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Effects of pirfenidone on increased cough reflex sensitivity in guinea pigs

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ABSTRACT

Pirfenidone, an antifibrotic drug with anti-inflammatory and antioxidant effects, delays fibrosis in idiopathic pulmonary fibrosis (IPF). Patients with IPF have a greater cough reflex sensitivity to inhaled capsaicin than healthy people, and cough is an independent predictor of IPF disease progression; however, the effects of pirfenidone on cough reflex sensitivity are unknown.

After challenge with an aerosolized antigen in actively sensitized guinea pigs, pirfenidone was administered intraperitoneally, and the cough reflex sensitivity was measured at 48 h after the challenge. Bronchoalveolar lavage (BAL) was performed, and the tracheal tissue was collected.

Pirfenidone suppressed the capsaicin-induced increase in cough reflex sensitivity in a dose-dependent manner. Additionally, increased levels of prostaglandin E_2, substance P, and leukotriene B_4, but not histamine, in the BAL fluid were dose dependently suppressed by pirfenidone. The decrease in neutral endopeptidase activity in the tracheal tissue was also alleviated by pirfenidone treatment. The total number of cells and components in the BAL fluid was not influenced.

These results suggest that pirfenidone ameliorates isolated cough based on increased cough reflex sensitivity associated with allergic airway diseases, and potentially relieve chronic cough in IPF patients who often have increased cough reflex sensitivity. Prospective studies on cough-relieving effects of pirfenidone in patients with IPF are therefore warranted.

1. Introduction

Chronic nonproductive dry cough is a complicating clinical feature in idiopathic pulmonary fibrosis (IPF) [1]. The cough is bothersome and has been described as “dry and nonproductive” or “hacking”, often accompanying a nagging desire to cough constantly, which is not relieved by coughing [2]. In an analysis of 242 patients with IPF, cough was present in 84% of subjects and was an independent predictor of disease progression [3]. Previous studies [4–6] have investigated the pathogenesis of cough in IPF; however, cough due to IPF is a diagnosis of exclusion after considering other common disorders. Additionally, reports have demonstrated that at least 50% of cough in IPF patients is not secondary to IPF [1,7]. B.D. Hope-Gill et al. [8] revealed that patients with IPF in whom gastroesophageal reflux disease (GERD), airway hyper-responsiveness, and other confounding influences were excluded showed enhanced cough reflex sensitivity to capsaicin and substance P (SP) compared with healthy subjects, suggesting a functional upregulation of respiratory tract sensory nerves.

Pirfenidone (5 methyl-1-phenyl-2-[1H]-pyridone) is an antifibrotic drug which delays fibrosis in IPF. In a bleomycin-induced lung fibrosis mouse model, pirfenidone showed antifibrotic and anti-inflammatory effects [9]. A phase III trial in Japan showed that pirfenidone caused a significant reduction in the decline of vital capacity and improved progression-free survival [10]. Pirfenidone has been reported to have anti-inflammatory [11,12] and antioxidant [13,14] effects, in addition to antifibrotic effects [15,16], in experimental models. Furthermore, the effects of pirfenidone on bronchial asthma have been investigated recently [17,18], and although the effects of pirfenidone on airway hyper-responsiveness remain controversial, these data suggest the possibility that pirfenidone may be used for the treatment of acute allergic airway diseases and may reduce or prevent airway remodeling. However, only one study [19] has clarified the inhibitory effects of

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pirfenidone on cough in patients with IPF, and no reports of the effects of pirfenidone on cough reflex sensitivity have been published.

Here, we investigated the effects of pirfenidone on increased cough reflex sensitivity to inhaled capsaicin accompanied by eosinophilic airway inflammation in actively sensitized guinea pigs with cough reflex sensitivity. We also examined the effects of pirfenidone on cell components, levels of sensory nerve mediators and neuropeptides in the bronchoalveolar lavage fluid (BALF), and neutral endopeptidase (NEP) activity in the tracheal tissue.

2. Material and methods

2.1. Study animals

Male albino Hartley strain guinea pigs weighing 200–220 g were obtained from the Sankyo Laboratory Service (Toyama, Japan) and were quarantined in the Animal Research Center of Kanazawa University. All animal procedures in this study complied with the standards specified in the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi Campus of Kanazawa University.

2.2. Study design

Guinea pigs ($n = 8$ per group) were assigned to the negative control (NC), positive control (PC), low-dose pirfenidone (PFD-low; 30 mg/kg), and high-dose pirfenidone (PFD-high; 300 mg/kg) groups (Shionogi Co., Ltd., Osaka, Japan). Animals in the PC and pirfenidone groups were actively sensitized and exposed to aerosolized antigen. On the other hand, animals in the NC group were exposed to aerosolized saline. Every 12 h after antigen challenge (a total of 3 times), 1.5 mL/kg pirfenidone solution dissolved in 0.5% of methylcellulose (MC; Wako Pure Chemical Ind., Osaka, Japan) at a concentration of 20 (PFD-low) or 200 (PFD-high) mg/mL (final dose: 30 mg/kg or 300 mg/kg, respectively) was administered intraperitoneally in the pirfenidone groups. Animals in NC and PC groups were intraperitoneally injected with vehicle (1.5 mL/kg of 0.5% MC) instead of pirfenidone. The cough reflex sensitivity to inhaled capsaicin (Sigma, St. Louis, MO, USA) was measured at 48 h after the antigen challenge. After measurement of the cough reflex sensitivity, bronchoalveolar lavage (BAL) was performed, and tracheal samples were collected for measurement of NEP activity.

