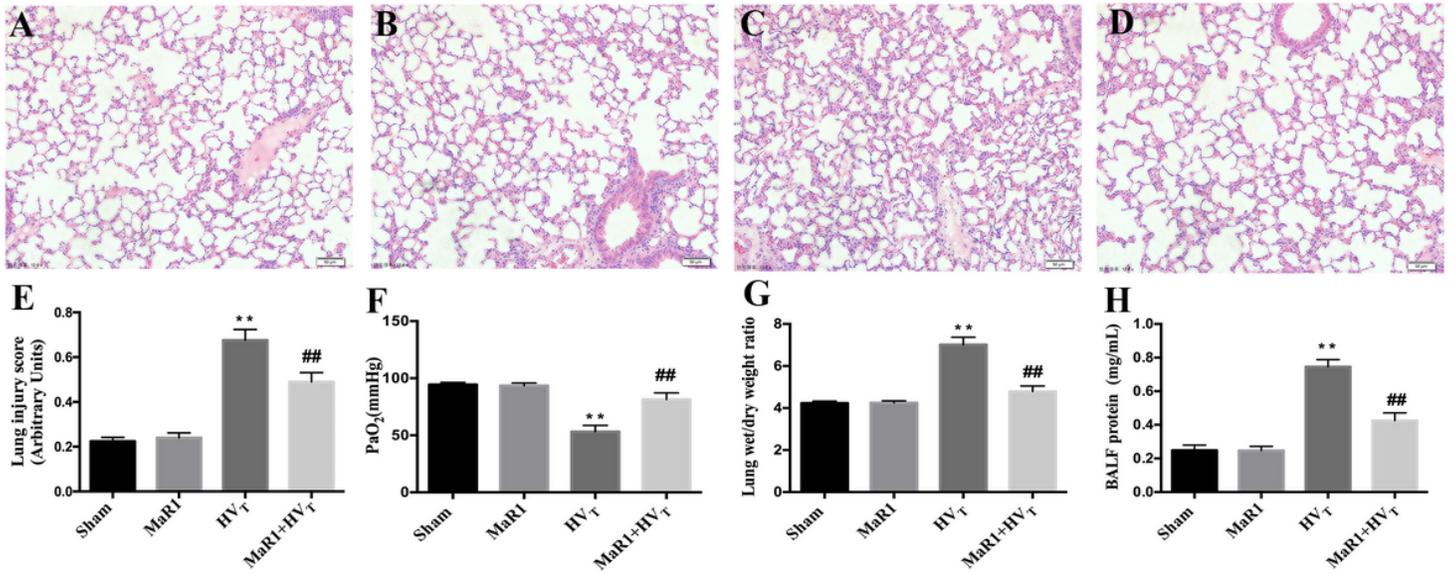
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