

Ovalbumin aerosol challenge in actively sensitized guinea pigs: relationship between airway microvascular leakage and airflow obstruction

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Airway inflammation is a common feature of asthma, and one of the cardinal features of inflammation is increased microvascular permeability. We investigated the characteristics of inhaled ovalbumin challenge-induced airflow obstruction and airway microvascular leakage *in vivo* in mechanically ventilated guinea pigs actively sensitized to ovalbumin. A method was used to quantify both airflow obstruction and airway microvascular leakage in order to investigate the relationship between these 2 pathophysiological features in the same animal. Airway microvascular leakage was assessed by Evans blue dye extravasation into airway tissues. Actively sensitized guinea pigs developed both acute airflow obstruction (increased lung resistance and reduced dynamic lung compliance) and Evans blue dye extravasation in response to exposure to aerosolised ovalbumin. Evans blue dye extravasation was preferentially distributed in the distal airways and correlated with airflow obstruction. The results show that inhaled allergen induced both acute airflow obstruction and airway microvascular leakage.

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A major pathological feature of asthma is an inflammation of the airways that can be found even in patients with mild asthma (4, 8). An increase in microvascular permeability is one of the cardinal features of acute inflammation (3, 10, 12) and would be associated with plasma exudation. The presence of airway microvascular leakage and plasma exudation would have several important consequences relevant in the pathophysiology of asthma (10). Nevertheless, there is little direct evidence of increased airway microvascular permeability and plasma exudation after allergen exposure in man. Many inflammatory mediators that may contribute to the pathophysiology of asthma, including histamine and leukotrienes, are active in causing airway smooth muscle contraction and microvascular leakage (5, 10, 11, 15). Airflow obstruction after exposure to inhaled allergen in animal models has been well studied, but there are no reports on the effect of exposure to aerosolised allergen on airway microvascular leakage. For airway luminal challenges, studies have mainly concerned the effects of topically administered allergen and inflammatory mediators (10, 11). By contrast, it is well known that intravenous challenge with allergen, as-

sociated with anaphylaxis, produces substantial airway microvascular leakage (1, 3, 4, 5, 10). Performing studies of airway allergy in animal models, it is important to remember that inhalation of allergen, rather than systemic administration, is the most common route of exposure to allergen in clinical asthma. Thus, we studied the effects of exposure to aerosolised allergen on concomitant changes in airway microvascular permeability and lung resistance in actively sensitized guinea pigs *in vivo*.

Methods

Ovalbumin sensitization

Male Dunkin-Hartley guinea pigs (250–300 g) were actively sensitized using a single intra-peritoneal injection of 0.5 ml of 0.9% (w/v) sodium chloride in distilled water (normal saline) containing 20 µg of chicken ovalbumin (Grade V) (Sigma, Dorset, UK) and 100 mg of aluminium hydroxide (Al(OH)₃) (1). Control (sham sensitization) animals were treated with a single intra-peritoneal injection of 0.5 ml of normal saline containing 100 mg of Al(OH)₃ (BDH Chemicals, Poole, UK). Both control and sensitized

groups of animals were kept under the same conditions in the same cage. Experiments were carried out with batches of animals, each batch included control and sensitized animals.

Protocol

Experiments were carried out 14 to 28 days after sensitization. Animals were anaesthetised with an intra-peritoneal injection of urethane (25% w/v in 0.9% saline; Sigma, Dorset, UK) and further doses were given as needed to maintain adequate anaesthesia. A carotid artery was cannulated (16G Portex, Kent, UK) for continuous blood pressure monitoring and a jugular vein was cannulated (17G; Portex) for intravenous drug administration. A cannula (9–11 mm length, 2.65 mm inner diameter) was inserted into the trachea through a high cervical tracheostomy, and then carefully tied to prevent air leak. The animal was promptly ventilated (Harvard, model 50-1718, Kent, UK) through the trachea cannula at a rate of 60 breaths/min and stroke volume of 1.2 ml/kg. A pleural catheter was inserted in a right lower intercostal space for measurement of pleural pressure. After baseline recordings of lung mechanics (see below) and blood pressure were made, Evans blue dye (30 mg/ml, 1 ml/kg) was slowly injected intravenously. Thirty seconds after injection of Evans blue dye, ovalbumin was aerosolised with an ultrasonic nebuliser (Model 2511, Pulmosonic, De Vilbiss Co., PA, USA; aerosolised mean mass aerodynamic diameter $4.2 \pm 1.25 \mu\text{m}$ ($\pm$ geometric SEM) as measured with a Malvern Instrument 2600C) and delivered to the airways by the ventilator for 30 breaths (output through the tracheal cannula; 20 μl /30 breaths). The volume of ovalbumin solution in the nebuliser chamber was kept at 3 ml and the concentration was varied from 1.25 to 10 mg/ml in doubling steps, using one concentration for each animal. Control experiments were performed with non-sensitized guinea pigs challenged with an ovalbumin solution concentration of 5 mg/ml. After the end of challenge, recordings of transpulmonary pressure, tidal volume and airflow (see below) were made at 30, 60, 120, 190, 240 and 300 s. After the last recording, the lungs were inflated by obstructing an expiratory breath to cause a doubling in tidal volume. A final recording of airway parameters was made 30 s later. The animal was then disconnected from the ventilator, and perfused for quantification of airway microvascular permeability as described below.

