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Saturated hydrogen saline attenuates endotoxin-induced lung dysfunction

*Yan Zhang, PhD, Yiming Liu, MD, and Jin Zhang, PhD**

Department of Anesthesiology, Shengjing Affiliated Hospital, China Medical University, Shenyang City, Liaoning Province, PR China

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ABSTRACT

Background: Acute lung injury induced by lipopolysaccharides (LPSs) is caused by pulmonary inflammation and pulmonary vascular permeability. Activation of p38 mitogen-activated protein kinase causes inflammation, and proinflammatory cytokines and oxidative stress induce autophagy, a catabolic mechanism responsible for protein degradation and recycling of damaged proteins and cytoplasmic organelles. If not controlled, excessive autophagy responses can result in cell death.

Materials and methods: In this study, we pretreated rats with saturated hydrogen saline, and examined the molecular mechanism by which saturated hydrogen saline attenuates LPS-induced acute lung dysfunction. Sixty-four male Sprague–Dawley rats were randomly assigned to one of three groups—a control group, an LPS group, or an LPS plus saturated hydrogen saline (LPS + H_2) group.

Results: Treatment with saturated hydrogen saline prolonged the median survival time of rats and reduced lung dysfunction induced by LPS. Moreover, saturated hydrogen saline significantly attenuated LPS-mediated induction of serum tumor necrosis factor α, interleukin 6, myeloperoxidase, and malondialdehyde ($P < 0.05$).

Conclusions: Autophagosomes were found in the cytoplasm of type II alveolar epithelial cells of LPS-treated rats, and light chain 3 protein (LC3)I/II was increased by LPS treatment. In contrast, saturated hydrogen saline decreased the number of autophagosomes and LC3I/II expression. Saturated hydrogen saline also attenuated the LPS-mediated increase in apoptosis and p38 expression. Taken together, saturated hydrogen saline may attenuate LPS-induced acute lung dysfunction in rats by reducing inflammation, autophagy, and apoptosis involving the p38 mitogen-activated protein kinase signaling pathway.

1. Introduction

Acute lung injury (ALI) can be caused by various internal and external factors and is characterized by excessive pulmonary inflammation and pulmonary vascular permeability [1]. Gram-negative bacterial infections are a common cause of ALI. The main active ingredient of bacterial endotoxin is lipopolysaccharide (LPS), which induces inflammation and thus contributes to diffuse lung tissue damage [2]. Sustained ALI can lead to acute respiratory distress syndrome or multisystem organ failure. In pathologic conditions, such as ischemia or inflamed ALI, the body produces large amounts of free radicals, which cause oxidative damage to cells. Excessive production of free radicals, including the highly toxic hydroxyl

* *Corresponding author*. Department of Anesthesiology, Shengjing Affiliated Hospital, China Medical University, No. 36, Sanhao Street, Heping District, Shenyang City, Liaoning Province, PR China.Tel: 008618940259646; fax: 008602423894455.
E-mail address: zhangj_1@sj-hospital.org (J. Zhang).

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radical (•OH), can cause oxidative damage to DNA, lipids, and proteins. To date, no endogenous pathway regulating •OH scavenging has been identified in mammals [3]. However, saturated hydrogen saline displays antioxidant properties by selectively removing cellular hydroxyl radicals and protecting cells and tissues from oxidative damage [4–10]. Some studies have suggested that saturated hydrogen saline is protective against ischemia-reperfusion–induced ALI by inhibiting oxidative stress and decreasing cytokine expression, apoptosis, autophagy, and lung tissue damage [11]. However, whether saturated hydrogen saline has any protective effect against LPS-induced lung injury remains unclear.

Systemic inflammation is increasingly prevalent among individuals in several countries [12] and is strongly associated with multiple organ dysfunction syndrome [13]. Both systemic inflammatory response syndrome and multiple organ dysfunction syndrome are characterized by excessive inflammation, accompanied by increased cell death in the affected organs [14]. Autophagy, an oxidative stress injury–induced form of non-apoptotic programmed cell death, is also likely to be involved in the development of inflammatory diseases and organ dysfunction [15–17]. The process of autophagy is homeostatic and generally occurs without affecting neighboring cells. This adaptive cellular response to nutrient deprivation ensures minimal housekeeping functions and nutrient recycling [18]. However, excessive autophagy is often associated with pathologic conditions that are characterized by an altered inflammatory response [19]. It can also contribute to the development of various lung diseases and infections [20]. Additionally, autophagy promotes inflammatory responses [21]. Thus, excessive autophagy responses may lead to increased cell death and pathologic conditions [22,23]. Saturated hydrogen saline has been shown to reduce the rate of apoptosis in liver tissue [2]. However, whether saturated hydrogen saline can protect against LPS-induced autophagy and apoptosis is unknown.

