Atomized Mutant Soluble Ectodomain of FGFR2IIIc Inhalation Treatment of Bleomycin-induced Pulmonary Fibrosis in Mice

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Abstract

We sought to determine the effect of atomized extracellular segment of FGFR2IIIc(msFGFR2c) inhalation treatment of pulmonary fibrosis in C57/BL6 mice treated with bleomycin. Sixteen female C57/BL6 mice were randomly divided into four groups: healthy control group, bleomycin model group, msFGFR2c control group, and msFGFR2c treatment group. Mice inhaled FGFR2IIIc extracellular segment proteins daily. After 14 days, all mice were sacrificed, and body weight changes and lung histopathological changes were observed. The results showed that inhaled nebulized FGFR2IIIc extracellular protein alleviated pulmonary fibrosis compared with untreated group in mice.

Keywords

Pulmonary fibrosis; Bleomycin; Mouse; FGFR2.

1. Introduction

In recent years, air pollution and other environmental factors have exacerbated the incidence of lung diseases such as lung cancer and pulmonary fibrosis in China. The situation is getting worse. Idiopathic pulmonary fibrosis (IPF) is a chronic, interstitial lung disease of unknown etiology. Its pathological features are mainly alveolar epithelial damage, inflammatory cell infiltration, alveolar wall thickening, abnormal accumulation of fibrotic tissue in the lungs. Clinical symptoms manifested as difficulty breathing eventually leading to respiratory failure, patients with high incidence and poor prognosis [1]. Although the pathogenesis of idiopathic pulmonary fibrosis (IPF) has not yet been fully clarified, past studies have reached an important milestone. Current studies have shown that repetitive micro-injury of the alveolar epithelium leads to persistently inflammatory responses that are the first drivers of pulmonary fibrosis, which subsequently lead to the activation and proliferation of lung fibroblasts, epithelial cells, and the conversion of fibroblasts to myofibroblasts. ECM deposition eventually leads to the formation of pulmonary fibrosis scars [2]. A number of pathological studies have shown that environmental factors are related to the pathogenesis of idiopathic pulmonary fibrosis (IPF). Smoking or contact with metals, wood, silicon, and stone dust are the causes of the disease. In addition, the survival rate of IPF patients with a history of smoking is lower than that of non-smokers [3]. Overall, IPF is a progressive pulmonary interstitial fibrosis disease induced by multiple factors.

The human respiratory tract has a huge surface area and administration through the respiratory tract can bypass the first metabolic pathway of the liver. In addition, the thickness of the respiratory epithelial barrier is thin, there are plenty of capillaries, proteolytic activity is low, and there is a thinner mucus layer compared to the gastrointestinal tract, making the airway suitable for systemic or local drug delivery [4]. FGF-2, also known as basic fibroblast growth factor (bFGF), acts to promote fibroblast proliferation and collagen synthesis in idiopathic pulmonary fibrosis (IPF) [5]. The administration of protein or peptide drugs through the lungs is also an important development direction for the treatment of major pulmonary diseases, and has great clinical application prospects. Our previous research have shown that mutant soluble extracellular segment msFGFR2c can bind to FGF2 with high affinity, thereby inhibiting FGF signaling and inhibiting the development of

pulmonary fibrosis[6]. This study evaluated the effect of aerosolized administration of extracellular domain of msFGFR2c on pulmonary fibrosis in mice.

2. Experimental Detail

2.1 Establishment of Pulmonary Fibrosis Mouse Model

Sixty- to Eight-week-old female C57/BL6 mouse(18-20 g) were purchased from Southern Medical University Experimental Animal Center. Each group of C57/BL6 mice was surgically modeled. The rats were anesthetized with isoflurane (500-700 ml/min), and 0.2 ml of bleomycin (BLM) diluted in physiological saline was drawn with an lml syringe. The syringe pierces the trachea through the two cartilage cartilage ring gaps towards the heart. After the rat was upright and continued to pierce the needle approximately 1.0-1.5 cm toward the heart, the air was drawn back without resistance, and the drug solution was injected into the trachea (3 mg/kg). The animals were rotated to distribute the drug solution uniformly in the lungs. Suture the wound. In the control group, the same volume of physiological saline was injected into the trachea under the same conditions.

2.2 Experiment Grouping

We randomly divided 16 C57/BL6 mice into four groups: healthy control group (A), msFGFR2c control group (B), bleomycin model group (C), and msFGFR2c treatment group (D). Four mice each. Groups A and B were normal healthy mice, and mice in groups C and D were all induced bleomycin-induced pulmonary fibrosis.

