Monitoring the Effects of Ligustilide on Mice with Idiopathic Pulmonary Fibrosis and Determining the Underlying Mechanism

Jin Wang*

Department of Physiology, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China

Abstract

Idiopathic pulmonary fbrosis is a chronic interstitial lung condition that currently lacks viable treatment options. One of angelica's key bioactive ingredients is ligustilide (LIG). The current study's objectives were to investigate the underlying mechanism and monitor the impact of LIG on mice with advanced pulmonary fbrosis lung fbrosis. After a single BLM instillation of 14 days, the mice received daily LIG treatment for 2 weeks. Then the efect of LIG on lung fbrosis was observed then. The impact of LIG on pulmonary fbrosis was assessed using the pulmonary function test, Hematoxylin-Eosin (H&E) and Masson's trichrome staining, immunofuorescence, and Western blot. Following in vitro therapy with transforming growth factor 1 (TGF-1), we looked into the underlying mechanism.

***Corresponding author:** Jin Wang, Department of Physiology, School of Medicine,

D : Chengdu herbpurify, Co., Ltd. was the seller of LIG (HPLC > 98%). Six groups of eight mice each were created using a random number generator: sham, BLM, BLM plus PFD (300 mg/kg), BLM plus 10 mg/kg of LIG, BLM plus 30 mg/kg of LIG, and BLM plus 90 mg/kg of LIG. In all groups save the sham group, the mice received 100 L of intratracheal bleomycin (2 mg/kg) following anaesthesia with pentobarbital sodium (1%, 50 mg/kg). e equal amount of saline was administered to the mice in the sham group. Mice received continuous treatment with LIG, PFD, or 0.5% CMC-Na solution for 14 days following BLM injection. Every day, the mice's body weight was recorded.

e mice were killed with pentobarbital sodium following a continuous 14-day treatment period in order to perform the pulmonary function tests as previously mentioned. e trachea of the mice was then exposed a er they had been put to death. A tracheal catheter had been implanted and secured to the trachea. e Forced Manoeuvres System was then used to examine the IC (Inspiratory Capacity, Volume Inspired During Slow Inspiration), ERV (Expiratory Reserve Volume), FVC (Forced Vital Capacity, Volume Exhausted During Fast Expiration), and TLC (Total Lung Capacity, FRC+IC) parameters (EMMS, Hants, UK). Each mouse was used three times for measurements. e last step was killing the mice and collecting lung samples for future investigation. Following their removal, the lung samples were immediately xed in 4% paraformaldehyde, cryoprotected, and then cut into 8-mm frozen sections using a freezing microtome. e sections were then stained as directed with Masson's trichrome (Biyun Tian, China) and hematoxylin-eosin to assess for lung damage. e pictures were seen using a microscope.

ScienCell was paid for the mouse lung broblast cell line (MLG). Fetal bovine serum (10%), penicillin-streptomycin solution, and Dulbecco's Modi ed Eagle Medium were added to the medium during cell culture, which took place at 37°C in an incubator with 5% CO2. Every two days the medium would be changed. Cells were treated to recombinant mouse TGF-1 to mimic brosis conditions in a test tube (Kingsley Biotechnology, China). Cells were treated with LIG (3, 10, 30 M) following 80-90% con uency, then TGF-1 was given for 24 hours. Cells were then utilised in a number of subsequent research.

R

LIG BLM- :

First, the toxicity of LIG was assessed to assure pharmacological safety. H&E staining revealed that no obvious lung histological damages were seen in either the Con- or LIG-treated animals, as shown in Supplementary Figure 2. Mice were stimulated with BLM (2 mg/kg) and then given the therapy depicted in gure to test the hypothesis that treatment with LIG at the late stage of pulmonary brosis reduces the e ects of BLM-induced lung damage. e LIG treatment groups (10, 30 or 90 mg/kg, for example), the PFD group, the vehicle group, and the sham group were the six treatment groups that were created. LIG therapy given two weeks a er BLM infusion enhanced the survival rate of mice that had received BLM. In addition, LIG kept mice's body weight stable in comparison to the vehicle group. Measurements of lung function revealed that LIG enhanced pulmonary function in comparison to the vehicle group (Figure 3).

