International Immunopharmacology 65 (2018) 96–107

Contents lists available at ScienceDirect

International Immunopharmacology

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

Berberine alleviates endothelial glycocalyx degradation and promotes glycocalyx restoration in LPS-induced ARDS

Lina Huang[a,1], Xiaohua Zhang[b,1], Xiaohong Ma[c,1], Dong Zhang[c,1], Dongxiao Li[c], Jiali Feng[c], Xinjie Pan[a], Junhong Lü[d], Xiaozhi Wang[c], Xiangyong Liu[a,*]

[a] Department of Cell Biology, Binzhou Medical University, Yantai 264003, Shandong Province, China
[b] Department of Biotechnology, Binzhou Medical University, Yantai 264003, Shandong Province, China
[c] Department of Respirator Medicine, Affiliated Hospital of Binzhou Medical University Binzhou, Binzhou 256603, Shandong Province, China
[d] Division of Physical Biology and CAS Key Laboratory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

ARTICLE INFO

Keywords:
Acute respiratory distress syndrome
Berberine
Endothelial glycocalyx
Matrix metalloproteinase 9
Heparanase
Lipopolysaccharide

ABSTRACT

In the pathogenesis of acute respiratory distress syndrome (ARDS), an increase in vascular endothelial permeability may trigger pulmonary edema and ultimately lead to respiratory failure. Endothelial glycocalyx damage is an important factor that causes an increase in vascular endothelial permeability. Berberine (BBR) is an isoquinoline alkaloid extracted from *Coptis chinensis*, a plant used in traditional Chinese medicine that exerts multiple pharmacological effects. In this study, pretreatment with BBR inhibited the increase in vascular endothelial permeability in mice with lipopolysaccharide (LPS)-induced ARDS. BBR pretreatment inhibited the shedding of syndecan-1 (SDC-1) and heparan sulfate (HS), which are important components of the endothelial glycocalyx that lessen endothelial glycocalyx damage. BBR further significantly inhibited increases in important endothelial glycocalyx damage factors, including reactive oxygen species (ROS), heparanase (HPA), and matrix metalloproteinase 9 (MMP9) in LPS-induced ARDS mice and in LPS-stimulated human umbilical vein endothelial cells. BBR pretreatment also decreased the production of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and inhibited NF-κB signaling pathway activation in LPS-induced ARDS. In addition, BBR promoted the recovery of SDC-1 and HS content in injured endothelial glycocalyx after LPS treatment and accelerated its restoration. This is the first report of BBR maintaining the integrity of endothelial glycocalyx. These results provide a new theoretical basis for the use of BBR in the treatment of ARDS and other diseases related to endothelial glycocalyx damage.

1. Introduction

Acute respiratory distress syndrome (ARDS) is a common critical respiratory disease that threatens human health with high mortality rates; moreover, no effective therapeutic drug has been developed for this disease. The pathogenesis of ARDS is complicated [1,2]. An increase in pulmonary vascular permeability may trigger pulmonary edema and ultimately lead to respiratory failure, which is the main cause of death in patients with ARDS [3,4].

The endothelial glycocalyx is a carbohydrate-rich layer that covers the luminal surface of the vascular endothelium. This layer is mainly composed of a core skeleton, which consists of proteoglycans, glycoproteins, and a side chain of glycosaminoglycans [5,6]. Additionally, a large number of other molecules are connected to endothelial glycocalyx, such as albumin, vascular endothelial growth factor, extracellular superoxide dismutase, and antithrombin III, are derived from the bloodstream or the endothelium [7,8]. The glycosaminoglycan side chain, a glycocalyx component, contains large amounts of carboxyl and sulfate groups; therefore, the glycocalyx surface is negatively charged. The glycocalyx is the first barrier and protects the vascular endothelial cells by size selectivity, steric hindrance, and electrostatic repulsion; this barrier plays an important role in maintaining vascular endothelial permeability [9,10].

