

Original article

Investigation of the measurement of murine airway hyperresponsiveness and the therapeutic effects of budesonide on ovalbumin sensitized and challenged mice

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Background Airway hyperresponsiveness (AHR) is one of the most important characteristics of asthma. This study investigated the parameters, by which assess the airway responsiveness under tidal ventilation.

Methods Female BALB/c mice were sensitized and challenged with ovalbumin (OVA) (group A), and part of them were treated with budesonide aerosol (group B). All the mice were anaesthetized and mechanically ventilated. The values of tidal volume (Vt), airway pressure (PA), airway flow (F), expiratory lung resistance (RL) and dynamic compliance of the thorax and lung (CT-L) were recorded by the AniRes2003 animal lung function system. In addition, the expiratory volume in the first 0.1 second after the start of expiration (EV0.1) was obtained according to the flow-volume (F-V) curve. The maximal or minimal values of EV0.1, RL and CT-L were documented after each dose of methacholine (MCH) and compared with values from negative control group (group C).

Results (1) When the dose of MCH reached 100 ng/g or 200 ng/g, the decrease of Vt in group A was much more significant than group C ($P=0.001$, <0.001 respectively), but not so between groups B and group C ($P=0.974$, 0.362 respectively). (2) With the dose of 25, 50, 100 or 200 ng/g MCH, the decrease in percentage of EV0.1 in group A was much higher than group C ($P=0.012$, 0.025 , 0.001 , 0.003 respectively), while that in group B showed no significant difference as compared with group C ($P=0.507$, 0.896 , 0.972 , 0.785). (3) RL and CT-L with the dose of 200 ng/g MCH, there was a statistically significant increase of RL in group A compared to group B or group C ($P<0.001$, <0.001 respectively), but no significant difference between groups B and C ($P=0.266$). With doses of 100 ng/g and 200 ng/g MCH, there was a statistically significant decrease of CT-L in group A compared to group B ($P=0.001$, <0.001) and group C ($P<0.001$, <0.001 respectively), but no significant difference between groups B and C ($P=0.775$, 0.310). (4) Histopathology: there were eosinophilic predominant peribronchial and perivascular inflammatory influx in murine lungs after OVA sensitizing and challenging, which could be counteracted by inhalation of budesonide in group B.

Conclusions The decline in EV0.1 in response to MCH challenge correlated with simultaneous changes in Vt, RL and CT-L, but more sensitively than all the other parameters. The decline in EV0.1 and inflammation in murine lung could be significantly alleviated by inhalation of nebulized budesonide solution, which indicated that EV0.1 to MCH is a valid measure of AHR in mice.

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Murine models currently serve as a very useful tool in research on the mechanisms and treatments of human respiratory diseases, including asthma, pulmonary interstitial fibrosis and chronic obstructive pulmonary diseases. Respiratory physiology, as one of the most important aspects in evaluating the lung function, has become an indispensable study. Much effort has been put into investigating ways to measure airway responsiveness in research on asthma using murine models. Measurements including invasive method^[1] and noninvasive method^[2,3] systemic administration of MCH^[4] and local administration of MCH^[5] have been employed to assess airway responsiveness in asthmatic models. However, no research has been reported on the sensitivity of the various parameters. Furthermore, there are few Chinese articles in this area.^[6] In this project, the measurement of murine respiratory physiology was explored by administration of methacholine (MCH) through jugular vein in an ovalbumin (OVA) sensitized and challenged murine model which was transtracheally incubated and mechanically ventilated. At the same time, the therapeutic effect of budesonide on airway hyperreactivity (AHR) and eosinophilic predominant, peribronchial and perivascular inflammatory response in OVA sensitized and challenged murine model was investigated.

METHODS

Animals and materials

Female BALB/c mice were bought from Animal Centre in Academy of Military Medical Sciences, and housed in Experimental Animal Centre (SPF grade) in Peking University People's Hospital. AniRes2003 animal lung function system was bought from Peking Biolab Company (China). Aluminium hydroxide gel was kindly gifted by Dr. Ted T Kung of Schering-Plough Research Institute (USA). Grade V OVA and MCH were from Sigma Inc. (USA). Budesonide mixture (Pulmicort Respules) was a kind gift from AstraZeneca Inc. (UK and Sweden).