2.3 Msfgfr2c Treatment

After 24 hours of injection of bleomycin or saline, mice of groups B and D were given inhaled msFGFR2c extracellular protein. Inhaled physiological saline was used in groups A and C. Each solution (5 mL) was charged into a nebulizer (China YUWELL) according to the manufacturer's instructions. The atomization rate of the atomizer was 0.2 ml/min. The mice were inhaled with an extracellular solution of msFGFR2c (1 mg/ml) or an equal volume of normal saline in a 5 L container once a day for 30 min, and each consumed 6 ml of the drug solution. When aerosolized, the mice were placed in the container and kept free to breathe and avoid external interference to maintain room temperature stability.

2.4 Sample Collection and Staining

After 14 days of administration, all mice were sacrificed. The mouse lung tissue was fixed in 4% paraformaldehyde, and pathological sections were made to evaluate the lung lesions.

H & E staining: HE staining, so that the structure of the tissue cells to facilitate observation. Hematoxylin (H) is an alkaline dye that dyes the nuclei and stains a blue-purple. Eosin (E) is an acid dye that stains the cytoplasm red or light red.

Dewaxing was performed before tissue staining. The staining procedure is as follows:

1) Immerse distilled water into slices of hematoxylin for 5 min.

2) Separation in acid water and ammonia water, fast.

3) Rinse with water for 1 hour and rinse with distilled water.

4) Dehydrate in 70% and 90% alcohol for 10 min each.

5) 5 into 0.5% alcohol eosin staining solution staining 2-3min.

6) Dehydration and dehydration: slices were dehydrated with 100% alcohol and soaked in xylene to make slices transparent.

7) Mounting: Apply neutral gel to transparent sections and cover with coverslips for mounting. After the gum has dried slightly, label it and observe it under a microscope.

Masson staining:

1. Tissues were fixed in 4% paraformaldehyde solution and dehydrated by routine dehydration; sections were deparaffinized to distilled water.

2. Weiger's iron hematoxylin dyeing 5-10min; running water slightly washed.

3.1% hydrochloric acid alcohol differentiation; rinse for a few minutes.

4. Ponceau Acid Fuchsin stain for 5-10min; rinse with distilled water.

5.1% phosphomolybdic acid aqueous solution was treated for about 5 minutes.

6. Do not wash with water and counterstain with aniline blue or green solution for 5 min.

7.1% glacial acetic acid treatment for 1 min.

8.95% alcohol dehydrated many times.

9. Alcohol dehydration, xylene transparent, neutral gum

2.5 Data Analysis

The experimental data were analyzed by SPSS statistical software. The data were expressed as mean ± standard deviation (Mean±SD). Differences between groups were analyzed using t test and analysis of variance. (*P<0.05 was considered statistically significant with significant differences)

3. Results and Discussion

3.1 Msfgfr2c Sample Preparation

According to the method previously studied by our group [7], the inclusion body of msFGFR2c expressed in prokaryotic fermentation was denatured, and the protein was recovered after high-salt gradient elution. High-salt gradient elution results in column refolded proteins. After dialysis for 12 hours, the renatured protein is taken out, the precipitate is removed by centrifugation. The target protein is further purified by heparin affinity chromatography to collect the target protein. By SDS-PAGE gel electrophoresis, it can be seen that there is a clear high-purity protein band at a molecular weight of about 24 KDa, indicating that the target protein has been successfully purified.

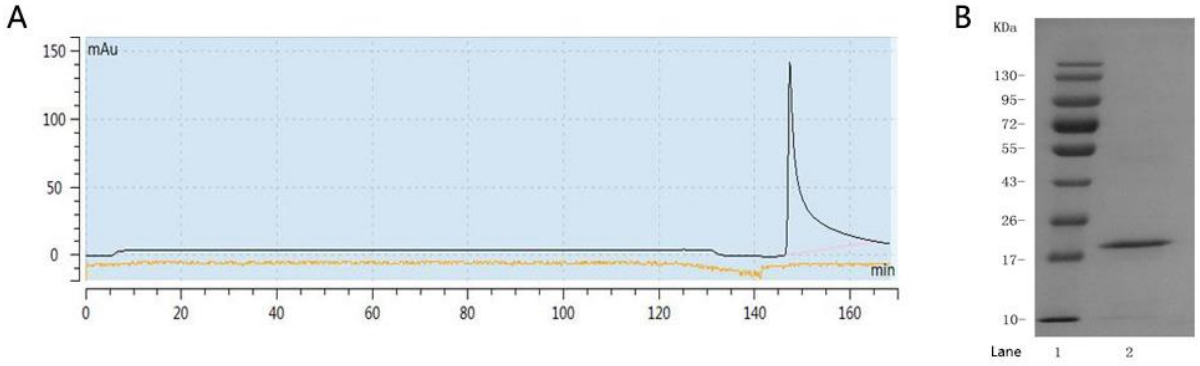

Fig 1. MsFGFR2c protein purification. (A) Purification of msFGFR2c protein and collection of proteins at elution peak (B) SDS-PAGE gel electrophoresis (Lane 1: Marker; Lane 2: target proteins)

