Life Sciences 185 (2017) 30–37

Contents lists available at ScienceDirect

Life Sciences

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

Resveratrol efficiently improves pulmonary function *via* stabilizing mast cells in a rat intestinal injury model

Xiaolei Huang[a,1], Weicheng Zhao[b,*,1], Dan Hu[c], Xue Han[d], Hanbin Wang[b], Jianyu Yang[a], Yang Xu[a], Yuantao Li[a], Weifeng Yao[e], Chaojin Chen[e,**]

[a] Department of Anesthesiology, Shenzhen Maternity and Child Healthcare Hospital, Southern Medical University. Shenzhen, China
[b] Department of Anesthesiology, The First People's Hospital of Foshan, 81 North of Rinlan Road, Foshan, China
[c] Department of Ophthalmology, The First People's Hospital of Foshan, China
[d] Department of Anesthesiology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China
[e] Department of Anesthesiology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

ARTICLE INFO

Keywords:
Acute lung injury
Intestinal ischemia/reperfusion injury
Resveratrol
Mast cells

ABSTRACT

Background: Intestinal ischemia/reperfusion (IIR) leads to acute lung injury (ALI) distally by aggravating pulmonary oxidative stress. Resveratrol is effective in attenuating ALI through its antioxidant capacity. This study aimed to determine the effects of resveratrol on IIR-induced ALI and to explore the role of mast cells (MCs) activation in a rat model of IIR.

Methods: Adult Sprague-Dawley rats were subjected to IIR by occluding the superior mesenteric artery for 60 min followed by 4-hour reperfusion. Resveratrol was intraperitoneally injected at a dose of 15 mg/kg for 5 days before IIR. MCs stabilizer/inhibitor cromolyn sodium and degranulator compound 48/80 were used to explore the interaction between resveratrol and MCs. Lung tissues were collected for pathological detection and MCs staining. Pulmonary protein expression of surfactant protein-C (SP-C), tryptase, $p47^{phox}$ and $gp91^{phox}$ (two NADPH oxidase subunits), ICAM-1(intercellular adhesion molecule-1) and P-selectin were detected. The levels of oxidative stress markers (SOD, MDA, H_2O_2 and MPO) and β-hexosaminidase were also measured.

Results: At the end of IIR, lung injury was significantly increased and was associated with decreased expression of SP-C and increased lung oxidative stress. Increased inflammation as well as activation of MCs was also observed in the lungs after IIR. All these changes were prevented or reversed by resveratrol pretreatment or MCs inhibition with cromolyn sodium. However, these protective effects of resveratrol or cromolyn sodium were reduced by MCs degranulator compound 48/80.

Conclusions: These findings reveal that resveratrol attenuates IIR-induced ALI by reducing NADPH oxidase protein expression and inflammation through stabilizing MCs.

1. Introduction

Intestinal ischemia/reperfusion (IIR) injury is a serious consequence which occurs following many clinical practices, such as bowel transplantation and liver transplantation [12]. IIR not only leads to intestinal injury but also causes remote organ damage, especially acute lung injury (ALI) [9,18]. IIR-induced ALI has been reported with high morbidity and mortality [14,19]. Numerous risk factors have been proposed in the pathogenesis of ALI induced by IIR, among them, oxidative stress has been considered as the main factor that contributes to the development of IIR-induced ALI [17]. Inhibition of oxidative stress has been shown to attenuate ALI in several models including IIR [17,38].

Many studies have shown correlation between mast cells (MCs) density and disease severity in pulmonary pathology such as ALI [10,20,21]. MCs are widely distributed in the lung which play major roles in inflammation and allergic reactions [5,13], and maintain homeostasis of respiratory function [17]. The role of MCs in non-allergic disorders including cardiovascular and renal disorders is well recognized [32,33]. Our previous studies have demonstrated that MCs activation exacerbated IIR-induced pulmonary oxidative stress and ALI [4,11], whereas inhibition of MCs by cell membrane stabilizer (*e.g.*, cromolyn sodium) or antioxidant (*e.g.*, *N*-acetylcysteine and

* Correspondence to: W. Zhao, Department of Anesthesiology, The First People's Hospital of Foshan, No.81 North of Rinlan Road, Foshan 528000, China.
** Correspondence to: C. Chen, Department of Anesthesiology, The Third Affiliated Hospital of Sun Yat-sen University, No.600 Tianhe Road, 510630 Guangzhou, China.
E-mail addresses: zwcheng@fsyyy.com (W. Zhao), chchjin@mail2.sysu.edu.cn (C. Chen).
[1] Xiaolei Huang and Weicheng Zhao contributed equally to this work.

http://dx.doi.org/10.1016/j.lfs.2017.07.018
Received 21 March 2017; Received in revised form 9 July 2017; Accepted 18 July 2017
Available online 19 July 2017
0024-3205/

sevoflurane) attenuated IIR or IIR-induced remote organ damage [40]. All these indicate the importance of MCs activation in oxidative stress-induced ALI. Thus, effective means that inhibit MCs activation directly or indirectly *via* reducing oxidative stress may attenuate ALI.

Resveratrol (trans-3, 5, 4′-trihydroxystilbene) is a polyphenolic compound in red wine that has antioxidative and organ protective effects in animal models including ALI [36]. Consumption of resveratrol reduced inflammatory response and oxidative stress in staphylococcal enterotoxin B-induced lung injury in mice [24]. Moreover, resveratrol has been shown to attenuate sepsis-induced ALI and bleomycin-induced ALI in rats through its antioxidant capacity [15,25]. In addition, resveratrol protects cerebromicrovascular endothelial cells from oxidative functional damages by reducing NADPH oxidase activity, the major source of oxidative stress in cerebromicrovascular endothelial cells (CMVECs) and astrocytes [26]. Given that activation of NADPH oxidase could lead to MCs degranulation/activation and leads to ALI [40], it is possible that resveratrol may attenuate IIR-induced ALI through inhibiting NADPH-induced MCs activation. Hence, we hypothesized that resveratrol may reduce ALI through inhibition of NADPH-mediated MCs degranulation during IIR in rats.

