Respiratory Physiology & Neurobiology 247 (2018) 12–19

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Heme oxygenase-1 participates in the resolution of seawater drowning-induced acute respiratory distress syndrome

Jia-jia Yuan[a], Xiao-tong Zhang[a], Yun-ting Bao[a], Xin-jie Chen[a], Yuan-zhao Shu[a], Jun-Liang Chen[a], Wei Chen[b], Bin Du[a], Qing-Feng Pang[a,*]

[a] Department of Pathophysiology, School of Wuxi Medicine, Jiangnan University, Wuxi, Jiangsu Province, 214122, China
[b] The Emergency Department, The Third People Wuxi Hospital, Wuxi, Jiangsu Province, 214041, China

ARTICLE INFO

Keywords:
Seawater
Drowning
Acute respiratory distress syndrome
Mice
Heme oxygenase-1

ABSTRACT

The aim of the present study was to investigate whether heme oxygenase-1(HO-1) participated in the resolution of seawater drowning-induced acute respiratory distress syndrome (ARDS). In this study, gross and microscopic morphology of pulmonary tissue, computed tomography images and biochemical indexes were continuously observed from 15 min to 15 day after seawater drowning. The content and activity of HO-1 were determined by western-blot and spectrophotometric method, respectively. The morphological and biochemical indexes indicated that the seawater drowning could lead to the serious pulmonary hemorrhage and edema. However, 6 h after drowning, these morphological and biochemical indexes gradually returned to basal level. Meanwhile, seawater drowning increased the HO-1 expression and activity while Zinc protoporphyrin (a HO-1 specific activity inhibitor) decreased the content of transforming growth factor beta-1 in lung tissue and hampered the repair process of seawater drowning-induced ARDS. Thus, HO-1 participates in the resolution of seawater drowning-induced ARDS.

1. Introduction

Drowning is one of the leading causes of accidental death. Although many effort have been employed to treatment drowned sufferers, the mortality rate from drowning is up to 6.8 per 100,000 person-years (Liu et al., 2014; Marik, 2014; Wallis et al., 2015). Therefore, it is urgent to look for the pathogenesis of seawater drowning-induced ARDS and to acquire suitable treatment.

Rabbits or dogs are common animal to investigate the pathogenesis of the drowning-induced ARDS through anesthetization and intratracheally instilled fresh or salt water (Chen et al., 2016a; Liu et al., 2015b; Ma et al., 2016). However, the method (intratracheally instilled water induced ARDS) has several apparent drawbacks. Firstly, intratracheally instilled seawater does not reproduce the real natural drowning scene. In fact, 10–20% drowners do not aspirates fluids and die from laryngeal spasm (i.e. Dry drowning) (Lunetta et al., 2004). Secondly, anesthetized animals are deprived of the stress response induced by drowning. In real natural condition, the drowners, especially nonswimmer, often produce the serious stress responses, such as the feeling of fear and despair (Sood et al., 2014). Lastly, instilled abundant seawater causes animals death in several hours so that it is impossible to reveal the key pathophysiological hallmarks of the disease and prognosis of drown victims during the several hours (too short experiment time).

In this study, we established the seawater drowning model by directly immersed the mice into water and found that the seawater aspiration-induced ARDS attenuated by itself on the third day and almost self-healed in 15 day. Furthermore, we found that heme oxygenase-1 (HO-1) might participate in the resolution of seawater drowning-induced ARDS.

2. Materials and methods

2.1. Reagents

The following reagents were purchased from the indicated sources: hemin, zinc protoporphyrin IX (ZnPP), Nicotinamide-Adenine-Dinucleotide Phosphate (β-NADPH, Sigma-Aldrich, St. Louis, USA); HO-1 rabbit polyclonal primary antibody (Abcam, USA); transforming growth factor beta-1(TGF-β1) rabbit polyclonal primary antibody (Abcam, USA); alkaline phosphatase (AP)-conjugated goat anti-rabbit secondary antibodies (Thermo Scientific, USA); Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, USA). Superoxide dismutase (SOD) and malondialdehyde (MDA) assay kit were all

* Corresponding author.
E-mail address: qfpang@jiangnan.edu.cn (Q.-F. Pang).

http://dx.doi.org/10.1016/j.resp.2017.08.016
Received 18 April 2017; Received in revised form 17 August 2017; Accepted 17 August 2017

obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The protein concentration assay kit was got from Pierce BCA Protein (Thermo scientific co., Ltd, USA). All other reagents were of analytical grade and obtained commercially.

2.2. Animals and seawater drowning model

Seawater was prepared according to the major compositions of the East China Sea provided by the Chinese Ocean Bureau (osmolality 1300 mmol/L, pH 8.2, specially weight 1.05, NaCl 26.518 g/L, MgSO4 3.305 g/L, $MgCl_2$ 2.447 g/L, $CaCl_2$ 1.141 g/L, KCl 0.725 g/L, and $NaHCO_3$ 0.202 g/L).

Adult ICR mice (male, 18–25 g) were provided by the Shanghai SLAC Laboratory Animal CO. LTD. All mice were allowed to habituate to the animal facilities for 5 days before initiation of the experiment. The present study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animal and was approved by the Animal Care and Use Committee of Jiangnan University.

