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Preventive effect of irbesartan on bleomycin-induced lung injury in mice

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ABSTRACT

Background: Idiopathic pulmonary fibrosis is a specific form of chronic fibrosing interstitial pneumonia that is limited to the lung. Angiotensin receptor blockers (ARBs) and peroxisome proliferator-activated receptor (PPAR) γ ligands have anti-inflammatory and anti-fibrotic effects. We investigated the effects of irbesartan—an ARB with PPAR γ activity—on the development of bleomycin-induced pulmonary fibrosis in mice.

Methods: Lung injury was induced in imprinting control region (ICR) mice by intratracheal instillation of 2 mg/kg of bleomycin. The treatment group orally received 20 mg/kg of irbesartan for 5 consecutive days before instillation. The mice were sacrificed and were evaluated 14 days after bleomycin instillation.

Results: Irbesartan reduced the fluid content and hydroxyproline level in the lung and improved the pathological findings as indicated by the Ashcroft score. Total cell counts, the numbers of macrophages, neutrophils, and lymphocytes, and the levels of transforming growth factor (TGF) β1 and monocyte chemotactic protein (MCP) 1 in the bronchoalveolar lavage fluid (BALF) were decreased. Treatment with a PPARγ antagonist GW9662 reversed some of the effects of irbesartan.

Conclusions: The results of this study indicated that irbesartan attenuated the development of bleomycin-induced pulmonary fibrosis in mice by decreasing TGF-β1 and MCP-1 via blocking of ATI, by binding to CCR2b, and by PPARγ-mediated inhibition of inflammation.

Abbreviations: IPF, idiopathic pulmonary fibrosis; AT1, angiotensin type 1 receptor; TGF, Transforming growth factor ; BALF, bronchoalveolar lavage fluid; PPARs, peroxisome proliferator activated receptors; ARBs, angiotensin receptor blockers; CCR2b, chemokine C–C motif receptor b; MCP-1, monocyte chemoattractant protein 1; ICR, imprinting control region; GW9662, 2-chloro-5-nitrobenzanilide; MIP-1α, macrophage inflammatory protein-1α; TNF-α, tumor necrosis factor-α; PGF2α, prostaglandin F2α; ELISA, enzyme-linked immunosorbent assay; CTGF, connective tissue growth factor; JNK, JUN N-terminal kinase; ANG1-7, angiotensin 1-7; R × Rs, retinoid × receptors; PPREs, PPARγ response elements

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1. Introduction

Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic fibrosing interstitial pneumonia that is limited to the lung. The etiology and pathogenesis of IPF are not known, and it remains a devastating disease with a 5-year mortality rate greater than 50%. Although several drugs have been used to attempt to treat IPF, an established treatment that definitely improves its outcome does not exist [1]. Thus, we await new therapies based on a new understanding of the pathogenesis of IPF.

Angiotensin II is a peptide that plays a crucial role in regulating blood pressure and sodium homeostasis. To date, 4 angiotensin receptors have been identified: angiotensin type 1 receptor (AT1), type 2 receptor, type 3 receptor, and type 4 receptor. The vast majority of angiotensin II actions are mediated via the AT1 receptor. It is widely accepted that AT1 is involved in organ fibrosis, and inhibition of AT1 can suppress fibrosis of the heart, kidney, and lung [2–5]. Transforming growth factor (TGF)-β plays a critical role in the pathogenesis of IPF and bleomycin-induced fibrosis. Furthermore, it was reported that AT1 antagonists simultaneously suppress the TGF-β1 level in bronchoalveolar lavage fluid (BALF) [3,4].

Peroxisome proliferator activated receptors (PPARs) are a family of ligand binding nuclear hormone receptors. The pleiotropic effects of PPARs include lipid and lipoprotein metabolism and adipogenesis, glucose homeostasis, cell cycle regulation, and cellular proliferation and differentiation. Three PPARs encoded by 3 separate genes have now been identified: α, β/δ, and γ. PPARγ plays a role in regulating cell differentiation and inflammation and is thus of high interest as a potential target for therapies for diseases involving dysregulated inflammation and/or differentiation. In hyperoxia-induced acute lung injury, PPARγ expression is dysregulated and PPARγ induction has an essential protective role [6]. PPARγ agonists also significantly reduce lung injury and fibrosis induced by bleomycin in mice [7].