Measurement of lung resistance (R_L) and dynamic compliance (C_L)

Transpulmonary pressure, measured as the pressure difference between the pleural space and the proxi-

mal end of the tracheostomy tube, was continuously monitored by a differential pressure transducer (Furness type FC040; Control Ltd, East Sussex, UK). Airflow was measured by a pneumotachograph (Model F1L; Mercury Electronics Ltd, Glasgow, UK) and tidal volume was derived by electronic integration of the airflow signal. Recordings were made on a 6-channel recorder (model MW 6148; Devices Ltd, Welwyn Garden City, UK). Lung resistance (R_L) and dynamic compliance (C_L) were calculated according to the method of von Neer-gaard & Wirz (14).

Airway microvascular permeability

Airway microvascular permeability was quantified by a method modified after that of Saria & Lundberg (13), using Evans blue dye as serum albumin marker. This method has been shown in our laboratory to correlate well with the leakage of radio-labelled albumin into airway tissue (11). After measurement of lung function was completed, the chest cavity was promptly opened for washing Evans blue dye out from the vascular space. Normal saline was perfused through the vascular system by inserting and clamping a 13-gauge blunt cannula through a left ventriculotomy to the aorta with the right atrium opened for perfusate outflow. The perfusion was maintained at a pressure of 100 mmHg, and 200 ml of normal saline was used for each perfusion. The airways and lung were then removed en bloc and cleared of extraneous connective tissue. The extra-pulmonary airway was separated into the trachea, which was itself divided into 2 equal lengths of upper and lower trachea, and the main bronchi. The intrapulmonary airways were stripped of parenchyma and separated into "proximal" (the proximal 3–4 mm portion) and "distal" components. Each tissue was blotted dry with filter paper and weighed. Evans blue dye was then extracted by overnight incubation in formamide (2 ml) at 40°C in a water bath. The amount of Evans blue dye extracted was quantified by measuring absorbance at 620 nm with a spectrophotometer (Model PU8630, Phillips, Cambridge, UK) and interpolation on a standard curve of absorbance of Evans blue dye with concentrations from 0.25 to 10 $\mu\text{g/ml}$. The amount of Evans blue dye extravasated into the tissue was then expressed as ng/mg of wet weight tissue.

Data analysis

Results are presented as means and their standard errors. Kruskal-Wallis one-way analysis of variance was used to test for differences between groups. Further comparison between any 2 groups was with the Mann-Whitney U-test. Spearman's correlation co-

efficient was used to assess the relationship between R_L or C_L and Evans blue dye extravasation. Statistical significance was accepted with $p < 0.05$.

