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Airway remodeling associated with cough hypersensitivity as a consequence of persistent cough: An experimental study

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ABSTRACT

Background: Chronic cough involves airway remodeling associated with cough reflex hypersensitivity. Whether cough itself induces these features remains unknown.
Methods: Guinea pigs were assigned to receive treatment with citric acid (CA), saline (SA), or CA+dextromethorphan (DEX). All animals were exposed to 0.5 M CA on days 1 and 22. On days 4–20, the CA and CA+DEX groups were exposed to CA, and the SA group to saline thrice weekly, during which the CA+DEX group was administered DEX pretreatment to inhibit cough. The number of coughs was counted during each 10-min CA or SA exposure. Terbutaline premedication was started to prevent bronchoconstriction. Bronchoalveolar lavage and pathology were examined on day 25. Average cough number for 10 CA exposures was examined as "cough index" in the CA group, which was divided into frequent (cough index>5) and infrequent (<5) cough subgroups for lavage and pathology analysis.
Results: The number of coughs significantly increased in the CA group from day 13 onwards. In the CA+DEX and SA groups, the number of coughs did not differ between days 1 and 22, while average number of coughs during days 4–20 was significantly lower than at days 1 and 22. Bronchoalveolar cell profiles were similar among the four groups. The smooth muscle area of small airways was significantly greater in the frequent-cough subgroup than in the other groups (in which it was similar), and highly correlated with cough index in CA group.
Conclusion: Repeated cough induces airway smooth muscle remodeling associated with cough reflex hypersensitivity.

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1. Introduction

Chronic cough is an important clinical problem. Asthma-related diagnoses such as cough-variant asthma involve eosinophilic airway inflammation [1,2]. Cough may also be ascribed to non-asthma-related diagnoses, such as gastro-esophageal reflux disease, but in some patients, it remains unexplained despite thorough investigations. Such non-asthmatic chronic cough (NACC) also involves airway inflammation characterized by increased neutrophils, mast cells, or lymphocytes [2–4].

Airway remodeling is another feature of asthma [5]. The pathological changes of remodeling in cough-variant asthma include subepithelial thickening, goblet-cell hyperplasia, vascular proliferation, and airway-wall thickening [2,6,7]. Eosinophilic bronchitis also involves similar changes [8]. These changes may be ascribed to eosinophil-derived fibrogenic mediators such as transforming growth factor (TGF)-beta, and may result in accelerated lung function decline in intractable cases [1], as in asthma [5]. Interestingly, cases of NACC without airway eosinophilia also show features of remodeling, such as subepithelial thickening, increase of airway smooth muscle (ASM), goblet cells and vessels, epithelial shedding, airway-wall thickening [2,3,7], and irreversible airflow obstruction [9]. Especially noteworthy is the increase of ASM despite absence of airway hyperresponsiveness [10].

The pathogenesis and functional consequences of airway remodeling are not precisely known in chronic cough, especially for NACC [10]. Airway epithelium exposed to compressive stresses, an effect mimicking acute bronchoconstriction in asthma, increases its expression of genes relevant to remodeling, such as TGF-beta 2. Synthesis of extracellular matrix by cocultured fibroblasts is also increased [11]. Cough may also exert mechanical stress on the airway mucosa [12]. In patients with NACC, TGF-beta 1 levels and neutrophil counts in bronchoalveolar lavage fluid (BALF), as well as subepithelial thickness, were increased [13]. TGF-beta 1 expression in the epithelium and ASM were also increased, and TGF-beta 1 levels correlated with subepithelial thickness [13]. In NACC patients, the degree of remodeling (as indicated by goblet-cell hyperplasia, epithelial shedding, and airway-wall thickening) correlated with cough reflex sensitivity to capsaicin [2,7]. Persistent cough may thus result in airway remodeling, in the presence or absence of inflammation. This may lead to cough reflex hypersensitivity that might induce further cough, creating a vicious cycle [10].

A guinea pig model of airway collapse mimicking cough has been reported, induced by rapid repetition of negative pressure applied to the airways of artificially ventilated animals [14]. Capsaicin sensitivity and BALF neutrophils transiently increased 6 hours after stimulus, and these features correlated with each other. However, this was a short-term experiment, failing to examine remodeling changes [14].

We investigated whether repeated induction of cough in awake guinea pigs is associated with airway remodeling and cough reflex hypersensitivity. Cough was induced by citric acid (CA) exposure, which is unlikely to induce tachyphylaxis and therefore useful for repeated cough induction and measurements [15–17].

2. Materials and methods

2.1. Animals

Male Dunkin-Hartley guinea pigs were obtained and quarantined. All animal procedures conformed to Kyoto University's regulations on animal experimentation (Med Kyo 04449; approval date: Nov 25, 2004).

2.2. Experimental design

Naïve guinea pigs were assigned to one of three treatment groups: CA group, saline (SA) group, or CA+dextromethorphan (DEX) group (Fig. 1). On days 1 and 22, guinea pigs in all groups were exposed to CA (0.5 M via nebulizer for 10 min) and the number of coughs was counted. From days 4 through 20, the CA and CA+DEX groups were exposed to 0.5 M CA, and the SA group to 0.9% saline for 10 min, three times weekly, at 2- or 3-day intervals (eight times in total). During this period, the CA+DEX group was treated with 30 mg/kg DEX intra-peritoneally (i.p.) 30 min prior to CA exposure, to inhibit cough. DEX was not administered on days 1 and 22. In the CA group, the mean number of coughs for 10 CA exposures was calculated as the "cough index" for each animal. The CA group was divided into a frequent cough subgroup (CA-F: Cough index ≥ 5) and an infrequent cough subgroup (CA-I: Cough index <5) for analysis of BAL and pathology results. The median number of cough index ($=5$) of the 18 animals in the CA group was used as the cut off level.

2.3. System for cough measurement

Guinea pigs were placed in a chamber allowing free movement and equipped with an internal microphone and a pressure transducer [18]. They were connected to an Amplifier Interface Unit series pre-amplifier (EMMS, Bordon, UK). The chamber was provided airflow by a Basic Flow Supplier AIR 200 (EMMS) at 1500 mL/min. The changes in airflow induced by respiration and cough were recorded by a pneumotachograph. Cough sounds were amplified and recorded

Abbreviations: CA, citric acid; ASM, airway smooth muscle; BAL, bronchoalveolar lavage; BALF, BAL fluid; PAS, periodic acid-Schiff; TGF, transforming growth factor; NACC, non-asthmatic chronic cough

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