Grouping, OVA sensitization and challenge

Eighteen female BALB/c mice (8 weeks old, 22 g to 24 g) were randomly divided into 3 groups: OVA sensitized and challenged asthma group (A), budesonide therapy group (B) and negative control group (C). The murine asthma model was established as previously described.^[7] Briefly, twelve mice were sensitized by 0.5 ml allergen solution (containing 15 µg OVA and 2 mg aluminium hydroxide gel) individually through intraperitoneal injection on day 0 and day 5. Twelve days after sensitization, the mice were challenged with aerosolized OVA solution (10 ml, NaCl 5 g/L, generated by a PARI Boy 37, Germany, nebulizer) for 1 hour twice daily on days 12 to 14. The aerosol was circulated through a glass chamber (30 cm×30 cm×45 cm). All mice were placed into the chamber for 1 hour in the morning and again in the afternoon (4 hours apart) on the same day. Six mice (group A) were treated with inhalation of 2 ml saline aerosol for 15 minutes per day while another 6 mice (group B) were treated with inhalation of 1 mg budesonide aerosol for 15 minutes per day on days 15 to 17. Six mice (group C) were given 0.5 ml aluminium hydroxide gel (2 mg in saline) through intraperitoneal injection on day 0 and day 5, then managed just as group A.

All mice were prepared for measuring their airway hyperresponsiveness to MCH on day 18, following which they were killed and their lungs were taken for histopathology.

Measurement of airway reactivity to methacholine

On day 18, mice were anaesthetized by an intraperitoneal injection of phenobarbital sodium (90 mg/kg), intubated with transtracheal (2.8 cm in length, 0.9 mm in outside diameter and 0.75 mm inside diameter) and jugular venous catheters and mechanically ventilated with AniRes2003 lung function system, a computer assisted ventilator. The ventilator's basic parameters were set as follows: tidal volume as 10 μ l/g, respiratory rate as 100 per minute and time ratio of inspiration/expiration as 1:1.5. To insure the comparability of the results, the same volume of saline was injected via jugular vein before MCH challenge. There were no changes in the values of RL or CT-L. Airway pressure (PA) and delivered volume (Vt) were measured continuously and airflow (F) was calculated from the derivative of volume. Expiratory lung resistance (RL) and dynamic compliance of the thorax and lung (CT-L) were calculated in every respiratory cycle on the base values of PA and Vt estimated by AniRes2003. The expiratory volume in 0.1 second at the beginning expiration of tidal volume (EV0.1) was obtained from flow-volume (F-V) curve. MCH doses were given approximately 5 minutes apart. The initial ventilation control under the preset tidal volume as 10 μ l/g was maintained during the experiment.

Histopathology

The lungs were perfused in situ by flushing the pulmonary vasculature with buffered saline and fixed in 4% phosphate buffered formalin. Paraffin sections of the lung tissues were then stained with haematoxylin and eosin and were observed under light microscope.

Statistical analysis

The increase or decrease of the values of RL or CT-L and Vt was their values after MCH challenge compared with their corresponding values before the same dosage of MCH challenge. Data were analysed with SPSS11.5 and presented as the mean $\pm$ standard error (SE) of measurement. Statistical significance was determined by one-way ANOVA (LSD test if homogenous variance, Dunnett's-T3 test if heterogeneous variance). P value less than 0.05 was accepted as statistical significance.