2. Materials and methods

2.1. Animals

Specific pathogen-free (SPF) male Sprague–Dawley rats (280–320 g) were obtained from animal center of Guangzhou University of Chinese Medicine. Rats were housed in individual cages with alternating 12-h light/dark cycles in a temperature-controlled SPF environment, in which rats were acclimated for one week before the experiment. Animals were fasted for 8 h but had free access to water before the experiments. All animal care and experimental protocols complied with the Guidelines of Sun Yat-sen University for Animal Experimentation and were approved by the Institutional Animal Care and Use Committee at The Third Affiliated Hospital of Sun Yat-sen University. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996).

2.2. Experiment design

A total of 56 rats were randomly assigned into seven groups ($n = 8$ *per* group) consisting of Sham, IIR, IIR + compound 48/80 (MCs degranulator) (IIR + CP), IIR + cromolyn sodium (MCs inhibitor) + CP (IIR + CS + CP), IIR + CS, IIR + resveratrol + CP (IIR + RESV + CP) and IIR + RESV.

2.3. Intestinal ischemia reperfusion in vivo and drug treatments

IIR was induced by superior mesenteric artery (SMA) occlusion for 60 min followed by 4-h reperfusion, as described previously [8]. Resveratrol (Sigma-Aldrich, USA) was intraperitoneally injected at dose 15 mg/kg for 5 days before operation [22]. Cromolyn sodium (Sigma-Aldrich, USA), a relative specific inhibitor of MCs degranulation, was injected *via* tail vein at 25 mg/kg 15 min before the operation [40]. Compound 48/80 (0.75 mg/kg, Sigma-Aldrich, USA) or equivalent volume of saline (vehicle) was injected *via* tail vein 5 min before the reperfusion.

Four hours after reperfusion, the rats were sacrificed and thoracotomy was performed. The right upper lung was fixed in 10% formaldehyde (Sigma-Aldrich, USA) and embedded in paraffin (Leica Biosystems, Germany) for sectioning. The middle lobe of the right lung was removed and used to measure the wet/dry (w/d) weight ratio. The inferior lobes of the right and left lungs were removed and preserved in liquid nitrogen (Guangzhou Pearl River Industrial Gases Co. Ltd., China) for ELISA and Western blotting experiment.

2.4. Pathological assessment

Lung sections which have been cut into 5-μm were used for hematoxylin and eosin (H & E) staining and examined by the pathologist who was blinded to the experiment. The morphological changes of the lung were scored as previously described by *Derks and Jacobovitz Derks* [35], which is a semi-quantitative scoring method. Lung damage was graded from score 0 (normal) to 4 (severe) in four categories, namely inflammatory cell infiltration, congestion, interstitial inflammation, and edema. Finally, the individual scores of each category were added up to a total score of lung injury.

2.5. Toluidine blue staining

As previously described [28], the slices (5 μm thick) were stained with 0.1% toluidine blue (Sigma-Aldrich, USA) in order to examine MCs. A light microscope (Nikon Eclipse 80i, USA) was used to exam toluidine blue-stained cells, which were counted as MCs. The density of MCs was calculated by dividing the number of nuclei in 5 fields at high magnification for every section.

2.6. Lung water content

The middle right lobes of lungs were firstly measured for wet weight to calculate lung water content. The dry weight was then recorded after the lung was placed in an oven at 80 °C for 24 h. The lung wet/dry (w/d) weight ratio was calculated by dividing the wet weight by the dry weight.

2.7. Enzyme-linked e immunosorbent assay (ELISA)

The concentrations of β-hexosaminidase in pulmonary homogenates were quantified with commercial ELISA assay kits (USCN business Co. Ltd., China), following the manufacturer's instruction.

2.8. Superoxide dismutase, hydrogen peroxide, myeloperoxidase, and malonaldehyde assay

Lung tissue homogenates were used to evaluate pulmonary superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), myeloperoxidase (MDA) and malonaldehyde (MPO) activity with SOD, H_2O_2, MDA and MPO detection kits (KeyGEN Biotech Company, China) according to the manufacturer's instructions.

2.9. Immunohistochemical staining

The expression of surfactant protein-C (SP-C) and $p47^{phox}$ were assessed by immunohistochemical staining. Sections (5 μm thick) were deparaffinized in xylene, rehydrated in graded alcohol, and washed three times with phosphate-buffered saline (PBS). Then endogenous peroxidase activity was blocked with 3% hydrogen peroxide (37 °C, 10 min) after heated in pH 6.0 citrate buffer for 20 min in a microwave oven. Next, sections were treated with normal goat serum (10%) in Tris-buffered saline (37 °C, 30 min). Subsequently, sections were, respectively, incubated overnight at 4 °C with anti-SP-C (1:1000; Santa Cruz Technology, USA) or anti-$p47^{phox}$ (1:1000; Santa Cruz Technology, USA). After washing three times with PBS, these sections were incubated with the biotin-labeled secondary antibody (mouse anti-rabbit IgG; Zhongshan Jinqiao Co., China) for 30 min at room temperature, and color was developed by reagent 3,3′-diaminobenzidine (DAB, Zhongshan Jinqiao Co., China).

2.10. Immunofluorescence staining

The expression of tryptase in MCs was conducted by immunofluorescence staining. Lung sections were incubated with tryptase