International Immunopharmacology 60 (2018) 202–210

Contents lists available at ScienceDirect

International Immunopharmacology

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

Fisetin inhibits the generation of inflammatory mediators in interleukin-1β–induced human lung epithelial cells by suppressing the NF-κB and ERK1/2 pathways

Hui-Ling Peng[a,1], Wen-Chung Huang[a,b,*,1], Shu-Chen Cheng[c,d], Chian-Jiun Liou[b,e,**]

[a] Graduate Institute of Health Industry Technology, Research Center for Food and Cosmetic Safety, Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, No.261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan
[b] Division of Allergy, Asthma, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, Linkou, Guishan Dist., Taoyuan City 33303, Taiwan
[c] Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[d] Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[e] Department of Nursing, Division of Basic Medical Sciences, Research Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, No.261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan

ARTICLE INFO

Keywords:
Fisetin
Anti-inflammation
ICAM-1
COX-2
ERK1/2
NF-κB

ABSTRACT

Fisetin, a flavone that can be isolated from fruits and vegetables, has anti-tumor and anti-oxidative properties and ameliorates airway hyperresponsiveness in asthmatic mice. This study investigated whether fisetin can suppress the expression of inflammatory mediators and intercellular adhesion molecule 1 (ICAM-1) in A549 human lung epithelial cells that were stimulated with interleukin-1β (IL-1β) to induce inflammatory responses. A549 cells were treated with fisetin (3–30 μM) and then with IL-1β. Fisetin significantly inhibited COX-2 expression and reduced prostaglandin E_2 production, and it suppressed the levels of IL-8, CCL5, monocyte chemotactic protein 1, tumor necrosis factor α, and IL-6. Fisetin also significantly attenuated the expression of chemokine and inflammatory cytokine genes and decreased the expression of ICAM-1, which mediates THP-1 monocyte adhesion to inflammatory A549 cells. Fisetin decreased the translocation of nuclear transcription factor kappa-B (NF-κB) subunit p65 into the nucleus and inhibited the phosphorylation of proteins in the ERK1/2 pathway. Co-treatment of IL-1β–stimulated A549 cells with ERK1/2 inhibitors plus fisetin reduced ICAM-1 expression. Furthermore, fisetin significantly increased the effects of the protective antioxidant pathway by promoting the expression of nuclear factor erythroid-2-related factor-2 and heme oxygenase 1. Taken together, these data suggest that fisetin has anti-inflammatory effects and that it suppresses the expression of chemokines, inflammatory cytokines, and ICAM-1 by suppressing the NF-κB and ERK1/2 signaling pathways in IL-1β–stimulated human lung epithelial A549 cells.

1. Introduction

Asthma is a common disorder that involves airway inflammation. Allergens, as well as bacterial and viral infections, can induce asthma attacks in patients [1]. Acute asthma attacks cause wheezing and coughing and involve mucus hypersecretion that plugs the airways and makes breathing difficult [2]. At the cellular level, a large number of inflammatory cells infiltrate into the airways and lungs of patients with chronic asthma, and these cells release inflammatory cytokines and chemokines that trigger more serious inflammation and damage cells. This exacerbates the symptoms of asthma and makes treatment difficult [1]. Research shows that activated macrophages in the lungs of asthmatic patients secrete IL-1β that stimulates airways and lung epithelial cells, which then release more proinflammatory cytokines that cause inflammation and release additional chemokines that attract immune cells and exacerbate inflammatory damage in the lungs [3,4]. Hence, many studies suggest that reducing the inflammatory response and inhibiting the activation of lung epithelial cells can ameliorate asthma

* Correspondence to: W.C. Huang, Graduate Institute of Health Industry Technology, Research Center for Food and Cosmetic Safety, Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, No.261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan.
** Correspondence to: C.J. Liou, Department of Nursing, Division of Basic Medical Sciences, Research Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, No.261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan.
E-mail addresses: wchuang@mail.cgust.edu.tw (W.-C. Huang), ccliu@mail.cgust.edu.tw (C.-J. Liou).
[1] Hui-Ling Peng and Wen-Chung Huang are equal contributors to the work.

https://doi.org/10.1016/j.intimp.2018.05.004
Received 17 January 2018; Received in revised form 4 May 2018; Accepted 8 May 2018

