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Interaction of tobacco smoke exposure and ovalbumin-sensitization promotes goblet cell and submucosal gland metaplasia in guinea pigs

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ABSTRACT

Exposure to irritants such as tobacco smoke (TS) causes acute airway inflammation. Chronic exposure may cause airway remodeling contributing to enhanced airway resistance. We hypothesize that combining airway sensitization and inhalation of irritants enhances the number of mucous producing cells beyond either agent alone. Guinea pigs were antigen sensitized or treated with its vehicle. These two groups were further divided into daily exposure to TS or air. After 3 months airway reactivity to ovalbumin (OA) was determined, airway and blood samples were examined and lung substance P quantified. Combining sensitization and TS exposure increased airway reactivity to OA, goblet cell and submucosal gland populations. Airway eosinophilia was greatest in the OA-sensitized group exposed to air rather than with its combination with TS exposure. Lung substance P levels were similarly elevated in both OA-sensitized groups. Airway irritant exposure in which airway sensitization exists enhances the potential of mucus production, airway resistance and mucus plugging of the airways through increasing the number of goblet cells and submucosal glands.

1. Introduction

TS exposure causes airway irritation resulting in increased coughing, airway secretions and resistance. Since the mucociliary escalator is the first defense barrier in the lungs (Rogers, 1994), mucus secretion from goblet cells is an important component of the response to airway irritation. Increased numbers of goblet cells are found in central and peripheral airways of smokers with chronic bronchitis and chronic airflow limitation (Cavallesco et al., 2000; Mullen et al., 1987; Saetta et al., 2000). In addition goblet cell metaplasia and hyperplasia are found in the airways of laboratory animals following prolonged cigarette exposure (Lamb and Reid, 1969; Jones et al., 1973; Coggins et al., 1980; Wright et al., 1992).

Asthmatic airways can become hyperresponsive to TS exposure enhancing airway irritation and airflow resistance (Gold, 2000). Increased airflow resistance occurs through enhanced mucus secretion and direct and reflex bronchoconstriction (Barnes, 2004). The combination of the two becomes problematic in that reduced airway diameter increases the possible formation of mucus plugs. Status asthmaticus may then result, a life threatening situation. Increased airway goblet cells with mucus accumulation are found in airways of patients dying of severe acute asthma attacks (Aikawa et al., 1992). However, the extent to which airway sensitization and TS exposure impact the number of mucous producing cells is unknown.

In awake ovalbumin (OA)-sensitized guinea pigs addition of prolonged tobacco smoke exposure causes spontaneous cough and enhanced coughing to capsaicin and bradykinin aerosol challenge but few coughs to either histamine or methacholine challenges despite increased Penh units (Bergren, 2001a). This suggests capsaicin-sensitive C-fiber sensitivity was enhanced in this model.

We hypothesize that combining airway sensitization and prolonged TS exposure increases the number of mucus secreting cells beyond that of either treatment alone. A possible mechanism may involve C-fiber activation. We previously showed that the combination of airway sensitization and TS exposure enhances C-fiber sensitivity to capsaicin and bradykinin (Bergren, 2001a,b,c). Enhance C-fiber sensitivity would be expected to enhance both central and axon reflex activity. That latter may elevate substance P (SP) release as C-fibers produce, store and release SP (Saria et al., 1988). Therefore we also hypothesize that TS increases SP content in the lungs. SP is a powerful mucus secretogogue.

2. Materials and methods

2.1. Animals

Guinea pigs are commonly used as a model of airway reactivity and asthma as well as of COPD resulting from TS exposure

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Day	1	12	28	60	90	120	121
Injections OA or Veh	X	X					
Aerosol Challenges OA or Veh			X	X	X	X	
Exposures TS or Veh	→	→	→	→	→	→	
Begin study termination							X

Fig. 1. Timeline of protocol (OA is ovalbumin and Veh is vehicle). See Section 2 for details.

(Andersson, 1980; Zosky and Sly, 2007; Canning and Chou, 2008; Ricciardolo et al., 2008). Twenty male Hartley guinea pigs (Harlan, Minneapolis, MN) weighing approximately 300 g at the time of purchase were housed in the Creighton University School of Medicine Animal Resource Facility with food and water ad libitum. Guinea pig weights were determined upon arrival and at 30, 60, 90 and 120 days after the initiation of the study. This study was performed with the approval of the Creighton University Animal Use Committee.

2.2. Airway sensitization

Two days after their arrival (considered as Day 1, see Fig. 1 for the protocol timeline) 10 guinea pigs were injected intraperitoneally with ovalbumin (OA, 10 μg) and aluminum hydroxide (100 mg) in distilled water (0.5 cc). A booster injection of OA (10 μg/0.5 cc distilled water) was administered at Day 12 as per Andersson, 1980. Another 10 guinea pigs were injected with the OA vehicle. Airway reactivity to OA was determined at Day 26 by OA aerosol challenge of the airways and determined every 30 days thereafter (described below).

