A NEW PARAMETER TO STUDY THE RESPIRATORY AIRWAYS HYPER-REACTIVITY IN AN OVALBUMIN-INDUCED RAT MODEL OF ASTHMA

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INTRODUCTION

Bronchial asthma is a chronic inflammatory disease of the respiratory airways, characterized by reversible bronchial obstruction produced by bronchospasm, mucosal edema and hypersecretion of adherent mucus that results from a complex interaction between inflammatory cells, chemical mediators and resident cells within the respiratory airways.¹

Allergic asthma is characterized by an allergen-specific immune response, the presence of airway...
inflammation, and airway hyperresponsiveness (AHR). In order to elucidate the contribution of each of these pathogenic components, there was a considerable interest in developing animal models that closely mimic the immunopathogenesis of the disease.\(^2\)

Table 1. Sensitizing protocols to ovalbumin (OVA)

<table>
<thead>
<tr>
<th>Intraportal administration of OVA</th>
<th>Aerosols administration of OVA</th>
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<tbody>
<tr>
<td>Lloyd et al, 2000 (3)</td>
<td>OVA+ aluminum hydroxide in days 0 and 12</td>
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<tr>
<td>McMillan et al, 2002 (20)</td>
<td>OVA+ aluminum hydroxide in days 0 and 12</td>
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<td>McMillan et al, 2005 (21)</td>
<td>OVA+ aluminum hydroxide in days 0 and 12</td>
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<tr>
<td>Hamelmann et al, 1999 (11)</td>
<td>OVA+ aluminum hydroxide in days 0 and 14</td>
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<tr>
<td>Tomkinson et al, 2001 (12)</td>
<td>OVA+ aluminum hydroxide in days 0 and 14</td>
</tr>
<tr>
<td>Hessel et al, 1995 (22)</td>
<td>7 days alternate</td>
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<tr>
<td>Janssen et al, 2000 (23)</td>
<td>7 days alternate</td>
</tr>
<tr>
<td>Henderson et al, 1996 (24)</td>
<td>OVA+ aluminum hydroxide in days 0 and 14</td>
</tr>
<tr>
<td>Blyth et al, 2000 (25)</td>
<td>7 days alternate</td>
</tr>
<tr>
<td>Sapong et al, 2003 (26)</td>
<td>Days 0 and 7</td>
</tr>
<tr>
<td>Kanehiro et al, 2001 (27)</td>
<td>OVA+ aluminum hydroxide in days 0 and 14</td>
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<tr>
<td>Choi et al, 2005 (7)</td>
<td>OVA+ aluminum hydroxide in days 0 and 10</td>
</tr>
<tr>
<td>Fernandez-Rodriguez et al, 2008 (14)</td>
<td>Days 0 and 5</td>
</tr>
<tr>
<td>Glaab et al, 2002 (6)</td>
<td>Day 0 OVA+ aluminum hydroxide+Bordetella Pertussis (subcutan)</td>
</tr>
<tr>
<td>Koerner-Rettberg, 2008 (9)</td>
<td>OVA+ aluminum hydroxide in days 1 si 7</td>
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</table>

Rodents develop AHR to several bronchoconstrictors after systemic and local allergen sensitization followed by allergen challenge via the airways. One of the largely used allergen is ovalbumin, and several protocols of sensitization have been used in inducing experimental models of asthma as summarized in Table 1.

The most commonly employed experimental models for studying AHR are using an \textit{in vitro} approach, namely organ bath studies of isolated tracheal strips harvested from the sensitized animals. An \textit{in vivo} model for studying AHR is particularly suitable since it will closely reflect changes in pulmonary response triggered by the allergen in the physiological environment.\(^3\)

**OBJECTIVE**

The primary objective of the present study was to evaluate the applicability of positive end-inspiratory pressure (PEIP) measurement as a valid indicator for the \textit{in vivo} assessment of airway hyperreactivity in a well-established model of allergic asthma in rats. The presence of AHR was further confirmed by the \textit{in vitro} evaluation of tracheal smooth muscle reactivity in isolated organ bath.

**MATERIAL AND METHODS**

**Animals**

Male Sprague-Dawley rats weighing 250-350 g fed ad libitum and housed at a 12 h light/dark cycle were divided into 2 groups: the control group (CON, n=8) and a group sensitized by ovalbumin (OVA, n=8). All investigations conformed to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health no. 85-23, revised 1996) and were approved by the Ethics and Deontology Committee of our university. The rats were killed at the end of the experiments by an overdose of intravenous sodium pentobarbital.

