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Effect of transient receptor potential vanilloid-1 on cough hypersensitivity induced by particulate matter 2.5

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ABSTRACT

Aims: The mechanism of cough hypersensitivity induced by particulate matter 2.5 (PM2.5) remains elusive. The current study was designed to explore the effect of transient receptor potential vanilloid-1 (TRPV1) on cough hypersensitivity in airway and central nervous system.
Main methods: The PM2.5-induced chronic cough model of guinea pig was established by exposure to different doses of PM2.5 for three weeks. After exposure, the animals were microinjected with TRPV1 agonist capsaicine, antagonist capsazepine in the dorsal vagal complex respectively. Cough sensitivity was measured by determining the provocative concentration of citric acid inducing 5 or more coughs (C5). Airway inflammation was detected by hematoxylin eosin (HE) staining and Evans blue fluorescence, and substance P (SP) and TRPV1 expressions in airway were observed by immunohistochemical staining. TRPV1 expressions in the dorsal vagal complex were observed by immunofluorescence. Retrograde tracing by pseudorabies virus-Bartha (PRV-Bartha) was conducted to confirm the regulatory pathway between airway and central nervous system.
Key findings: PM2.5 induced TRPV1 expressions in both of airway and dorsal vagal complex and airway neurogenic inflammation. Airway vascular permeability increased after being exposed to PM2.5. The expressions of SP in the airway and airway inflammation was increased after microinjecting TRPV1 agonist, and decreased after microinjecting TRPV1 antagonist. PRV infected neurons in medulla oblongata mainly located in the dorsal vagal complex.
Significance: These findings show that TRPV1 in the dorsal vagal complex could promote airway neurogenic inflammation and cough reflex sensitivity through neural pathways of vagal complex-airways, which indicate the therapeutic potential of specific inhibition of TRPV1 for chronic cough induced by PM2.5.
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1. Introduction

The impact of air pollution on human health has become an important global public health problem [1–2]. PM2.5, with small size, high toxicity, long-distance transmission and long resident time, plays an important role in air pollution. Therefore, after entering the body along with breathing, PM2.5 can lead to multi-system diseases, especially respiratory diseases [3]. Epidemiological survey has shown that air pollution may induce chronic cough. Cough is a physiological-defensive reflex which can exclude secretions and foreign bodies in airway. However, when microbes, pollutants and allergens act on airway, cough may become excessive and nonproductive, and is potentially harmful to the airway mucosa. Chronic cough can be attributed to increased cough reflex sensitivity, which is referred to cough hypersensitivity. There is a close relationship between neurogenic inflammation and cough reflex sensitivity [4]. Transient receptor potential vanilloid-1 (TRPV1), a non-selective cation channel, widely present in sensory nerve endings, plays an important role in cough hypersensitivity. Since TRPV1's main inducements, such as low pH, ethanol [5], oleoylethanolamide [6] or cigarette smoke [7] show similar effect to the chemical compositions of PM2.5, it would be important to explore the role of TRPV1 in chronic cough induced by PM2.5.

Would PM2.5 stimulate TRPV1 expressions and then cause increased SP expressions, leading to airway neurogenic inflammation and cough hypersensitivity? The stimulus signals input to sensory center which can classify and handle the signals and integrate it to advanced nervous center [8–9]. Eventually, the integrated information could impact, modify or inhibit the cough reflex. TRPV1 was found to be distributed in the dorsal vagal complex [10]. It is unclear whether TRPV1 in the dorsal vagal complex would be activated and play a regulatory role on airway

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neurogenic inflammation and cough reflex sensitivity induced by PM2.5.

Therefore, on the basis of establishing the model of guinea pigs exposed to PM2.5, cough reflex sensitivity, airway inflammation and TRPV1 expressions in airway and dorsal vagal complex would be measured, with TRPV1 agonist or antagonist microinjecting into the dorsal vagal complex.

2. Materials and methods

2.1. Animal

The Animal Care and Use Committee in Medical School of Southeast University approved all experiments described in this study. Male Hartley guinea pigs (300–500 g, housed in the Experimental Animal Center of Medical School in Southeast University.) were on a 14 h/10 h light/dark cycle and were given a standard guinea pig chow diet/tap water randomly.

2.2. The collection of PM2.5 and the preparation of suspensions

Five sampling sites in Nanjing were selected in accordance with the urban function: Hunan Road (the northern exit of Lion Bridge), Maigaoqiao (Gaojia village), Shanxi Road, industrial area along the Yangtze River (the junction of Nanpu Road, Puzhou Road) and Xianlin University which respectively represented business areas, commercial areas, heavy traffic areas, industrial areas and the suburbs. There was no shelter around within 100 m at each sample point. Sampling days were from December 15, 2014 to April 1, 2015. Particles were sampled by TH-150C air sampler with PM2.5 cuter (Beijing Granville Record Co Ltd., China) at a constant flow (3.5 L/min) for 8 h once. After sampling, particles on filter membrane were put into the deionized water bathing in the ultrasonic oscillator to elute and shocked 30 min before the supernatant was taken. Then they were put into the deionized water bathing in the ultrasonic oscillatory again for the second time by double distilled water. Both liquids were combined into an erlenmeyer flask. Particles were gained after being filtered by six layers of sterile gauze and dried.

