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Long-term administration of simvastatin reduces ventilator-induced lung injury and upregulates heme oxygenase 1 expression in a rat model

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ABSTRACT

Background: Simvastatin reduces ventilator-induced lung injury and is regularly used in clinical practice. This study aimed to test the hypotheses that long-term use of simvastatin could affect the incidence and severity of ventilator-induced lung injury after mechanical ventilation, and the process may involve heme oxygenase-1 (HO-1).

Materials and methods: Forty healthy adult Sprague–Dawley rats were randomly divided into four groups, namely control, ventilation, simvastatin, and simvastatin + ventilation groups. Saline (control and ventilation groups) or 10 mg kg^{-1} d^{-1} simvastatin (simvastatin and simvastatin + ventilation groups) was administered by gavage to the animals for 4 wk. Mechanical ventilation (tidal volume 50 mL/kg) was then applied for 4 h to the ventilation and simvastatin + ventilation groups. Lung tissues were harvested for hematoxylin-eosin staining and pathologic examination, and HO-1 contents were measured by immunoblotting and polymerase chain reaction.

Results: A severe pathologic damage was observed in rats that underwent mechanical ventilation. Interestingly, protein concentration, wet/dry weight ratio, myeloperoxidase activity, and malondialdehyde level were increased, and superoxide dismutase activity decreased, in lung tissues after mechanical ventilation. The pathologic damage was substantially alleviated in rats treated with simvastatin before mechanical ventilation: reduced protein concentration, wet/dry weight ratio, myeloperoxidase activity, and malondialdehyde level, and increased superoxide dismutase activity in lung tissues, compared with the ventilation group. Both mechanical ventilation and simvastatin administration induced HO-1 messenger RNA and protein expression in lung tissues.

Conclusions: Long-term administration of simvastatin significantly reduces the inflammatory response and pulmonary injury induced by mechanical ventilation, potentially by upregulating HO-1 in lung tissues.

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1. Introduction

Mechanical ventilation is often required in the management of acute lung tissue injury (ALI) and acute respiratory distress syndrome. However, ventilation by itself may aggravate lung injuries [1]. Ventilator-induced lung injury (VILI) describes ALI resulting from the interaction between the underlying pulmonary diseases and mechanical ventilation [2]. The mechanical forces in VILI not only may directly induce tissue damage but also can promote the activation of immune cells, including neutrophils and macrophages [3,4], causing the release of cytokines and other inflammatory factors [5–7]. Prolonged mechanical ventilation has also been shown to increase oxidative stress in epithelial cells, endothelial cells, and vascular smooth muscle cells, causing cell edema and death [7–9].

Statins, a class of lipid-lowering drugs, can reduce the incidence and severity of coronary heart diseases. In recent years, accumulating evidence suggests various additional functions for statins, including inhibition of platelet aggregation and coagulation, oxidation lowering, stabilization of atherosclerotic plaques, inhibition of mesangial cell proliferation, and inflammation reduction [10]. Therefore, statins are widely used to treat osteoporosis, renal disease, Alzheimer's disease, tumors, coagulation system disorders, and cardiovascular and cerebrovascular diseases [11]. Animal experiments have previously demonstrated that statins could reduce the severity of ALI [12–20], improving outcomes in pneumonia and sepsis [21]. Clinical studies have also suggested that statins may reduce the acquisition of pneumonia and ameliorate the symptoms of severe pneumonia [21] and pulmonary arterial hypertension [22]. However, despite reducing endothelial dysfunction and exerting antithrombotic effects in animal models, statins do not ameliorate the increased capillary permeability that occurs after cardiac or major vascular surgery [21,23].

The heat shock protein heme oxygenase-1 (HO-1) is induced in response to stress *in vivo*. Its cellular level can be increased by over 100-fold, mediating cytoprotection against oxidative injury and cellular stress [24]. Indeed, HO-1 expression has been associated with the cellular response to oxidative stress [25]; in addition, increased HO-1 expression protects against VILI [26]. Studies of lung transplantation and hyperoxic lung injury have reported that elevated HO-1 expression is associated with the severity of organ damage [27,28], suggesting that HO-1 induction may represent a potential prophylactic clinical strategy to prevent VILI [29]. In a mouse model, HO-1 expression was reported to modulate the cytoprotective response to lung injury [30].

Interestingly, a previous study in mice demonstrated that high doses of the statin simvastatin administered 24 h before mechanical ventilation attenuate VILI by reducing pulmonary inflammation [14,31]. In addition, it was demonstrated that simvastatin inhibits the proliferation of pulmonary artery smooth muscle cells through upregulation of HO-1 and p21WAF1, therefore protecting against pulmonary hypertension [32]. Indeed, statins such as simvastatin are widely used in clinical practice, with a significant proportion of patients regularly taking simvastatin. Therefore, we hypothesized that long-term use of simvastatin could affect the incidence and severity of VILI after mechanical ventilation, with this process possibly involving HO-1. Accordingly, this study aimed to assess the effects of long-term simvastatin use on VILI after mechanical ventilation. In addition, we investigated whether the efficacy of simvastatin might be related to HO-1 induction in lung tissues.

2. Materials and methods

2.1. Animals

Forty healthy adult Sprague–Dawley rats (220–300 g) were purchased from the animal center of Xuzhou Medical College. Animals were housed at 24 ± 2°C with 12 h light–dark cycle and free access to food and water.

Rats were randomly divided into 4 groups of 10, including the control, ventilation, simvastatin, and simvastatin + ventilation groups. Control and ventilation group rats received gavage with saline for 4 wk. Rats in the simvastatin and simvastatin + ventilation groups were treated orally with 10 mg kg^{-1} d^{-1} simvastatin (dissolved in 1-mL saline) for 4 wk. Rats in the ventilation and simvastatin + ventilation groups underwent mechanical ventilation for 4 h (tidal volume 50 mL/kg).

Ten percent chloral hydrate (320 mg/kg) was intraperitoneally injected 30 min after administration of normal saline or simvastatin on the 28th day to anesthetize the rats. Animals in the control and simvastatin groups were allowed to breathe spontaneously for 4 h, whereas those of the ventilation and simvastatin + mechanical ventilation groups underwent tracheotomy. For mechanical ventilation, the tracheal catheter was fixed by thread and connected to a ventilator (DH-150; Medical instrument factory, Zhejiang University, Hangzhou, China) for 4 h, with breath frequency of 50 per min, inhalation/exhalation rate of 1:3, positive end-expiratory pressure of 0, tidal volume of 50 mL/kg, and fraction of inspired oxygen of 21%.

The study was approved by the Committee of Animal Usage and Care of the Affiliated Hospital of Xuzhou Medical College, Xuzhou 221004, China.

2.2. Pathologic examinations and enzyme activity measurements

After 4 h of spontaneous breath or mechanical ventilation, rats were sacrificed by bloodletting from the abdominal aorta. The upper right pulmonary lobe was harvested and fixed in 10% paraformaldehyde for 48 h, then paraffin embedded, and sliced (3–4 μm). Hematoxylin-eosin (H&E) staining was performed following standard protocols, and tissue samples were observed by light microscopy. The severity of diffuse alveolar damage (DAD) was evaluated by measuring alveolar fibrin exudation, pulmonary interstitial edema, alveolar bleeding, alveolar neutrophil count, and pulmonary interstitial neutrophil count, which were each scored 0, 1, 2, and 3 for normal, mild, moderate, and severe, respectively. The sum of these five scores was calculated to indicate the severity of