

Consecutive allergen challenges resulted in decreased bronchial responsiveness

HE Jianxin 贺建新, JIANG Zaifang 江载芳

HE Jianxin 贺建新 (Respiratory Department, Beijing Children's Hospital, Capital University of Medical Sciences, Beijing 100045, China)

JIANG Zaifang 江载芳 (Respiratory Department, Beijing Children's Hospital, Capital University of Medical Sciences, Beijing 100045, China)

Correspondence to: (Tel: . Fax: . E-mail:)

Keywords

asthma;SD rat;airway responsiveness

Abstract

Objective To investigate bronchial responsiveness to acetylcholine in allergic airway inflammation of SD rats.

Methods SD rats were immunized and challenged by chicken ovalbumin (OVA). Airway responsiveness, acetylcholine (Ach) provocation concentration needed to increase baseline airway resistance by 200% (PC 200) were measured.

Results The value of baseline airway resistance in asthma group was significantly higher than that in control group (2.282 ± 0.128 vs 3.193 ± 0.239 ; $P < 0.01$). After multiple ovalbumin exposures, airway responsiveness to intravenous injection of acetylcholine decreased significantly ($-\text{LogPC } 200 : 4.006 \pm 0.554$ vs 2.059 ± 0.262 ; $P < 0.01$). Bronchial alveolar lavage fluid (BALF) and lung tissue specimen analysis indicated that airway allergic inflammation was present.

Conclusions The study demonstrates a dissociation between the bronchoconstrictor response and bronchial hyper-responsiveness and indicates that multiple ovalbumin exposures induces persistent bronchoconstriction with airway hypo-responsiveness despite airway allergic inflammation.

Patients with asthma have characteristic symptoms or intermittent airway obstruction, airway hyper-responsiveness and chronic airway inflammation. The exact mechanisms of airway responsiveness in asthmatic responses are not well understood. Currently, airway hyper-responsiveness is often regarded as a consequence of airway inflammation. Researches on airway hyper-responsiveness in in vivo animal models have not been reported in China. The authors used SD rats to establish allergic airway inflammation and to investigate the changes of airway responsiveness.

METHODS

Rats

Twenty-eight male SD rats, weighing 180-200 g, were randomly divided into two groups (control group, Group C; asthma group, Group A). Each group included 14 animals.

Laboratory methods

Asthma group rats were actively sensitized by intraperitoneal injection of 100 mg ovalbumin together with 100 mg $\text{Al}(\text{OH})_3$ in 1 ml 0.9% NaCl on the first day of study (day 0). As a second adjuvant, concentrated preparations of 6.4×10^9 heat-killed Bordetella pertussis bacilli were given intraperitoneally at the same time. Control group rats were injected peritoneally with 1ml 0.9% NaCl. Fourteen days after sensitization (day 14), rats were placed in a purpose-designed box ($50 \times 40 \times 30$ cm) and exposed to aerosolized 1% ovalbumin saline solution, which was generated with an ultrasonic medical nebulizer, for consecutive 7 days (once/day, 20 min/once). Control groups were challenged by 0.9% sterile NaCl.

Measurements of bronchial responsiveness to acetylcholine and airway resistance

Twenty-four hours after the last ovalbumin challenge, all animals were anaesthetized intraperitoneally with urethane (1 mg/kg). A tracheal cannula was inserted into the lumen of the trachea. A catheter was inserted into the right jugular vein for the administration

of intravenous drugs. Animals were placed in a closed chamber (5500 cm³) and connected to a small animal respirator and ventilated with 40% oxygen and air mixture (ventilator parameter: PIP 8 cmH₂O, RR 60 bpm, Ti 0.4s, Ti/Te 1:1.5). Airway pressure was measured with a pressure transducer, with the terminal side attached to a side port of the intratracheal cannula. Tidal volume and air flow were measured with a plethysmograph connected to a pressure transducer. The signals from the transducer were digitalized with a 12-bit analogue-digital board connected to an IBM personal computer and analyzed with software which was programmed to instantaneously calculate airway resistance. Airway resistance = $\Delta P / \Delta V / \Delta T = \Delta P / \Delta F$.

Animals were initially injected with suxamethonium (1 mg/kg) to stop spontaneous breathing. Increasing concentrations of acetylcholine (1×10^{-2.5} mol/L, 1×10⁻² mol/L) were given by intravenous injection, with the initial concentration set at 1×10⁻³ mol/L. The provocation concentration to produce an increase in airway resistance of 200% over baseline (PC 200) was calculated by log-linear interpolation of concentration-response curves from individual animals.

Bronchoalveolar lavage and histological analysis of lung tissue

The left lung was lavaged with three volumes of 3 ml each of 0.9% sterile NaCl. Lavage fluid was centrifuged (1250 r/min) and the cell pellet was washed in 1ml phosphate buffered saline (PBS). The total numbers of leukocytes in the bronchoalveolar lavage fluid and differential cell counts, from cytospin preparations stained by May-Grünwald-Giemsa, were determined under an optical microscope. The right-middle lobe was sliced and embedded in paraffin for histologic analysis. Statistical analysis

Data were expressed as mean±s PC 200 measurements were Log 10 -transformed. An increase in mean -LogPC 200 represents an increase in airway responsiveness. A probability value of less than 0.05 was regarded as significant.