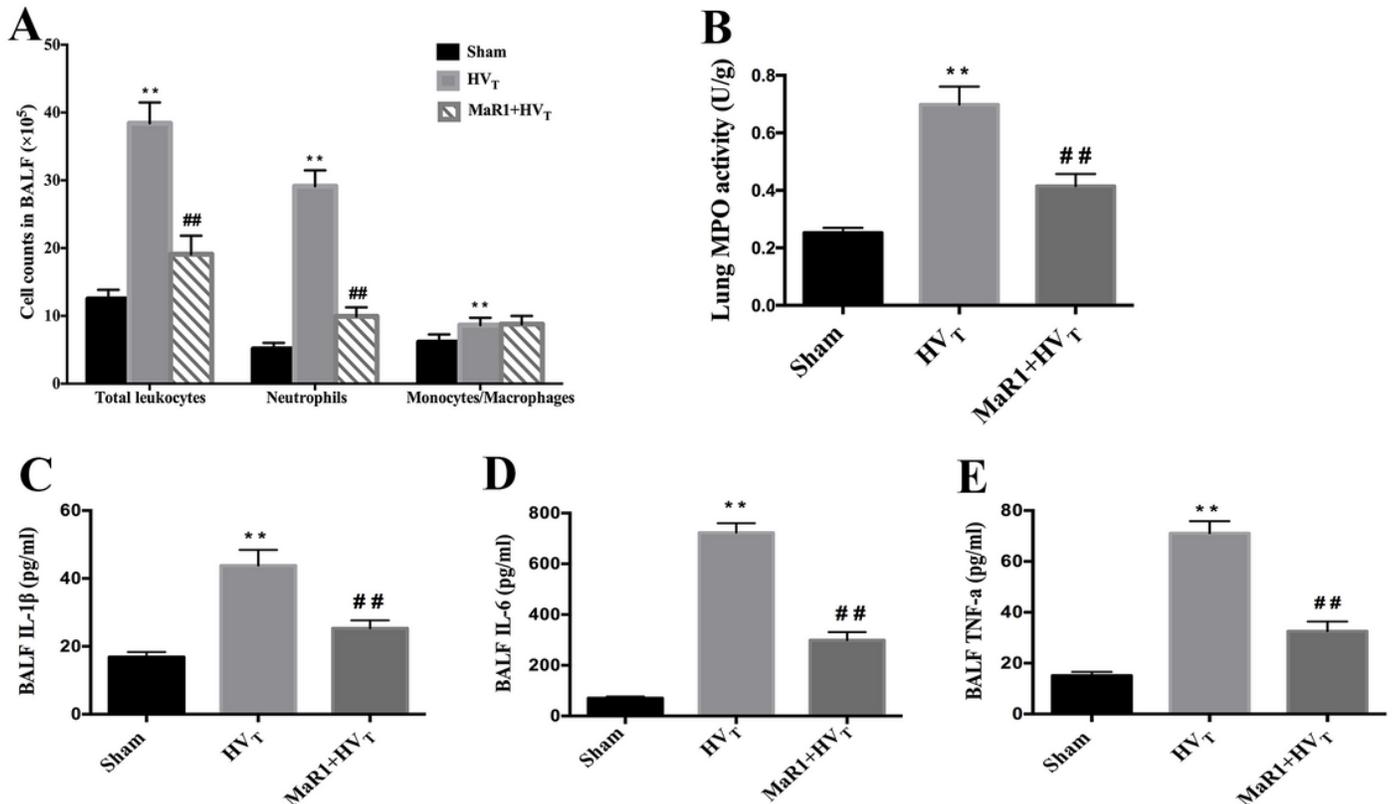
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## Figures



