International Immunopharmacology 23 (2014) 649–657

Contents lists available at ScienceDirect

International Immunopharmacology

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

Hydroxysafflor yellow A ameliorates lipopolysaccharide-induced acute lung injury in mice via modulating toll-like receptor 4 signaling pathways

Ya-Li Liu [a], Ya-Juan Liu [b], Yang Liu [a,*], Xue-Song Li [a], Shuan-Hu Liu [a], Yong-Gui Pan [a], Jian Zhang [a], Qiang Liu [c], Ying-Yan Hao [a]

[a] Department of Critical-Care Medicine, Tangshan Gongren Hospital, Tangshan, Hebei 063000, PR China
[b] Department of Ultrasonography, Tangshan City Ninth Hospital, Tangshan, Hebei 063000, PR China
[c] Department of Neurosurgery, Tangshan Gongren Hospital, Tangshan, Hebei 063000, PR China

ARTICLE INFO

Article history:
Received 1 September 2014
Received in revised form 3 October 2014
Accepted 19 October 2014
Available online 27 October 2014

Keywords:
Hydroxysafflor yellow A
Acute lung injury
Lipopolysaccharide
Toll-like receptor 4

ABSTRACT

Hydroxysafflor yellow A (HSYA) is a main bio-active compound important of a traditional Chinese medicine named *Carthamus tinctorius* L. and has been shown to possess various effects, especially anti-inflammatory benefits and potential protections against acute lung injury (ALI) in previous studies. Therefore, in this present study, we aimed to evaluating effects of HSYA on lipopolysaccharide (LPS)-induced ALI in mice. ALI was induced by intratracheal instillation of LPS into lung, and dexamethasone was used as a positive control. Results demonstrated that HSYA abated LPS-induced pathological change and attenuated lung vascular permeability and edema. HSYA down-regulated both the ability of myeloperoxidase (MPO) in lung tissues and levels of inflammatory mediators including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IFN(interferon)-β in serum. Moreover, HSYA prevented toll-like receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF) protein up-expressions. In addition, the activations of mitogen-activated protein kinases including p38, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) were blocked by HSYA. And also, the phosphorylations of interferon regulatory factor 3 (IRF3), translocation of nuclear factor kappa B (NF-κB)/p65 and inhibitory kappa B (IκB)-α were inhibited by HSYA. In conclusion, HSYA attenuated inflammatory response in ALI mice through inhibition of TLR 4-dependent signaling pathways.

1. Introduction

Acute lung injury (ALI) is characterized by uncontrolled hyper-inflammatory responses in the lung airspaces and lung parenchyma involving alveolar-capillary membrane damage, vascular permeability increase, neutrophils recruitment, pulmonary edema and respiratory failure [1,2]. Clinically, ALI is supported to be the first step in the development of the multiple organ dysfunctions syndrome (MODS) during the early sepsis and remains one of the most frequent causes of morbidity and mortality in intensive care units (ICU) [2]. Although several technical developments and advanced supportive treatments have been used for ALI, there is still no effective therapy strategy but relatively noteworthy mortality rates [2,3]. Therefore, to develop novel effective therapies for ALI is urgently required [4,5].

Lipopolysaccharide (LPS), the primary component of outer membrane of Gram-negative bacteria, is regard as the predominant microbial initiators of inflammatory responses and is responsible for the overwhelming innate immune responses in ALI patients [6]. Therefore, the host defense responses to LPS could include excess and uncontrolled productions of inflammatory mediators. Although various factors and molecular activities are involved in these responses, LPS recognition by the host cells has been considered as a critical step to initiate inflammatory process [7,8]. Toll-like superfamily receptors (TLRs) are transmembrane proteins and act as signal transduction molecules [9]. TLRs signaling pathway play a crucial role in the innate immune system as the first line of defense against pathogens [8]. Among TLRs, TLR4 has been regarded as the main sensors for recognition of LPS and transmits its associated downstream regulators [8]. Once stimulated by LPS in the LPS-induced ALI model, TLR4 can activate nuclear factor (NF)-κB protein via two major pathways: myeloid differentiation factor 88 (MyD88)-dependent pathway and the Toll/interleukin (IL)-1 receptor domain, containing the adaptor protein-inducing interferon (IFN)-β (TRIF) pathway, which finally induce the excess productions of inflammatory mediators [10]. Hence, drugs focusing on down-regulating the TLR4 signaling pathways would provide potential therapeutic effects for ALI [11].

