Effect of thalidomide on the alveolar epithelial cells in the lung fibrosis induced by bleomycin in mice

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Abstract

Introduction: Idiopathic pulmonary fibrosis (IPF) is a relentlessly progressive and usually fatal lung disease of unknown etiology for which no effective treatments currently exist. In the adults type I and II pneumocytes, forms Components of the alveolar epithelial cells. In this study, we investigated the effect of thalidomide on the alveolar epithelial cells (type I and II pneumocytes) in the lung fibrosis induced by bleomycin in mice.
Materials and methods: In this research, 32 male adult C57BL/6 mice were randomly divided into 4 groups. Mice received in group Bleomycin, bleomycin sulfate, in group Bleomycin+Thalidomide, bleomycin besides thalidomide, in group Thalidomide, only thalidomide and in group carboxymethylcellulose, carboxymethylcellulose via intraperitoneum. At the end of experiment, mice lung samples were prepared and histological studies were performed on them. After the investigation of tissue slides, number of type I and II pneumocytes were calculated and results were analyzed by using one-way ANOVA.
Results: Histological studies showed a significant decrease in the number of type I pneumocytes and a significant increase in the number of type II pneumocytes in the Bleomycin group in comparison with the carboxymethylcellulose group ($p<0.001$). But number of these cells (type I and II pneumocyte) in the Bleomycin+Thalidomide group increased and decreased respectively compared to the Bleomycin group ($p<0.001$).
Conclusion: The results of this study showed that Thalidomide decreases number of pneumocytes II and increases pneumocytes I and Thereby reduces pulmonary fibrosis in the mice.

Keywords: Bleomycin, Lung fibrosis, Type I and II Pneumocyte, Thalidomide

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive and often lethal lung disorder. The histopathological features of IPF are usual interstitial pneumonia, which consists of honeycombing, patchy fibrosis, lung epithelial cell dysfunction, lung fibroblast activation and proliferation, fibroblastic foci and hyperplasia of type II pneumocytes, excessive collagen deposition and subsequent destruction of the normal lung architecture with loss of alveolar spaces (1,2). IPF begins with repeated injury to the lining of the alveoli, which type II alveolar epithelial cells (AEC II) undergo hyperplasia and become suppliers of fibrogenic cytokines that induce the proliferation and migration of lung fibroblasts and eventually leads to extensive remodeling of the distal airspace of lung, stiffening it and making breathing

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difficult. The prognosis for IPF patients is poor and current therapies are ineffective in preventing or even delaying the onset of respiratory failure. Novel therapeutic approaches include molecular targeting of specific signaling pathways activated during fibrotic processes (3,4). Thalidomide, a derivative of glutamic acid, was synthesised originally as a sedative and an anti-emetic. Subsequently the drug was withdrawn because of its teratogenic effects (5). Thalidomide was found effective first in erythema nodusum leprosum lesions (6), and later in rheumatological diseases (7), Crohn's disease (8), tuberculosis (9), cutaneous sarcoidosis (10) and lung transplantation (11). In addition, the ability of thalidomide to inhibit angiogenesis has led to its use in malignancy, particularly multiple myeloma (12). Previous studies have shown that thalidomide can inhibit the production of inflammatory cytokines such as TNF-α, IL-12 from peripheral blood mononuclear cells (PBMCs) and alveolar macrophages (Ams) (13–16) and IL-6 and TGF-β from lung fibroblasts (17). However, the mechanisms underlying the effects of thalidomide in interstitial lung disease are still unclear. In this study, we investigated the effect of thalidomide on the alveolar epithelial cell in the lung fibrosis induced by bleomycin in mice by morphometric theqniq and have shown that thalidomide has an antifibrotic effect in the lung fibrosis by Reduction in type II alveolar epithelial cells and increase in type I alveolar epithelial cells.

Materials and methods

Ethical approval: Thirty two male adult C57BL/6 mice were used in this study. The study was carried out in strict accordance to guidelines of the 1975 Declaration of Helsinki.

Model of pulmonary fibrosis: Male C57BL/6 mice were injected intraperitoneally with bleomycin sulfate (2 mg/ mouse).