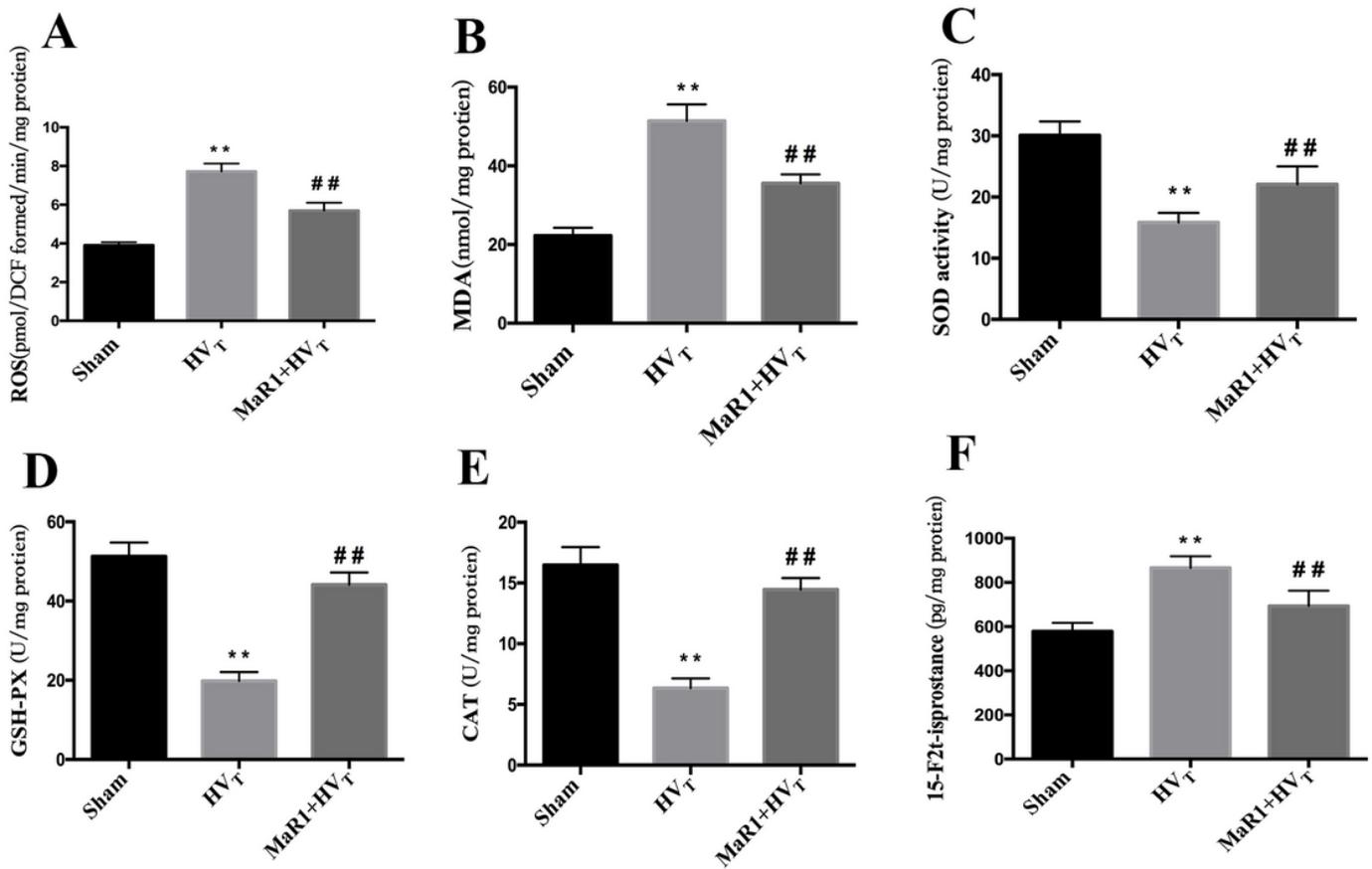
**Figure 1**

Maresin1 significantly improved the degree of lung injury in the VILI model. Fig2A-D are the representative pictures of H-E staining lung tissues (magnification 200×). A: The sham group B: The MaR1 group C: The HVT group D: The MaR1 + HVT group. E: The lung injury scores of all groups. F: The arterial partial pressure of oxygen G. The lung wet/dry weight ratio. H. The protein concentration in BALF. Data was presented as means ± SEM, n = 7. \*\*P < 0.01 versus the sham group; ##P < 0.01 versus the HVT group.