Hydroxysafflor yellow A (HSYA, the chemical structure is shown in Fig. 1) is the main important bioactive compound of a traditional

* Corresponding author at: Department of Critical-Care Medicine, Tangshan Gongren Hospital, No. 27, Wenhua Road, Lubei District, Tangshan 063000, PR China. Tel./fax: +86 315 2305112.
E-mail address: YangLiu_TSGRH@163.com (Y. Liu).

http://dx.doi.org/10.1016/j.intimp.2014.10.018
1567-5769/

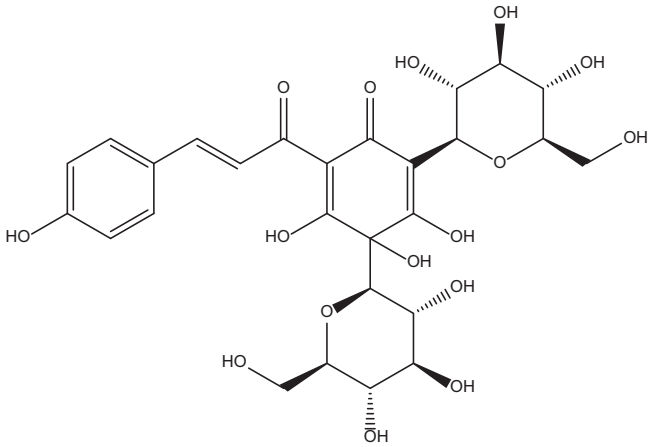

Fig. 1. Chemical structure of hydroxysafflor yellow A.

Chinese medicine named *Carthamus tinctorius* L., and is also the principal efficiency ingredient of Safflor Yellow Injection treating for several ischemic cardio-cerebrovascular diseases [12]. Previous studies indicated that HSYA possessed various kinds of bio-activities, including oxygen-free radicals scavenging, anti-inflammatory actions, anti-platelet aggregation, anti-tumor effects and reducing myocardial-infarct sizes [13–15]. In addition, researches also improved that this compound could improve the neurological behavioral conditions in sepsis rats induced by transient cerebral ischemia/reperfusion [15–17]. Moreover, previous research also demonstrated that HSYA have the protective effects against ALI induced by intraperitoneal application of LPS associated with the inhibition of mitogen-activated protein kinases including (MAPK)/p38, nuclear factor kappa B (NF-κB)/p65 activation and alteration of inflammatory cytokine expressions. Although HSYA has been shown benefits intraperitoneal application of LPS-induced ALI, the more explicit underlying mechanisms against ALI by HSYA and whether HSYA possessed regulation effects in the TLR 4 signaling pathways still remained unclear [15,17]. Nowadays, with clinical relations to the process of ALI, the ALI model that induced by intratracheal instillation of LPS to experimental animals has been demonstrated to be a well-suited and reproducible model for preliminarily pharmacological researches of novel drugs or other therapeutic agents [18,19]. Therefore, in this present study, we aimed to evaluate the effect of HSYA on LPS-induced ALI in mice by intratracheal instillation of LPS. And for mechanisms elucidations, the TLR4 signaling pathways and associated downstream regulators were also explored.

2. Materials and methods

2.1. Materials

Hydroxysafflor yellow A (purity: >98% by HPLC) from National Institutes for Food and Drug Control (Beijing, China). Lipopolysaccharide (LPS, from *Escherichia coli 0111:B4*) was purchased from Sigma Co. Ltd. (St. Louis, USA). Dexamethasone (Dex) was purchased from Guangdong Huanan Pharmaceutical Group Co., Ltd (Dongguan, Guangdong, China). All other chemicals were of the reagent grade.