RESULTS

Baseline airway resistance and bronchial responsiveness of acetylcholine

Intravenous acetylcholine caused a concentration-dependent increase in airway resistance. Baseline airway resistance of the asthma group was significantly higher than that of the control group. After multiple ovalbumin exposures, the mean -LogPC200 (2.059±0.262) was significantly lower than that in control SD rats (4.006±0.554) (Table 1).

Bronchoalveolar lavage

After multiple ovalbumin exposures, the numbers of total white blood cells, and percentage of eosinophils, neutrophils and lymphocytes (5.928±1.046, 1.338±0.254, 1.267±0.257 and 24.836±2.517, respectively) in bronchoalveolar lavage fluid increased as compared with control group (0.533±0.169, 0.080±0.045, 0.217±0.088 and 8.492±0.573, respectively;P<0.01). (Table 2).

Histological analysis of lung tissue

An inflammatory condition of the airways and vessels (small, median and large) in which there was tissue eosinophilia and a predominance of lymphocytes and neutrophils was recognized. The bronchi were in a constricted condition.

DISCUSSION

The present study has shown that following consecutive 7 days exposures to ovalbumin, there was a significant degree of bronchoconstriction. In contrast, airway responsiveness to acetylcholine was found to be reduced significantly. Despite the attenuation of airway responsiveness in the repeatedly exposed SD rats, there were significant increases in eosinophils, neutrophils and lymphocytes in bronchoalveolar lavage fluid and in bronchial airway tissue. Thus, there was a dissociation between the bronchoconstrictor response and bronchial hyper-responsiveness and between the onset of bronchial hyper-responsiveness and allergic inflammatory cells influx in the airways in the response to multiple exposures to ovalbumin.

In this study, baseline airway resistance increased significantly after multiple ovalbumin exposure. Our lung tissue specimen analysis indicated that bronchoconstriction was mainly due to airway wall smooth muscle constriction. Contraction of airway smooth muscle itself may directly decrease airway compliance. Another element that could affect airway responsiveness is changes in the components of the extra-cellular matrix in the airways. By increasing airway wall stiffness, changes in the extra-cellular matrix might represent an attempt to oppose bronchial hyper-responsiveness.[1] It is reported that after prolonged allergen exposure, changes in extra-cellular matrix can enhance or protect against airway hyper-responsiveness depending on the extent and distribution of remodeling despite persistent airway inflammation.[2] So it appears unlikely that the inflammatory cells are playing modulatory roles in bronchial hypo-responsiveness. Except for the above factors, the reason why airway hyper-responsiveness was not observed in our study can at least be partly explained by differences in the strains of rats and in the sensitization and exposure protocols used in these studies and our own. As high-IgE response rats, BN rats expressed Th2 cytokine (protein and mRNA) but SD rats expressed Th1 cytokine as low-IgE response rats.[3] It is possible that hypo-responsiveness may relate to the relatively higher levels of ovalbumin for the SD rats or that the SD rats may release more bronchoprotective agents on repeated exposures to ovalbumin. The loss of protective mechanisms could contribute to the persistent bronchial hyper-responsiveness observed in human asthma.

The mechanisms underlying the decrease in airway responsiveness following multiple exposures to ovalbumin are not clear. There is an alternative hypothesis that the basic abnormality in asthma might be loss of normal mechanisms restricting airway narrowing rather than amplification of mechanisms of narrowing. Restraints on luminal narrowing depend on the mechanical properties of the total airway wall and of the surrounding lung tissue. For the intra-pulmonary airways, the most important factor stabilizing the airway wall against the effects of airway smooth muscle contraction is the attachment of alveolar walls to the external perimeter of the airway wall. Amplifying factors might include the thickening of the airway wall on the internal side of the contracting muscle, increased airway wall compliance and reduced effects of extra-airway extension. In the present animal model, the value of airway responsiveness from acetylcholine must be derived from the balance between these restricting and amplifying factors. In conclusion, we have described an in vivo SD rat model that displays characteristics of allergic airway inflammation. We postulate that changes in airway smooth muscle condition, lung tissue compliance and extra-cellular matrix components contribute to the development in loss of BHR.

REFERENCES

1. Sun J, Chung KF. Airway inflammation despite loss of bronchial hyper-responsiveness after multiple ozone exposures. *Respir Med* 1997;91:47-55.
2. Palmans E, Kips JC, Pauwels RA. Prolonged allergen exposure induces structural airway changes in sensitized rats. *Am J Respir Crit Care Med* 2000;161:627-635.
3. Renzi PM, Asaad AS, Yang J, et al. Cytokine expression in the presence or absence of late airway responses after antigen challenge of sensitized rats. *Am J Respir Cell Mol Biol* 1996;15:367-373.