Angiotensin receptor blockers (ARBs) were developed for the treatment of high blood pressure to antagonize increased angiotensin II-dependent vasoconstriction. In addition to their AT1 blocking properties, several ARBs function as partial agonists of PPARγ; for example, telmisartan, candesartan, losartan, and irbesartan serve as PPAR ligands *in vitro* [8]. Irbesartan also has the beneficial effects of binding to chemokine C–C motif receptor b (CCR2b) to block monocyte chemoattractant protein 1 (MCP-1) binding [9] and inducing adiponectin by PPARγ activation [10,11]. The present study was designed to investigate the effect of irbesartan, an ARB with CCR2b binding and PPARγ activity, on the development of bleomycin-induced lung injury in mice.

2. Materials and methods

2.1. Mice and reagents

All mice received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication 8523, revised 1985; http://www.nyu.edu/uawc/Forms/Guideexcerpts). The Ethics Committee for animal experiments of Niigata University, Niigata, Japan, approved the study protocol (June 4th, 2009, Niigata University Research #32). Specific 8-week-old, pathogen-free, male imprinting control region (ICR) mice were obtained from Japan SLC (Niigata, Japan) and housed in the animal facility of Niigata University. Bleomycin was purchased from Nippon Kayaku (Tokyo, Japan). Dainip pon Sumitomo Pharma Co., Ltd. (Osaka, Japan) kindly sup plied us with irbesartan. The treatment group orally received 20 mg/kg of irbesartan for 5 consecutive days before bleomycin instillation. The PPARγ selective antagonist, 2-chloro-5-nitrobenzanilide (GW9662), was purchased from Sigma (St Louis, MO, USA).

2.2. Experimental design to determine the activity of irbesartan as an inhibitory agent in the bleomycin model

An experimental design to determine the activity of irbesartan in the bleomycin model is shown in Fig. 1. We used ICR

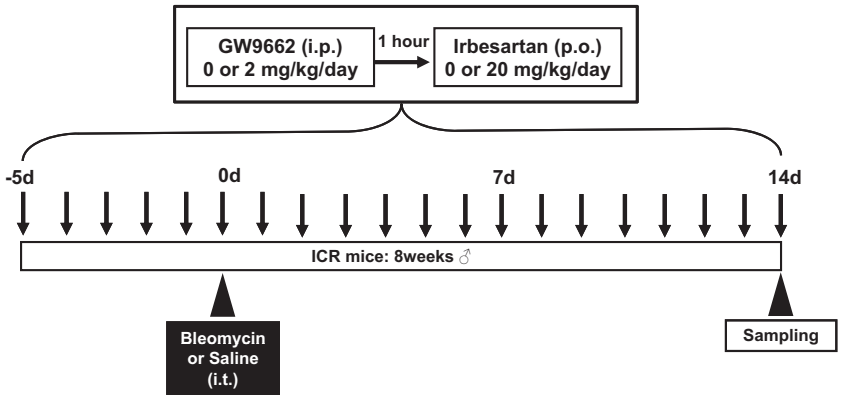

Fig. 1 – Experimental design to determine the activity of irbesartan in the bleomycin model. **Male ICR mice (8 weeks of age) were instilled intratracheally (i.t.) with bleomycin (2 mg/kg in 50 mL of saline) under anesthesia. The treatment group orally (p.o.) received irbesartan (20 mg/kg) for 5 consecutive days before bleomycin instillation. GW9662 (2 mg/kg) was injected intraperitoneally (i.p.) 1 h before irbesartan administration. The mice were sacrificed and evaluated 14 days after bleomycin instillation.**