Tropical Journal of Pharmaceutical Research May 2023; 22 (5): 1031-1036
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i5.14

Original Research Article

Protective effect of baicalin against lipopolysaccharide-induced acute lung injury in rats, and the mechanism of action

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Sent for review: 4 February 2023 *Revised accepted: 3 May 2023*

Abstract

Purpose: *To study the protective influence of baicalin (BA) against LPS-mediated acute lung injury (ALI) in a rat model.*
Methods: *Twenty-seven Sprague-Dawley (SD) rats were assigned (9 rats each) to groups as follows: normal control (NC) treated intraperitoneally with normal saline; lipopolysaccharide (LPS) group treated with 10 mg/kg LPS; and lipopolysaccharide and baicalin (LPS + Ba) group treated with 100 mg/kg LPS, followed 15 min later by 10 mg/kg of baicalin. Pathological changes were assessed by H & E staining. Different types of cells were identified and counted using Rayleigh Giemsa staining. Levels of IL-6 and TNF-α and IL-1β in pulmonary tissue were determined by enzyme-linked immunosorbent assay (ELISA). Matute-Bello scoring method was applied to evaluate the extent of lung injury, while pulmonary expression levels of Nrf2, HO-1 and NQO1 were assayed with Western blot.*
Results: *Lung injury score, and numbers of neutrophils and macrophages were significantly higher in LPS than in NC group. Inflammatory score, and numbers of neutrophils and macrophages in LPS + BA group were significantly lower than those in LPS group ($p < 0.05$). Compared with NC group, lung tissue levels of IL-6, TNF-α and IL-1β in LPS group were significantly increased, but were significantly reduced in LPS + BA rats, relative to LPS rats ($p < 0.05$). Expression levels of Nrf2, HO-1 and NQO1 in lung tissue were significantly lower in LPS group than in NC group ($p < 0.05$) but higher in lung tissue of LPS + BA group, relative to LPS group.*
Conclusion: *Baicalin (BA) protects rats from LPS-induced ALI by enhancing the expressions of Nrf2 and HO-1 through activation of Nrf2/ARE signal pathway. These findings may be useful in developing novel BA-based anti-inflammatory drugs.*

Keywords: *Baicalin, Acute lung injury, Nrf2/ARE, Matute-Bello score, Neutrophils, Macrophages, Inflammatory score*

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INTRODUCTION

It has been reported that ALI is a serious pulmonary infectious disease due to various pathogenic causes within and outside the lungs [1]. It may progress to diffuse lung injury and acute respiratory distress syndrome (ARDS). The late stage of ARDS causes multiple organ

dysfunction syndrome which is fatal. Acute lung injury (ALI) is the basic pathophysiological manifestation of ARDS [2]. The disease results in enhanced permeability of lung vessels, alveolar capillary lesions, appearance of alveolar fibrin, infiltration of lung tissue by inflammatory cells, and pulmonary edema [3]. Despite recent studies on ALI/ARDS, there is still a lack of effective methods for reducing the high mortality associated with the disease. Therefore, it is necessary to discover new drugs and develop appropriate treatment strategies for ALI/ARDS.

Baicalin (BA) is a major bioactive component of *S. baicalensis* roots, which is widely used for treating pneumonia, hepatitis and atherosclerosis [4]. Studies have shown that BA exerts several pharmacological effects due to its anti-inflammatory, anti-oxidative stress, neuroprotective and antibacterial properties [5]. Other studies have shown that BA exerted a protective effect against lipopolysaccharide (LPS)-induced ALI [6]. Although Nrf2/ARE signal route is implicated as a likely mechanism involved in ALI or ARDS [7], it is not known whether BA exerts a regulatory effect on ALI via this route. The present research was carried out to study the influence of BA on LPS-mediated ALI in rats, and the mechanism involved in this process.

EXPERIMENTAL

Animals

Twenty-seven healthy male SD rats were bought from Guangdong Medical Laboratory Animal Center (batch no. SCXK Guangdong; 2020-0019). The animals had a mean weight of 230 ± 30 g, and they were fed adaptively for 2 - 3 days before commencement of the experiment.

Reagents and equipment

Lipopolysaccharide (LPS) was purchased from Beijing Kairiji Biotechnol. Co. Ltd., while BA was a product of Nanjing Saihongrui Biotechnol. Co. Ltd. Bicinchoninic acid (BCA) protein concentration assay kit was product of Shanghai Rongbai Biotechnology Co. Ltd. Rat ELISA kits for IL-6 and TNF-α were bought from Shanghai Yaji Biotechnology Co. Ltd. Staining kit for H&E kit was supplied by Jinklon (Beijing) Biotechnology Co. Ltd. Rabbit anti-mouse monoclonal antibodies for Nrf2, HO-1 and NQO1 were purchased from Wuhan Pure Biotechnology Co. Ltd. Goat anti-Rabbit IgG secondary antibody was obtained from Wuhan Finn Biotechnology Co. Ltd.

