International Immunopharmacology 25 (2015) 55–64

Contents lists available at ScienceDirect

International Immunopharmacology

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

The protective effect of CDDO-Me on lipopolysaccharide-induced acute lung injury in mice

Tong Chen [a,1], Yi Mou [a,1], Jiani Tan [a], Linlin Wei [a], Yixue Qiao [a], Tingting Wei [a], Pengjun Xiang [b], Sixun Peng [a], Yihua Zhang [a], Zhangjian Huang [a,*], Hui Ji [a,**]

[a] State Key Laboratory of Natural Medicines, China Pharmaceutical University, No. 24 Tongjiaxiang, Nanjing 210009, Jiangsu, China
[b] School of Pharmacy, China Pharmaceutical University, No.24 Tongjiaxiang, Nanjing 210009, Jiangsu, China

ARTICLE INFO

Article history:
Received 3 November 2014
Received in revised form 10 December 2014
Accepted 12 January 2015
Available online 19 January 2015

Keywords:
CDDO-Me
LPS
ALI
RAW 264.7 cells

ABSTRACT

CDDO-Me, initiated in a phase II clinical trial, is a potential useful therapeutic agent for cancer and inflammatory dysfunctions, whereas the therapeutic efficacy of CDDO-Me on LPS-induced acute lung injury (ALI) has not been reported as yet. The purpose of the present study was to explore the protective effect of CDDO-Me on LPS-induced ALI in mice and to investigate its possible mechanism. BalB/c mice received CDDO-Me (0.5 mg/kg, 2 mg/kg) or dexamethasone (5 mg/kg) intraperitoneally 1 h before LPS stimulation and were sacrificed 6 h later. W/D ratio, lung MPO activity, number of total cells and neutrophils, pulmonary histopathology, IL-6, IL-1β, and TNF-α in the BALF were assessed. Furthermore, we estimated iNOS, IL-6, IL-1β, and TNF-α mRNA expression and NO production as well as the activation of the three main MAPKs, AkT, IκB-α and p65. Pretreatment with CDDO-Me significantly ameliorated W/D ratio, lung MPO activity, inflammatory cell infiltration, and inflammatory cytokine production in BALF from the *in vivo* study. Additionally, CDDO-Me had beneficial effects on the intervention for pathogenesis process at molecular, protein and transcriptional levels *in vitro*. These analytical results provided evidence that CDDO-Me could be a potential therapeutic candidate for treating LPS-induced ALI.

1. Introduction

ALI is a clinical syndrome characterized by a disruption of epithelial integrity, neutrophil accumulation, noncardiogenic pulmonary edema, severe hypoxemia and an intense pulmonary inflammatory response with a wide array of increasing severity of lung parenchymal injury [1]. Previous studies have shown that lots of pathogenesis contribute to ALI, such as oxidant/antioxidant dysfunction, dysregulation of inflammatory/anti-inflammatory pathway, upregulation of chemokine production and adhesion molecules [2]. However, to date there is no effective medicine to control ALI [3]. Lipopolysaccharide (LPS) is a main component of the outer membrane of Gram negative bacteria. It has been reported to activate toll like receptors 4 (TLR4) and to stimulate the release of inflammatory mediators inducing ALI-like symptoms. Intratracheal administration of LPS has been used to construct animal models of ALI [4].

The biological importance of naturally occurring triterpenoids has long been recognized. Oleanolic acid, exhibiting modest biological activities, has been marketed in China as an oral drug for the treatment of liver disorders in humans. Among its derivatives, bardoxolone methyl (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid methylester) CDDO-Me, had completed a successful phase I clinical trial for the treatment of cancer and started a phase II trial for the treatment of patients with pulmonary arterial hypertension. For its broad spectrum antiproliferative and anti-tumorigenic activities, CDDO-Me has also been reported to possess a number of pharmacological activities such as antioxidant, anti-tumor and anti-inflammatory effects [5,6]. However, the mechanisms by which CDDO-Me exerted its anti-inflammatory effects on macrophage were insufficiently elucidated. More importantly, there is no available report to evaluate its therapeutic effect on acute lung injury.