Studies have found that the endothelial glycocalyx is destroyed under the pathological conditions of ARDS and other diseases, such as atherosclerosis [11], diabetes [12], ischemia/reperfusion [13], and

* Corresponding author.
E-mail address: liuxiangyong81@gmail.com (X. Liu).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.intimp.2018.10.001
Received 27 June 2018; Received in revised form 30 September 2018; Accepted 2 October 2018

acute kidney injury [14]. Reactive oxygen species (ROS), heparanase (HPA), and matrix metalloproteinases (such as MMP9) are the main substances that can directly destroy the glycocalyx [15,16].

Berberine (BBR), a major active ingredient extracted from the rhizome of *Coptis chinensis* (*Rantus chinensis*, Ranunculaceae), is an isoquinoline alkaloid [17]. BBR exerts various pharmacological effects and protects against hyperlipidemia [18], diabetes mellitus [19], tumors [20], and fatty liver [21]. BBR also exerts protective effects on lung injuries caused by various factors. BBR ameliorates lipopolysaccharide (LPS)-induced lung injury by reducing pro-cytokine TNF-α production and down-regulating cytosolic phospholipase A2 expression [22]. Furthermore, BBR can alleviate cigarette smoke-stimulated acute lung injury by inhibiting inflammatory responses and NF-κB signaling pathway activation [23].

However, the effect of BBR on the endothelial glycocalyx has not been extensively studied. Therefore, in the present study, we analyzed the effect of BBR on the endothelial glycocalyx in LPS-induced ARDS and its possible related mechanisms. This study provides a new theoretical basis for drug development that targets the endothelial glycocalyx and uses BBR to treat ARDS and other diseases involving endothelial glycocalyx damage.

2. Materials and methods

2.1. Drugs and reagents

BBR (purity: > 98%) was obtained from Shanghai Ziqi Biology Technology Co., Ltd. (Shanghai, China). Lipopolysaccharide (LPS; from *Escherichia coli* 055:B5), dimethyl sulfoxide (DMSO), Triton X-100, 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazoliumbromide (MTT), and 2′,7′-dichlorodihydrofluoresceindiacetate (DCFH-DA) were provided by Sigma- Aldrich (Shanghai, China). The kit for determining malondialdehyde (MDA) content was purchased from Abcam (Cambridge, USA). Rabbit monoclonal antibodies to syndecan-1, rabbit polyclonal antibody to MMP9, NF-κB/P65, IκBα, p-IκBα, Lamin B1 and β-actin were purchased from Abcam (Cambridge, USA). Mouse polyclonal antibody to HS was purchased from AMS Biotechnology (Switzerland). Rabbit polyclonal antibody to human HPA1 was purchased from InSight Biopharmaceuticals Ltd. (Israel). DyLight 488 goat anti-mouse lgG, and Alexa Fluor 594 goat anti-rabbit lgG were purchased from Bioss Biotechnology Ltd. (Beijing, China).

2.2. Cell culture and experimental groups

Human umbilical vein endothelial cells (HUVECs) were obtained from AllCells Biotechnology Ltd. (Shanghai, China) and grown to confluence in 25 cm^2 flasks supplemented with complete culture medium, which was provided by AllCells. The cells were incubated under 95% humidity and 5% CO_2 at 37 °C.

For BBR pretreatment, the HUVECs were separated into five groups: the control group, the LPS group, and LPS + BBR (1.25, 2.5, and 5 μM) groups. The control group was cultured with complete culture medium without LPS treatment. In the LPS group, HUVECs were treated with LPS (1 μg/mL) for 6 h. In the LPS + BBR groups, HUVECs were pretreated with BBR (1.25, 2.5, and 5 μM) for 1 h prior to LPS (1 μg/mL) stimulation for 6 h. For BBR post-treatment, HUVECs were separated into six groups: the control group, LPS group, self-repairing group, and BBR-repairing (1.25, 2.5, and 5 μM) groups. HUVECs in the LPS group, self-repairing group, and BBR-repairing (1.25, 2.5, and 5 μM) groups were stimulated with LPS (1 μg/mL) for 6 h. The control cells were not exposed to LPS. After LPS treatment for 6 h, the HUVECs in the self-repairing group were replaced with complete medium, whereas the HUVECs in the BBR-repairing groups were replaced with complete medium containing BBR (1.25, 2.5, and 5 μM) for an additional 12 h incubation. The experimental schedule is shown in Fig. 1A and Fig. 7A.

