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Luteolin Ameliorates Experimental Lung Fibrosis Both *in Vivo* and *in Vitro*: Implications for Therapy of Lung Fibrosis

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Lonicera japonica (*Caprifoliaceae*) has been known as an anti-inflammatory herb in traditional Chinese medicine for thousands of years and is used constantly for upper respiratory tract infections. Luteolin, an active flavonoid compound isolated from *Lonicera japonica*, has a spectrum of biological activities, especially with antioxidative and anti-inflammatory properties. However, whether luteolin has a direct inhibitory effect on lung fibrosis has not been established. In this study, we examined the effects of luteolin on lung fibrosis both *in vivo* and *in vitro*. We found that oral administration of luteolin (10 mg/kg) efficiently suppressed the neutrophil infiltration as well as TNF-α and IL-6 elevation in the bronchoalveolar lavage fluid in bleomycin-instilled C57BL/6J mice. Luteolin also alleviated collagen deposition, TGF-β1 expression, and lung fibrosis upon bleomycin instillation. A similar tendency was observed in both early and delayed luteolin-treated groups. Next, our *in vitro* studies showed that luteolin inhibited TGF-β1-induced α-SMA, type I collagen, and vimentin expression in primary cultured mouse lung fibroblasts. Moreover, luteolin significantly blocked TGF-β1-mediated epithelial marker (E-cadherin) downregulation and mesenchymal cell markers (fibronectin and vimentin) upregulation, as well as retaining epithelial morphology in human alveolar epithelial-derived A549 cells. Additionally, luteolin could attenuate TGF-β1-induced Smad3 phosphorylation in both lung fibroblasts and A549 cells. These findings suggest that luteolin has a potent antifibrotic activity; this effect was mediated, at least in part, by inhibition of lung inflammation and suppression of myofibroblast differentiation as well as epithelial-to-mesenchymal transition.

KEYWORDS: Luteolin; bleomycin; lung fibrosis; TGF-β1; epithelial-to-mesenchymal transition

INTRODUCTION

Interstitial lung disease (ILD) is a broad category of lung diseases that includes more than 130 disorders which are characterized by fibrosis and inflammation of the lungs. Much work has been done in an attempt to explain the etiology of ILDs, such as occupational/environmental factors, drugs, hypersensitivity reactions and infections (*1*). Although ILDs can develop from a variety of sources, the disease processes, including cell repair, immune responses, fibroblast proliferation and increased secretion of collagen and extracellular matrix, all share the same characteristics with formation of lung fibrosis (*2*). Previous studies reported that several factors play important roles in the development of fibrosis including profibrotic cytokines, chemokines, eicosanoids, fibrinolytic/fibrinogenic factors, oxidative stress, matrix metalloproteinases and their inhibitors (*2*). Among them, transforming growth factor beta 1 (TGF-β1), a potent profibrotic cytokine, is one of the central mediators which induce the phenotypic modulation of fibroblasts to myofibroblasts, which have the potential to play important roles in the pathogenesis of pulmonary fibrosis and to contribute to the recruitment of inflammatory cells (*3*). Histological evaluation of lung tissue further shows evidence of inflammation and disorder of lung mesenchymal cells in patients and experimental animals with lung fibrosis (*4*, *5*). Moreover, a number of studies on the pneumonia/lung fibrosis mechanism have documented that inflammatory cells such as macrophages, lymphocytes and neutrophils play a key role in secretion of a variety of cytokines and growth factors that regulate proliferation, chemotactism and secretory activity of fibroblasts (*6*). These observations suggested that the inflammatory process results in lung injury and lung fibrosis and emphasized that effective therapies for these disorders must be given early in the natural history of the disease, prior to the development of extensive lung destruction and fibrosis. The drug therapies, such as steroids, immunosuppressor, and antifibrosis drug (colchicine), that are currently available for ILDs can have serious side effects and often are not effective. Hence, new drugs

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