学 位 論 文 の 要 旨

氏名　　新堀　智子

学 位 論 文 名　The Role of Cysteinyl Leukotrienes in the Pathogenesis of Pulmonary Fibrosis in Mice

発 表 雑 誌 名
（巻，初頁～終頁，年）
著 者 名

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論 文 内 容 の 要 旨

INTRODUCTION

Pulmonary fibrosis is the consequence of pathophysiological responses to injury that are induced by various factors, such as drugs, radiation, infection, and exposure to toxic particles, but the cause of idiopathic pulmonary fibrosis (IPF) is unknown. IPF is a specific form of chronic interstitial pneumonia characterized by progressive fibrosis and destruction of the normal lung architecture, and currently, there is no established therapeutic strategy to prevent the development of pulmonary fibrosis. Although the pathogenesis of pulmonary fibrosis is unknown, cysteinyl leukotrienes (CysLTs) have been implicated in the etiology of pulmonary fibrosis. The CysLTs such as leukotriene (LT) C_4, LTD_4 and LTE_4 are a family of lipid mediators derived from arachidonic acid through the action of 5-lipoxygenase, and exert their effects by activating at least two subtypes of receptors: cysteinyl leukotriene type 1 ($CysLT_1$) receptor and cysteinyl leukotriene type 2 ($CysLT_2$) receptor. CysLTs are known as proinflammatory mediators that promote bronchoconstriction, mucus secretion, tissue edema, and leukocyte infiltration. $CysLT_1$ receptor antagonists have been established as therapeutic agents for asthma. In addition to inflammatory reactions, recent in vitro studies showed that CysLTs act directly on fibroblasts to promote proliferation, differentiation, and collagen synthesis thereof. Furthermore, CysLTs also elicit a wide range of responses by modulating various cytokines and growth factors, some of which are involved in the profibrotic response. These results suggest that CysLTs participate in the pathogenesis of pulmonary fibrosis. However, the precise role of CysLTs in the pathogenesis of pulmonary fibrosis is unclear. In the present study, we elucidated the role of CysLTs in pulmonary fibrosis using two types of murine models: bleomycin-induced pulmonary fibrosis model showing acute but not persistent lung injury, and silica-induced model showing chronic and persistent pulmonary fibrosis.

MATERIALS AND METHODS

We performed the following two studies to reveal contribution of CysLTs in the pathogenesis of pulmonary fibrosis. Firstly, we evaluated the effects of a selective $CysLT_1$ receptor antagonist on the development of bleomycin-induced pulmonary fibrosis in mice during acute phase (study 1: reference 1). Secondly, we investigated the involvement of CysLTs in silica-induced pulmonary fibrosis in mice during both acute and chronic phases (study 2: reference 2).

Study 1: Effect of $CysLT_1$ receptor antagonist on bleomycin-induced pulmonary fibrosis in mice

Pulmonary fibrosis was induced in female C57BL/6J mice (8-week-old) by single intratracheal instillation of bleomycin (2 mg/kg). The bleomycin-instilled mice were divided into 2 experimental groups. One group was orally administered a $CysLT_1$ receptor antagonist, montelukast sodium (10 mg/kg once daily), for 3 days before and 14 days after bleomycin instillation. The other group received vehicle over the same period. Age-matched mice, each of which had a single intratracheal instillation of sterile saline and daily oral administration of vehicle, served as normal controls. After treatment with montelukast or vehicle, the mice were euthanized using a lethal dose of sodium pentobarbital (100 mg/kg), and lung lobes and bronchoalveolar lavage fluid (BALF) were collected for histological and biochemical analyses. We evaluated the extent of pulmonary fibrosis by measuring hydroxyproline content and fibrotic area. The number of inflammatory cells in BALF was counted with a hemocytometer. CysLT content in BALF was measured using enzyme-linked immunosorbent assay (ELISA). We also investigated the expression of various cytokines (TNF-α, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-13, and TGF-β1) and two types of CysLT receptors in lungs by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

Study 2: Involvement of CysLTs in the pathogenesis of silica-induced pulmonary fibrosis in mice

Pulmonary fibrosis was induced in female C57BL/6J mice (8-week-old) by intratracheal instillation of crystalline silica particles (0.1 g/kg). On days 3, 7, 14, 28, and 56 after intratracheal silica instillation, the mice were euthanized using a lethal dose of sodium pentobarbital (100 mg/kg); lung lobes and BALF were collected for histological and biochemical analyses. Age-matched mice, which received saline-instillation, served as normal controls. We evaluated the extent of pulmonary fibrosis, the number of inflammatory cells, and CysLT content as mentioned in study 1. We also investigated the expression of profibrotic cytokines (TNF-α and TGF-β1) and two types of CysLT receptors by quantitative RT-PCR and immunohistochemical analyses.