Particles were collected and mixed with deionized water before smearing. Its morphology, original scale and scattered forms in the solution were measured by eyepiece and objective micrometers of microscopes. The distributions of the particles were mostly made up of circular particles <2.5 μm except some few gathered particles, so the particles met the criteria of dispersion of PM2.5. The proportions of main chemical composition of PM2.5 we collected were examined via the method of chemical mass balance (CMB) and the result was shown in Fig. 1. These qualified 0.6 g and 2.4 g PM2.5 were dissolved in 3 mL saline respectively make low and high dose of PM2.5 which were stored in refrigeration at 4 °C and used up in one week.

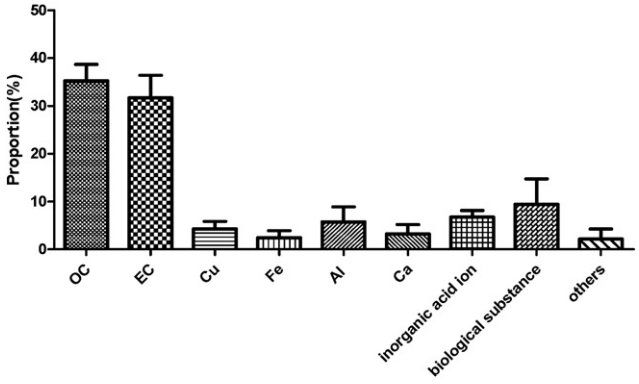

Fig. 1. The proportions of main chemical composition of PM2.5. OC: organic carbon, EC: elemental carbon.

2.3. Establishment of animal model and grouping

Guinea pigs were put into the sound recorder of cough (Jiangsu Jintan Medical Instrument Factory, China) linked four physiological signal acquisition process systems (RM6240B, Chengdu instrument factory, China) and the normal respiratory waveforms were recorded for 10 s before. Then the ultrasonic nebulizer (402AI, Diving Medical Equipment Co Ltd., China) was put on and citric acid (900 mmol/L) went through a plastic pipe into the sound recorder of cough for 15 s and the cough times of guinea pigs in 20 min were recorded later which included 3 aspects: 1) 15 min in air-tight smoking environment, 2) 5 min in normal environment after the smog, 3) latent time from the beginning to the first cough. Guinea pigs which were over-sensitive or not sensitive would be excluded according to the standard of exclusion as follows: (1) the times of cough in 5 min ≤ 10 and ≥50 and/or (2) latent time ≤ 10 s and ≥120 s [11–13].

The process of grouping, preparing models and experiment was shown as Fig. 2a and b. A total of 144 guinea pigs which met the criteria above were selected and randomly divided into group A ($n = 64$), group B ($n = 72$) and group C ($n = 8$). The guinea pigs in group A were randomly divided into four subgroups, with sixteen animals in each subgroup. The guinea pigs in group B were randomly divided into nine subgroups, with eight animals in each subgroup. Eight guinea pigs in group C were used for retrograde tracing. All the animals were nasally-instilled with the corresponding liquid at a dose of 0.4 mL/kg once a day for 3 weeks. In addition to being nasally-instilled high dose of PM2.5, CPZ group would be interfered by daily intraperitoneal injection of TRPV1 specific antagonist capsazepine (Sigma) at a dose of 1 μmol/kg.

2.4. Cough sensitivity to citric acid

Method A: Cough reflex sensitivity was measured according to previous literatures [14–16]. The original solution of citric acid (900 mmol/L) was diluted by deionized water, ultimately forming the double-diluted concentrations as follows: 450 mmol/L, 225 mmol/L, 113 mmol/L, 57 mmol/L, 29 mmol/L, 15 mmol/L, 8 mmol/L respectively. After inhaling saline by the ultrasonic nebulizer as a base value, the citric acid was inhaled from the lowest concentration for 15 s and transferred to next concentration after 5 min, the times of cough in 3 min were recorded. The cough challenge test would end when guinea pigs cough ≥5 times within 3 min. This concentration would be recorded as C5. The logC5 was used to compare cough reflex sensitivity in different groups [14–17].

Methods B: Guinea pigs in group B were anesthetized with urethane (1 g/kg, intraperitoneal, i.p.). The extra thoracic trachea was exposed by a midline incision in the neck and cannulated at its caudal-most end with a bent 15 gauge Luer stub adaptor [18]. Care was taken not to damage the tracheal vasculature or the recurrent laryngeal nerves, as these nerves carry the afferents that regulate the cough reflex evoked from the trachea and larynx. The tracheal cannula was attached to a small length of tubing that terminated inside a water-jacketed organ bath that was continuously filled with warmed (37 °C) and humidified room air at a rate of 50 L/h. The tracheal mucosa (rostral to the caudal) was then exposed by a midline incision in the ventral tracheal wall. The tracheal mucosa (rostral to the caudal) was then exposed by a midline incision in the ventral tracheal wall. In all of the experiments described, this segment of trachea was superfused (3 mL/min) continuously with warmed (37 °C), oxygenated Krebs-bicarbonate buffer. Indomethacin and the neurokinin receptor antagonists were included in the buffer to prevent formation of neuromodulatory prostanoids and to prevent the local actions of tachykinins released from airway *C*-fibers upon stimulation, respectively. The buffer was recovered from the trachea using a gentle suction source positioned at the level of the larynx. We evoked cough by applying double-diluted solutions of citric acid (900 mmol/L, 450 mmol/L, 225 mmol/L, 113 mmol/L, 57 mmol/L, 29 mmol/L,

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