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Salbutamol improves markers of epithelial function in mice with chronic allergic pulmonary inflammation

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ABSTRACT

We investigated the effects of salbutamol on the markers of epithelial function in a murine model of chronic allergic pulmonary inflammation by recording the ciliary beat frequency (CBF) and the transepithelial potential difference (PD) in vivo. Mice were sensitized and received four challenges of ovalbumin (OVA group) or 0.9% saline (control group). Forty-eight hours after the 4th inhalation, we observed eosinophilia in the bronchoalveolar lavage and epithelium remodeling with stored acid mucus in the OVA group ($P < 0.001$). No difference in the baseline CBF was noticed between the groups; however, the OVA group had a significantly lower baseline PD ($P = 0.013$). Salbutamol increased the CBF in all groups studied, and the dose response curve to salbutamol increased the PD in the OVA group from 10^{-4} M to 10^{-2} M. We suggest that salbutamol affects the CBF and the depth of the periciliary layer, which, in great part, determines the ability of the cilia to propel the mucus layer. This effect may have a positive impact on airway mucociliary transport in asthma and may have clinical implications.

1. Introduction

Mucociliary transport in the airways is governed by three primary factors: (1) the ciliary beat frequency (CBF), which depends on the coordination of the cilia (2) the quantity and quality of mucus as well as the rheologic characteristics of airway secretions derived from goblet cells and submucosal glands and (3) the interaction between cilia and mucus, which is modulated by periciliary fluid and determined by ion and water transport in the airway epithelium (Bennett, 2002).

Mucociliary transport is decreased in experimental models of asthma and in allergic asthmatics (Wanner et al., 1975; Pavia et al., 1985; O'Riordan et al., 1992; Daviskas et al., 1995, 2005) and contributes to an increase in infection susceptibility. In asthmatics, changes of the bronchial mucosa accompanied by epithelial thickening, goblet cell hyperplasia with altered rheological properties and changes in ion and water transport across airway epithelium (Galietta et al., 2002; Davies, 2009) are involved in the impairment of mucus transport.

β_2-Adrenoceptor agonists (β_2-agonists) are widely used as bronchodilators in the treatment of asthma because of their potent bronchodilating effects on airway smooth muscle. In addition to being bronchodilators, they may also have anti-inflammatory properties, including inhibition of granulocyte functions (Yasui et al., 2006). β-agonists also have multiple effects on airway epithelial cells, including stimulation of CBF (Verdugo et al., 1980; Devalia et al., 1992; Frohock et al., 2002) and stimulation of chloride secretion toward the airway lumen, which, theoretically, could improve mucus hydration by promoting water secretion onto the airway surface. Stimulation of CBF (Frohock et al., 2002) and chloride secretion (Davis et al., 1979) could contribute to the enhanced mucociliary transport observed after β-adrenergic inhalation (Laube et al., 2007).

A previous study showed that β-adrenergic agonists, such as salbutamol and salmeterol, could reverse human neutrophil elastase-induced mucociliary dysfunction in vivo in the trachea of sheep (Sabater et al., 2005). Several studies have reported a stimulating effect of beta-2-agonists on the CBF, but most studies are based on *in vitro* data (Verdugo et al., 1980; Sanderson and Dirksen, 1989; Devalia et al., 1992) and, to our knowledge, there were no previous studies performed in an experimental model of asthma in vivo.

The purpose of our study was to evaluate the effects of salbutamol in the airway epithelial barrier in a murine model of allergic

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pulmonary inflammation by recording the CBF and transepithelial potential difference (PD) in vivo.

2. Materials and methods

2.1. Experimental groups

The experimental protocol was approved by the Institutional Ethics Committee of the School of Medicine of the University of São Paulo. The animals received care in compliance with the "Principles of Laboratory Animal Care" published by the National Institutes of Health (NIH publication 86-23, revised 1985). Male Swiss mice (6–8 weeks old, 40–50 g) were divided at random into five groups: (a) naïve (subjected to the CBF protocol); (b) control (subjected to the saline sensitization and CBF protocols); (c) OVA (ovalbumin, subjected to the OVA sensitization and CBF protocols); (d) control (subjected to the saline sensitization and PD protocols) and (e) OVA (subjected to the OVA sensitization and PD protocols).

2.2. Sensitization protocol

Male Swiss mice were immunized intraperitoneally by an injection of 50 μg of ovalbumin (OVA) (Advanced Nutrition Ltd., Rio de Janeiro, Brazil) in the presence of 6 mg of $Al(OH)_3$ adjuvant (Pepsamar, Sandei-Synthelabo SA, Rio de Janeiro, Brazil) diluted in a 0.2-ml saline solution on days 1 and 14. Aerosolized OVA (1% challenges) were started 1 week after the second immunization, on days 22, 24, 26 and 28. The mice were placed in a Plexiglas box (30 cm × 15 cm × 20 cm) coupled to an ultrasonic nebulizer (US-1000; ICEL, São Paulo, SP, Brazil) and exposed to 1% OVA for 30 min. Control mice received an intraperitoneal injection of the adjuvant and were exposed to a nebulized aerosol of saline (0.9% NaCl) at the same time points. Mice were subjected to the CBF or PD protocols 48 h after the last challenge.