2.3. Animals and experimental groups

Adult male C57BL/6 mice weighing approximately 18 g to 20 g were purchased from Jinan Pengyue Laboratory Animal Center (Shandong, China). Mice were housed in a temperature-controlled room (12 h/12 h dark and light cycles) and given free access to food and water. All animal studies were performed in accordance with the guide for the Care and Use of Laboratory Animals established by the US National Institutes of Health and permitted by the Binzhou Medical University Institutional Review Board.

For BBR pretreatment, mice were randomly separated into five groups (n = 8): the control group, LPS group, and LPS + BBR (50, 100, and 200 mg/kg) groups. The BBR concentration used in this study is based on pre-experimental results and previous reports [24–26]. The LPS + BBR groups received BBR administered orally for 7 days, and the control and LPS groups were given an equal volume of sterile saline instead. Then, mice in the LPS group and LPS + BBR (50, 100, and 200 mg/kg) groups were intraperitoneally injected with LPS (20 mg/kg) to induce ARDS as previously described [27–29]. Mice in the control group received an equal volume of normal saline. After infusion of LPS for 6 h, mice were anesthetized with 4% chloral hydrate and killed. For BBR post-treatment, mice were randomly separated into six groups (n = 8) as follows: the control group, LPS group, self-repairing group, and BBR-repairing (50, 100, and 200 mg/kg) groups. The mice in the LPS group, self-repairing group, and BBR-repairing (50, 100, and 200 mg/kg) groups were intraperitoneally injected with LPS (20 mg/kg) to induce ARDS. The control was given an equal volume of sterile saline instead. At 6 h after LPS treatment, the mice in the LPS group were sacrificed, and those in the BBR-repairing groups received BBR (50, 100, and 200 mg/kg) administered orally for 3 days. The mice in the control and self-repairing groups received an equal volume of sterile saline each instead of BBR. The experimental schedules are shown in Fig. 1A and Fig. 7A.

2.4. Lung wet/dry (W/D) ratios

Mice were narcotized after LPS administration for 6 h. The left lung tissues were removed and weighed to obtain the wet weight. Subsequently, the tissues were placed in an oven at 80 °C for 48 h to gain the dry weight. The ratio of the wet lung to the dry lung was used to assess the severity of pulmonary edema.

2.5. Pulmonary histopathology evaluation

After experimental treatment, the lung tissues were removed and fixed with 4% paraformaldehyde for 48 h. Then, the tissues were dehydrated in graded alcohol and embedded in paraffin. Finally, the tissues were sliced into 5 μm sections and stained with hematoxylin and eosin (H&E), and the slides were observed under an optical microscope (Olympus Optical, Tokyo, Japan) to see histopathological changes. The lung injury scores (LISs) were obtained by combined assessments of inflammatory cell infiltration in the airspace or vessel wall, alveolar congestion, hemorrhage, alveolar wall thickness, and hyaline membrane formation [28]. The degree of lung injury was scored as follows: 0, minimum; 1, mild; 2, moderate; 3, severe; and 4, maximum. For each sample, six fields were selected randomly and then graded for average LIS [30].

2.6. Lung vascular permeability assay

To assess the pulmonary vascular leakage, we used the Evans Blue dye (EBD) extravasation. At the end of LPS challenge, EBD (20 mg/kg, Sigma-Aldrich) was administered via the tail vein. After circulating for 30 min, the lungs were perfused with normal saline. Then, the lungs were excised and imaged. After imaging, the lungs were blotted dry, weighed, and homogenized in formamide. Following overnight