Ultra-low temperature (-80 ℃) refrigerator was supplied by Qingdao Haier Biological Medical Co. Ltd. Low-temperature centrifuge was product of Beijing Qianming Gene Technology Co. Ltd.

Animal grouping and treatments

Twenty-seven healthy male SD rats were assigned at random to normal control (NC) group, lipopolysaccharide (LPS) and LPS + BA groups, each with 9 rats. Rats in LPS and LPS + BA groups received LPS intraperitoneally at doses of 10 and 100 mg/kg, respectively. In the LPS + BA group, the rats were first intraperitoneally injected with LPS at a dose of 100 mg/kg, followed 15 min later by injection with BA (10 mg/kg). The NC rats received an equivalent volume of saline intraperitoneally in place of LPS.

Hematoxylin and eosin (H&E) staining

Pathological changes in lung tissues of rats in each group were determined using H&E staining. The different types of cells in BALF of rats in each group were determined using Ray-Giemsa staining. The BALF was examined under a microscope for determination of the numbers of macrophages and neutrophils.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-6, TNF-α, and IL-1β in pulmonary tissues of rats were determined with ELISA. The extent of lung damage was evaluated using the scoring method of Matute-Bello. The higher the score, the more serious the degree of lung tissue damage.

Oxidative and protein expression levels

An automated biochemical analyzer was used to assay pulmonary activities of SOD and GPx and ROS levels, while the protein expression levels of Nrf2, HO-1 and NQO1 in lung tissue homogenates were assayed with Western blotting.

Statistical analysis

SPSS 22.0 software package was used for statistical analyses of data. Data from measurements are presented as mean ± standard deviation (SD). Paired comparison was done with *t*-test, while one-way analysis of variance was used for comparison amongst multiple groups. Values of $p < 0.05$ were considered indicative of statistically significant differences.

RESULTS

Pathological changes in lung tissues

Pulmonary tissue structure in NC rats was normal, and there were no neutrophils, red blood cells and edema fluid. In contrast, the alveolar wall thickness of rats in LPS group was significantly increased, and a large number of neutrophils and red blood cells were exuded intravascularly, accompanied by edema fluid. However, there was significant reduction in the degree of damage to lung tissue structure of rats in LPS + BA group, as well as marked reductions in neutrophils, red blood cells and edema fluid exudation.

Inflammatory score and numbers of inflammatory cells in BALF

Pulmonary tissue injury score and the number of neutrophils and macrophages were higher in LPS rats than in NC rats. Inflammatory score and the number of neutrophils and macrophages in LPS + BA group were significantly lower than those in LPS group ($p < 0.05$). These results are shown in Table 1.

Pulmonary levels of inflammatory mediators

Relative to NC rats, levels of IL-6, TNF-α and IL-1β in lung tissue of LPS group were significantly increased. However, these cytokines were significantly down-regulated in lung tissue of rats in LPS+BA group, relative to LPS group ($p < 0.05$). These data are presented in Table 2.

Levels of oxidative indices

The levels of ROS in lung tissue of rats were higher in LPS-treated rats than in NC rats, but SOD and GPx activities were lower in LPS group than in NC rats ($p < 0.05$). However, ROS levels in lung tissue of rats were lower in LPS + BA group than in LPS-treated rats, but SOD and GPx activities were significantly lower in LPS group. These results are presented in Table 3.

Expression levels of Nrf2/ARE signal route-related proteins

Table 4 shows that the protein expression levels of Nrf2, HO-1 and NQO1 in lung tissue of rats in LPS group were significantly decreased when compared with those in NC group ($p < 0.05$). In contrast, the pulmonary protein expression levels of Nrf2, HO-1 and NQO1 were significantly higher in rats in LPS + BA group than in LPS rats.

DISCUSSION

Acute lung injury (ALI) results in non-cardiogenic lung edema due to increased permeability of endothelial and epithelial cells [8]. There is infiltration of a variety of inflammatory cells, especially neutrophils, resulting in pulmonary cytokine storm that ultimately damages the lungs [9].

Table 1: Comparison of inflammatory scores and number of inflammatory cells in each group (n = 9)

Group	Lung tissue injury score (points)	Neutrophils ($\times10^7$/mL)	Macrophages ($\times10^7$/mL)
NC	2.57±0.18	1.73±0.27	0.14±0.03
LPS	14.29±2.27[a]	4.876±0.31[a]	0.36±0.07[a]
LPS+BA	5.32±0.21[b]	2.76±0.30[b]	0.20±0.04[b]

[a]$P < 0.05$, vs NC; [b]$p < 0.05$, vs LPS group

Table 2: Levels of inflammatory mediators in lung tissue homogenates (pg/mg; n = 9)

Group	IL-6	TNF-α	IL-1β
NC	32.84±4.96	183.67±56.95	14.29±2.43
LPS	276.48±21.86[a]	943.75±84.68[a]	52.38±5.69[a]
LPS+BA	173.57±16.64[b]	383.29±68.62[b]	32.64±4.23[b]