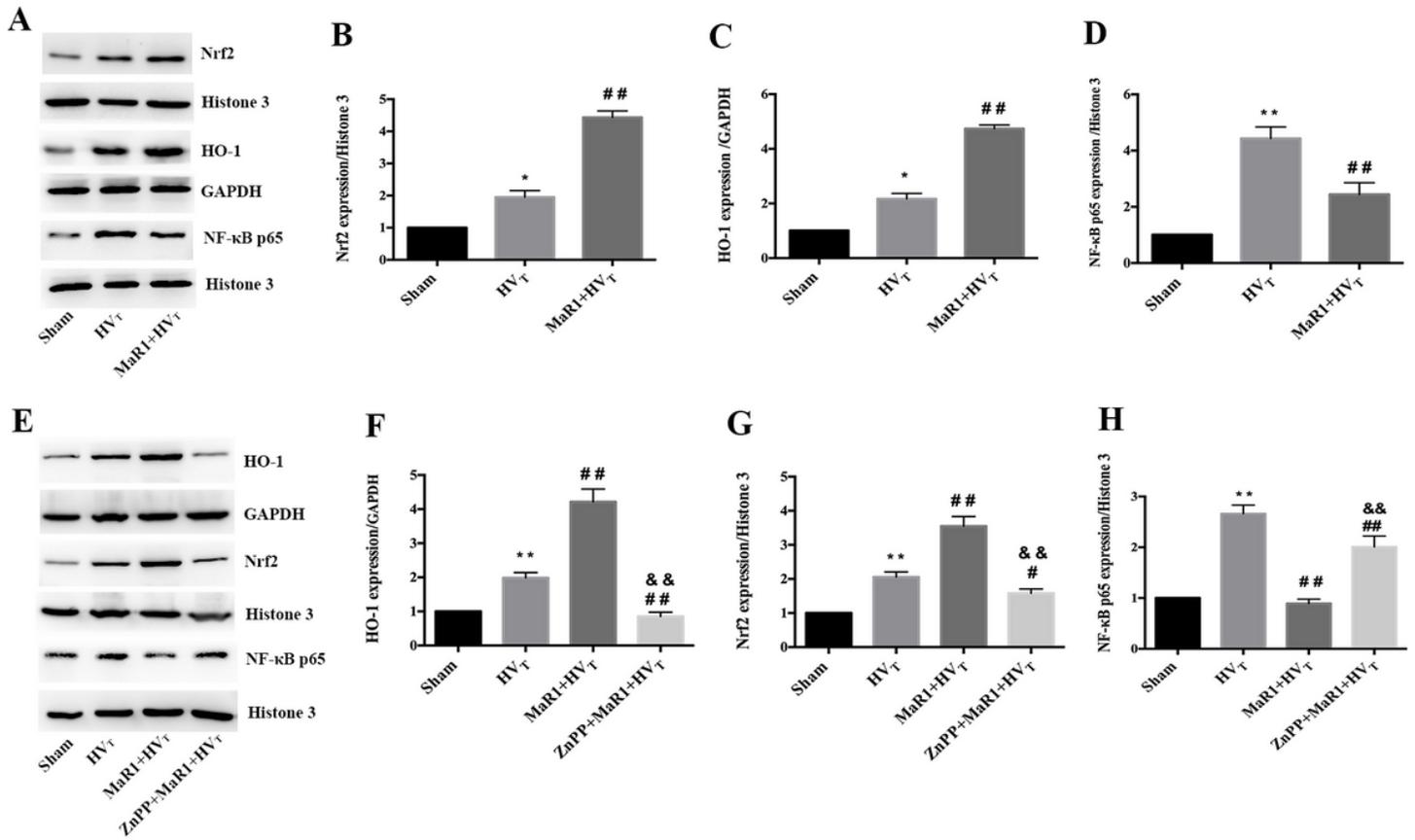
**Figure 2**

Maresin1 inhibited the excessive inflammation response in VILI. A: The cell count in BALF B: The MPO activity C: IL-1 $\beta$  D: IL-6 E: TNF- $\alpha$ . Data was presented as means  $\pm$  SEM, n = 7. \*\*P < 0.01 versus the sham group; ##P < 0.01 versus the HVT group.