RESULTS

Murine respiratory physiology after OVA sensitization and challenge

F-V curves

The changes of the F-V curve in the three experimental groups before and after administration of MCH are shown as [Fig. 1](#). With increasing doses of MCH, the tidal ventilation F-V circle shrunk gradually since the decrease of Vt resulted from bronchial constriction. The decrease in percentage of EV0.1 (the minimum value after each dose of MCH compared with the basement value) calculated from the F-V curve served as an index of bronchoconstriction ([Fig. 2](#)). With the dose of 25, 50, 100 or 200 ng/g MCH, the decrease percentage of EV0.1 was (30.52 $\pm$ 11.22)%, (53.85 $\pm$ 20.78)%, (73.68 $\pm$ 14.21)% and (93.49 $\pm$ 4.98)% in group A respectively, (6.83 $\pm$ 2.08)%, (17.47 $\pm$ 3.15)%, (37.59 $\pm$ 11.08)% and (67.11 $\pm$ 13.89)% in group B and (9.65 $\pm$ 4.81)%, (20.36 $\pm$ 10.92)%, (37.88 $\pm$ 16.37)% and (60.39 $\pm$ 13.71)% in group C. The decrease percentage of EV0.1 was much higher in group A than in group C at each dose (P=0.012, 0.025, 0.001, 0.003), while there was no statistical difference between groups B and C (P=0.507, 0.896, 0.972, 0.785). Therefore, the expiratory volume in the first 0.1 second after the start of expiration was reduced because of the bronchoconstriction to MCH, being smallest in asthma group but

ameliorated in the budesonide therapy group.

Change of Vt

After murine airways were challenged with MCH intravenously, the tidal volume (Vt) was decreased with the functional residual volume increasing. Compared with its basic value, Vt was reduced inversely with the increasing dosage of MCH (Fig. 3). When the dosage of MCH was increased to 100 ng/g, murine Vt was decreased by (58.82 $\pm$ 17.08)% in group A, (28.22 $\pm$ 10.41)% in group C and (28.46 $\pm$ 9.07) % in group B. There was a statistically significant difference in the decrease of Vt between group A and group C (P=0.001) but no difference between groups B and C (P=0.974). When the dosage of MCH was 200 ng/g, murine Vt was decreased by (88.97 $\pm$ 9.05)% in group A, (48.38 $\pm$ 11.72)% in group C and (54.72 $\pm$ 13.75)% in group B. As above, there was a statistically significant difference in the decrease of Vt between group A and group C (P<0.001), and no difference between groups B and C (P=0.362). In other words, the tidal volume of OVA sensitized and challenged mice was significantly decreased compared with that of negative control mice, which could be antagonized by inhalation of nebulized budesonide.

The change of expiratory lung resistance and dynamic compliance of the thorax and lung to methacholine

When the dosage of MCH was at 200 ng/g, RL was increased by (2171.79 $\pm$ 582.58) %, (717.46 $\pm$ 695.12) % and (358.32 $\pm$ 217.15) % in groups A, B and C respectively. RL in group A was significantly increased when compared with that in group B or group C (P<0.001 and P<0.001) but there was no significant difference in RL between group B and group C (P=0.266) (Fig. 3). With the dosage of 100 ng/g and 200 ng/g, CT-L was decreased by (69.40 $\pm$ 6.42)% and (92.66 $\pm$ 2.23)% in group A, (37.85 $\pm$ 4.17)% and (65.77 $\pm$ 5.57)% in group B and (35.68 $\pm$ 4.95)% and (59.19 $\pm$ 4.78)% in group C. CT-L in group A was significantly decreased when compared with group B (P=0.001 and P=0.001) and group C (P<0.001 and P<0.001 respectively), but no significant difference between group B and group C (P=0.775, 0.310) (Fig. 3). Therefore, RL increased and CT-L decreased more significantly in the OVA sensitized and challenged group than in the negative control group. The above abnormalities could be alleviated by administration of nebulized budesonide. Changes of RL and CT-L in budesonide therapeutic group were similar to those in control group.

In addition, the basic values of RL and CT-L before MCH challenge were recorded and analysed. There was no difference among the three groups.

There was no statistical difference in the other parameters with dosages of 25 ng/g or 50 ng/g MCH (except the decrease percentage of EV0.1) among the three groups.

Histopathology

Murine airway epithelia in group C were smooth with no inflammatory cells infiltration. It was found that there was peribronchial and perivascular EOS predominant infiltration in group A. Compared with OVA sensitized and challenged group, EOS predominant infiltration was greatly attenuated in budesonide treated group (the data not shown).