Signaling *via* the p38 mitogen-activated protein kinase (p38MAPK) pathway regulates multiple cellular processes, including apoptosis, cell cycle arrest, and autophagy [24,25]. However, a potential role of p38MAPK in mediating the effects of saturated hydrogen saline on apoptosis and autophagy in ALI has not been explored.

Here, we examine if saturated hydrogen saline can protect against LPS-induced lung injury by inhibiting excessive autophagy responses and apoptosis. We also explore the mechanism by which p38 signaling may regulate this process.

2. Materials and methods

2.1. Reagents

Saturated hydrogen saline was provided by the Department of Diving Medicine, Faculty of Navy Medicine, Second Military Medical University (Shanghai, China). LPS (*Escherichia coli* 055:B5) was purchased from Sigma (St. Louis, MO). Myeloperoxidase (MPO) and malondialdehyde (MDA) detection kits were purchased from the NanJing Jiancheng Bioengineering Institute (China). Rabbit polyclonal antibodies against light chain 3 (LC3)I and LC3II were obtained from Abcam (Cambridge, MA). Rabbit polyclonal antibodies to p38 and p-p38 were obtained from Abcam. Enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor (TNF)-α and interleukin (IL)-6 were obtained from Abcam. Caspase 3 colorimetric activity assay kit was obtained from Chemicon International (Temecula, CA). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit was purchased from Roche (Nutley, NJ).

2.2. Animal studies

A total of 64 specific pathogen-free male Sprague–Dawley rats (body weight 220–250 g) were provided by the Laboratory Animal Center at the Affiliated Shengjing Hospital of China Medical University in Shenyang, China. The rats were housed in a controlled environment at 24 ± 2°C with a 12-h light–12-h dark cycle. Animals had free access to food and water. Animals were randomly assigned to one of the following three treatment groups: control group ($n = 8$), LPS group ($n = 28$), or LPS plus saturated hydrogen saline (LPS + H_2) group ($n = 28$). Animals in the LPS group received a single caudal vein injection of LPS (10 mg/kg), and animals in the control group received the same dose of normal saline using the same method. The saturated hydrogen saline was stored under atmospheric pressure at 4°C in an aluminum bag without dead volume. Hydrogen-rich saline (H_2) was freshly prepared every week and stored at 4°C to ensure a constant concentration of >0.6 mM. Rats in the LPS + H_2 group were given 8 mL/kg saturated hydrogen saline by caudal vein injection 20 min before LPS treatment and then every 1 h saturated hydrogen saline was injected once for six continuous hours after LPS administration. This dosing regimen was repeated six consecutive times. The first caudal vein injection of hydrogen-rich water was given 20 min before LPS treatment and every hour for six continuous hours after LPS administration. Rats in both the LPS and the LPS + H_2 groups were monitored for 72 h after the final injection to assess animal survival; all remaining rats were anesthetized *via* intraperitoneal injection of 15% urethane (3.75 mL/kg body weight), 6 h after the final injection. Arterial blood gas analysis was performed hourly (Alpha-Stat, Rapidlab 865 Blood Gas Analyzer; Bayern, Mijdrecht, The Netherlands). After sacrifice, tissue samples from each rat were collected for examination. All efforts were made to minimize both animal suffering and the number of animals used. All animal procedures were approved by the Animal Use and Care Committee of the Affiliated Shengjing Hospital of China Medical University.

2.3. Wet-to-dry lung weight ratio

To quantify the magnitude of pulmonary edema, we evaluated the wet-to-dry (W/D) lung weight ratio. The harvested wet lung was weighed. It was then placed in an oven at 80°C for 24 h and reweighed once dried.

2.4. Optical microscopy

Lung tissues were carefully removed from animals 6 h after the final injection. Paraffin-embedded lung tissues were sectioned into 5 μm slices, then fixed in 4% paraformaldehyde, deparaffinized, and rehydrated. Samples were stained with hematoxylin and eosin, mounted, and evaluated by