RESULTS AND DISCUSSION

First, we investigated the involvement of CysLTs using acute pulmonary fibrosis model, bleomycin-induced pulmonary fibrosis mice. Hydroxyproline content in lung tissues was significantly increased by bleomycin instillation with the concurrent increase of CysLT content in BALF. Treatment with a $CysLT_1$ receptor antagonist, montelukast, for 2 weeks significantly attenuated the development of pulmonary fibrosis. Montelukast treatment also decreased mRNA levels of profibrotic cytokines such as IL-6, IL-13, and TGF-β1, all of which were elevated in fibrotic lungs. Furthermore, $CysLT_1$ receptor mRNA levels were increased, whereas $CysLT_2$ receptor mRNA levels were decreased in fibrotic lungs,

and montelukast treatment induced the recovery of $CysLT_2$ receptor mRNA levels to normal control levels but no change from the elevated levels of $CysLT_1$ receptor mRNA. These results suggest that montelukast exhibits its beneficial effects by inhibiting the overexpression of profibrotic cytokines and by modulating the homeostatic balance between the $CysLT_1$ and $CysLT_2$ receptors. $CysLT_1$ receptor-mediated effects of CysLTs may have major role in the pathogenesis of bleomycin-induced pulmonary fibrosis. However, several important points should be noted regarding models of pulmonary fibrosis in rodents. The bleomycin model is the most common and best-characterized pulmonary fibrosis model, and thus very useful for assessing fibrotic responses at the acute stage and for exploring the triggering mechanism that induces pulmonary fibrosis. Nevertheless, several reports showed that the fibrotic response to bleomycin reached its peak around 3 to 4 weeks; thereafter the resolution of fibrosis began and got completed as early as 6 weeks. Therefore, we considered that alternative models showing chronic and irreversible fibrosis are needed to further elucidate the involvement of CysLTs in the pathogenesis of human pulmonary fibrosis.

In the second study, we established silica-induced pulmonary fibrosis mice as a chronic pulmonary fibrosis model. We showed that silica-induced pulmonary fibrosis in mice is persistent and progressive. Furthermore, histological study showed some similarities between the murine model of silica-induced pulmonary fibrosis and human pulmonary fibrosis, such as alveolar wall thickening, obliteration of alveolar space, scattered fibroblastic foci, and a loss of architectural integrity. Thus, our results confirmed that the silica model is suitable for analyzing the pathogenesis of pulmonary fibrosis particularly in the chronic phase. Utilizing this model, we studied the contribution of CysLTs in the pathogenesis of pulmonary fibrosis during both acute and chronic phases. CysLT content in BALF and lung tissue was markedly increased in the acute phase. Noteworthy is that, in the chronic phase, CysLT content in BALF returned to the control level, whereas the tissue content of CysLTs remained increased at as late as the day 56. TGF-β1 and TNF-α mRNA levels in lung tissues were also increased during both acute and chronic phases of pulmonary fibrosis. Furthermore, strong immunohistochemical staining for $CysLT_1$ receptor, TGF-β1, and TNF-α, but not for $CysLT_2$ receptor was co-detected in the pathological lesions during both acute and chronic phases. These results suggest that the activated production of CysLT in tissues triggers and maintains the fibrosis directly as well as indirectly *via* increases in TGF-β1 and TNF-α expression. Additionally, changes in the ratio of $CysLT_1$ receptor to $CysLT_2$ receptor may also be involved in the persistent progression of fibrosis in the lung. These results strongly suggest that CysLTs play an important role in the pathogenesis of pulmonary fibrosis during not only acute phase but also chronic phase.

CONCLUSION

In conclusion, we showed that CysLTs participate in the pathogenesis of pulmonary fibrosis in two different experimental models: bleomycin-induced pulmonary fibrosis model and silica-induced pulmonary fibrosis model. Increased production of CysLTs and the change in the ratio of $CysLT_1$ receptor to $CysLT_2$ receptor may be involved in the pathogenesis of not only the acute profibrotic response, but also the persistent progression of pulmonary fibrosis.

別紙

氏名　　新堀　智子

論文名　1. Effects of montelukast, a cysteinyl-leukotriene type 1 receptor antagonist, on the pathogenesis of bleomycin-induced pulmonary fibrosis in mice

2. Involvement of leukotrienes in the pathogenesis of silica-induced pulmonary fibrosis in mice.

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著者名　1. Chiko Shimbori, Naotaka Shiota, and Hideki Okunishi

2. Chiko Shimbori, Naotaka Shiota, and Hideki Okunishi