Treatment groups: Thirty two healthy male C57BL/6 mice weighting 20-25 g on average at the age of 6-8 weeks were randomly assigned into bleomycin (Bleo), bleomycin plus thalidomide (Bleo + Thal), thalidomide (Thal) and carboxymethylcellulose (CMC) groups (n=8 per group). Pulmonary fibrosis models were established in groups Bleo and Bleo + Thal by the intraperitoneal injection of 2 mg/mouce bleomycin sulfate (Nippon Kayaku, Japan) dissolved in 0.1 ml carboxymethylcellulose(CMC) (Sigma, USA) 0.5% in days 1, 8 and 15. Group Bleo + Thal received bleomycin as was mentioned for the Bleo group in addition 4 mg thalidomide (Sigma, USA) dissolved in 0.1 ml CMC 0.5% via intraperitoneal injections five times a week for 4 weeks. Group Thal received 4 mg thalidomide dissolved in 0.1 ml CMC 0.5% via intraperitoneal injections five times a week for 4 weeks, and group CMC received 0.1 ml CMC 0.5% via intraperitoneal injections five times a week for 4 weeks (17,18). When the experiment finished, the mice were killed by high doses of ketamine anesthetic. Thorax was opened and lungs were removed, after being washed with PBS, the lungs were placed in formalin 10%. The tissues were dehydrated at different levels and were embedded in paraffin.

H&E staining: After molding, transverse sections of 6 µm thickness were prepared. Haematoxylin and eosin staining agents were purchased from Sigma-Aldrich. The prepared sections were stained with haematoxylin and eosin following the manufacturer's standard protocol for morphological analysis. The slides were then investigated under a light microscope .

PAS staining: Periodic acid schiff (PAS) is a staining method used to detect polysaccharides such as glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues. The number of type I and II pneumocyte in this staining was detectable and ratio of them was calculated. In this method, for each

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group, 10 fields of 10 sections were examined with 40X magnification .

Statistical analysis

The results were analyzed in SPSS, version 20 were presented as the mean ± standard error. The groups were compared using one-way ANOVA. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Pathological changes: Four weeks after bleomycin instillation, in the Bleo group, there was pathological changes including thickening of alveolar walls, infiltration of inflammatory cells into the interstitium, disorganization of the alveolar structure, impairment of alveolar septa, development of cystic changes in alveoli, and increased amount of connective tissue (Figure 1A). The number of cuboidal type II pnoumocyte increased which project into the lumen often occupying a niche in the corner of the alveoli and was shown by PAS staining (Figure 2A). In the group Bleo + Thal that received thalidomide besides bleomycin, compared to the Bleo group, The severity of changes diminished such as relative reduction in the inflammatory reaction, decreased thickness of alveolar septa, better organization of the alveolar structure, and fewer changes consistent with alveolar impairment were observed (Figure 1B), The number of type II pnoumocytes decreased in the PAS staining (Figure 2B). In the Thal and CMC groups No pathological changes were observed in the lungs tissue of animals (Figure 1C, D).