3.2 Changes of Body Weight in BLM-Induced Mouse Pulmonary Fibrosis Model

From the first day of bleomycin modelling, mice were weighed every other day. After lung fibrosis mice were modeled with bleomycin (BLM), body weights of mice in groups C and D gradually decreased with time, and weight loss of mice in group C treated with extracellular segment protein of msFGFR2c was slower. In group C and group D, body weight began to increase after 14 days. There was no significant difference in body weight between group A and group B.

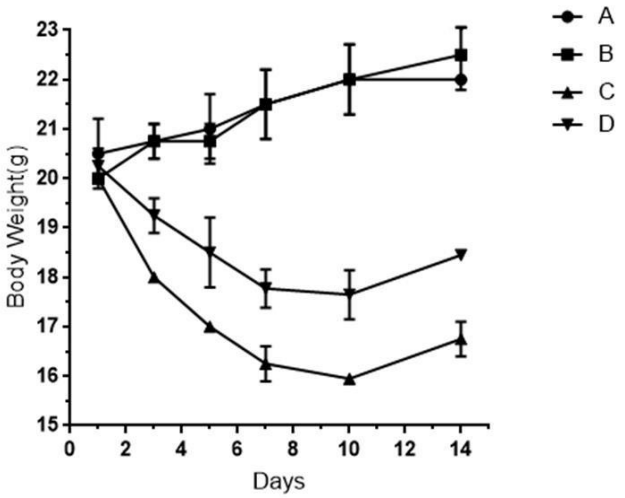

Fig 2. Weight change in mice. Group A is the healthy control group. Group B was healthy msFGFR2c control group. Group C are mouse with pulmonary fibrosis and no treatment. Group D is the msFGFR2c treatment group.

3.3 Msfgfr2c Extracellular Protein Alleviates Pathological Features of Pulmonary Fibrosis in Mice

3.3.1 Observation of Mouse Lung Tissue HE Staining

Pulmonary tissue sections of group A and group B showed clear alveolar structures and normal lung function. C group of mouse lung tissue sections showed extensive alveolar interstitial thickening, the formation of a substantial organization, so that the alveolar volume decreased, affecting gas exchange, alveolar septum widening and fibrosis, a large number of fibroblasts proliferation, alveolar atrophy, some alveolar the alveolar epithelial cells are fused into large vesicles with a large number of inflammatory cells infiltrating to form extensive and distinct fibrosis, which is a severe fibrosis symptoms. D group of mouse lung tissue sections showed alveolar wall thickening, a small amount of alveolar structure collapse, alveolar septum visible cellulose-like exudate, belonging to moderate fibrosis, indicating that msFGFR2c can alleviate bleomycin-induced pulmonary fibrosis in mice.

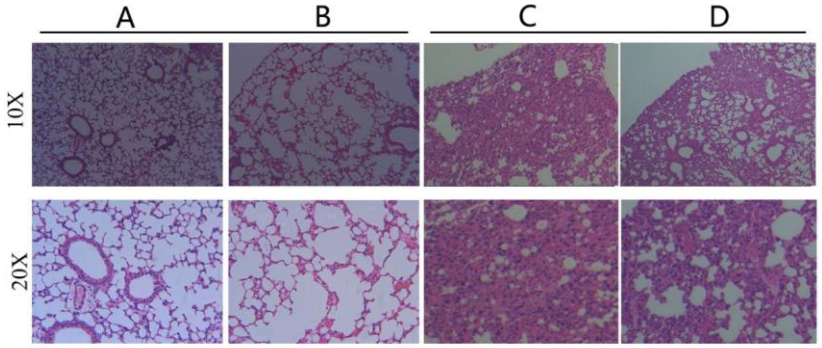

Fig 3. HE staining of mouse lung tissue. Group A is the healthy control group. Group B was healthy msFGFR2c control group. Group C are mouse with pulmonary fibrosis and no treatment. Group D is the msFGFR2c treatment group.

