International Immunopharmacology 57 (2018) 91–101

Contents lists available at ScienceDirect

International Immunopharmacology

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

New thiazolidinedione LPSF/GQ-2 inhibits NFκB and MAPK activation in LPS-induced acute lung inflammation

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ARTICLE INFO

Keywords:
Acute lung injury
Thiazolidinediones (TDZs)
NFκB
MAPKs

ABSTRACT

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are responsible for high mortality rates in critical patients. Despite > 50 years of intensive research, there is no pharmacologically effective treatment to treat ALI. PPARs agonists, chemically named thiazolidinediones (TZDs) have emerged as potential drugs for the treatment of ALI and ARDS due to their anti-inflammatory efficacy. The present study aims to evaluate the potential anti-inflammatory effects of new TZDs derivatives, LPSF/GQ-2 and LPSF/RA-4, on ALI induced by LPS. BALB/c mice were divided into five groups: 1) Control; 2) LPS intranasal 25 μg; 3) LPSF/GQ-2 30 mg/kg + LPS; 4) LPSF/RA-4 20 mg/kg + LPS; and 5) DEXA 1 mg/Kg + LPS. BALF analyses revealed that LPSF/GQ-2 and LPSF/RA-4 reduced NO levels in BALF and inflammatory cell infiltration induced by LPS. MPO levels were also reduced by the LPSF/GQ-2 and LPSF/RA-4 pre-treatments. In contrast, histopathological analyses showed better tissue protection with LPSF/GQ-2 than DEXA and LPSF/RA-4 groups. Similarly, LPSF/GQ-2 reduced inflammatory markers (IL-1, iNOS, TNFα, IL-1β, IL-6) better than LPSF/RA-4. The LPSF/GQ-2 anti-inflammatory action could be attributed to the inhibition of NFκB, ERK, p38, and PARP pathways. In contrast, LPSF/RA-4 had no effect on the expression of p38, JNK, NFκB. The present study indicates that LPSF/GQ-2 presents a potential therapeutic role as an anti-inflammatory drug for ALI.

1. Introduction

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome (ARDS), are considered important causes of death in critical patients. Epidemiological studies estimate that ALI/ARDS is associated with hospital mortality ranging from 30 to 60% [1–3].

The first description of ALI/ARDS was made in 1967 by ASHBAUGH and collaborators [4]. In general, ALI/ARDS is defined by acute-onset tachypnea, hypoxemia, diffuse pulmonary infiltrates, and loss of lung compliance, and characterized by high short-term mortality in adults. The heterogeneous nature of ALI/ARDS and the presence of additional risk factors makes difficult to assess its molecular basis [5].

Lipopolysaccharide (LPS) is an important agent used to induce ALI/ARDS in animals models due to its relation with sepsis [6,7]. LPS is a principal component of the outer membrane of Gram-negative bacteria, and an important inductor agent to elicit inflammatory responses, shock, and death [6]. In monocytes, the activation of Toll-Like Receptors (TLR4) by LPS, activates transcription factors like NFκB and AP-1 [8].

The NFκB pathway regulates the expression of several inflammatory mediators such as cytokines, chemokines, adhesion molecules, COX-2 and iNOS [9]. The excessive activation of NFκB is associated with the

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https://doi.org/10.1016/j.intimp.2018.02.011
Received 11 September 2017; Received in revised form 26 January 2018; Accepted 14 February 2018

severity of respiratory dysfunction in patients with ALI/ARDS [10]. Classically, the activation of the NFκB pathway depends on its inhibitory protein degradation (IκB) by a mechanism that involves phosphorylation by IKKs and polyubiquitination [11]. The IKK activators englobe MAPKs and represent a convergence point for numerous stimuli, like the TLRs, IL-1β, IL-18, TNFα, and cell B and T receptors [11].

Since the inflammatory process is a key role in the development of ALI and contributes to the dysfunction of the airways and tissue remodeling [12], new therapeutic agents are being investigated to treat ALI. PPARs agonists have emerged as potential drugs due to their anti-inflammatory efficacy on T cell regulation, macrophages differentiation, dendritic cells, airway smooth muscle cells, and cytokines expression [13,14].

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor (NR) superfamily that have evolved to become the biological sensors of an lipid metabolism, in particular, the intracellular fatty acid levels [15]. Currently, PPARα agonists (fibrates) are clinically used for treating dyslipidemia, and PPARγ agonists (thiazolidinediones – TZDs) are used for treating type 2 diabetes mellitus [16,17].

Recently, two new thiazolidinediones (TZDs) compounds, LPSF/GQ-2 and LPSF/RA-4, showed anti-inflammatory activity in models of pleurisy and atherosclerosis, respectively [18,19]. However, no studies have yet evaluated the effect of these molecules on LPS–induced ALI. The present study aims to evaluate the mechanisms of action of LPSF/GQ-2 and LPSF/RA-4 on LPS–induced ALI.