DISCUSSION

Precisely identifying the properties of AHR is an essential prerequisite in the experimental study of murine asthma models for characterizing AHR in human asthma and investigating therapies

for asthma. Several methods, including invasive and noninvasive ones, with different parameters have been employed in evaluating airway responsiveness of murine asthma models abroad. The parameters most commonly chosen for assessment of the airway reactivity are mainly focused on lung resistance and lung compliance.

It is well known that FEV₁ (the forced expiratory volume in the 1st second of expiration) is one of the most sensitive indices in human challenge and dilation test. It is quite natural to extend this to the measure of airway reactivity in murine asthma models. However, it is impossible to make the mouse expire forcefully. Lai and his associates suggest using the forced expiratory volume in 0.1 second (FEV_{0.1}) as a useful indicator for evaluating the murine airway constriction. Briefly, the lungs of the anaesthetized mice were inflated to total lung capacity and deflated with a negative pressure of 40 cmH₂O, which produced the maximal expiratory flow. The changes in flow, volume and airway pressure were recorded and the MEFV (maximal expiratory flow volume) curve was obtained at both baselines (before MCH challenge) as well as at the time of the maximal response to MCH. The value of FEV_{0.1} was calculated from the MEFV curve. FEV_{0.1} could serve as an indicator for evaluating airway constriction in that it resembles the FEV₁ in humans. However, MEFV manoeuvre adopted in Lai's experiment was complex and difficult. In addition, the time to test FEV_{0.1} after MCH challenge is hard to determine.

It has been reported that the analysis of tidal flow patterns is a useful indicator of bronchial obstruction in adults and children and has also been employed in toxicological studies with mice and guinea pigs. So in this investigation, the measurement of murine respiratory physiology was under tidal ventilation and the value of EV_{0.1} was easily obtained (from the normal respiratory cycle) in addition to the classical parameters RL, CT-L and V_t. As a result, the EV_{0.1} to MCH challenge coincided with simultaneous changes in V_t, RL and CT-L, but was more sensitive than all the other parameters (statistically differentiate the three groups at an MCH dose of 25 ng/g while other indices at more than 100 ng/g). This may be because the expiration is no more than 0.36 second and the EV_{0.1} is about 90% of V_t before MCH challenge. So the change during the first 0.1 second is more obvious than other parameters. F-V curves were used to represent the airway responsiveness to different doses of MCH. There was no significant difference in F-V curves among three groups before MCH challenge, which suggested that the OVA sensitized and challenged mice did not suffer from the obvious expiratory obstruction as allergic asthma patients do before systemic nonspecific challenges. This might also be associated with the properties of murine airway constitution with relatively thin epithelium and wide airway lumen compared with humans. When stimulated with MCH, the expiratory flow decreased more rapidly in OVA sensitized and challenged mice than that in negative controls (Fig.1-A, data shown here is the smallest tidal volume after each dosage and its F-V curve). With the increasing dosage of MCH, this trend became clearer. The value of EV_{0.1}, as an index reflecting the bronchospasm, markedly decreased in OVA sensitized and challenged mice than in negative controls. After intravenous MCH, EV_{0.1} was obviously decreased per F-V curves in all experimental groups. However, EV_{0.1} in asthma model group exhibited the greatest response among the three experimental groups. When the value of EV_{0.1} was normalized by their weight, tidal volume became comparable in different individuals and groups.

The values of RL, CT-L, V_t and EV_{0.1} were all ameliorated in the budesonide treated mice compared with OVA sensitized and challenged mice, and the eosinophilic dominant inflammatory infiltration was also attenuated. This indicated that budesonide alleviated the AHR and the inflammation of lung, which agrees with the former report. And this, in turn, illustrates

that the method and parameters employed here could be effective in distinguishing between different levels of airway responsiveness.