To avoid the influence of inhaled capsaicin on substance P (SP) levels in the BAL fluid (BALF), different guinea pigs were allocated to NC, PC, PFD-low, and PFD-high groups ($n = 8$ for each group), and all animals were maintained under the same conditions, except that inhalation of capsaicin and BAL was performed to measure SP levels without cough measurement in certain groups. Moreover, to investigate the effects of pirfenidone on naïve guinea pigs, 8 guinea pigs were treated in the same manner as NC groups and were intraperitoneally injected with pirfenidone (300 mg/kg) instead of 0.5% MC. Cough reflex sensitivity to inhaled capsaicin was then measured (this was termed the NC300 group).

2.3. Active sensitization and antigen challenge

The guinea pigs were actively sensitized by the method reported by Muraki et al. [20]. Each animal was intraperitoneally injected with 2.0 mg ovalbumin (OA; Sigma) and 100 mg aluminum hydroxide ($Al(OH)_3$; Wako Pure Chemical Ind.) 2 days after an intraperitoneal injection of 30 mg/kg of cyclophosphamide (Shionogi Co., Ltd.) to increase Th2-dominant bronchial reactivity. Three weeks later, a booster injection of 0.01 mg OA and 100 mg $Al(OH)_3$ was administered intraperitoneally. The guinea pigs in the NC and NC300 groups were maintained under the same conditions as the actively sensitized animals.

Three weeks after the booster injection, actively sensitized guinea pigs were challenged with an aerosolized OA solution under spontaneous breathing at 30 min after intraperitoneal administration of 20 mg/kg diphenhydramine (Wako Pure Chemical Ind.) and 0.1 mg/kg procaterol (Otsuka Pharmaceutical Co., Ltd., Osaka, Japan) to prevent anaphylaxis and asthma attack. Conscious guinea pigs were challenged with 10 mg/mL OA aerosol for 90 s by a method previously described [21–23]. Animals in NC and NC300 groups were not injected with diphenhydramine or procaterol, but they did inhale saline aerosol by the same method as that described for the sensitized guinea pigs.

2.4. Cough reflex sensitivity

Each conscious guinea pig was placed in an airtight custom-built transparent plastic box consisting of a head chamber (volume, 1600 mL) isolated from the body chamber. The pressure in the body chamber was recorded by the polygraph system (Model RMT-1000, NIHON KOHDEN Co., Tokyo, Japan) and Lab Chart 7 software (AD Instruments Co., Colorado Springs, CO, USA). Coughs were detected as a transient specific change in the pressure in the body chamber (a rapid inspiration followed by rapid expiration) (See Fig. 1). We also carefully listened to the sound of the cough and visually monitored the movements of the animal to be sure that all motion- and sneeze-related changes in the pressure were observed. Each guinea pig inhaled 10^{-4} M capsaicin solution for 2 min from a Devilbiss 646 nebulizer (Devilbiss Co., Somerset, PA, USA) operated by compressed air at 1.6 L/min (Iwaki Air Pump AP-115AN, Iwaki Co., Ltd., Tokyo, Japan). The nebulizer output was 0.037 mL/min. Coughs were counted during a 2-min inhalation of each capsaicin solution and for an additional 1-min observation (total: 3 min) by a trained observer and recognized by changes in pressure, characteristic animal posture, and sound.

2.5. Bronchoalveolar lavage (BAL)

BAL was performed immediately after the measurement of cough reflex sensitivity by previously described methods [23,24]. Briefly, the guinea pigs were anaesthetized by intraperitoneal injection of 75 mg/kg sodium pentobarbital (Abbot Laboratories, North Chicago, IL, USA) and were placed in the supine position. The

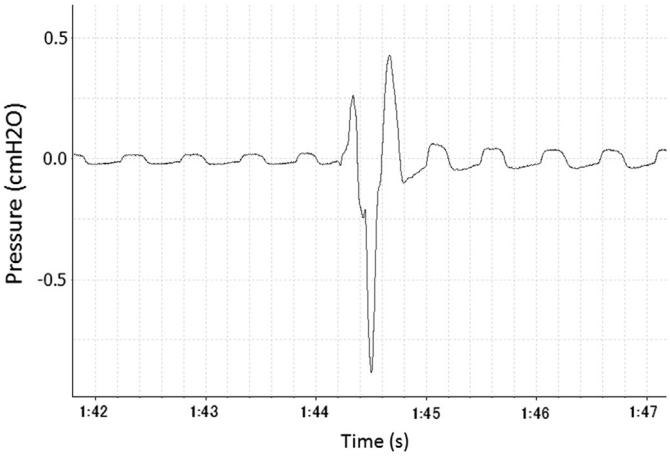

Fig. 1. An example of the change in pressure in the body chamber when the animal coughed. Coughs were detected as a transient specific change in pressure in the body chamber. A change in pressure with rapid inspiration followed by rapid expiration occurred when the animal coughed.