2.3. Experimental design

Forty-eight hours after the last challenge, the animals were anesthetized with 50 mg/kg ketamine (Ketamine, Cristália, Brazil, 50 mg/ml) and 40 mg/kg xylazine (Rompum, Bayer, Brazil, 2% solution) by intramuscular injection. Supplemental doses were administered as needed. The animals were kept in spontaneous respiration, and the humidity and temperature of the room air were controlled (60% and 25 °C, respectively). The mice were put on a rigid surface, the anterior cervical region was dissected, and a window of about 3 mm of the frontal tracheal wall was removed in order to expose the posterior tracheal epithelium for CBF or PD measurements, as described below. At the end of the protocol, the abdominal aorta was dissected, and the animals were killed by exsanguination. The animals were then submitted to bronchoalveolar lavage. Finally, lung, trachea and the nose of each animal were removed for histological analysis.

2.4. Ciliary beat frequency

After anesthesia, the tracheal epithelium was exposed, and CBF was measured by a modification of a videoscopic technique described previously (Pazetti et al., 2008). Briefly, the technique consists of focusing on a group of cilia through an optical microscope (10× objective, 10× ocular) connected to a video camera (Sony, Model 3CCD Iris), with the resulting image being sent to a monitor (Sony Trinitron). A stroboscopic light (Machine Vision Strobe, Model 5000, USA) is placed in front of the ciliary epithelium and emits flashes (0–30 Hz). The incident light illuminating the ciliated epithelium is reflected from the cilia, which is packed together, and from the thin layer of mucus covering the cilia. This reflection is cyclic because its direction changes according to the movements of the underlying cilia. By manual control, the frequency of the stroboscopic light is changed until it is the same as the CBF, and the observer cannot distinguish the ciliary beat. The CBF was measured in the trachea of anesthetized and tracheostomized mice (breathing spontaneously) from naïve, control and OVA groups at baseline and after 5 min of salbutamol (3.5×10^{-3} M or 3.5×10^{-4} M) inhalation in a Plexiglas box (30 cm × 15 cm × 20 cm) coupled to a Devilbiss nebulizer.

2.5. Transepithelial electric potential difference (PD)

A technique previously described (Festa et al., 1985a,b) was used as follows. After anesthesia, the tracheal epithelium was exposed, and the PD was measured using two flexible agar bridges saturated with potassium chloride (Lacaz-Vieira and Procópio, 1988) and connected to a calomel half-cell – one for reference and the other, experimental; both were connected to a grounded electrometer. Because of the saturated agar bridges, a correction for diffusion potential was not necessary when measuring at different ion concentrations. The reference electrode was placed in the submucosa of the mice, and the test electrode was placed on the epithelial surface of the trachea and palate. Measurements were performed on the palate of the animals as a positive control. The PD was computed as the mean of the three measurements.

The PD was measured in the control and OVA groups after baseline measurement, saline inhalation and during a dose–response curve to salbutamol inhalation (10^{-4} M, 10^{-3} M and 10^{-2} M, $n = 8$ for the control group and $n = 7$ for the OVA group) to determine the effect of salbutamol in the generation of the tracheal PD in vivo. In each salbutamol challenge, tracheostomized and anesthetized mice were placed in a Plexiglas box (30 cm × 15 cm × 20 cm) coupled to a Devilbiss nebulizer during 5 min of spontaneous tidal breathing; after that, the PD was measured. There was a 5 min interval between challenges.

2.6. Bronchoalveolar lavage

After the CBF or PD measurements, bronchoalveolar lavage (BAL) was performed to evaluate inflammatory cells in control and OVA groups ($n = 6$ for both groups) by introducing 0.5 ml of sterile saline into the lungs via a tracheal cannula and withdrawing the fluid into a test tube on ice. The recovery volume was over 95% of the instilled fluid. This procedure was repeated 3 times. The fluid collected was centrifuged at 900 × *g*, for 8 min, at 5 °C, and the cell pellet was re-suspended in 1 ml of physiological saline. Total cells were counted using a Neubauer hemocytometer chamber and an optical microscope with a magnification of 1000×. BAL differential cell counts were performed using a cytocentrifuge; slides were prepared by centrifugation of each sample at 900 × *g* for 6 min (Cytospin 2, Shandon Scientific, Pittsburgh, PA). These slides were stained by Giemsa stain, and differential counts of at least 200 cells were made according to standard morphologic criteria ($n = 6$ for the control group and $n = 7$ for the OVA group).

2.7. Histological analysis of the nasal cavity, trachea and lung

After BAL, the head of the animal was removed, and the nasal cavity was flushed in a retrograde manner through the nasopharyngeal orifice with 5 ml of 10% neutral buffered formalin. Excess soft tissue and the lower jaw were removed, and the head was immersed in formalin for 1 day and then decalcified in 5% EDTA for 14 days. After this period, 5-μm-thick sections were taken from the nasal cavity immediately posterior to the upper incisor teeth. This approach permits examination of the major epithelial types in the nasal cavity and accurate interpretation of changes in the epithelial