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Emodin alleviates bleomycin-induced pulmonary fibrosis in rats

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HIGHLIGHTS

- Emodin improves pulmonary function in BLM-treated rats.
- Emodin inhibits BLM-induced expression of profibrotic molecules, including TNF-α, IL-6, TGF-β1, α-SMA and HSP-47 in the lungs.
- Emodin attenuates TGF-β1-induced myofibroblast differentiation and ECM deposition in human embryo lung fibroblasts.
- Emodin suppresses TGF-β1-activated Smad2/3 signaling pathway.
- Emodin suppresses TGF-β1-induced STAT3 phosphorylation.

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a lethal lung disease with few treatment options and poor prognosis. Emodin, extracted from Chinese rhubarb, was found to be able to alleviate bleomycin (BLM)-induced pulmonary fibrosis, yet the underlying mechanism remains largely unknown. This study aimed to further investigate the effects of emodin on the inflammation and fibrosis of BLM-induced pulmonary fibrosis and the mechanism involved in rats. Our results showed that emodin improved pulmonary function, reduced weight loss and prevented death in BLM-treated rats. Emodin significantly relieved lung edema and fibrotic changes, decreased collagen deposition, and suppressed the infiltration of myofibroblasts [characterized by expression of α-smooth muscle actin (α-SMA)] and inflammatory cells (mainly macrophages and lymphocytes). Moreover, emodin reduced levels of TNF-α, IL-6, TGF-β1 and heat shock protein (HSP)-47 in the lungs of BLM-treated rats. *In vitro*, emodin profoundly inhibited TGF-β1-induced α-SMA, collagen IV and fibronectin expression in human embryo lung fibroblasts (HELFs). Emodin also inhibited TGF-β1-induced Smad2/3 and STAT3 activation, indicating that Smad2/3 and STAT3 inactivation mediates emodin-induced effects on TGF-β1-induced myofibroblast differentiation. These results suggest that emodin can exert its anti-fibrotic effect via suppression of TGF-β1 signaling and subsequently inhibition of inflammation, HSP-47 expression, myofibroblast differentiation and extracellular matrix (ECM) deposition.

1. Introduction

As the most common and severe type of the idiopathic interstitial pneumonias, idiopathic pulmonary fibrosis (IPF) is a chronic, aggressive and fatal disease (Raghu et al., 2011). The incidence of IPF is estimated to be 14.0–42.7 per 100,000 people in the United States alone (King et al., 2011). The survival time of patients with IPF is approximately 3–5 years once diagnosed, making it a global challenge (Xaubet et al., 2014).

IPF is characterized by abnormal lung tissue remodeling, interstitial inflammation, collagen deposition and aberrant re-epithelization, with consequent progressive destruction of lung architecture and irreversible loss of pulmonary function (Gross and

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Hunninghake, 2001; Raghu et al., 2011). The poor prognosis of IPF is ultimately associated with the proliferation of fibroblastic foci, which consist of massive activated myofibroblasts (King et al., 2001). Myofibroblasts, characterized by expression of α-smooth muscle actin (α-SMA), are mainly derived from the differentiation of fibroblasts and are the principal cell type responsible for the production of extracellular matrix (ECM) in humans and animals with IPF (Darby and Hewitson, 2007). In addition, heat shock protein-47 (HSP-47), a collagen-specific molecular chaperone, is localized primarily in endoplasmic reticulum of collagen-producing cells and participates in the intracellular processing of procollagen (Kakugawa et al., 2004). The expression of HSP-47 is elevated in various fibrotic organs, including kidney, liver and lung (Xiang et al., 2015). Inhibition of excessive HSP-47 expression and/or myofibroblast differentiation is therefore an effective method for IPF treatment.

Transforming growth factor-β1 (TGF-β1), a primary fibrogenic cytokine, is involved in the development of pulmonary fibrosis (Chen et al., 2013). Enhanced level of TGF-β1 in the lungs has been observed in animal models of pulmonary fibrosis and in humans with IPF (Kakugawa et al., 2004; Khalil et al., 1991), while inhibition or down-regulation of TGF-β1 ameliorates bleomycin (BLM)-induced pulmonary fibrosis (Ji et al., 2013). Further, fibroblasts treated with TGF-β1 elevate HSP-47 expression and would differentiate to a myofibroblast phenotype primarily through a canonical Smad-dependent mechanism (Kottmann et al., 2015; Nakayama et al., 2008). Hence, given the established function of TGF-β1 on HSP-47 expression, myofibroblast differentiation and ECM deposition, drugs capable of disrupting TGF-β1 production and/or blocking the related signaling pathway may have a therapeutic potential for pulmonary fibrosis.

Current available therapies for IPF, including corticosteroids, immunosuppressive agents and cytotoxic agents, are generally nonspecific and accompanied by serious adverse effects (Noth and Martinez, 2007). Thus, novel therapies for IPF with improved efficacy and fewer side effects are urgently desired, and one ideal approach would be to explore therapeutic compounds from natural resources.