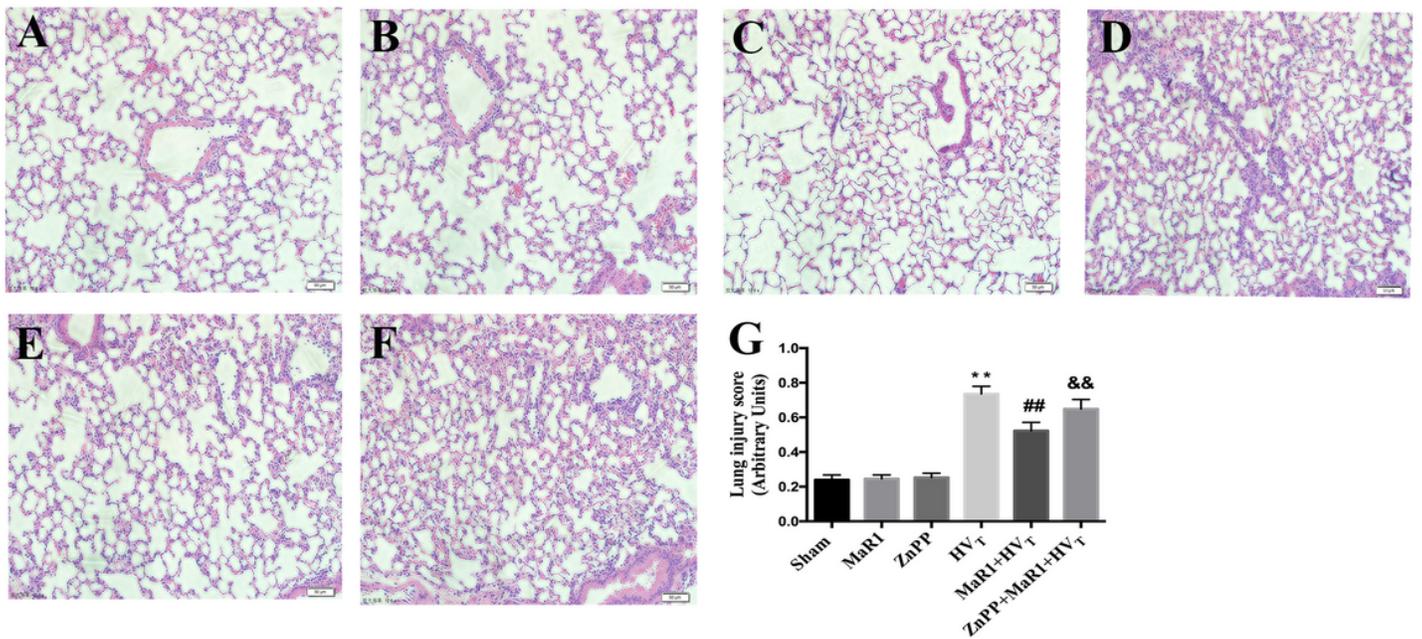
**Figure 3**

Maresin1 significantly inhibited the oxidative stress response in VILI. A: ROS B: MDA C: SOD D: GSH-PX E: CAT F: 15-F2t-isprostane Data was presented as means  $\pm$  SEM, n = 7. \*\*P < 0.01 versus the sham group; ##P < 0.01 versus the HVT group.