LIG reduced BLM-induced widespread alveolar collapse and wall thickening in the lung tissue, according to Hematoxylin-Eosin (H&E) staining. Additionally, LIG decreased collagen accumulation in the lungs of mice treated with BLM, according to Masson's trichrome staining. e ndings demonstrated that LIG increased mice's survival rates and lung function a er receiving BLM infusions and protected against BLM-induced lung damage, suggesting that LIG may be a potential medication for the treatment of pulmonary brosis.

LIG BLM:

Extracellular matrix is deposited together with the development of pulmonary brosis, hence the impact of LIG on this process was assessed. LIG reduced the deposition of collagen I and -SMA as compared to the vehicle group. Additionally, a Western blot demonstrated how LIG

treatment, as seen in. To gauge the ability of the treated cells to migrate, a 2D scratch test was used. LIG prevented cell migration in response to TGF-1. We investigated if LIG therapy may prevent broblast proliferation following TGF-1 injection because cell proliferation is necessary for broblast activation. e LIG-treated group's broblast proliferation was reduced according to the data. e protective e ect of LIG on pulmonary brosis may be connected to the reduction of broblast activation following TGF-1 exposure, according to our research, which indicated that LIG decreased TGF-1 induced broblast activation.

LIG' N 2 ROS :

We examined whether LIG's e ect on broblast activation involves the antioxidant action of LIG in light of the possibility that oxidative stress is the cause of broblast activation. We anticipated the route associated to oxidative stress triggered by TGF-1 based on a combined investigation of online public databases and literature publications. According to the molecular docking results, LIG ts reasonably into the cavity of the Keap1 protein, and their strong connection is indicated by their binding energy of -6.7 kJ/mol. However, Nrf2 was insu cient to engage with LIG (data not shown). e Nrf2 pathway, which plays a key role in transcription under oxidative stress, was studied, along with the expression of its target antioxidant genes, NQO1 and HO-1. As demonstrated, upon TGF-1 exposure, LIG increased the protein levels of Nrf2, HO-1, and NQO1. e impact of LIG on ROS synthesis was then studied. According to data, LIG decreased ROS generation in broblasts exposed to TGF-1. LIG's impact on the generation of ROS was diminished by the Nrf2 inhibitor ML385, indicating that LIG's impact on oxidative stress may be in uenced by the control of the Nrf2 pathway.

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In this study, we discovered that LIG demonstrated survival rate and lung function following BLM infusion for two weeks, and it also provided protection against lung injury and collagen deposition. LIG also decreased TGF-1-induced ROS formation, broblast activation, including cell migration, proliferation, and collagen I protein levels, as well as activation of the Nrf2 pathway. us, LIG stimulated the Nrf2 pathway to lessen the oxidative stress response, which helped to diminish the activation of myo broblasts in pulmonary brosis.

Pulmonary brosis is characterized by the di erentiation of broblasts to myo broblasts followed by excessive ECM deposition. A er pulmonary brosis, respiratory function is substantially compromised, as evidenced by a dry cough and growing dyspnea. As the illness and lung damage worsen, patients' respiratory function continues to decline. Idiopathic pulmonary brosis is becoming more common and has a higher death rate each year, however there are currently no viable treatments available. Finding novel medications to treat pulmonary brosis is so crucial. BLM is a chemotherapy drug, one of which side e ects is to lead pulmonary brosis [23]. As a result, BLM is a frequently utilised inducer for the development of pulmonary brosis in animal models. Being a pro brotic cytokine, TGF-1 was shown to be much higher following BLM therapy, indicating that it plays a role in the pathophysiology of the pulmonary brosis brought on by BLM [24]. Numerous cells, including alveolar macrophages, broblasts, and activated alveolar epithelial cells, generate TGF-1 in the lungs [11,25]. According to research, preventing TGF-1-induced broblast activation may lessen lung brosis.

C

Numerous studies have demonstrated the physiological e ects of natural bioactive compounds, which are found in a broad variety of plants, animals, marine creatures, and microbes. ese chemicals also have anti-in ammatory, anti-cancer, antioxidant, and other physiological e ects. e primary active ingredient of the volatile oil of the Chinese umbrella plant Angelica sinensis is LIG, commonly referred to as Angelica phthalide. According to reports, LIG has a wide range of pharmacological properties, including antioxidant and anti-in ammatory properties, and it also acts as a preventative against cardiovascular disease. For instance, LIG reduced in ammatory pain

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