When binded with LPS, the host receptor TLR4 is activated, which consequently initiates the PI3K–Akt pathway. Moreover, the phosphorylation and degradation of IκBαresult in the activation of nuclear factor-kappa B (NF-κB) [7]. Meanwhile, recent research indicated that mitogen-activated protein kinase (MAPK) pathways motivated by LPS participated in the pathogenesis of inflammation [8]. Both the above pathways lead to systemic dysregulated response marked by excessive accumulation of various mediators such as interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and nitric oxide (NO). Particularly, appropriate amount of NO participates in regulating considerable physiological condition, whereas excessive level of NO synthesized by inducible nitric synthase (iNOS) are believed to be involved in the inflammatory disorder [9]. Furthermore, the inhibition of NO is associated with the inactivation of nuclear factor-kappa B

* Corresponding author. Tel./fax: +86 25 83271072.
** Corresponding author. Tel./fax: +86 25 86205849.
E-mail addresses: cpudahuang@163.com (Z. Huang), huijicpu@163.com (H. Ji).
[1] Tong Chen and Yi Mou contributed equally to this work.

http://dx.doi.org/10.1016/j.intimp.2015.01.011
1567-5769/

(NF-κB) and Akt in LPS-induced RAW 264.7 cells [10]. On the basis of these evidences, we hypothesized that CDDO-Me had pharmacological effects on ALI elucidated the potential anti-inflammatory mechanism in this study.

2. Materials and methods

2.1. Reagents and antibodies

CDDO-Me (Fig. 1A, purity >99%) was provided by Pro. Yihua Zhang, and dissolved in DMSO at a concentration of 200 mM. The final concentration of DMSO was less than 0.1% [*v*/*v*] in all the experiment. Dexamethasone (DEX) was purchased from the National Institutes for Food and Drug Control (Beijing, China). LPS (*E. coli* 055:B5) and NO kit (catalog no. S0021) was supplied by Beyotime Institute of Biotechnology (Nanjing, China). Mouse IL-6, IL-1β, and TNF-αenzyme-linked immunosorbent assay (ELISA) kits were purchased from Biolegend (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Life Technologies (Carlsbad, CA, USA). Primary antibodies against p65, p-IκB-α, IκB-α, β-actin and horseradish peroxidase-conjugated anti-rabbit antibody were supplied by Bioworld Technology Inc. (MN, USA). The anti-phospho-ERK, anti-phospho-p38MAPK, anti-phospho-JNK, anti-ERK, anti-JNK, anti-p38MAPK, and horseradish peroxidase-conjugated anti-rabbit antibody were supplied by Cell Signaling Technology (Beverly, MA, USA). Chemiluminescence detection reagents were supplied by KeyGEN Technology Company (Nanjing, China). Trizol reagent was purchased from Bios Tech. (Nanjing, China). M-MLV reverse transcriptase was provided by Invitrogen Co. (CA, U.S.). Gene-specific PCR primers were synthesized by Genray Biotechnology (Shanghai, China). iQSYBR Green PCR Master Mix was supplied by Bio-Rad Laboratories (Herculues, CA, U.S.)

2.2. Animals

Specific pathogen-free male BALB/c mice (8–10 weeks old, 18–20 g each), acquired from Suzhou Industrial Park Aiermaite Technology Co., Ltd. (Suzhou, China), were maintained in an animal facility under standard laboratory conditions for 3 days prior to experiments. Mice were provided with water and standard chow ad libitum. All the experimental procedures were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of China.

2.3. Experimental protocol for acute lung injury model

All mice were randomly divided into five groups: control group, model group, dexamethasone (5 mg/kg) group, and CDDO-Me (0.5 mg/kg, 2 mg/kg) group ($n = 13$). CDDO-Me and dexamethasone were given intraperitoneally for continuous 3 days. Simultaneously, control and model group were pretreated with an equal volume of vehicle. 1 h later the last administration, mice were anesthetized by intraperitoneal injection of 10% chloral hydrate 4 mg/kg and then acutely intratracheally instilled with 10 μg LPS dissolved in 50 μl PBS. Animals in control group were given 50 μl PBS without LPS. Six hours after administration, all animals were sacrificed by diethyl ether asphyxiation.