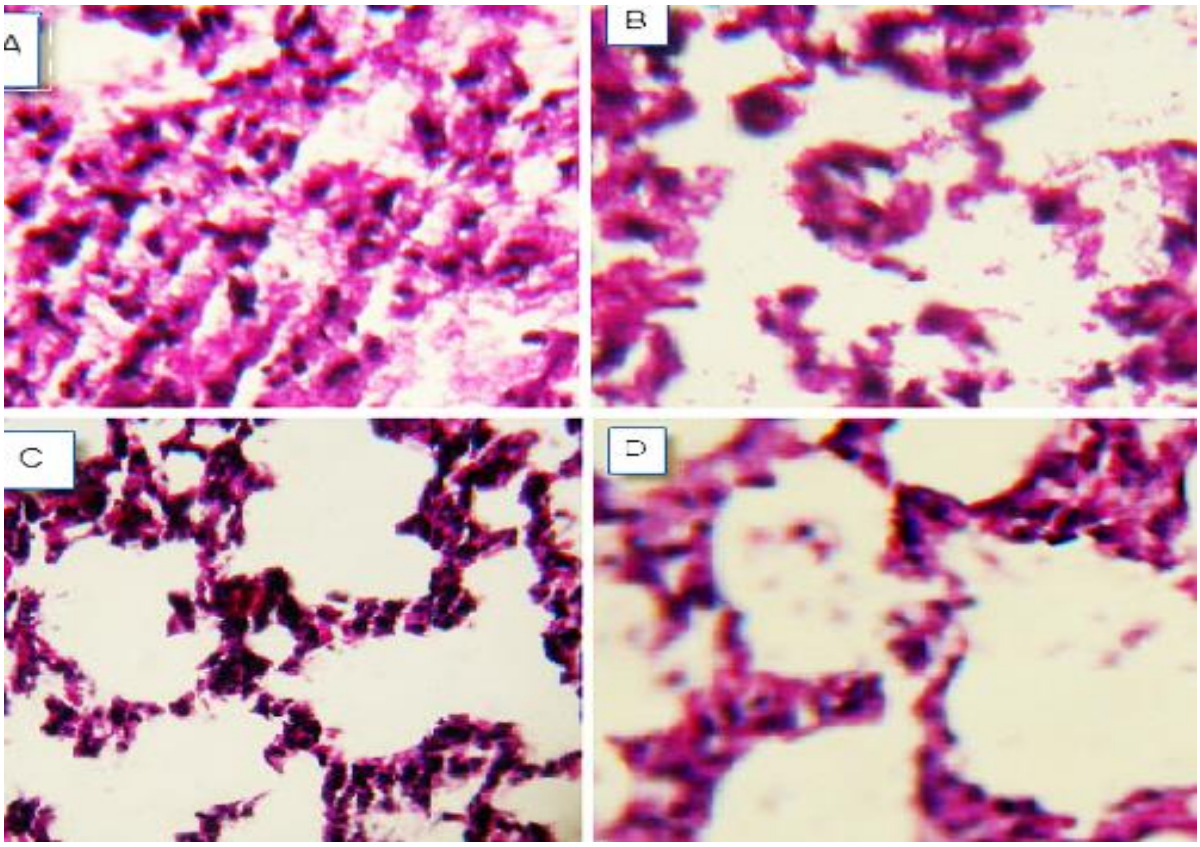


Figure 1. Effect of thalidomide on bleomycin induced lung fibrosis in mouse lung tissue. male adult C57BL/6 mice were injected ip with bleomycin in the Bleo group (A). Mice in the Bleo + Thal group (B) received thalidomide besides bleomycin and in the Thal (C) and CMC (D) groups received thalidomide and carboxymethylcellulose respectively. After 4 weeks histological changes of lung tissue samples were shown by H&E staining.

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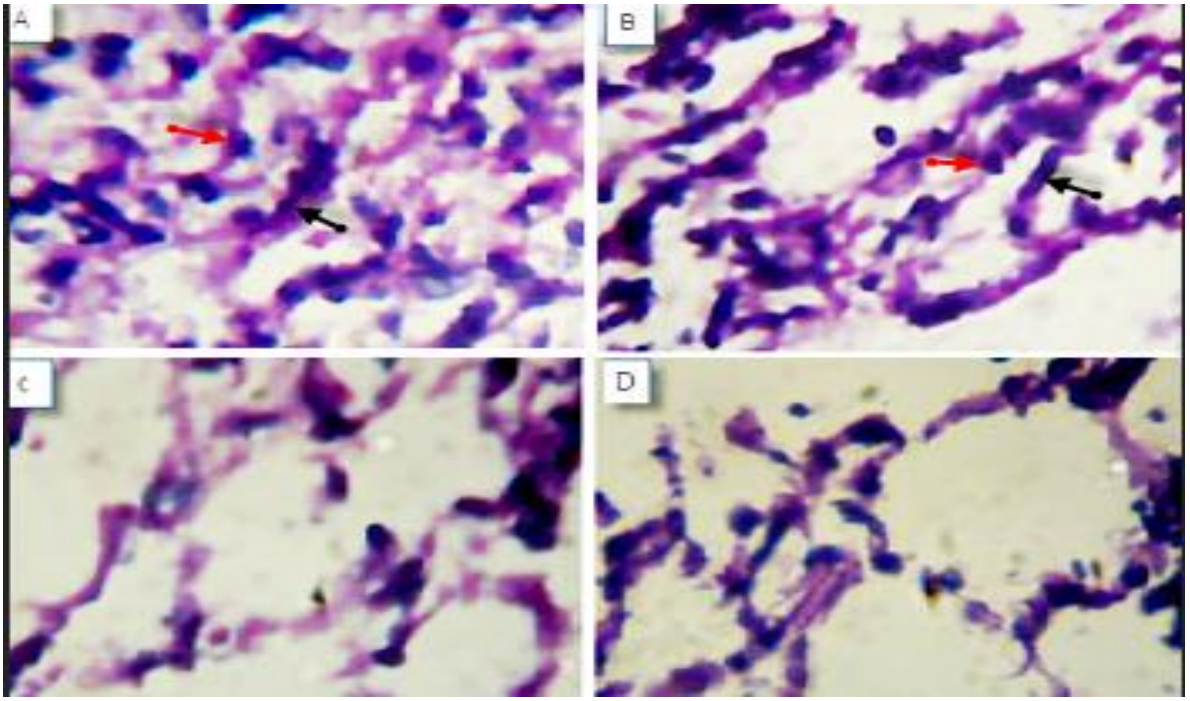


