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Submicron emulsion of cinnamaldehyde ameliorates bleomycin-induced idiopathic pulmonary fibrosis via inhibition of inflammation, oxidative stress and epithelial-mesenchymal transition

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ABSTRACT

Aims: Idiopathic pulmonary fibrosis (IPF) is the most frequent and severe form of idiopathic interstitial pneumonias. The pathogenesis is associated with inflammation and oxidative stress and epithelial-mesenchymal transition (EMT). Cinnamaldehyde exhibits antiinflammatory and antioxidant properties, but its effect on IPF is unknown. The present study is to investigate the anti-fibrotic effect and action mechanism of cinnamaldehyde on IPF.

Materials and methods: IPF was induced by intratracheal bleomycin in mice. Submicron emulsion of cinnamaldehyde was given by intraperitoneal injection once everyday for 7 or 21 continuous days after bleomycin administration. Lung histological and injury indexes were analyzed. The protein expressions of inflammation and oxidative stress as well as EMT markers alpha-smooth muscle actin (α-SMA) and E-cadherin in mice and cultured A549 cells were measured.

Results: Cinnamaldehyde attenuated the bleomycin-induced histological injury, reduced hydroxyproline level and improved pulmonary function by the inhibiting inflammatory cytokines and reactive oxygen species production as well as enhancing total superoxide dismutase activity in bleomycin-induced mice. Cinnamaldehyde also inhibited EMT in both bleomycin-induced mice and TGF-β1-stimulated A549 cells.

Conclusions: Cinnamaldehyde ameliorated bleomycin-induced IPF via inhibition of inflammation and oxidative stress and EMT.

1. Introduction

Fibrosis can be defined as the excessive accumulation of extracellular matrix (ECM) particularly fibrillar collagens. Fibrosis is a vital factor of progressive organ dysfunction and damage in various inflammatory and metabolic-associated diseases, such as pulmonary fibrosis, advanced kidney disease and advanced liver disease [1–8]. Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal interstitial lung disease. The pathogenesis of IPF is still unknown but thought to be associated with excessive inflammation, oxidative stress and abnormal epithelial-mesenchymal transition (EMT) [9,10]. There have been no fundamental breakthroughs in drug treatment for IPF. Lung transplantation remains the viable therapeutic option for patients with IPF [11]. Despite many drugs have been applied to treat IPF, accumulated evidence demonstrated their intervention is not completely effective [12]. The anti-fibrotic agents of pirfenidone and nintedanib were approved for treatment of IPF in 2014, but they received only a conditional recommendation for use and neither one had a clear advantage on mortality outcomes [12]. Methylprednisolone could partly reduce IPF induced by bleomycin in animal model, but the main disadvantage is its strong immunity inhibition. Hence, there is a pressing need to develop novel therapeutic agents with minimal side effect for prevention and treatment of IPF.

Cinnamaldehyde is a plant secondary metabolite isolated from the stem bark of Cinnamomum trees [13]. Previous studies indicated that cinnamaldehyde exhibited a wide range of biological activities including anti-tumor, anti-bacterial and anti-mutagenic properties [14,15]. Our previous studies demonstrated that cinnamaldehyde

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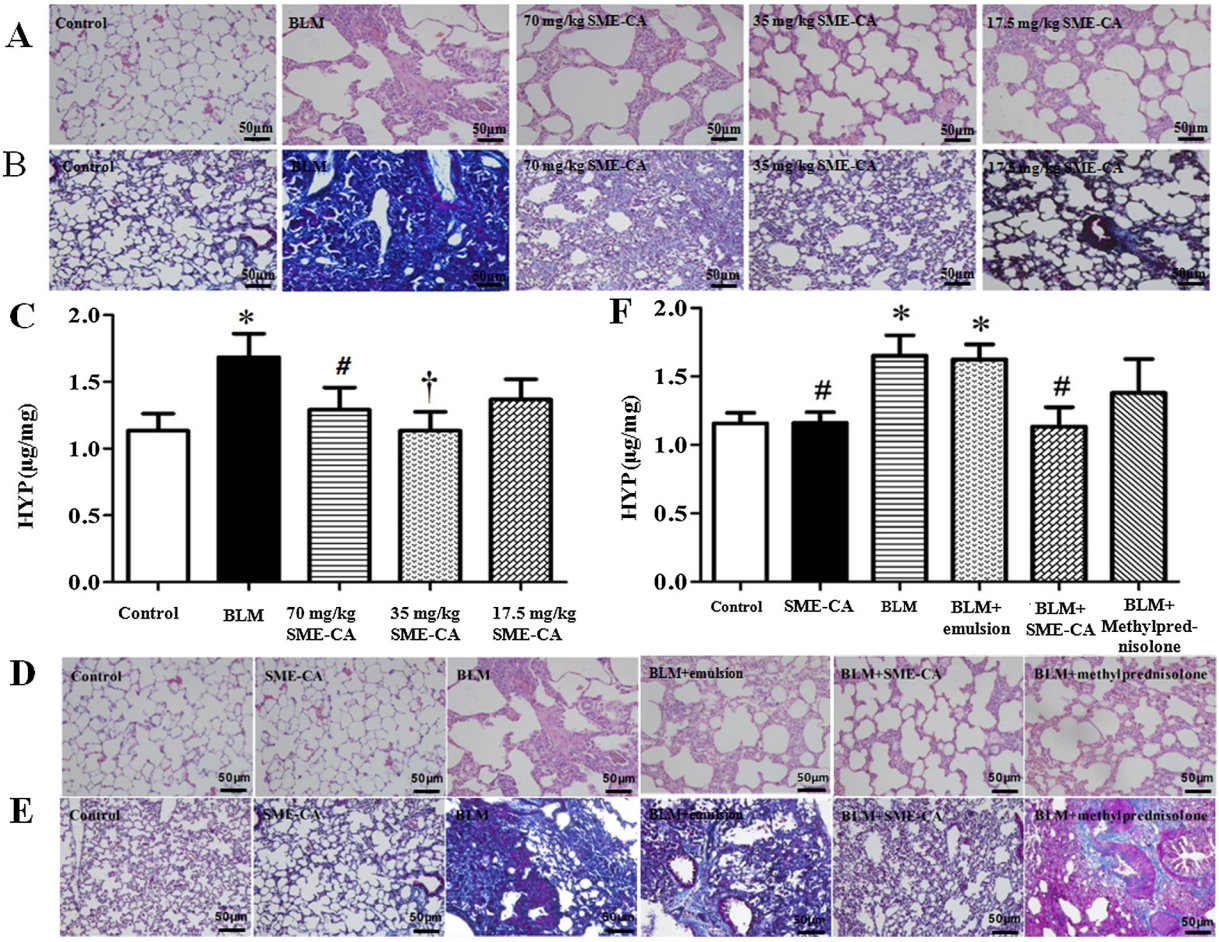