2.4. Collection of bronchoalveolar lavage fluid (BALF) and cell counting

6 h after LPS or PBS administration, the collection of BALF was performed three times through atracheal cannula with autoclaved PBS and instilled up to a total volume of 1.3 ml. Then BALF samples were centrifuged at 3000 rpm for 10 min at 4 °C and the cell-free supernatants were stored in −80 °C for analysis of cytokine concentrations.

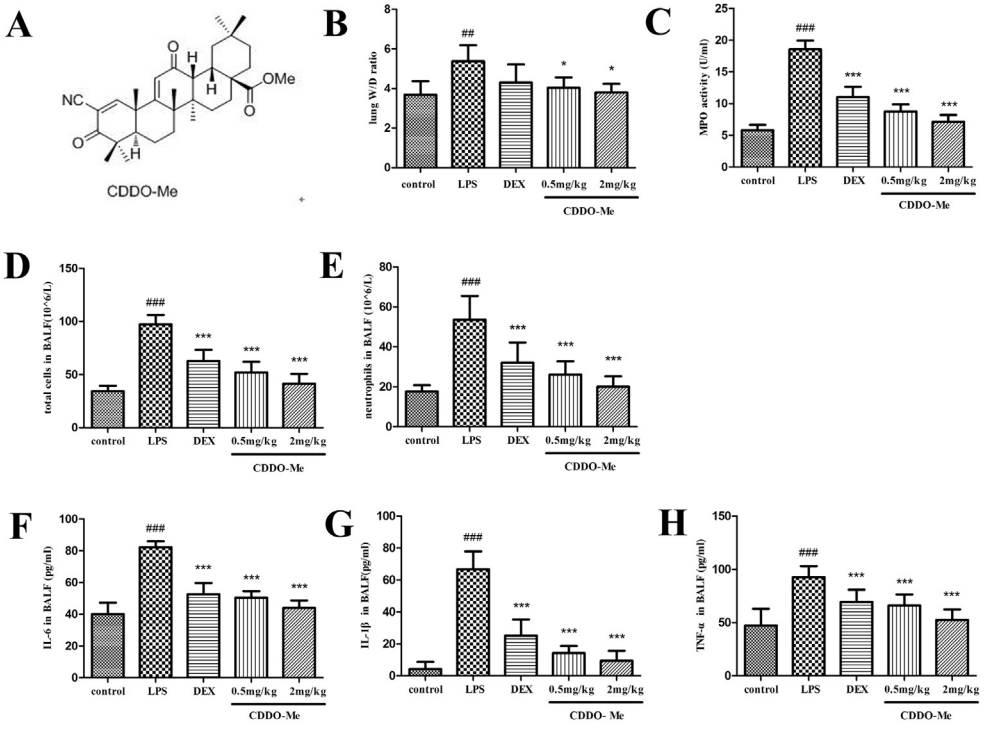

Fig. 1. Effects of CDDO-Me on acute lung injury. (A) The chemical construction of CDDO-Me. Mice were given an intraperitoneal injection of CDDO-Me (0.5 mg/kg, 2 mg/kg) and DEX (5 mg/kg) for a continuous 3 days. 1 h after the last administration, mice received an intratracheal instillation of LPS (8 mg/kg) or the same volume of PBS. The mice were sacrificed at 6 h after LPS challenge. The values presented mean ± SD, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with control group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with LPS group.(B) Effects of CDDO-Me on W/D ratio of lung tissues. (C) Effects of CDDO-Me on MPO activity of lung tissues. Effects of CDDO-Me on the number of total cells (D) and neutrophils (E) in BALF. Effects of CDDO-Me on the production of IL-6 (F) and IL-1β (G), TNF-α (H) in BALF.