2. Methods

2.1. Synthesis of thiazolidines derivatives — LPSF/GQ-2 and LPSF/RA-4

LPSF/GQ-02 represents the compounds 5-(4-chloro-benzylidene)-3-(4-methyl-benzyl)-thiazolidine-2,4-dione and LPSF/RA-4 represents the structure of 5,3-Benzyl-5-(1H-Indol-3-Metileno)- thiazolidine-2,4-dione. See the chemical structure in Fig. 1A. All the compounds were synthesized and purified at the Department of Antibiotics of the Universidade Federal de Pernambuco (Brazil) following the methodology described by Mouro et al. [20].

2.2. Reagents and antibodies

All the chemicals and reagents used were of analytical grade. LPS (*Escherichia coli* serotype, 0111-B4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone was obtained from Aché (Guarulhos, SP, Brazil). The antibodies were obtained from: anti-IL-1β (GenWay, USA); anti-iNOS (BD Biosciences, USA); anti-TNFα (Abcam, USA); anti-IL6 (Abcam, USA); anti-NFκB (SCBT, USA); anti-Phospho NFκB (Abcam, USA); anti- IκB (SCBT, USA); anti-Phospho IκB (Abcam, USA); anti-p38 (SCBT, USA); anti-phospho p38(SCBT, USA)' anti-JNK (SCBT, USA); anti-Phospho JNK (SCBT, USA); anti-ERK(SCBT, USA); anti-PhosphoERK(SCBT, USA); and anti-PARP (Abcam, USA).

2.3. Animals

Male adult BALB/c mice, weighing 25–35 g, were obtained from the Aggeu Magalhães Institute, Fiocruz (Brazil). The animals were kept at an environmentally controlled room temperature (21 ± 2 °C) in a light/dark cycle of 12 h, with free access to food and water. All experimental procedures were approved by the Ethics Committee for Animal Experimentation, protocol n° 101/2016 (CEUA/FIOCRUZ/IAM).

2.4. Experimental design

The mice were randomly divided into five groups ($N = 10$) as described below:

I. CONTROL: Mice received water for injection (100 μL) + 30 μL Tween20 by oral gavage. After 1 h, they received 0.9% NaCl (25 μL) by the intranasal route.
II. LPS: Mice received water for injection (100 μL) + 30 μL Tween 20 by oral gavage. After 1 h, they received 25 μL of LPS (1 μg/1 μL) by the intranasal route.
III. LPSF/GQ-2: Mice received pre-treatment with LPSF/GQ-2 (30 mg/kg) dissolved in water (100 μL) + 30 μL Tween 20 by oral gavage. After 1 h, they received 25 μL of LPS (1 μg/1 μL) by the intranasal route.
IV. LPSF/RA-4: Mice received pre-treatment with LPSF/RA-4 (20 mg/kg) dissolved in water (100 μL) + 30 μL tween 20 by oral gavage. After 1 h, they received 25 μL of LPS (1 μg/1 μL) by the intranasal route.
V. DEXA: Mice received pre-treatment with dexamethasone sodium phosphate, (1 mg/kg) dissolved in water 100 μL) + 30 μL Tween 20 by oral gavage. After 1 h, they received 25 μL of LPS (1 μg/1 μL) by the intranasal route.

The selection of the dosages of LPSF/GQ-2 and LPSF/RA-4 was based on previous studies, which evaluated the best dose and efficacy of these molecules in inflammation models [18,19].

2.5. Acute lung inflammation protocol

Mice were anesthetized intraperitoneally with a combination of 10% ketamine hydrochloride (115 mg/kg) and xylazine 2% (10 mg/kg). After confirming analgesia, 25 μg of LPS was dissolved in 25 μL (0.9% NaCl) and instilled intranasally (i.n.) to induce lung injury. After 24 h, mice were euthanized, lung tissues and BALF were collected for the subsequent experiments. Fig. 2A shows the experimental design.

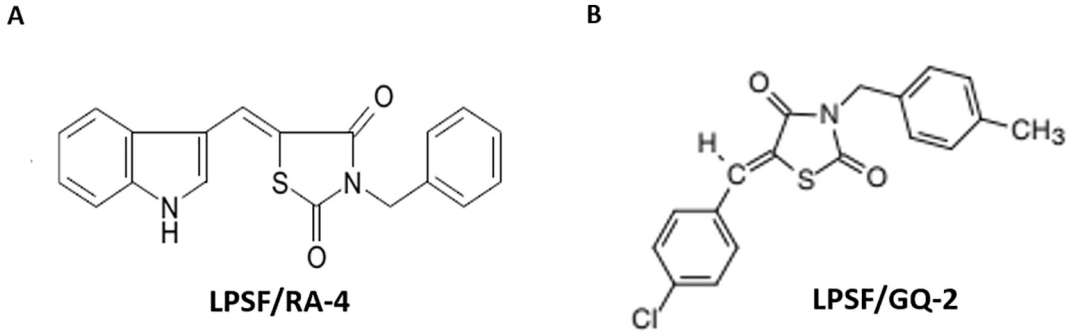

Fig. 1. The molecular structure of the new thiazolidine derivatives LPSF/RA-4 and LPSF/GQ-2.