For seawater drowning model, mice were packed into the mice holder and immersed into a water tank containing 6 cm depth and 25 ± 2 °C temperature seawater, using a metal bucket (diameter 3 cm, height 10 cm) for 28 s. After the drowning procedure, mice were immediately resuscitated through chest cardiopulmonary resuscitation and then survivals returned to their housing cages. In the preliminary experiment, if the merged time exceed more than 30 s, the mortality rate was about 60%; if the merged time was less than 20 s, there was no serious ARDS in every drowned mice.

2.3. Experimental design

2.3.1. Part I

After resuscitating, the drowned mice were randomly sacrificed at 15 min, 30 min, 1 h, 6 h, 1 day, 3 days and 15 days after drowning (6 mice at each time point). The right lung was harvested for biochemical analysis and the left lung for microscopy examination.

2.3.2. Part II

Experimental protocol for the effect on HO-1 in seawater-induced mild ARDS. Mice were randomly divided into 4 groups (n = 8): normal group, normal + Zinc protoporphyrin (ZnPP, the HO-1 enzymatic activity inhibitor) group, drowning group and drowning + ZnPP group. Mice were returned to their cages without future manipulation in normal group. In normal + ZnPP and drowning + ZnPP group, Mice were given intraperitoneal injection of ZnPP (40 mg/kg) each 48 h (Cheng et al., 2016). Mice were sacrificed at 3 days after drowning, and lung tissue samples were collected and evaluated.

2.4. Histopathology

2.4.1. Hematoxylin-eosin staining

Left lungs were moved, fixed immediately in 4% formaldehyde for 2 days, and embedded in paraffin. Tissue was cut into 4 μm thick sections and stained with hematoxylin & eosin. Lung injury scores based on categories of infiltration of inflammatory cells, edema, congestion, and intra-alveolar hemorrhage was undertaken as previously described (Chen et al., 2016b).

2.4.2. Prussian blue stain

Left lungs were moved, fixed immediately in 4% formaldehyde for 2 days, and embedded in paraffin. Tissue was cut into 4 μm thick sections and stained with Prussian blue staining. Prussian blue staining is specific to detect the presence of Fe^{3+} in histologic specimens. Iron deposits in tissue are visualized as blue deposits.

2.5. Pulmonary computed tomography image

A quantum Fx micro computed tomography (CT) imaging system (SOMATOM Sensation 64) was used to obtain CT image data of lung phantoms in the mice. Each phantom was scanned using 64 mA 120 Kv with a voxel of 1.0 mm.

2.6. Measurement of concentration of methane dicarboxylic aldehyde and superoxide dismutase in lung tissue

The superoxide dismutase (SOD) activity and the methane dicarboxylic aldehyde (MDA) concentration in pulmonary tissue were determined using assay kits (Nanjing Jiancheng Corp., China) following the manufacturer's recommendations.

2.7. Measurement of heme oxygenase-1 enzyme activity in lung tissue

The HO-1 activity was measured by the spectrophotometric determination of bilirubin production, as described previously (Bauer et al., 2016). Briefly, tissue was homogenized in normal saline and then was centrifuged (14,000 × g, 4 °C, 10 min). The supernatant was transferred to tubes and was used to determine HO enzyme activity in a reaction mixture containing rat liver cytosol (as a source of biliverdin redutase, 80 μl), hemin (0.5 μl,10 mM), desferrioxamine (12.5 μl, 80 mM), p450 (0.6 μl) and NADPH (10 μl, 20 mM) in a total volume of 200 μl. The reaction was incubated at 37 °C for 60 min in the dark. Finally, the formed bilirubin was extracted with 200 μl of chloroform by vigorous vortexing 3 times for 10 s. The optical density at 464 nm and 530 nm of organic phase was measured.

2.8. Immunological histological chemistry

The sections were incubated in 3% H_2O_2 to block endigenous peroxidase and blocked in 5% BSA for 1 h followed by staining using rabbit polyclonal primary antibody for TGF-β1 overnight at 4 °C. Then the sections were washed three times in PBS and were incubated in secondary antibody. Slides were incubated in DAB (BOSTER, AR1022), counterstained in Mayer's Haematoylin, dehydrated, cleared in xylene and in Depex mounting medium.

2.9. Western blotting

Lung tissues homogenates were prepared by grinding frozen tissue and placed in a RIPA lysis buffer containing a protease inhibitor mix. The samples were centrifuged at 14,000 × g for 10 min at 4 °C and the supernatants were removed. The total protein content of the resulting supernatants was measured by Pierce BCA Protein Assay Kit. Then, the protein was denatured, loaded and run on 10% SDS-PAGE gel. The gel was first run at 80 V for 15 min and then changed to 120 V using an electrophoresis system. The proteins were then transferred to a nitrocellulose membrane and incubated with 5% defatted milk for 30 min. The membrane was then exposed to mouse polyclonal primary antibodies for HO-1 at 1/1000 dilution, rabbit polyclonal primary antibodies for GAPDH at 1/10,000 dilution and rabbit polyclonal primary antibodies for TGF-β1 at 1/1000 dilution overnight at 4 °C. The membrane was washed and incubated with anti-mouse and anti-rabbit antibodies at a 1/5000 dilution for 2 h. The protein bands were subsequently visualized with a BeyoECL Star (Beyotime).

2.10. Statistical analysis

Statistical analysis was performed using SPSS 10.0 software package (SPSS, Chicago, IL, USA). Data are expressed as means ± SD. Sample sizes were indicated in figure legends. Differences between the two groups were assessed by One-way ANOVA and followed by a Student–Neuman–Keuls. A value of $P < 0.05$ was considered