Results

Airway function

Control and test groups of guinea pigs did not differ significantly in body weight or in baseline R_L , C_L or systolic blood pressure (Table 1). Exposure to ovalbumin concentration of 1.25 mg/ml ($n = 6$) showed that there was a slight but insignificant increase in R_L and C_L compared to the control group (Fig. 1). At ovalbumin concentrations of 2.5 ($n = 7$), 5 ($n = 12$) and 10 ($n = 6$) mg/ml, there was an immediate increase in R_L with a peak effect within 2 min after the end of nebulization ($p < 0.005$); partial spontaneous recovery was observed by 5 min with further recovery by lung inflation. Ovalbumin 5 mg/ml caused higher changes in R_L than 2.5 mg/ml, but ovalbumin of 10 mg/ml was equivalent to that of 2.5 mg/ml. There was a corresponding fall in C_L at each concentration of ovalbumin. However, falls in C_L were similar at the 3 higher concentrations of ovalbumin. The fall in C_L at the 3 highest ovalbumin concentrations was more persistent with time (Fig. 2); there was little spontaneous recovery at the end of 5 min. Unlike changes in R_L , lung inflation only improved recovery of C_L at ovalbumin concentration of 2.5 mg/ml, and not for concentrations of 5 and 10 mg/ml. At higher concentrations of ovalbumin aerosol, lung inflation failed to completely reverse the increase of R_L or C_L .

Airway microvascular leakage

There was no significant increase in Evans blue dye content at any of the airway levels studied at the lowest microvascular concentration of 1.25 mg/ml. However, increases were seen in main bronchi, and central and distal intrapulmonary airways at ovalbumin concentrations of 2.5, 5 and 10 mg/ml (Fig. 3). Mean Evans blue dye extravasation at the distal

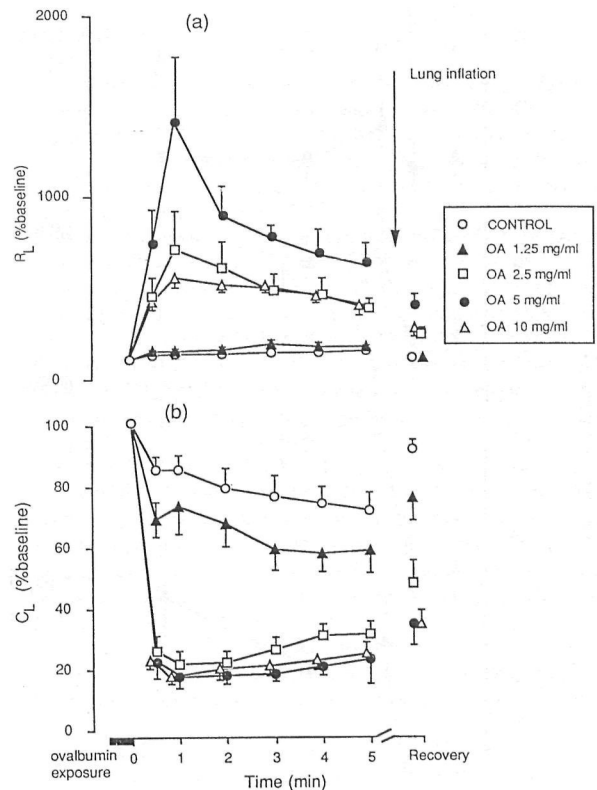


Fig. 1. Time course of the changes in (a) lung resistance (R_L) and (b) dynamic compliance (C_L) after aerosolized ovalbumin challenge. Control experiments were performed in non-sensitized guinea pigs challenged with ovalbumin 5 mg/ml. Data shown as mean $\pm$ SEM.

intrapulmonary airways was 135% of control for ovalbumin concentration of 1.25 mg/ml, 361% for 2.5 mg/ml, 318% for 5.0 mg/ml and 399% for 10 mg/ml. There were no significant changes in Evans blue extravasation in the upper and lower trachea at any concentration. Evans blue dye extravasation in the intrapulmonary airways (proximal or distal) and main bronchi significantly correlated with the peak and recovery (following lung inflation) changes of lung functions (Table 2, Fig. 4).

Table 1. Baseline values of lung resistance (R_L , $\text{cmH}_2\text{O} \cdot \text{ml}^{-1} \cdot \text{s}$), lung dynamic compliance (C_L , $\text{ml}/\text{cmH}_2\text{O}$), trans-pulmonary pressure (TPP, $\text{cm H}_2\text{O}$), systolic blood pressure (BP-S, mm Hg), weight (WT, g) and number (n) of control and sensitized groups, challenged with various ovalbumin (OVA) concentrations. There is no significant difference in the variables for all groups by analysis of variance. Values are means $\pm$ SEM