Figure 2. Effect of thalidomide on the alveolar epithelial cells in the lung fibrosis induced by bleomycin in mice. male adult C57BL/6 mice were injected ip with bleomycin in the Bleo group (A). Mice in the Bleo + Thal group (B) received thalidomide besides bleomycin and in the Thal (C) and CMC (D) groups received thalidomide and carboxymethylcellulose respectively. After 4 weeks, PAS staining were done on the lung tissue samples and the the alveolar epithelial cells (I and II pneumocytes) were observed. red arrows indicates the type II pneumocytes and black arrows indicates the type I pneumocytes.

Quantitative results: The quantitative results related to the alveolar epithelial cells (type I and II pnoumocyte) of the mice lung simples in different groups were obtained by morphometric method. This data analysed using SPSS and one-way ANOVA. The mean values of type I pnoumocyte in the Bleo, Bleo + Thal, Thal and CMC groups were 2.83 ± 1.18 , 3.18 ± 0.98 , 12.5 ± 4.85 , 10 ± 1.18 respectively (Figure 3). The mean values of type II pnoumocyte in the Bleo, Bleo + Thal, Thal and CMC groups were 8.30 ± 2.5 , 4.37 ± 1.36 , 3.30 ± 1.25 and 2 ± 2.56 respectively (Figure 4).

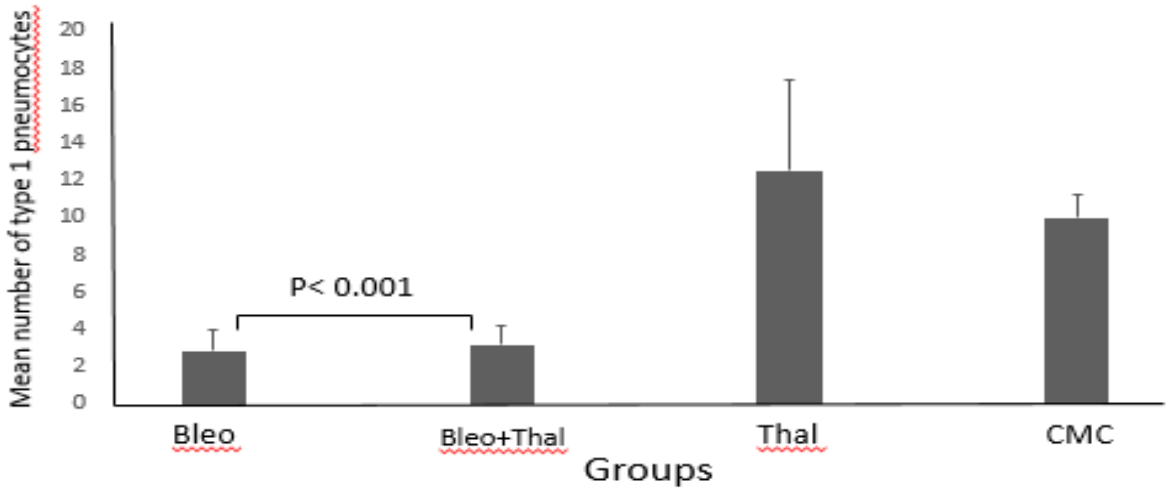


Figure 3. Mean number of type 1 pneumocytes in different groups.

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In this experiment mice recieved in the Bleo, Bleo+Thal, Thal and CMC groups bleomycin, bleomycin plus Thalidomide, Thalidomide and carboxymethylcellulose respectively. At the end of experiment, PAS staining were done on the lung tissue samples and the mean number of type 1 pneumocytes in Different groups was calculated by SPSS and one-way ANOVA.