2.2. Animals

Male ICR mice (20–25 g) were purchased from Laboratory Animal Center of Hebei United University. All animals were kept on 12-hour light/12-hour dark cycles under regular temperature (22 ± 2 °C) and humidity (50 ± 10%) with standard diets and clean water ad libitum. All animals were sacrificed by lethal sodium pentobarbital injection. All experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. LPS-induced ALI

LPS-induced ALI was performed as follows: after fasted for 12 h, mice were anesthetized via intraperitoneally injecting pentobarbital sodium (30 mg/kg) and received a single intratracheal instillation with either 20 μL/10 g body weight of LPS (2.5 mg/mL, dilution with PBS) to conduct the LPS-induced ALI. In the meanwhile, the control group was anesthetized and received the same volume of PBS.

2.4. Experimental design and specimen collection protocol

To conduct survival experiments and assess mortality rate, mice (n = 24 per group) were injected intraperitoneally (i.p.) with HSYA (HSYA groups, 40, 80 and 120 mg/kg, dilution with normal saline) or normal saline (vehicle group) at 0 h or 0 and 12 h, respectively before the LPS instillation. After this procedure, mice of all groups were monitored and the time when any animal died was recorded every 6 h up to 120 h. Then the survival rate of each group within 120 h was calculated and compared using the Kaplan Meier methods.

In the further experiments, mice were randomly assigned to the following 6 groups (n = 24): control group (vehicle treated normal mice), LPS group (LPS-induced ALI mice treated with vehicle), HSYA + LPS groups (LPS-induced ALI mice treated with HSYA of 80 and 120 mg/kg) and Dex + LPS group (LPS-induced ALI mice treated with dexamethasone of 10 mg/kg). Before ALI operations, HSYA + LPS groups were given HSYA (80 and 120 mg/kg, i.p.) at 0 and 12 h before LPS instillation operations while the Dex + LPS group was given dexamethasone (10 mg/kg, i.p.) as a positive control group at 1 h before LPS instillation operations. During this period, control group and LPS group were given equal volumes of normal saline.

For the specimen collection, 24 h after LPS instillation, 24 mice of each group were randomly divided into 3 parts, 8 mice per part. Part 1 was used for the bronchoalveolar lavage fluid (BALF) preparation and the lung wet/dry weight ratio measurement. In brief, after anaesthetization, mouse was surgically exposed the trachea and clamped the right main bronchus. Then the left lung of each animal was lavaged with ice-cold PBS (1.5 mL in 3 times) using a venous indwelling needle. The BALF recovery rate was kept at more than 90%. Then the mouse was sacrificed, and right lung was harvested for the wet/dry ratio measurement. Part 2 was used for the histopathologic evaluation and lung MPO determination. In brief, after the mouse was sacrificed, the left lung was taken, placed in appropriate amount of pre-cold PBS immediately, and homogenized using. Then the homogenate was centrifuged at 4 °C. 100 μL of the supernatant was used for protein measurement and the rest was immediately collected and stored at −80 °C for further analysis of MPO. At the same time, the right lung was harvested for the histopathologic examination. Part 3 was left for ELISA assay and western-blot assay. In brief, mouse was sacrificed, blood samples were collected from the abdominal inferior vena cava, and then the lung tissues were harvested. All of these samples were frozen, and stored in liquid nitrogen immediately.

2.5. Histopathologic analysis

Biopsies of right lungs were fixed with 4% paraformaldehyde and imbedded in paraffin. After deparaffinization and dehydration, the lung tissues were cut into 4 μm sections and stained with hematoxylin and eosin subsequently. The severity of lung injury was scored by a blinded observer according to lung pathological changes, including infiltration or aggregation of neutrophils in air spaces or vessel walls; alveolar congestion; hemorrhage and thickness of alveolar wall/hyaline membrane formation. Each indicator was graded according to a five-point scale: 0 = minimal damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage and 4 = maximal damage. Thus, the severity of lung injury was evaluated according to the sum of the four criteria.