	Control	Sensitized			
		OVA 1.25 mg/ml	OVA 2.5 mg/ml	OVA 5 mg/ml	OVA 10 mg/ml
R_L	0.218 ± 0.012	0.275 ± 0.036	0.248 ± 0.017	0.228 ± 0.017	0.215 ± 0.012
C_L	0.797 ± 0.18	0.631 ± 0.04	0.731 ± 0.056	0.746 ± 0.049	0.948 ± 0.13
TPP	7.7 ± 0.26	7.9 ± 0.4	7.9 ± 0.4	6.8 ± 0.7	7.4 ± 0.3
BP-S	48.8 ± 3.6	48.4 ± 6.8	46.4 ± 8.5	40.2 ± 1.3	49.1 ± 2.2
WT	472.8 ± 16.1	437.3 ± 1.9	440.7 ± 11.0	426.3 ± 6.9	447.2 ± 8.3
n	8	6	7	12	6

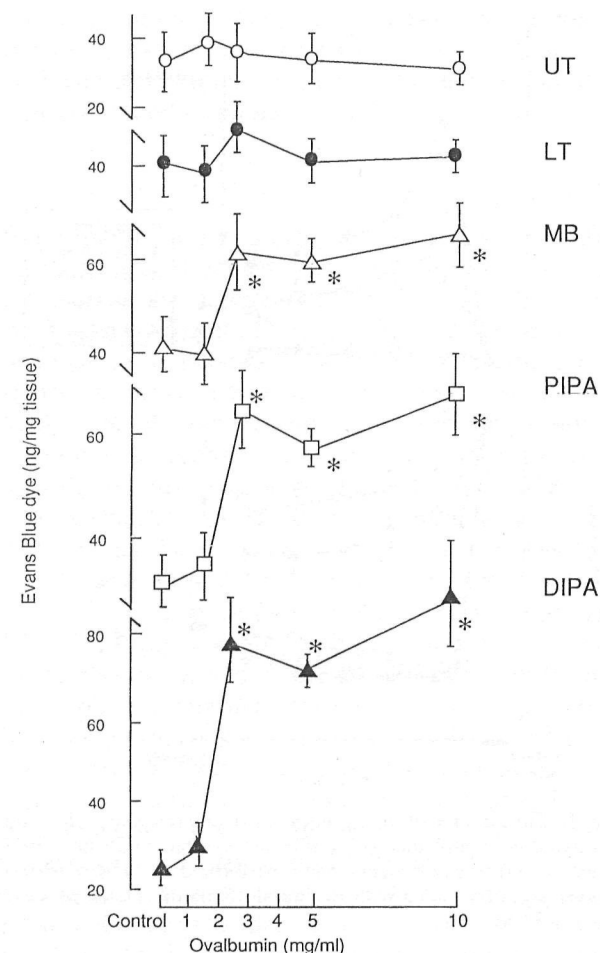


Fig. 2. Effect of ovalbumin challenge on airway microvascular permeability as measured by Evans blue dye extravasation into airway tissues. No significant dye extravasation was seen in the upper trachea (UT) or lower trachea (LT) at any dose. There was significant dye extravasation at 2.5, 5 and 10 mg/ml of ovalbumin in the main bronchi (MB), proximal and distal intrapulmonary airways (PIPA and DIPA). Data shown as mean $\pm$ SEM, * p < 0.05.

Table 2. Correlation coefficient (Spearman's) between Evans blue dye extravasation at different airways levels, and lung resistance and dynamic compliance after aerosolised ovalbumin challenge at peak and recovery (after hyperinflation) times

Airways	Lung resistance		Dynamic compliance	
	Peak	Recovery	Peak	Recovery
UT	0.17	0.004	-0.18	-0.048
LT	0.2*	0.27	-0.17	-0.25
MB	0.49**	0.52**	-0.46*	-0.51*
PIPA	0.71**	0.72**	-0.72**	-0.70**
DIPA	0.81**	0.75**	-0.80**	-0.77**

UT: upper trachea; LT: lower trachea; MB: main bronchi; PIPA: proximal intrapulmonary airway; DIPA: distal intrapulmonary airway. * p < 0.05, ** p < 0.005.