In addition to the invasive methods mentioned above, there is still a noninvasive method for measuring AHR in murine asthmatic models. Noninvasive whole body plethysmographic (WBP) technology was applied to document a dimensionless variable, enhanced pause, for monitoring bronchoconstriction in conscious animals by Hamelmann and his colleagues.² However, the results can be influenced easily by the temperature and humidity changes caused by inspired and expired air of mice placed in a WBP chamber.¹⁵ It is worthwhile to mention that respiratory mechanics could be influenced by respiratory frequency and volume.¹⁶ Further, the variation of tidal volumes can also influence the balance of serum acid/base levels, which in turn influence the respiratory function.¹⁷ It is reported that this balance can be maintained at a good level when the tidal volume is within the range 8 to 10 $\mu\text{l/g}$.^{18,19} So in our experiment to measure airway reactivity, ventilating rate and tidal volume were set at 100 times per minute and 10 $\mu\text{l/g}$ respectively at the beginning of the measurement of AHR in each mouse.

In conclusion, the decline in EV0.1 to MCH challenge correlated with simultaneous changes in V_t , RL and CT-L, but was more sensitive than all the other parameters. The allergy induced decrease in EV0.1 and airway inflammation in murine lung could be significantly alleviated by inhalation of nebulized budesonide solution, which indicated that EV0.1 to MCH is a valid measure of AHR in mice.

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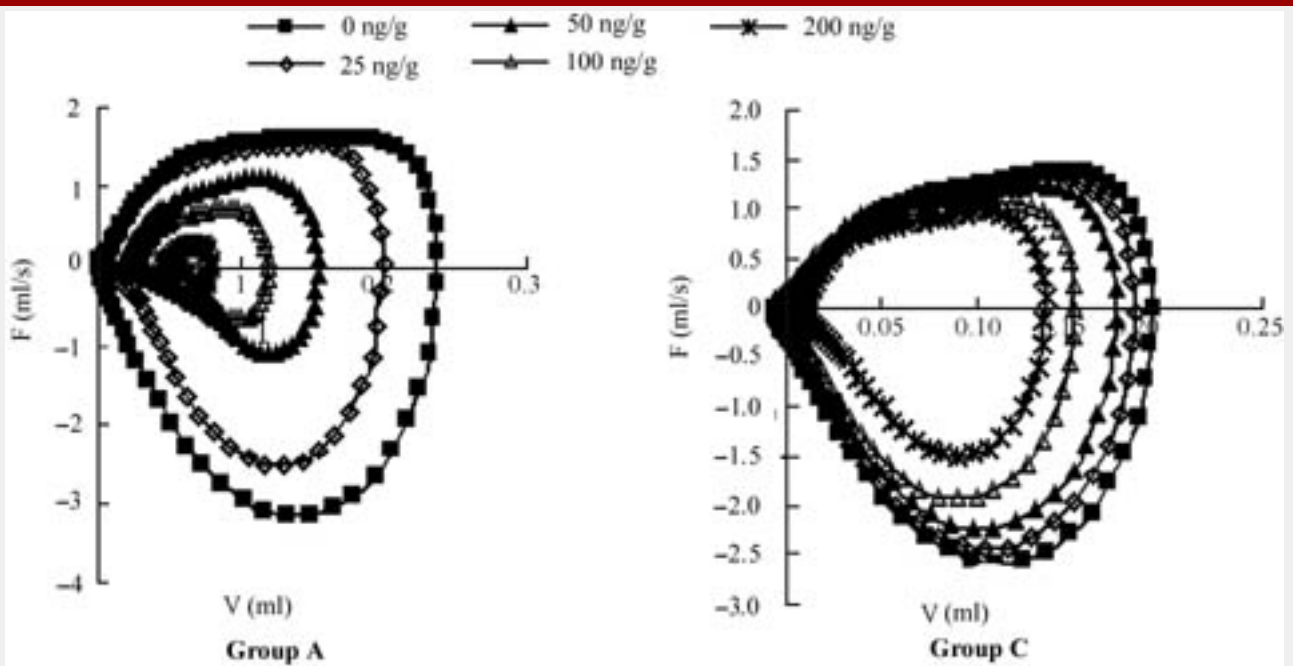


Fig. 1. Example of F-V curve of group A and C. The diagram shows airway flow plotted against volume in anaesthetized mice. The curve above the X axis is inspiration and below the X axis is expiration. The ring of F-V was shrunk with the increasing doses of MCH, and which is most obvious in group A and least in group C with group B in between (figure not shown here) to the increasing doses of MCH.

