International Immunopharmacology 63 (2018) 198–203

Contents lists available at ScienceDirect

International Immunopharmacology

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

Morin attenuates cigarette smoke-induced lung inflammation through inhibition of PI3K/AKT/NF-κB signaling pathway

Baoning Cai, Xiangfeng Gan, Jinxi He, Wei He, Zhixiong Qiao, Bo Ma, Yuning Han*

Department of General Thoracic Surgery, General Hospital of Ningxia Medical University, Ningxia, China

ARTICLE INFO

Keywords:
Cigarette smoke
Lung inflammation
Inflammatory cytokines
P13K/ATK/NF-κB signaling pathway

ABSTRACT

Cigarette smoke (CS) is a major factor that leads to lung inflammation. The prevalence of CS-induced lung injury has continuously increased worldwide. Morin exists in a large member of plants and fruits that has been reported to have antioxidant and anti-inflammatory properties. In the present study, we tested the mechanism and protective effects of morin on CS-induced lung inflammation in mice. The mice were exposed to CS for 2 h twice a day for 4 weeks. Morin (10, 20, and 40 mg/kg) was treated to mice through oral gavage 1 h before CS administration. 24 h after the last CS exposure, the mice were euthanized. The lung tissues were collected and the pathological changes, wet/dry ratio, MPO activity, MDA levels, and P13K/ATK/NF-κB signaling pathway expression were detected. The bronchial alveolar lavage fluid (BALF) was obtained and the levels of inflammatory cells and inflammatory cytokines were measured. The results showed that morin treatment significantly inhibited lung pathological changes, wet/dry ratio, MPO activity, and MDA level. The levels of total cells, neutrophils, macrophages, as well as the production of inflammatory cytokines in the BALF induced by CS were also suppressed by morin. Further research showed that morin dramatically suppressed the activation of P13K/ATK/NF-κB singling pathway induced by CS. This study highlights the protective effects of morin on CS-induced lung inflammation, which may, at least part, be mediated through inhibiting P13K/ATK/NF-κB signaling pathway. These finding demonstrated that morin could be a potential drug for CS-induced lung injury.

1. Introduction

Cigarette smoke (CS)-induced pulmonary disease, especially lung inflammation, is the major leading cause of mortality among worldwide [1,2]. It is characterized by the increased levels of inflammatory cytokines, including TNF-α, and IL-1β. These cytokines lead to the activation and recruitment of neutrophils [3,4]. CS exposure also induced the destruction of alveolar tissues, the impairment of endothelial functions, and the loss of surface area for gas exchange [5–7]. Previous studies showed that increased NF-κB activation were observed in mice of CS-induced lung inflammation and pharmacological inhibition of P13K/ATK/NF-κB signaling pathway could protect mice against CS-induced lung inflammation [8,9]. However, there are still few effective drugs for the treatment of lung inflammation. Thus, the safety and effectiveness medicine for curing CS-induced lung inflammation are urgently needed.

Morin (3,5,7,2′,4′-pentahydroxyflavone), a major component of traditional medicinal herbs, was extracted from *Cudrania tricuspidata*, Osage orange, *Artocarpus heterophyllus* Lam., fig, and other Moraceae family members [10,11]. Previous studies have shown that morin had antioxidant and anti-inflammatory activities. Morin inhibited the production of TNF-α, IL-4, IL-13, matrix metalloproteinase-9 through suppressing ROS/MAPK signaling pathway in goblet cell stimulated by OVA [11]. Morin treatment inhibited the inflammatory cytokines TNF-α, IL-1β, and IL-6, and inflammatory mediator NO production, as well as NF-κB signaling pathway activation in MSU-crystals stimulated macrophage cell [12]. Evidence also suggested that morin mainly exerted its anti-inflammatory effects by inhibiting the activation of NF-κB and NF-κB-regulated gene expression [13]. Although morin has been reported in the treatment of a number of inflammatory diseases, it remains unclear whether it has protective effects against CS-induced lung inflammation. The aim of our study was to test the mechanism and protective effects of morin on CS-induced lung inflammation in mice. The results showed morin attenuated CS-induced inflammatory response through inhibiting P13K/AKT/NF-κB signaling pathway activation.

* Corresponding author.
E-mail address: 807696299@qq.com (Y. Han).

https://doi.org/10.1016/j.intimp.2018.07.035
Received 25 April 2018; Received in revised form 26 July 2018; Accepted 27 July 2018

2. Materials and methods

2.1. Animals

C56BL/6N male mice, weighted 20–25 g, were purchased form Experimental Animal Center of Ningxia Medical University. The mice were used for experiments after one week of acclimatization, and were provided with sufficient water and food. All procedures were approved by the care and use of laboratory animal manual published by the US National Institutes of Health.