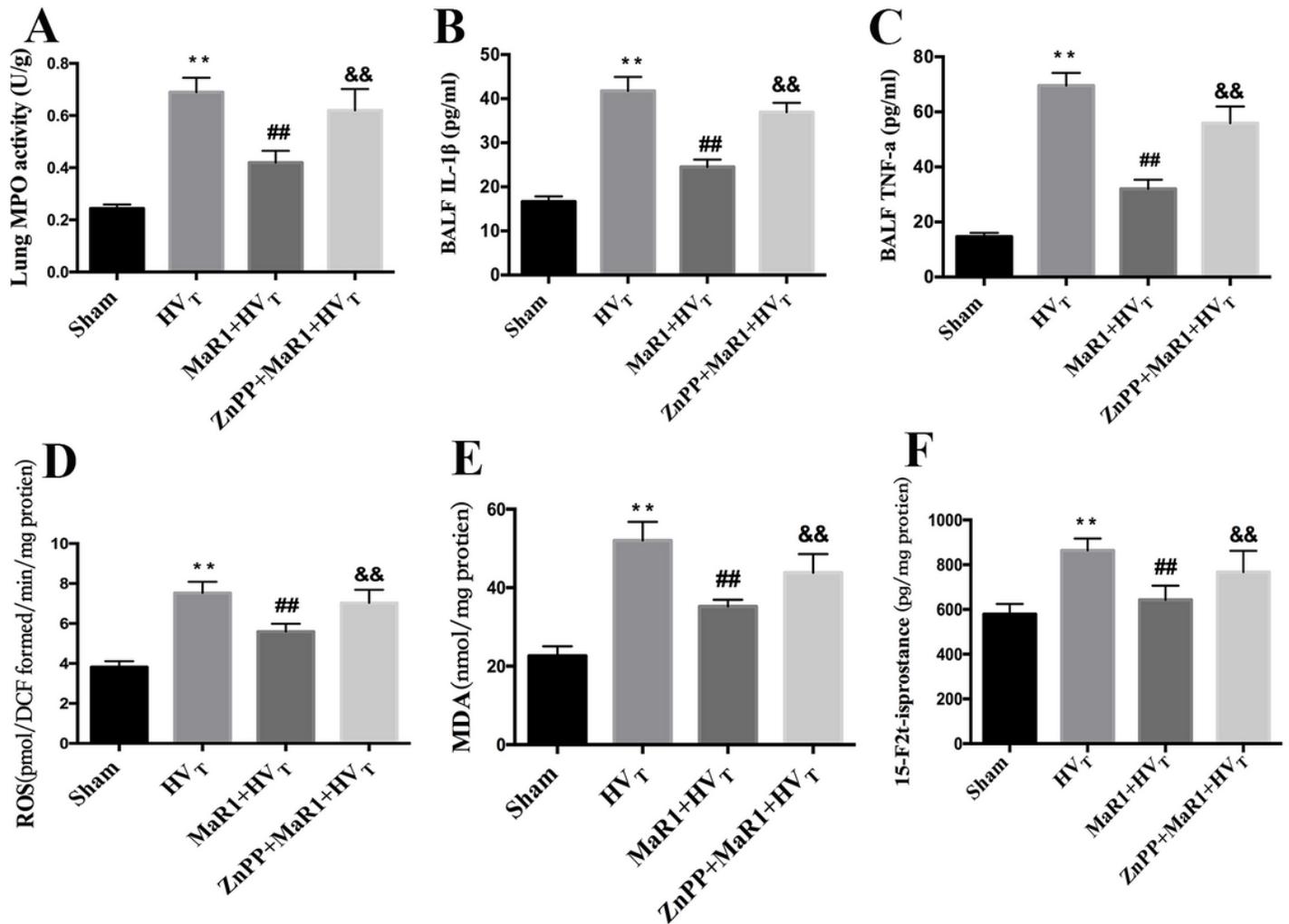
**Figure 4**

Maresin1 activated the Nrf2/HO-1 pathway and suppressed the NF-κB in VILI. A and E are the expression level of Nrf2, Histone 3, HO-1, GAPDH, and NF-κB p65 proteins of the lung tissues. B-D and F-H are the statistical tables corresponding to A and E respectively. Data was presented as means ± SEM, n = 7. \*P < 0.05 versus the sham group; \*\*P < 0.01 versus the sham group; #P < 0.05 versus the CLP group; ##P < 0.01 versus the CLP group; && P < 0.01 versus the MaR1 + HVT group.



**Figure 5**

ZnPP dampened the protective effect of Maresin1 on lung tissue in VILI. Fig6A-F are the representative pictures of H-E staining lung tissues (magnification 200×). A: The sham group B: The MaR1 group C: The ZnPP group D: The HVT group E: The MaR1 + HVT group. F: The ZnPP + Maresin1 group G: Lung injury scores of all the groups. Data was presented as means  $\pm$  SEM, n = 7. \*\*P < 0.01 versus the sham group; ##P < 0.01 versus the HVT group ;&& P < 0.01 versus the MaR1 + HVT group.



**Figure 6**

ZnPP weakened the inhibition effect of Maresin1 on the inflammation response and the oxidative stress response. A: MPO activity B: IL-1 $\beta$  C: TNF- $\alpha$  D: ROS E: MDA F: 15-F2t-isprostane. Data was presented as means  $\pm$  SEM, n = 7. \*\*P < 0.01 versus the sham group; ##P < 0.01 versus the HVT group;&& P < 0.01 versus the MaR1 + HVT group.