**Sensitization protocol**

Animals were sensitized to ovalbumin (OVA) according to the following protocol: (i) day 1 and 7 – intraperitoneal administration of 100 mg OVA emulsified with aluminum hydroxide in normal saline since aluminum hydroxide is widely used as an adjuvant to induce Th2 response and stimulate IgE synthesis; (ii) day 21 – induction of respiratory airways hyperreactivity by continuous nebulisation for 15 min with 5 ml OVA solution 5% (rate 5-6 L/min) and (iii) day 28 – induction of bronchospasm by continuous nebulisation for 1 h with 10 ml OVA solution 0,2 % (rate 5-6 L/min). (Fig.1)
Endotracheal intubation and mechanical ventilation

In vivo experiments in rodents require an adequate anesthesia and ventilation. Anesthesia inhibits respiratory musculature causing a shortage of oxygen and accumulation of carbon dioxide, thus leading to acidosis and eventually death. Without external respiratory assistance the animal would not survive the surgical shock. Accordingly ventilation is mandatory to compensate for the loss of the respiratory muscle function and provide gas exchange throughout the experiment.

![Figure 1. Sensitizing protocol with ovalbumin (intraperitoneal and nebulisation).](image1)

General anesthesia was induced using 50 mg/kg sodium pentothal (Sigma) intraperitoneally (without reported effects on tracheo-bronchial musculature). Pancuronium bromide (Pavulon 2 mg i.v), a non-depolarizing blocker of acetylcholine action at the motor end-plate of neuromuscular junction was also administered.

After local anesthesia of hipopharynx, epiglottis and glottis with xylene 4%, animals were intubated by means of a custom-made laryngoscope.4 (Fig. 2)

![Figure 2. Endotracheal intubation](image2)

Rats were mechanically ventilated throughout the experiments with a custom-made pressure-controlled ventilator with electronically preset respirations.5 Respiratory parameters were as follows: respiratory rate 80-90 breaths/min, tidal volume 10 ml/body weight), positive end-expiratory pressure (PEEP, 3 cmH₂O), positive end-inspiratory pressure (PEIP, cmH₂O). We maintained PEEP at 3 cmH₂O in order to prevent the collapse of pulmonary alveoli. Ventilatory parameters were continuously adjusted according to arterial blood gases and acide-base parameters (AVL 995 blood gas analyzer).

The left common carotid artery was dissected free for the insertion of a fluid-filled catheter connected to a pressure transducer (APT 300 Hugo Sachs Elektronik-Harvard Apparatus GmbH) to monitor arterial blood pressure and to allow blood sampling for acide-base status assessment. (Fig. 3) A standard limb lead II electrocardiogram (ECG) was recorded from subcutaneous limb leads. Both ECG and mean arterial blood pressure (MABP) were continuously recorded (Digidata 1440A, Axoscope 10 software, Molecular Devices Ltd).

![Figure 3. Catheterization of left common carotid artery.](image3)

Rectal temperature was recorded via a precalibrated steel thermistor probe and core temperature was maintained at 37-38°C with the aid of a heating pad. (Fig. 4)

In vivo assessment of airways hyperresponsiveness (AR)

An ultrasound nebulizer with metacholine (MCh, 1 mg/ml) was placed along the inspiratory limb of the ventilation circuit. MCh was administered at a rate of 5-6 L/min for 8-12 minutes until a steady-state level of bronchoconstriction was obtained. Since no changes of ventilatory parameters (set in beginning of
we postulate that PEIP strictly depends on pulmonary compliance or pneumatic resistance of respiratory airways. This resistance is inversely related to the diameter of respiratory airways. Accordingly, in the case of bronchoconstriction, the pneumatic resistance of respiratory airways will increase together with a proportional, correspondent increase of PEIP. Monitoring of respiratory function was started after 5-10 min of stabilization of individual measurements with the basal recording of PEIP and the further increase after challenging the animals with MCh aerosols (1 mg/mL).

**In vitro assessment of airways hyper-responsiveness (AR).**

After the in vivo experiments, tracheas were removed and cut spirally into strips 15 mm long and 3 mm wide tracheal spiral strips of 15 mm/2-3 mm in order to investigate their reactivity in isolated organ bath. Animals were sacrificed with a dose of sodium pentobarbital 50 mg/body weight. After isolation, tracheal strips were washed and kept in Krebs-Henseleit solution at 4°C for a maximum period of 15 minutes before being vertically mounted in a 10 mL water-jacketed Krebs-Henseleit-filled tissue bath. The composition of Krebs-Henseleit solution was: NaCl 118 mM, KCl 4.7 mM, CaCl\(_2\) 2.5 mM, MgSO\(_4\) 1.2 mM, KH\(_2\)PO\(_4\) 1.2 mM, NaHCO\(_3\) 25 mM, glucose 5.55 mM. The solution was aerated with a mixture of 95% O\(_2\) and 5% CO\(_2\) pH at 37°C and the pH (7.4) was checked every 30 minutes.