[a]$P < 0.05$, compared with NC group; [b]$p < 0.05$, compared with LPS group

Table 3: Levels of oxidative indices (n = 9)

Group	ROS	SOD (U/mg)	GSH-Px
NC	41.63±4.63	24.38±2.69	37.96±3.49
LPS	57.48±4.87[a]	14.15±2.07[a]	18.68±2.63[a]
LPS+BA	46.72±5.38[b]	20.66±2.15[b]	29.84±2.74[b]

[a]$P < 0.05$, vs NC; [b]$p < 0.05$, vs LPS

Table 4: Levels of Nrf2/ARE signal route proteins in each group (n = 9)

Group	Nrf2	HO-1	NQO1
NC	1.13±0.14	1.27±0.16	1.18±0.22
LPS	0.68±0.15[a]	0.42±0.12[a]	0.45±0.17[a]
LPS+BA	0.89±0.16[b]	0.83±0.14[b]	0.78±0.16[b]

[a]$P < 0.05$, compared with NC group; [b]$p < 0.05$, compared with LPS group

In the present research, it was seen that LPS-induced lung tissue injury score was significantly increased, and alveolar wall thickness was significantly increased. Moreover, there was exudation of large numbers of neutrophils and red blood cells, accompanied by edema fluid. However, after administration of BA, there was a significant reduction in pulmonary lesions in ALI rats. At the same time, after BA treatment, ROS levels were decreased, while GPx, SOD and expression levels of inflammatory cytokines were increased. These results indicate that BA reduced oxidation-induced damage and inflammation. Moreover, BA activated the Nrf2/ARE signal pathway, as was evident in increased expressions of the associated proteins in ALI rats.

Recently, it was found that BA exerted a positive effect on LPS-induced ALI through TLR4/NF-κB/PI3K/Akt/mTOR signal routes [10]. Lipopolysaccharide binds to TLR4 moiety on the cell membrane and transmits signals to immunocytes. Activations of MyD88-reliant signaling route and TLRIF-reliant signaling route lead to activation of mitogen-activated protein kinase (MAPK) and NF-κB, thereby activating the MAPK/NF-κB signal pathway. The activated NF-κB enters the nucleus, interacts with the promoters of related genes, promotes the release of inflammatory factors or chemokines, and causes inflammatory reactions [11]. Other studies have found that baicalin liposome attenuates Lipopolysaccharide-mediated ALI in mice by suppressing TLR4/JNK/ERK/NF-κB route [12]. Baicalin inhibits BLM-induced pulmonary fibrosis by upregulating A2aR [13]. Hence BA inhibits the occurrence and development of LPS-induced ALI by regulating inflammatory response.

During ALI/ARDS, the injured tissues produce excessive reactive oxygen species (ROS). Superoxide dismutase (SOD), quinone-1 (NQO1), CAT, GPx and HO-1 are involved in antioxidant functions [14,15]. Excessive ROS leads to oxidation of unsaturated FAs in cell membranes, and it decreases the fluidity of membranes while making them more permeable, resulting in pulmonary edema and pulmonary expansion. Reactive oxygen species also damage alveolar and pulmonary vascular endothelial cells as well as microvascular barrier and aggravate pulmonary edema [16].

It is known that Nrf2 binds to Kelch-like ECH-related protein 1 (KEAP1) in the cytoplasm. At this stage, Nrf2 has no biological activity. When phosphorylated, Nrf2 becomes activated and is translocated to the nucleus where it binds to endogenous antioxidant response elements (AREs). This results in increased expression of antioxidant genes such as SOD, NQO1, GSH-PX and HO-1, thereby maintaining cellular redox homeostasis and normal metabolism [17,18]. In addition, Nrf2 regulates NLRP3 inflammasome and MAPK/NF-κB signal routes pathways, thereby preventing inflammatory reactions and oxidative damage [19]. These data show that Nrf2 is a crucial link for ALI/ARDS therapy.

Studies have also shown that Nrf2 protects the lungs from ROS-induced lesions, and also from damage arising from pulmonary diseases [20,21]. When Nrf2 is translocated to the nucleus, it enhances activation and translation of associated genes for defense against oxidative damage and for cytoprotection [22,23]. In addition, on activation, Nrf2 translocates to the nucleus where it regulates expressions of HO-1-associated genes, resulting in suppression of inflammation cascades and attenuation of ALI-mediated inflammation [24]. In the present research, BA significantly enhanced nuclear translocation of Nrf2, indicating that BA mitigated ALI via Nrf2/ARE route.

CONCLUSION

Baicalin (BA) protects pulmonary tissue in ALI rats and plays an antioxidant role by enhancing the expressions of Nrf2 and HO-1 through regulation of Nrf2/ARE signal pathway. These findings may be useful in developing novel BA-based anti-inflammatory drugs.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Xiaozhen Chen conceived and designed the study, Fei Xiao, Shanwu Dong and Yongli Chen collected and analyzed the data, Jiwu wang wrote the manuscript.

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