Archives of Medical Research 47 (2016) 275–284

Archives of Medical Research

ORIGINAL ARTICLE

Budesonide Attenuates Ventilator-induced Lung Injury in a Rat Model of Inflammatory Acute Respiratory Distress Syndrome

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Received for publication March 22, 2016; accepted July 22, 2016 (ARCMED-D-16-00184).

Background and Aims. Patients with acute respiratory distress syndrome (ARDS) are particularly susceptible to ventilator-induced lung injury (VILI). This study investigated the effect of budesonide on VILI in a rat model of inflammatory ARDS.

Methods. Forty eight rats were randomized into three groups ($n = 16$ each): sham group (S), endotoxin/ventilation group (LV), endotoxin/ventilation/budesonide group (LVB). Rats in the S group received anesthesia only. Rats in the LV and LVB groups received endotoxin to simulate ARDS and were mechanically ventilated for 4 h (tidal volume 30 mL/kg). Rats in the LVB group received budesonide 1 mg, and rats in the LV group received saline in airway. PaO_2/FiO_2, lung wet-to-dry weight ratios, inflammatory factors in serum and bronchoalveolar lavage fluid (BALF), histopathologic analysis of lung tissue, and survival were examined.

Results. PaO_2/FiO_2 was significantly increased in rats in the LVB group compared to the LV group. Total cell count, macrophages, and neutrophils in BALF, and levels of intercellular adhesion molecule (ICAM)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8 in BALF and serum were significantly decreased in rats in the LVB group compared to the LV group, whereas levels of IL-10 in BALF and serum were significantly increased. Histopathological changes of lung injury and apoptosis were reduced, and survival was increased in rats in the LVB group compared to the LV group.

Conclusions. Budesonide ameliorated VILI in a rat model of inflammatory ARDS. © 2016 IMSS. Published by Elsevier Inc.

Key Words: Budesonide, Ventilator-induced lung injury, Acute respiratory distress syndrome.

Introduction

Approximately 39% of patients in intensive care units (ICUs) require mechanical ventilation (MV) (1). However, MV can lead to ventilator-induced lung injury (VILI). In fact, an estimated 24% of all patients mechanically ventilated develop VILI (2). Although lung protective strategies (3), anti-inflammatory drugs (4), liquid ventilation (5), alveolar recruitment (6), glucocorticoids (7,8), hypercapnia (9), and inhalation of TNF-α inhibitor (10) have been utilized in an attempt to prevent the development of VILI, it still remains a major problem in ICUs (11).

Patients with acute respiratory distress syndrome (ARDS) are particularly susceptible to VILI. Importantly, these patients often need large volumes of MV and increase of airway pressure to maintain oxygenation (12,13). Currently, lung protective strategies used in MV of ARDS patients include the use of limited pressure and limited volume; however, mortality remains substantial.

Evidence suggests that an inflammatory response to MV-associated trauma plays a key role in the development of VILI. MV exposes the lungs to high pressures or high volumes, which provokes the activation of inflammatory cells and the release of pro-inflammatory factors. The

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0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2016.07.012

inflammatory response leads to impairment of alveolar-capillary permeability, decreased compliance, lung edema, and severe hypoxemia (14−18). Therefore, effective control of inflammation is a potential strategy in the prevention of VILI.

Budesonide is a steroid that has been shown to reduce lung injury in healthy lungs (19−24). However, 4.5−7% of patients admitted to the ICU have prior lung injury or ARDS; therefore, it is important to assess the efficacy of budesonide in patients with prior lung injury. In a previous study we demonstrated that inhalation of budesonide ameliorated endotoxin-induced lung injury (23). However, that study was conducted in healthy animals with a normal tidal volume. This study investigated the effect of budesonide on VILI in a rat model of inflammatory ARDS.

Materials and Methods

Study Design

Adult (275−375 g) male Wistar rats (n = 48) were purchased from the Second Affiliated Hospital of Harbin Medical University, Harbin, China. All experiments were performed in accordance with the Institutional Animal Care and Use Committee of Harbin Medical University. Animals were treated according to national guidelines. Rats were fasted for 24 h before the study, but water was provided *ad libitum*.