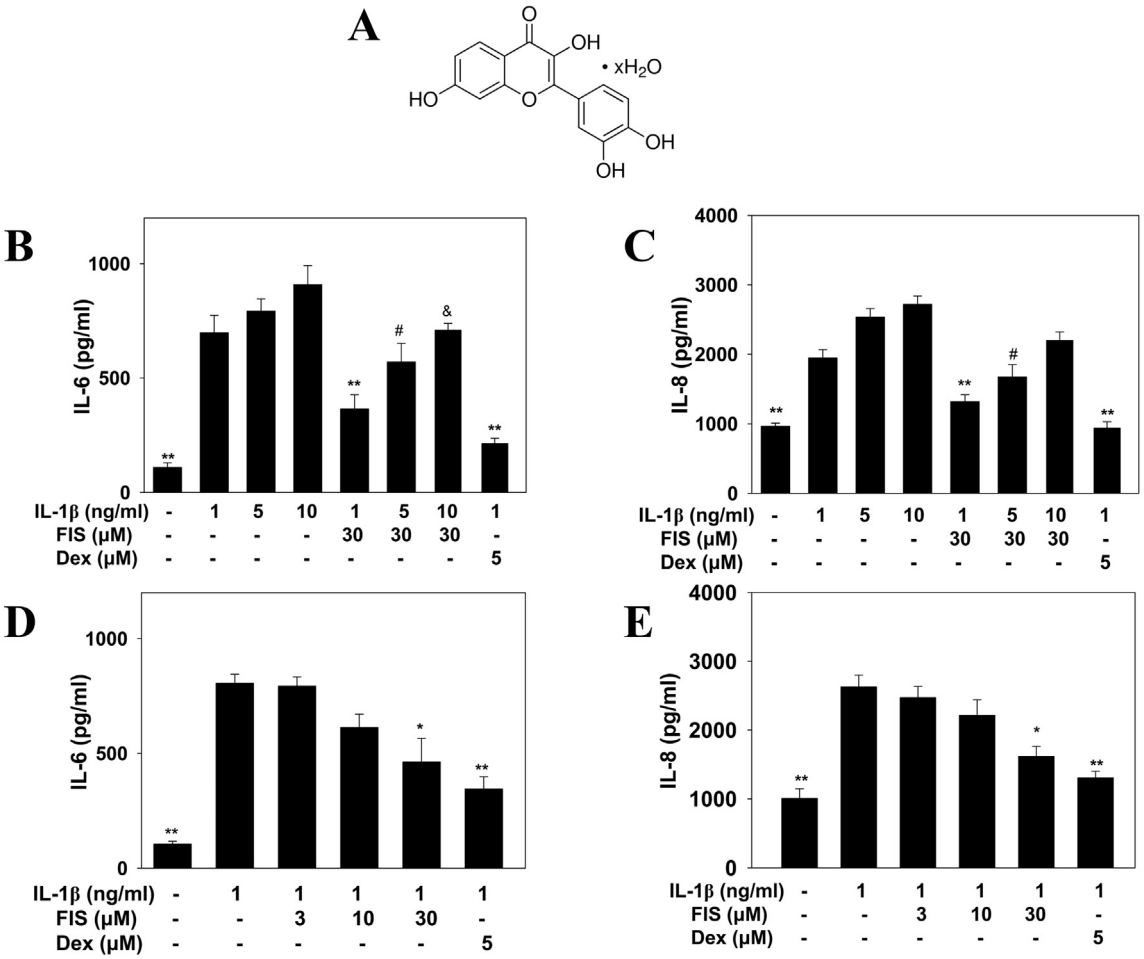

Fig. 1. The effects of fisetin (FIS) on IL-1β–induced IL-6 and IL-8 production. (A) The chemical structure of fisetin. A549 cells were treated with fisetin or dexamethasone (Dex) for 1 h and then stimulated with the indicated doses of IL-1β (1–10 ng/ml) for 24 h. IL-6 (B) and IL-8 (C) levels were measured by ELISA. We also treated A549 cells with fisetin plus IL-1β at the same time and then cultured the cells for 24 h. IL-6 (D) and IL-8 (E) levels were measured by ELISA. The data are presented as means ± SDs of three independent experiments (n = 6); *$p < 0.05$, **$p < 0.01$ compared with the (1 ng/ml) IL-1β–treated group. #$p < 0.05$, compared with the (5 ng/ml) IL-1β–treated group, $^{\&}p < 0.05$, compared with the (10 ng/ml) IL-1β–treated group.

symptoms, reduce cell damage, and improve respiratory function [5,6].

Steroids are anti-inflammatory drugs that are often used to prevent or treat asthma attacks [7]. Previous studies showed that steroids have a remarkable ability to inhibit the inflammatory response of airway epithelial cells in asthmatic patients and to reduce the secretion of pro-inflammatory cytokine into bronchoalveolar lavage fluid [8]. Experiments in which lung epithelial cells were stimulated with IL-1β and treated with dexamethasone found that dexamethasone inhibited inflammatory cytokine and IL-8 expression and reduced cyclooxygenase (COX)-2 expression by decreasing the activity of nuclear transcription factor kappa-B (NF-κB) or mitogen-activated protein kinase (MAPK) pathways [9]. Previous studies also found that glucocorticoids reduce the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), in lung epithelial cells, which increase inflammatory cell infiltration into lung tissue [10]. Unfortunately, treatment with steroids does not improve the asthma symptoms of patients with steroid-resistant asthma [8,11]. One study found that patients with steroid-resistant asthma have more neutrophils and macrophages, but not more eosinophils, infiltrating their airways and lung tissue [11]. Inflammatory neutrophils and macrophages also secrete more IL-1β, which causes severe pulmonary inflammatory reactions in the lungs of patients with asthma [12]. Patients with steroid-resistant asthma cannot use steroids to prevent or treat their asthma. Therefore, researchers are looking for other drugs or natural compounds that can improve airway inflammation in asthmatic patients.

Fisetin is a flavone that can be isolated from fruits and vegetables [13]. Previous work showed that fisetin reduces the inflammatory response in lipopolysaccharide-treated RAW264.7 cells by suppressing the NF-κB and JNK pathways [14]. Fisetin also improves acute lung injury in rats by regulating Toll-like receptor 4 and the NF-κB pathway [15]. In addition, fisetin attenuates airway inflammation in asthmatic mice by suppressing the NF-κB pathway [16]. However, it is not clear how fisetin regulates the inflammatory response of lung epithelial cells. This study investigated the anti-inflammatory effects of fisetin and its effects on MAPK and NF-κB pathway regulation in IL-1β–stimulated A549 human lung epithelial cells.

2. Materials and methods

2.1. Materials

Fig. 1A shows the chemical structures of fisetin (≥98% purity by HPLC; Sigma-Aldrich, St. Louis, MO, USA) (Fig. 1A). A stock solution of 100 mM fisetin was prepared in DMSO. The final DMSO concentration did not exceed 0.1% in the culture medium.

2.2. Cell line and the cell viability assay

A549 human lung epithelial cells were obtained from the Bioresource Collection and Research Center (BCRC, Taiwan) and grown