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Sinapic acid ameliorates bleomycin-induced lung fibrosis in rats

Mohammad Raish[a,*], Ajaz Ahmad[b], Mushtaq Ahmad Ansari[c], Abdul Ahad[a], Fahad I. Al-Jenoobi[a], Abdullah M. Al-Mohizea[a], Altaf Khan[c], Naushad Ali[d]

[a] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[c] Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[d] Quality Research Unit, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

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ABSTRACT

Background: Pulmonary fibrosis is a multifaceted disease with high mortality and morbidity, and it is commonly nonresponsive to conventional therapy.
Purpose: We explore the possible discourse of sinapic acid (SA) against the prevention of bleomycin (BLM)-instigated lung fibrosis in rats through modulation of Nrf2/HO-1 and NF-κB signaling pathways.
Design/Methods: Lung fibrosis was persuaded in Sprague-Dawley rats by a single intratracheal BLM (6.5 U/kg) injection. Then, these rats were treated with SA (10 and 20 mg/kg, p.o.) for 28 days. The normal control rats provided saline as a substitute of BLM. The lung function and biochemical, histopathological, and molecular alterations were studied in serum, bronchoalveolar lavage fluid (BALF), and the lungs tissues.
Results: SA treatment significantly restored BLM-induced alterations in body weight index and serum biomarkers [lactate dehydrogenase (LDH) and alkaline phosphatase (ALP)]. SA (10 and 20 mg/kg) treatment appeared to show a pneumoprotective effect through upregulation of antioxidant status, downregulation of inflammatory cytokines and MMP-7 expression, and reduction of collagen accumulation (hydroxyproline). Nrf2, HO-1, and TGF-β expression was downregulated in BLM-induced fibrosis model, while the reduced expression levels were significantly and dose-dependently upregulated by SA (10 and 20 mg/kg) treatment. We demonstrated that SA ameliorates BLM-induced lung injuries through inhibition of apoptosis and induction of Nrf2/HO-1-mediated antioxidant enzymes via NF-κB inhibition. The histopathological findings also revealed that SA treatment (10 and 20 mg/kg) significantly ameliorated BLM-induced lung injury.
Conclusion: The present results showed the ability of SA to restore the antioxidant system and to inhibit oxidative stress, proinflammatory cytokines, extracellular matrix, and TGF-β. This is first report demonstrating that SA amoleriates BLM induced lung injuries through inhibition of apoptosis and induction of Nrf2 and HO-1 mediated antioxidant enzyme via NF-κB inhibition. The histopathological finding reveals that SA treatment (10 and 20 mg/kg) significantly ameliorates BLM induced lung injuries.

1. Introduction

The progression of pulmonary fibrosis results in pulmonary letdown and poor diagnosis in patients with various illnesses of known and unknown origin. The advancement of pulmonary fibrosis is regulated by a complex network involving several cytokines, chemokines, inflammatory cells, endothelial cells, alveolar type II cells, and growth factors [1]. Interstitial pulmonary fibrosis is described by changed cellular arrangement of alveolar region with unequal disposition of collagen, and pulmonary inflammation is well-thought-out the root cause of lung fibrosis [2]. Bleomycin (BLM) intratracheal instillation induces lung fibrosis and is a normally used rodent model of fibrosis [3]. The increase in number of activated inflammatory cells in lower airways induces the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can cause pulmonary injury and fibroblast proliferation in alveolar walls [4,5]. Epithelial apoptosis a

Abbreviations: SA, sinapic acid; BLM, bleomyci; BALF, bronchoalveolar lavage fluid; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; ROS, reactive oxygen species; RNS, reactive nitrogen species; TNFα, tumor necrosis factor-alpha; IL-β, interleukin-1β; MPO, myeloperoxidase; MDA, malondialdehyde; GPX, reduced glutathione; CAT, catalase; TGF-β, transforming growth factor beta; Nrf-2, nuclear factor erythroid-derived 2; HO-1, heme oxygenase; MMP-7, matrix metalloproteinase-7

* Corresponding author at: Department of Pharmaceutics, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia.
E-mail address: mraish@ksu.edu.sa (M. Raish).

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key phase in progression of lung fibrosis. Implication of NADPH oxidase (NOX4) and IL-6 is established fibroblast differentiation in lung fibrosis [6]. Implication of pro-inflammatory factors in lung fibrosis are regulated by NOX derived ROS. Release of proinflammatory cytokine IL-1β, IL-6 and TNF-α in response to ROS induced oxidative stress via activation redox-sensitive transcription factors including hypoxia-inducible factor 1 (HIF-1), nuclear factor-kappa B (NF-κB) [7–10].