2.2. Materials

Morin was purchased from sigma (St Louis, MO, USA). Cigarettes were purchased from Tobacco Group Company Limited. MPO and MDA determination kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). TNF-α and IL-1β enzyme-linked immunesorbent assay kits were purchased from eBioscience (San Diego, CA, USA). Antibodies against NF-κB p65 (6956), p-NF-κB p65 (3033, Ser536), IκBα (4814), p-IκBα (2859, Ser32), ATK (9272), p-ATK (4060, Ser473), PI3K (4255), and p-PI3K (13,857, Ser249) were purchased from Cell Signaling Technology Inc. (Beverly, MA). All other chemicals were of reagent grade.

2.3. Animal treatment

Seventy-two mice were divided into six groups and each group contained twelve mice, including control group, morin group (40 mg/kg), CS group, and morin (10, 20, 40 mg/kg) + CS groups. The mice model of CS-induced lung inflammation was administrated by previous described [14,15]. Mice were placed in a 20-liter Perspex chamber, and exposed to cigarette smoke for 2 h twice a day for 4 weeks. Mice were exposed to air served as control group. Mice in groups of CS + morin were treated with morin 1 h before CS administration. 24 h after the last CS exposure, mice were euthanized and lung tissues and bronchial alveolar lavage fluid (BALF) were collected to store at −80 °C until used.

2.4. Lung histology assay

The right lobe of lung tissues were collected and fixed in 10% formaldehyde for 48 h. Tissues were exposed to a series of graded ethanol for dehydration, embedded in paraffin, cut into section (4 μm), and stained with hematoxylin and eosin (H & E) (Solarbio, Beijing, China). Each slide was assessed with light microscope (Olympus, Japan).

2.5. Lung wet/dry weight ratio assay

The lung wet/dry ratio is an index of pulmonary edema. Immediately following lung perfusion, the inferior lobe of the right lung was excised and then recorded before placement in an oven at 60 °C for 72 h to obtain the dry weight. The dates were to calculate lung wet/day weight ratio.

2.6. Lung MPO activity and MDA levels assay

Lung tissues obtained from each group were weighted and homogenized with cold saline and the MPO reaction substrates were added. The mixture was boiled in 60 °C water bath for 10 min, and added other reagents according to the manufacturer's instruction. The Lung MDA, a lipid peroxidation marker, was evaluated using MDA kit (Jiancheng Bioengineering Institute, Nanjing, China) in accordance to the manufacturer's instruction.

2.7. Inflammatory cytokines and inflammatory cells assay

BALF was obtained 24 h after of CS-induced lung inflammation. The samples were centrifuged at 3000 rpm at 4 °C for 10 min. The supernatant was used to test the levels of inflammatory cytokine TNF-α and IL-1β by ELISA according to the manufacturer's protocols. The cell pellet was suspended in 0.2 mL PBS. The total cells were counted using a hemacytometer, and the neutrophils and macrophages counts were performed by cytocentrifugation at 800 rpm for 5 min followed by Wright's stain.

2.8. Western blot assay

Total protein from lung tissue was extracted using the protein extraction reagent (Thermo, USA). The concentration of protein was measured using BCA kit (Beyotime Institute of Biotechnology, China) according to the manufacturer's instruction. Equal amounts of protein from each sample were separated on a 12% SDS-polyacrylamide gel and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 3% BSA for 2 h and incubated with anti-NF-κB p65 antibody (1:1000), anti-NF-κB p-p65 antibody (1:1000), anti-IκBα antibody (1:1000), anti-pIκB antibody (1:1000), anti-ATK antibody (1:1000), anti-p-ATK antibody (1:1000), and anti-β-actin (1:1000) antibody at 4 °C overnight. The membranes were washed with TBS-T for three times and incubated with secondary antibody (1:5000) at room temperature for 1 h. After washing by TBS-T for three times, the members were developed with the ECL Plus Western Blotting Detection System.

2.9. Statistical assay

Data were analyzed using SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL). Values are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for multiple comparisons among groups. The results were considered statistically significant at $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Effect of morin on lung pathological change

Lung pathological changes were tested by H & E staining. As shown in Fig. 1A and F, lung tissues of the control group and morin alone group showed normal alveolar structure and no any pathological damage. In comparison, CS exposed to mice lead to severe inflammatory cell infiltration and obvious alveolar wall thickening (Fig. 1B). However, morin treatment significantly inhibited the pathological changes induced by CS (Fig. 1C, D, E).

3.2. Effect of morin on lung wet/dry ratio

Lung wet/day ratio is a marker of lung edema. The results showed that compared with the control group, the lung wet/dry ratio markedly increased in the CS-stimulated group, and morin dose-dependently reduced the lung wet/dry ratio induced by CS (Fig. 2).

3.3. Effect of morin on inflammatory cytokines levels in the BALF

The levels of inflammatory cytokines TNF-α and IL-1β in BALF were tested by ELISA. As shown in Fig. 3, the levels of TNF-α (Fig. 3A) and IL-1β (Fig. 3B) in the BALF were higher in CS group than in the control group. However, morin dose-dependently inhibited the levels of TNF-α and IL-1β induced by CS.

3.4. Effect of morin on inflammatory cells count in BALF

The counts of total cells, neutrophils, and macrophages in BALF were evaluated in the present study. As shown in Fig. 4, treatment of CS induced a significantly increase in total cells (A), neutrophils (B), and