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Ghrelin ameliorates catabolic conditions and respiratory dysfunction in a chronic obstructive pulmonary disease model of chronic cigarette smoke-exposed rats

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ABSTRACT

Cigarette smoking, which is a well-known major risk factor for chronic obstructive pulmonary disease (COPD), causes both pulmonary and extrapulmonary abnormalities. Ghrelin is a gastric peptide that regulates energy homeostasis. In the present study, we investigated the effects of ghrelin on the catabolic changes, respiratory function and emphysema in an animal model of COPD induced by chronic exposure to cigarette smoke. Rats were exposed to cigarette smoke, and they were administered human ghrelin (0.1 or 1 mg/kg, subcutaneous, twice daily) for 12 weeks. Compared with air-exposed rats, body weight gain, food intake, food efficiency, tidal volume, peak expiratory flow rate, and forced expiratory volume at 100 ms were significantly lower, while functional residual capacity, lung capacity, and neutrophils in bronchoalveolar lavage fluid were significantly higher in cigarette smoke-exposed rats. These indicated that the systemic abnormalities associated with COPD developed after the exposure to cigarette smoke. Ghrelin significantly and dose-dependently increased the body weight gain and food efficiency in cigarette smoke-exposed rats. In ghrelin-treated rats, skeletal muscle strength, which tended to be lowered by cigarette smoke exposure, was improved. Ghrelin ameliorated respiratory function and emphysema in a dose-dependent manner, but did not inhibit the increase in neutrophils in the bronchoalveolar lavage fluid. The respiratory functional parameters and lung capacity were significantly correlated with body weight gain. These results suggest that ghrelin inhibited the development of the catabolic changes, respiratory dysfunction, and emphysema that were induced by cigarette smoke exposure in rats, at least in part, through the amelioration of nutritional status.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitations due to a mixture of small airway disease and emphysema (Vestbo et al., 2013). Recently, in addition to the pulmonary pathology of COPD, several systemic manifestations, such as inflammation, malnutrition, body weight loss, and skeletal muscle wasting, have been observed in patients with COPD (Agusti et al., 2003; Celli et al., 2004; Wouters, 2002). The prevalence of cachexia has been reported as 20–40% in patients with COPD (Wagner, 2008). Weight loss or a low body mass index is an independent risk factor for mortality apart from the airflow limitations in patients with COPD (Schols et al., 1998). Weight loss in patients with COPD is associated with peripheral muscle dysfunction and exercise intolerance (Agusti et al., 2002). Intriguingly, pulmonary emphysematous changes and/or respiratory dysfunction have been observed in malnourished patients, including those with anorexia nervosa, and in starved animals (Coxson et al., 2004; Dias et al., 2004; Massaro et al., 2004). These findings suggest that corrections of the catabolic conditions in metabolic energy balance are critical, particularly in the treatment of COPD cachexia.

Ghrelin is a growth hormone releasing peptide which was originally discovered in the stomach (Kojima et al., 1999), and it is now known to stimulate food intake and regulate energy homeostasis (Kojima and Kangawa, 2005). The production of ghrelin, which occurs predominantly in the stomach, is upregulated under conditions of negative energy balance, such as fasting, anorexia, and cachexia (Ariyasu et al., 2001). In underweight patients with COPD, plasma ghrelin levels are significantly increased, and these increase levels are associated with a cachectic state and abnormal pulmonary function (Itoh et al., 2004; Uzum et al., 2014), which suggests a compensatory function of ghrelin against catabolism. Ghrelin improves body weight gain and food intake in animal models with body weight loss that is induced by various stimuli, such as tumor

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inoculation (Hanada et al., 2003), pulmonary fibrosis with bleomycin (Imazu et al., 2011), and cachexia from angiotensin II (Sugiyama et al., 2012). These results suggest that ghrelin might improve the catabolic changes in COPD cachexia.

Chronic exposure to cigarette smoke causes pathological changes in the respiratory tract and lung parenchyma due to inflammation, which results in airflow limitations and systemic abnormalities in patients with COPD (Calverley and Walker, 2003). Cigarette smoke-induced respiratory dysfunction or pathophysiological changes in animals resemble the clinical features of COPD (Wright et al., 2008). Cigarette smoke-exposed animals exhibit decreased body weight, increased plasma ghrelin levels, and skeletal muscle atrophy (Gosker et al., 2009; Tang et al., 2010; Tomoda et al., 2012). However, the pharmacological effects of ghrelin have not been investigated in cigarette smoke-exposed animals. In the present study, we examined the systemic effects of ghrelin on COPD in chronic cigarette smoke-exposed rats and the effects of ghrelin on respiratory function and emphysema.

2. Materials and methods

2.1. Animals

Five-week-old male Wistar rats (Japan SLC Inc., Shizuoka, Japan) were housed individually in polycarbonate cages at 19–25 °C with a 12-h light/dark cycle. Rats received regular chow (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*. This study was approved by the Committee for Ethics in Animal Experiments of Asubio Pharma Co., Ltd.