Rats were randomized into three groups (n = 16 each): sham group (S), endotoxin/ventilation group (LV), endotoxin/ventilation/budesonide group (LVB). Rats were anesthetized by intraperitoneal (i.p.) injection of 3% pentobarbital sodium (30 mg/kg). Anesthesia was maintained for 4.5 h. Rats in the S group received anesthesia only. Rats in the LV and LVB groups were intubated, and the caudal vein and artery were canulated. Endotoxin 500 μg/kg (Escherichia coli endotoxin, 0111:B4, Sigma, St. Louis, MO) was administered by intravenous injection. After 30 mins, rats were mechanically ventilated for 4 h (tidal volume 30 mL/kg, respiratory rate: 50/min, inspiratory expiratory ratio: 1:1, FiO_2:50%) after injection of rocuronium 0.6 mg/kg. Budesonide 1 mg (2 mL) (24) was instilled in the airways of rats in the LVB group when MV was started, and rats in the LV group received 2 mL saline immediately.

Eight rats from each group were sacrificed for tissue analyses after MV. The remaining eight rats in each group were used for survival analyses.

Tissue Analyses

Arterial blood gases. Arterial blood gases were analyzed using the Bayer Rapidlab 348 (Bayer Diagnostics, Germany) at baseline, 30 min after endotoxin injection, and after MV.

BALF and serum. Peripheral blood was sampled at baseline (T0), 30 min after endotoxin injection (T1), and after MV (T2). Bronchoalveolar lavage fluid (BALF) was collected by infusing the left lung of sacrificed rats with 4°C saline (15 mL/kg). BALF was centrifuged at 1,000 g for 15 min at 4°C. After centrifugation, BALF was stored at −80°C until analysis. Total protein in BALF was measured using the Bradford method. Total cells, macrophages and neutrophils in BALF were counted using a cell counter chamber. The neutrophils elastase levels in BALF were detected.

Intercellular adhesion molecule (ICAM)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and IL-10 were measured in serum and BALF using commercially available ELISA kits, according to the manufacturer's instructions (Wuhan Boster Bio-Engineering Limited Company, Wuhan, Hubei, China) (Sensitivities: ICAM-1, 31.2 pg/mL; TNF-α, $<$1 pg/mL; IL-1β, $<$1 pg/mL; IL-10, $<$ 2 pg/mL; neutrophil elastase, 31.2 pg/mL).

Pulmonary alveolocapillary permeability. Pulmonary alveolocapillary permeability was measured by sampling a portion of the right lung from sacrificed rats. The wet weight of this lung tissue was recorded immediately, and the dry weight was recorded after drying for 48 h at 60°C. The wet/dry weight (W/D) ratio was calculated.

Histopathological analysis of lung tissue. A portion of the right lung from sacrificed rats was fixed with 10% formalin and embedded in paraffin. Four-μm-thick sections were cut and stained with hematoxylin and eosin. An independent pathologist blinded to the grouping evaluated lung injury under light microscopy based on an assessment of alveolar congestion, edema, neutrophil infiltration in the airspace or vessel wall, hemorrhage, thickness of the alveolar wall, and hyaline membrane formation. Lung injury was scored according to the following scale: 0, minimum damage; 1, mild damage; 2, moderate damage; 3, severe damage; and 4, maximum damage.

Apoptosis. Apoptosis in lung tissue from sacrificed rats was investigated with TUNEL staining using the Apoptosis Assay kit according to the manufacturer's instructions (Roche Diagnostics GmbH, Science, Mannheim, Germany). Briefly, sections of the right lung from sacrificed rats were digested with proteinase K, rinsed twice with phosphate-buffered saline, and immersed in TUNEL reaction mixture (TdT and biotinylated dUTP) for 60 min at 37°C. Endogenous peroxidase activity was quenched with 0.3% H_2O_2. Sections were covered with extra-avidin peroxidase and immersed in diaminobenzidine solution. Sections were counterstained with Mayer-hematoxylin, dehydrated,