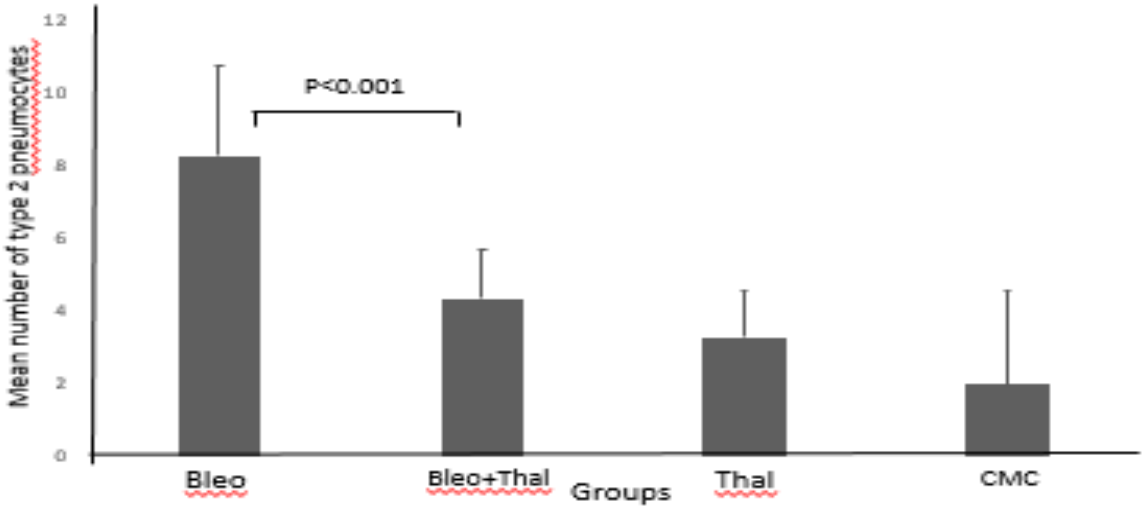


Figure 4. Mean number of type 2 pneumocytes in different groups.

The results indicate an increase in the type I pnoumocyte in the Bleo + Thal group in comparison with the Bleo group. Statistical analysis showed that the groups have significant difference with each other in the mean value of type I and II pnoumocyte (P<0.001).

Discussion

In the current study, the effect of thalidomide on the alveolar epithelial cells (type I and II pnoumocyte) in the lung fibrosis induced by bleomycin was investigated. We demonstrated the relationship between thalidomide and pulmonary fibrosis. IPF is a chronic, progressive, and usually lethal lung disorder of unknown etiology. Although the pathogenic mechanisms have not been elucidated, aberrantly activated alveolar epithelial cells, which produce a variety of mediators during the development of the disease, seem to play a key role (19-21). We treated mice with bleomycin by intraperitoneal administration instead of intratracheal administration, as usually carried out in many reports, because study the effect of the drug when added to the whole body as this administration resembled more clearly the use of the drug in human cancer therapy. Bleomycin is an effective chemotherapeutic agent which its repeated systemic or high dose administration often leads to lung injury and fibrosis (22). Bleomycin-induced pulmonary fibrosis in animal models is very applicable for studying the cellular and molecular mechanisms of interstitial lung fibrosis (23) .

Here, we found that thalidomide could inhibit lung fibrosis by preventing the proliferation of type II pneumocyte and increasing the type I pneumocyte in the alveolar epithelial cells. Researchers have studied the anti-fibrotic effect of thalidomide and reported that thalidomide could relieve the symptoms and stop the progression of lung fibrosis in the human and animal samples. In the study Tabeta and his colleagues in 2007 showed that thalidomide has inhibitory effect on bleomycin-induced pulmonary fibrosis in mice (17). They showed that thalidomide have prevention effect on synthesis of collagen, TGF-β, IL-6, and VEGF which

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these marker have an important role in the lung fibrosis. Zhao and his colleagues in the 2009 demonstrated that thalidomide could be effective in treatment of lung fibrosis via its inhibitory effect on hydroxyproline (HYP) and α-SMA protein (24). Maureen et al. (2012) demonstrated that oral thalidomide in patients with lung fibrosis can considerably alleviate the patients' cough (25). In summary all of the study mentioned above including our experiments demonstrates that thalidomide has an antifibrotic effect in the lung fibrosis. However the molecular mechanism of thalidomide is largely unknown and more research needs to be done .

Conclusion

In summary, we demonstrated that bleomycin induse the lung fibrosis and thalidomide by decrease of type II pneumocyte caused the decrease of lung fibrosis .

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Conflicts of interest

The authors declare no conflict of interest.

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