2.3. Tobacco smoke (TS) exposure

Beginning Day 1 and after the initial OA or vehicle injection, 5 OA-sensitized and 5 non-sensitized guinea pigs were exposed to mainstream TS from 5 standard 2R4F reference cigarettes with the filters removed (Tobacco and Health Research Institute, Lexington, KY) drawn into an exposure chamber (36 L in volume). Exposures were 30 min/day and 7 days/week for 120 days. The TS concentration inside the chamber averaged 5 mg/L during the exposure. The temperature inside the chamber was monitored and remained at room temperature during the 30 min exposure. Guinea pigs not exposed to TS were exposed to air in an identical manner. Bias flow in the chamber was 25 L/min, which is well above the estimated combined estimated minute volume of 5 L of the 10 guinea pigs housed in the chamber. This prevented development of either a hypoxic or hypercapnic environment or increased temperature within the chamber. Carbon dioxide content of the exhaust air from the chamber was monitored (LB-2 CO_2 monitor, Beckman, Schiller Park, IL). CO_2 content remained less than 1% of the exhaust air.

2.4. Assessment of airway reactivity to ovalbumin (OA)

OA solution (400 μg/ml) was aerosolized by ultrasonic nebulizers (Devilbiss 65, Somerset, PA). The output of nebulizer was attached to the input ports of small animal plethymographs (PLY4215, Buxco Electronics, Wilmington, NC). The aerosol was delivered for 30 s at 0.375 ml/min. Airway reactivity to OA aerosol challenge was determined by monitoring Penh units obtained from plethysmographs. The Penh unit is a dimensionless product of respiratory pause and peak expiratory pressure/peak inspiratory pressure, where respiratory pause is expiratory time (Te) minus expiratory relaxation time (Tr = 36% of Te) divided by Tr (Hamelmann et al., 1997). In guinea pigs Penh units and specific airway resistance are correlated measurements of airway reactivity (Hamelmann et al., 1997; Bergren, 2001a,b,c). Penh units were recorded for 1 min before the OA aerosol challenge and then for 8 min after the aerosol administration.

2.5. Histological studies

At the termination of the study guinea pigs received an intraperitoneal injection of pentobarbital (65 mg/ml) to induce stage four anesthesia. After cardiac arrest as determined by auscultation, the trachea was exposed and cannulated with polyethylene tubing connected to a 2-way stop cock. Then the thorax was opened. The lower left lobe was ligated and removed for analysis of its SP content (methods are detailed below). Paraformaldehyde solution in phosphate buffer (5 ml of 4%, pH 7.4) was injected into the lungs via the tracheal cannula. The stopcock was closed to the lungs. The lungs were removed from the chest, sectioned and placed in fresh paraformaldehyde solution and stored over night at 4 °C. Three tracheal rings from each group were removed near ring 10 below the larynx for microscopic examination. The rings were immediately placed in vials of the paraformaldehyde solution.

The tissues were processed into paraffin blocks. The tracheal sections were positioned so that the airway was cut through its circumference and sectioned at 5 μm. The slides were stained using hematoxylin and eosin, Alcian blue or toluidine blue standard staining procedures and cover-slipped. The tracheal epithelium was placed in the mid-microscopic field. Goblet cells were counted in the epithelium in 5 different fields of high power (400×), numbers averaged and reported as cells per mm. Polymorphonuclear leukocytes was also counted in the fields as those inside blood vessels, in the sub-epithelium and within the epithelium and reported as the total number present.

Tracheal sections were photographed at low magnification (40×) in order to see the entire tracheal ring. Using the ImageJ software (available free at NIH.gov) the area of the submucosa and submucosal glands was determined. The percentage of the area of the submucosal glands/submucosa was calculated. Positive identification of a submucosal gland included circular cuboidal cellular orientation with an obvious lumen, location within the submucosa and positive mucus staining by the Alcian blue or toluidine blue (Widdicombe et al., 2001).

Blood samples were taken from the jugular vein for blood smears on slides. The preparation was dried and then stained by Diff Quick as per the manufacturer's recommendation (Dade Behring, Deerfield, IL, USA). Polymorphonuclear leukocytes (PMNs) were counted per field at 400×. Five fields were counted for each individual and averaged. Counting was conducted where the cells were one thick in confluence.

2.6. Analysis of SP content of lung tissue

The ligated lower left lobe was placed in 5 ml of ice chilled normal saline and homogenized. The sample was then placed in sealed containers and stored at −70 °C until analysis by enzyme immunoassay (Cayman, Ann Arbor, MI, USA) The procedure was as directed by the manufacturer. Colorimetric assay of the samples in a 96-microwell plate was accomplished using a microplate reader (Dynatech MR5000, Chantilly, VA, USA).

2.7. Statistical analysis

The mean, standard deviation and the standard error of the mean of each variable were determined. Testing the null hypothesis for differences among means of weights, peak Penh units to OA aerosol challenge, goblet cell, submucosal gland and eosinophil populations in the airways and PMNs in blood smears and SP