BLM induces the release of ROS and RNS by binding with DNA and iron, resulting in DNA damage [11]. BLM and DNA interaction is suggested to initiate inflammatory response and fibro proliferative changes via cytokines resulting in collagen augmentation in the lungs [12]. Moreover, BLM causes the depletion of endogenous antioxidant defenses, which in turn leads to ROS- and RNS-induced alveolar tissue damage [13]. Alveolar tissue is particularly exaggerated by BLM as it deficiencies of the enzyme that hydrolyzes the carboxyamide bond in the β-amino alanine moiety of BLM and inhibits the binding of its metabolites with metals like iron [14]. Approaches intended at decreasing oxidative stress to ameliorate or prevent BLM-induced pulmonary damage and fibrosis have been extensively studied [15,16]. There has been an increasing interest to investigate the possible anti-inflammatory and antioxidant agents like N-acetyl cysteine (NAC), erdosteine, porphyrins, vitamin E, and polyphenols in ameliorating or preventing BLM-induced lung fibrosis [17–19]. Nuclear factor erythroid 2–related factor 2 (Nrf2), a cap'n'collar transcription factor, has been implicated as a crucial factor in the synchronized induction of antioxidant and phase II enzymes under the control of antioxidant response element (ARE) [20,21]. Nrf2 plays pivotal roles in defense against oxidative stress-induced pulmonary inflammation and fibrosis [22,23]. Several studies have established that Nrf2 also alters T-helper 1 (Th1)/T-helper 2 (Th2) skewing by regulating oxidative stress. Nrf2 is a crucial factor in determining the proneness of individuals to lung fibrosis.

Polyphenols are a multifaceted group of bioactive phytoconstituents extensively spread in the plants. These phytoconstituents are implicated in defense mechanism against oxidative damage owing to their potent antioxidant behavior or free radical scavenging property, wide range of therapeutic activity, and chemoprotective ability. Sinapic acid (SA or 3,5-dimethoxy-4-hydroxycinnamic acid) is a well-known polyphenolic compound [24–27]. SA possesses several pharmacological and chemoprotective properties such as antioxidant, anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial, and anti-tumor effects [28–30]. However, studies investigating its potential pneumoprotective activity are limited. Present work is designed to explore implication of in vivo modulation of Nrf2/HO-1 signaling pathway, oxidative stress, apoptosis, and inflammation via NF-κB downregulation by SA in BLM-induced lung fibrosis in rats.

2. Materials and methods

2.1. Chemicals and reagents

SA, hydroxyproline, and bleomycin hydrochloride (BLM) were obtained from Sigma-Aldrich. Tumor necrosis factor-alpha (TNFα), interleukin-1β (IL-β), and myeloperoxidase (MPO) ELISA Kits were procured from R&D Systems Inc. (Minneapolis, USA). Malondialdehyde (MDA), reduced glutathione (GPX), and catalase (CAT) kits were obtained from My BioSource. Antibodies against transforming growth factor beta (TGF-β), Nrf-2, heme oxygenase (HO)-1, caspase-3, Bcl-2, β-actin, and horseradish peroxidase (HRP)-conjugated secondary antibody were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The NE-PER Nuclear and Cytoplasmic Extraction Kit was obtained from Pierce Biotechnology (Rockford, IL, USA). All other chemicals were of analytical grade.

2.2. Animals

Sprague-Dawley rats (weight: 200–220 g) were procured from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were housed in cages at room temperature (23 ± 2 °C), 60 ± 10% humidity, and 12: 12 h light-dark cycle. The rats were provided ad libitum access to water and pallet diet. This study was approved by the animal Ethics Committee, King Saud University, Riyadh, Saudi Arabia.

2.3. Experimental model of pulmonary fibrosis

The animal model of BLM-induced lung fibrosis was established as described previously [31,32]. The doses of SA (10 and 20 mg/kg body weight) were selected based on their ability to curb oxidative stress in various animal models of disease in rodents [33,34].

2.4. Animal study protocol

The rats were divided into four groups of six rats each as follows:

Group I: Normal saline-instilled rats (normal control).

Group II: BLM-instilled rats (6.5 U/kg body weight).

Group III: BLM-instilled rats treated intraperitoneally with SA (10 mg/kg body weight) after 6 h of BLM instillation, once daily throughout the study period.

Group IV: BLM-instilled rats treated intraperitoneally with SA (20 mg/kg body weight) after 6 h of BLM instillation, once daily throughout the study period.

After 28 days, blood was collected in clean sterilized tubes and serum was separated. All rats in all the groups were euthanized. Broncho alveolar lavage fluid (BALF) was obtained from both the lungs. Lungs were incised in several pieces. Then, the lungs were blended in 0.1 M Tris–HCl buffer (pH 7.4) and used for analysis. All animals were weighed before and at the end of the investigations. The alteration in body weight and lung tissue was estimated to calculate the lung index.

2.5. Estimation of serum markers for lung injuries, oxidative stress and collagen deposition

On day 28, Estimation of serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The activities of GPx and CAT, as well as the contents of lipid peroxidation marker MDA, hydroxyproline, MPO, and MMP-7 in rat lung tissues were measured with ELISA kits (R&D system), according to the manufacturer's protocols.

2.6. Measurement of inflammatory cytokines

TNF-α and IL-lβ levels in the lung tissue were analyzed by ELISA Kits (MyBioSource). The standard curve was generated using the TNF-α and IL-1β standard supplied with the kit.

2.7. Western blot analysis

An western blot was carried out as per the protocol of Towbin et al. [35]. A detailed protocol has reported. [36].

2.8. Histological examination

The pulmonary tissues were dehydrated in graded alcohol and fixed in paraffin blocks. Each was sliced into 5-μm thick slices. Fallowed by dewaxing in xylene, rehydrated in gradient ethanol, and washed with distilled water. These slices were keep with hematoxylin for 6 min and then with eosin for 4 min.

2.9. Statistical analyses

The data values were presented as mean ± standard error of mean (± SEM). The significance was assessed by one-way analysis of variance as appropriate; p values of < 0.05 were considered significant