2.2. Cigarette smoke exposure

Rats were randomly divided into 4 groups (10 rats per group) based on body weight just before cigarette smoke exposure. Three out of 4 groups were exposed to cigarette smoke that was generated from 20 cigarettes (Peace®, 28 mg of tar and 2.3 mg of nicotine per cigarette, Japan Tobacco Inc., Tokyo, Japan) twice a day at around 10 a.m. and 4 p.m. for 12 weeks with a smoking apparatus (INH06-CIG01A, M.I.P.S. Inc., Osaka, Japan). One group was exposed to smoke-free air with the same protocol and used as a control (sham).

2.3. Ghrelin treatment

Human ghrelin that was synthesized as described previously (Makino et al., 2005) was dissolved in a 5% D-mannitol solution and injected subcutaneously at a dose of 0.1 or 1 mg/kg into cigarette smoke-exposed rats twice daily (before the cigarette smoke exposure in the morning and after the cigarette smoke exposure in the afternoon) for 12 weeks. The sham group and one of the three groups that was exposed to cigarette smoke (control) were subcutaneously administered the D-mannitol solution without ghrelin (1 ml/kg).

2.4. Body weight and food intake

Body weight and food intake were measured throughout the study, and cumulative values were calculated for each. Food efficiency, which was an index that converts ingested calories into body weight, was calculated by dividing the cumulative weight gain by the total food intake. On Days 21 and 77, food intake was measured for 1 h after the ghrelin or vehicle injection in the morning.

2.5. Grip strength

In order to evaluate skeletal muscle strength, a grip strength meter (MK-380CM/R, Muromachi Kikai Co., Ltd., Tokyo, Japan) was applied to the forelimb (Meyer et al., 1979). Briefly, the rat was gently lifted onto the stage and made to grab the pull-gauge by holding its tail. The gauge was then pulled horizontally at a constant rate by the researcher. Grip strength was recorded when the rat released the gauge. Five sequential trials were performed in each animal. The average of three trials, excluding the maximum and the minimum values, was taken as the representative value for each animal. The test was performed in the morning on Day 84 before the ghrelin injection.

2.6. Respiratory function

In the morning on Day 77 before the ghrelin injection, the tidal volume and peak expiratory flow rate were measured with a whole-body plethysmograph with a respiratory function analysis system (Biosystem XA, Data Sciences International, St. Paul, MN, USA) when the rats were awake. In order to record functional residual capacity and forced expiratory volume at 100 ms as indexes of respiratory function, rats were anesthetized with intraperitoneal injections of urethane (1.2 g/kg, Sigma-Aldrich Co. LLC, St. Louis, MO, USA), and a cannula was placed in the trachea on Day 85. These functional parameters were measured with a forced maneuvers system (Bio-System for Maneuvers, Data Sciences International).

2.7. Collection and analysis of plasma

After the measurement of respiratory function, blood was taken from the abdominal vein and transferred into tubes containing ethylenediaminetetraacetic acid and aprotinin (Bayer Yakuhin, Ltd., Osaka, Japan). After centrifugation (1500*g*, 4 °C, 15 min), plasma was collected and stored at −80 °C. Plasma levels of leptin as an index of fat mass (Ostlund et al., 1996) and C-reactive protein as an index of systemic inflamation (Clyne and Olshaker, 1999) were determined with a leptin-high sensitivity enzyme-linked immunosorbent assay kit (Yanaihara Institute Inc., Shizuoka, Japan) and a mouse high sensitivity C-reactive protein enzyme-linked immunosorbent assay (Kamiya Biomedical Co., Seattle, WA, USA), respectively.

2.8. Collection and analysis of bronchoalveolar lavage fluid

After the collection of venous blood, the rat was exsanguinated by incision of the abdominal aorta, and the chest of the rat was opened. The right main bronchus was ligated, and the trachea was cannulated. The left lung was lavaged twice with 4 ml of saline that was administered through the cannula. The fluid that was obtained was considered bronchoalveolar lavage fluid and stored on ice. The bronchoalveolar lavage fluid samples were centrifuged at 500*g* at 4 °C for 10 min. The pellet was suspended in 1 ml of saline. Turk's solution was added to the bronchoalveolar lavage fluid pellet suspension, and the number of white blood cells per 1 μl was counted with a hemocytometer. This suspension was centrifuged again as described above. An aliquot of the cell suspension was mounted on a glass slide and stained with Wright–Giemsa solution. The numbers of neutrophils, monocytes/macrophages, lymphocytes, and eosinophils were counted under a microscope, and the ratio of these numbers to the number of total white blood cells was calculated. The numbers of each leukocyte subpopulation per 1 μl were determined based on the ratio.

2.9. Stereology and immunohistochemistry of the lung

The chest was opened, and the lung was instilled with 10% buffered formalin through the cannula at a constant pressure of 25 cmH_2O for 4 h. The volume of instilled formalin was determined as the lung capacity. Then, the right lower lobe was excised and immersed in formalin. The paraffin-embedded lobe was sectioned