Fig. 1. Effect of SME-CA on the bleomycin-induced pulmonary fibrosis. Histopathological evaluation of the doses of SME-CA in bleomycin-induced pulmonary fibrosis mice. Sections of pulmonary tissue were subjected to H&E staining on day 7 (A and D) and Masson trichrome staining on day 21 (B and E). Hydroxyproline (HYP) levels in lungs on day 21 (C and F). $^{*}P < 0.05$ vs Control; $^{\#}P < 0.05$ vs BLM.

Table 1
Lung-to-body weight ratio in the bleomycin-induced mice.

	Control	SME-CA	BLM	BLM + emulsion	BLM + SME-CA	BLM + methylprednisolone
7d	0.69 ± 0.03	0.68 ± 0.03$^{\#}$	1.53 ± 0.10*	1.49 ± 0.11*	1.10 ± 0.12$^{\#}$	1.06 ± 0.09$^{\#}$
21d	0.70 ± 0.04	0.73 ± 0.03$^{\#}$	1.17 ± 0.09*	1.15 ± 0.11*	0.90 ± 0.14$^{\#}$	1.12 ± 0.12

The wet lung-to-body weight ratio is an indicator of lung inflammation. We found that the bleomycin-treated mice increased lung-to-body weight ratios, which could be ameliorated by SME-CA. Each value represents the Mean ± SD. $N = 6$ mice per group.

* $P < 0.05$ vs Control.

$^{\#}$ $P < 0.05$ vs BLM.

showed protective effects on viral myocarditis, myocardial ischemia, cardiac inflammation and fibrosis [16–19]. However, the therapeutic effect of cinnamaldehyde on IPF is not completely clear. Because cinnamaldehyde is easily oxidized to cinnamic acid and unstable in blood, recently we developed the submicron emulsion of cinnamaldehyde to promote its application. The present study is to investigate the effect of submicron emulsion of cinnamaldehyde (SME-CA) against IPF in bleomycin-induced mice and its underlying mechanism.

2. Materials and methods

2.1. Materials

Bleomycin was purchased from Nippon Kayaku. Cinnamaldehyde (99.0% purity) was purchased from Yuancheng Pharmaceutical Co., Ltd. Diff-Quick reagents, anti-rabbit or anti-rat conjugated to horseradish peroxidase and GAPDH were purchased from Solarbio Biological Technology Company. Tumor necrosis factor α (TNF-α), Interleukin-1β (IL-1β), Malondialdehyde (MDA) and total superoxide dismutase (T-SOD) test kits were purchased from Wuhan Boshide Biological Technology Company. A549 cells were purchased from American Type Culture Collection. Transforming growth factor-β1 (TGF-β1) recombinant was obtained from Peprotech EC. Rabbit polyclonal anti-TGF-β1,α-SMA and E-cadherin antibody was obtained from Abcam (Cambridge, UK). ECL-Kit was purchased from Millipore. SME-CA was prepared as described previously [20].

2.2. Animal experiments

Mice were randomly divided into eight groups (n = 12/group): 1) saline control (Control); 2) saline + SME-CA (SME-CA); 3) bleomycin model (BLM); 4) bleomycin + blank emulsion (BLM + emulsion); 5)