3.3.2 Masson Staining of Lung Tissue in Mice

The collagen fibers stained by the Masson method are blue, the nucleus is black, and the cytoplasm is purple. The Masson staining method can clearly identify the tissue morphology, and has important value in the pathological diagnosis, differential diagnosis and research work of many diseases. The lung tissue sections of mice in groups A and B showed clear structure of alveoli, no collagen deposition in alveolar space, and no abnormal lesions. The lung tissue sections of mice in group C

showed perivascular, bronchial basement membranes with a large amount of collagen fibers deposited, and the alveolar space was occupied by collagen fibers and fibroblasts. The lung tissue sections of group D showed decreased alveolar septum and collagen fibers deposition in the bronchial basement membrane, and the symptoms of fibrosis were alleviated.

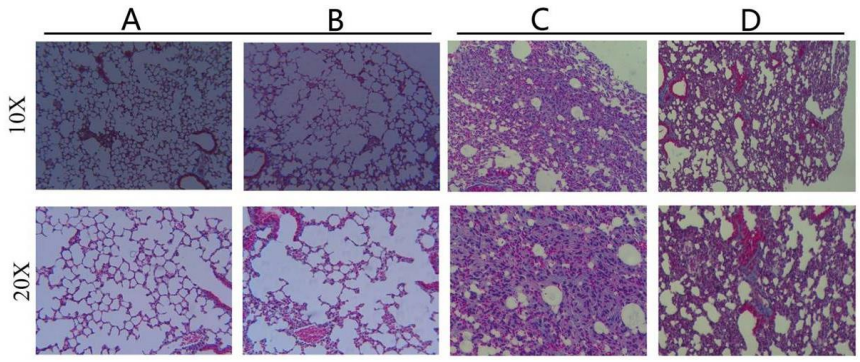

Fig 4. Masson staining of mouse lung tissue. Group A is the healthy control group. Group B was healthy msFGFR2c control group. Group C are mouse with pulmonary fibrosis and no treatment. Group D is the msFGFR2c treatment group.

4. Conclusion

The natural presence of proteins in living organisms makes them a drug with high therapeutic potential for a variety of diseases. The main method of administration of peptide drugs is injection, which brings great pain to patients. However, oral peptide drugs are easily digested by gastrointestinal fluid to make protein biological activity inactive. Secondly, the first pass effect of liver is also one of the difficulties. The absorption of polypeptides through the mucosa of the respiratory tract is an emerging treatment strategy. In this study, we used msFGFR2c protein to treat mice with pulmonary fibrosis by aerosolization. The results showed that msFGFR2c protein can alleviate the symptoms of pulmonary fibrosis in mice. It is a potential drug for the treatment of pulmonary fibrosis. Next we will continue to study the better dosage form and administration of the msFGFR2c protein in the treatment of pulmonary fibrosis. In order to promote the clinical application of protein drugs, the new strategy for treating pulmonary fibrosis lays a solid foundation.

References

[1] Raghu G, Collard H R, Egan J J, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management[J]. Am J Respir Crit Care Med, 2011, 183(6): 788-824.

[2] Sgalla G, Iovene B, Calvello M, et al. Idiopathic pulmonary fibrosis: pathogenesis and management[J]. Respir Res, 2018, 19(1): 32.

[3] Spira A, Beane J, Shah V, et al. Effects of cigarette smoke on the human airway epithelial cell transcriptome[J]. Proc Natl Acad Sci U S A, 2004, 101(27): 10143-8.

[4] Agu R U, Ugwoke M I, Armand M, et al. The lung as a route for systemic delivery of therapeutic proteins and peptides[J]. Respiratory research, 2001, 2(4): 198.

[5] Thannickal V J, Aldweib K D, Rajan T, et al. Upregulated expression of fibroblast growth factor (FGF) receptors by transforming growth factor-β1 (TGF-β1) mediates enhanced mitogenic responses to FGFs in cultured human lung fibroblasts[J]. Biochemical and biophysical research communications, 1998, 251(2): 437-441.

[6] Ju W, Zhihong Y, Zhiyou Z, et al. Inhibition of α-SMA by the ectodomain of FGFR2c attenuates lung fibrosis[J]. Molecular Medicine, 2012, 18(1): 992.

[7] Liu X T, Zhi-Hong Y U, Shui-Lian H E, et al. Expression,Refolding,and Activity Studies of the FGFR2IIIc Soluble Ectodomain Mutant P253R[J]. Pharmaceutical Biotechnology, 2011.