Emodin (Fig. 1A), an active and predominant component extracted from rhubarb, has diverse biological activities, including laxative, immunosuppressive, anticancer and anti-inflammatory effects (Xiao et al., 2014). Currently, emodin is reported to alleviate liver fibrosis, pancreatic fibrosis and pulmonary fibrosis (Chen et al., 2009; Wang et al., 2007; Zhan et al., 2000). Chen et al. (2009) demonstrated that emodin attenuates BLM-induced pulmonary fibrosis through inhibition of myeloperoxidase activity (a marker for neutrophil influx into tissue) in mice, as well as suppression of cell proliferation and the expression of TGF-β1 and collagen I in lung fibroblasts (Chen et al., 2009). However, the precise mechanisms by which this compound offers protection against pulmonary fibrosis remain largely unknown. In the present study, we demonstrate that emodin inhibits TGF-β1-induced myofibroblast differentiation and ECM deposition via a blockage of TGF-β1-activated profibrotic signaling in pulmonary fibroblasts. We also provide *in vivo* evidence that emodin dramatically depresses BLM-induced pulmonary inflammation and fibrosis, and consequently improves pulmonary function by inhibiting the expression of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), TGF-β1, HSP-47 and the infiltration of inflammatory cells (mainly macrophages and lymphocytes) and myofibroblasts.

2. Materials and methods

2.1. Chemicals and reagents

Emodin was purchased from Shanghai future industry Limited by Share Ltd (Shanghai, China). BLM was purchased from Nippon Kayaku (Tokyo, Japan). TRIzol Reagent was from Invitrogen Corporation (Carlsbad, USA). The ReverTra Ace qPCR RT Kit and SYBR Green Real-time PCR Master Mix were purchased from TOYOBO (Osaka, Japan). Diff-quick staining kit was purchased from Zhuhai Baso Bio-technology limited (Zhuhai, China). IL-6 and TNF-α ELISA kits were purchased from Shanghai Weiao Biotech Ltd (Shanghai, China). Rabbit anti-α-SMA, anti-HSP-47 and anti-collagen IV antibodies were purchased from Abcam Biotechnology (Cambridge, MA, USA). Rabbit anti-p-Smad2, anti-p-Smad3, anti-Smad2 and anti-Smad3 antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Rabbit anti-p-STAT3 antibody was purchased from Absci (MD, USA). Rabbit anti-fibronectin and anti-STAT3 antibodies were purchased from Proteintech (Chicago, IL, USA). Mouse anti-GAPDH polyclonal antibody, HRP-labeled Goat Anti-Rabbit/Mouse IgG(H + L) and RIPA lysis buffer were purchased from Beyotime Institute of Biotechnology (Haimen, China). The poly-vinylidene fluoride (PVDF) membranes were from Millipore Corporation (Billerica, MA, USA). ECL-Plus detection kit probed was from Tiangen Biotech Co. Ltd (Beijing, China). The other chemicals and reagents used in the experiment were of analytical grade.

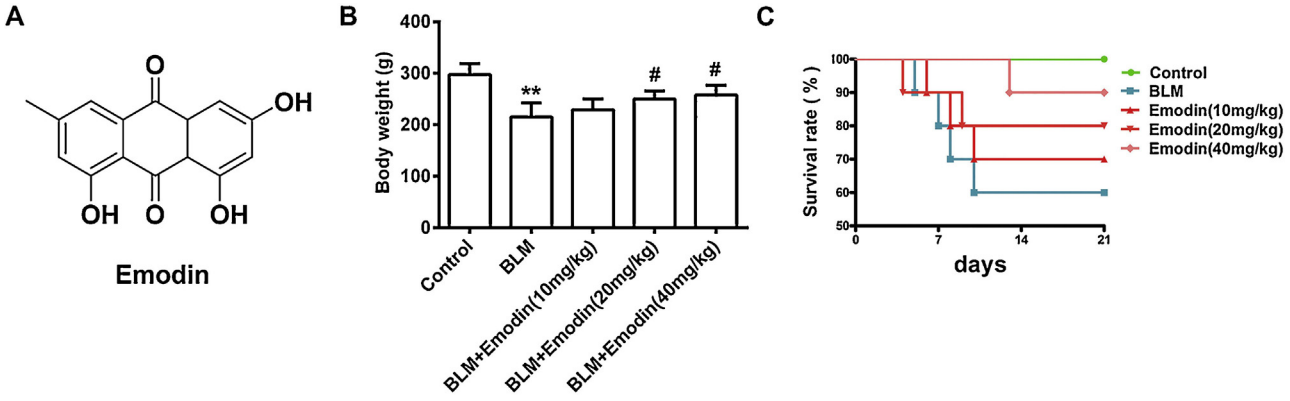

Fig. 1. Emodin reduced body weight loss and increased the survival rate of BLM-treated rats. (A) Chemical structure of emodin. (B) Average body weight of rats in each group was compared, three weeks after BLM administration. As data shown, BLM treatment caused a significant weight loss and emodin ameliorated BLM-induced weight loss in a dose dependent manner. Data are presented as mean ± SD (n = 6). $^{**}P < 0.01$ versus control group. $^{\#}P < 0.05$ versus BLM group. (C) Survival rates were shown over a 21-day observation period.