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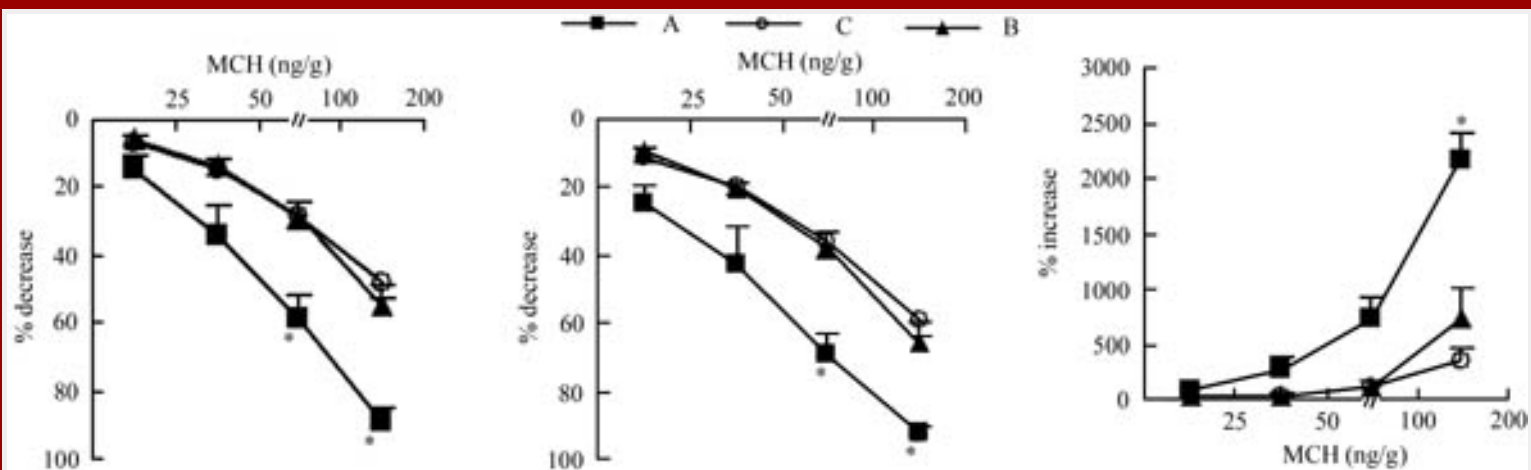


Fig. 3. Decrease of Vt (%) or CT-L (%) and increase of RL(%) to different doses of MCH in three experimental groups. Values represent mean $\pm$ SE (n=6 per group) of percent variation in tidal volume (Vt), lung resistance (RL) and compliance of thorax and lung (CT-L). *P<0.05 compared with group C and B.

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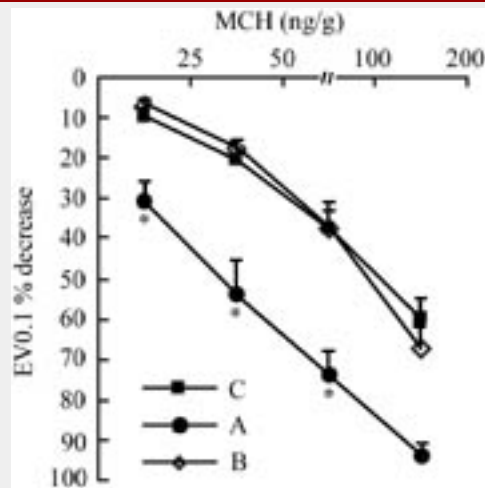


Fig. 2. The decrease percentage of EV0.1 with different doses of MCH. Values represent mean $\pm$ SE (n=6 per group) of percent decrease in EV0.1. *P<0.05 compared with group C and group B.

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