Spirally cut tracheal strips were suspended in an organ bath, and one end was fixed to the bottom of the bath while the other was connected to an isometric force transducer (FORT 10, World Precision Instruments, WPI Inc).

Strips were pretensioned (1.5 g), allowed to equilibrate for 60-90 minutes and washed every 15 minutes with Krebs-Henseleit solution before the isometric tension was recorded. In order to perform reproducible experiments, the contractile response of tracheal strips using acetylcholine (10\(^{-5}\) M) was assessed and only strips with two similar contractions were further studied.

Contractile response of tracheal strips to a submaximal dose of MCh (10\(^{-5}\) M) harvested from the OVA group were compared with those from the CON group. After every step of experiment the strips were washed with Krebs-Henseleit solution, 3 times, at 1-2 minutes interval and a 5-10 minutes period for the recovery of basal tone was allowed.

The data were recorded and displayed using BIOPAC Systems’ AcqKnowledge and further analysed using the Acqknowledge version 3.7.2 software.

**Statistics**

Results are expressed as the mean ± standard deviation (SD). All statistical analyses (Student “t” test and ANOVA) were performed with GraphPad Prism v. 4.0 (GraphPad Software, USA). A \(p\) value < 0.05 was considered significant.

**RESULTS**

**In vivo assessment of AR**

In the presence of metacholine 10\(^{-5}\) M, PEIP values were significantly higher in tracheal strips collected from OVA group as compared to those from the CON group (OVA: 39.5 ± 4.95 cmH\(_2\)O vs. CON: 24.75 ± 2.54 cmH\(_2\)O, \(p < 0.001\)). (Fig.5)

**In vitro assessment of AR**

The results of *in vitro* study showed that submaximal contractile response induced by 10\(^{-5}\) M MCh was significantly enhanced in tracheal strips with OVA group after administration of MCh 1mg/mL.
collected from OVA group than in those from CONs, respectively (OVA: 1.16 ± 0.15 g force, CON: 0.93 ± 0.25 g force, \( p < 0.05 \)). (Fig.6)

Figure 6. Submaximal contractile response on action of MCH 10\(^{-5}\) at CON group comparative with OVA group

DISCUSSIONS

Ovalbumin is the most used allergen in experimental models of allergic bronchial asthma on rats. In our experimental model, sensitization to the allergen was induced via the intraperitoneal administration of ovalbumin+ aluminum hydroxide in day 1 and 7, followed by the administration of aerosols with ovalbumin in day 21 to induce airways hyperreactivity, and in day 28 to induce bronchospasm, adapted from Glaab et al and Choi et al.\(^6,7\)

Several methods for the \textit{in vivo} assessment of airway responsiveness and of bronchconstriction in laboratory animals are reported in the literature. Bellofiore et al have evaluated pulmonary resistance by assessing the methacoline dose requested to obtain a 200% increase of pulmonary resistance.\(^8\) Several authors used parameters obtained from a plethysmographic method such as: the enhanced pause (\( P_{enh} \)) and pulmonary resistance in the presence of metacholine, the mid expiratory flow (EF50) after acetylcholine administration, whereas ten Berge et al measured the pleural pressure.\(^9,10\) Misawa et al used a pneumotachograph connected with an integrator in order to determine the ventilation overflow in the presence of acetylcholine.\(^11\) Di Giovanni et al used a small animal ventilator and measured the pulmonary resistance after administration of metacholine.\(^12\) We propose the \textit{in vivo} assessment of PEIP as a new parameter for studying AR in rodent since it is easy to be determined, does not require expensive equipment, and represents an reliable approach to study experimental asthma in anesthetized rodents.

In our model we used for anesthesia sodium pentothal with no reported effects on respiratory airways. However, different anesthetic regimens have been used (halothane -Glaab et al, ketamine - Zosky et al and Koerner-Rettberg et al, and urethane by Misawa et al; all these drugs are reported to differentially impact on the function of trachea and bronchial musculature, thus interfering with an appropriate assessment of pulmonary function.\(^9,13,17,19\)

Limits of the study

Experimental animal models represent the first research step in the attempt to describe pathophysiology of a disease, in this case, allergic asthma. However, asthma in rodents might not present with all the characteristics of human disease, since the large majority models (including ours) of experimental induced asthma evaluate the acute response after a short period of exposure to allergen. In order to assess the progression of AR and of allergic asthma as well, animal models of chronic/repetitive exposures to allergen are definitely requested.

CONCLUSIONS

In the rat model of ovalbumin induced-asthma, positive end-inspiratory pressure is a valid index of airways hyperreactivity as a valid measure of \textit{in vivo} airway hyperresponsiveness in allergic rodents.

REFERENCES

13. Denis M, et al. Chronic intranasal administration of mould spores or extracts to unsensitized mice leads to lung allergic inflammation, hyper-reactivity and remodeling. Immunology 2007;122:268-78.