Discussion

The present study has demonstrated that exposure to aerosolised ovalbumin in actively sensitized guinea

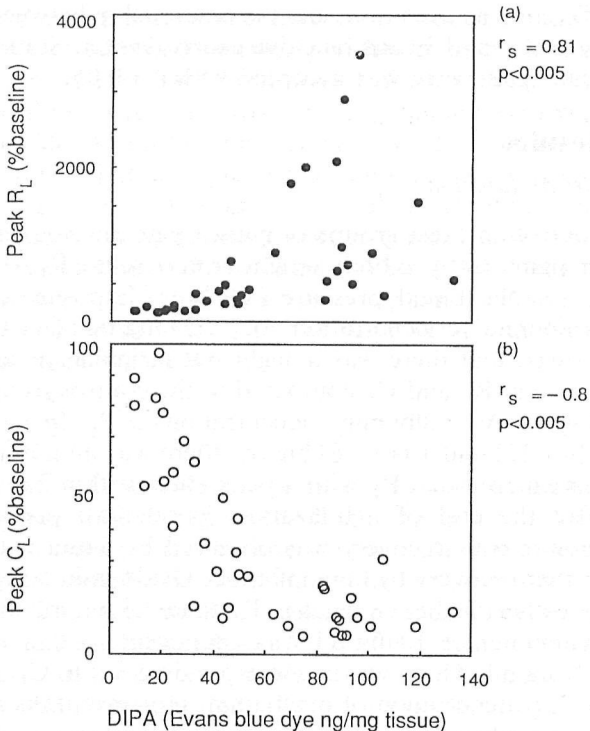


Fig. 3. Scatter diagrams showing the relationships between peak lung resistance (R_L , upper panel) or dynamic compliance (C_L , lower panel) and Evans blue dye extravasation in the distal intrapulmonary airway (DIPA). Spearman's correlation coefficients were 0.81 (p < 0.005) and -0.80 (p < 0.005), respectively.

pig *in vivo* caused airflow obstruction and increased extravasation of Evans blue dye into airways. The increase in Evans blue dye extravasation is preferentially distributed in the peripheral airways, and was correlated with changes in lung resistance or dynamic compliance.

The technique used in our study allowed measurement of both airflow obstruction and airway microvascular leakage in the same animal. Previous investigators have only examined these parameters separately in similar models of guinea pig anaphylaxis, usually with the ovalbumin challenge administered by the intravenous route (1, 2, 9, 10, 15). Because most mediators, such as histamine and the leukotrienes, are capable of constricting smooth muscle and increasing airway microvascular leakage, it is highly likely that these 2 events happen concomitantly in the tracheobronchial tree. It is, therefore, not surprising that we found a significant positive relationship between airflow obstruction and Evans blue dye extravasation in the intrapulmonary airways. A recent study showed that ovalbumin aerosol challenge did not cause an increase in extravascular water content in the trachea in sensitized guinea pigs, but the trachea was the only airway tissue examined (7). This is in agreement with the findings in this study, as extravasation of Evans blue dye was found mainly in the bronchi.

The relatively steep dose-response to allergen challenge for measurements of airflow obstruction and Evans blue dye extravasation is interesting. Surprisingly few studies have evaluated the dose response of the anaphylactic airway response to inhaled allergen, considering the number of investigations into the pharmacological modulation of this model. Woodward et al. (15) found a dose-related dependent increase in albumin extravasation in the airway of sensitized guinea pigs over a 1000-fold range of ovalbumin given intravenously, but did not achieve a plateau response at the highest dose used. It is possible that their results differ from ours because of the different methods of ovalbumin sensitization and route of challenge. Our method of sensitization is similar to that of Andersson (1) and favours the production of IgE antibodies. In contrast, Woodward et al. (15) used high-dose ovalbumin sensitization which would preferentially lead to development of IgG antibodies. Flint et al. (6) found that the release of histamine from human mast cells recovered by bronchoalveolar lavage from extrinsic asthmatics rapidly achieved a maximal response over a 3- to 5-fold range of dilution of anti-IgE, similar to the responses observed here.

In summary, we have described a method for examining changes in airflow and microvascular leakage in the same animal *in vivo*. Actively sensitized guinea pigs developed both acute airflow obstruction and increased airway microvascular leakage in response to exposure to aerosolised allergen. Inhaled allergen-induced airway microvascular leakage appeared to be preferentially distributed in the distal airways, and correlated with airflow obstruction. This model may be more useful than those previously described, where ovalbumin was administered intravenously, in the further investigation of allergen-induced events in the airway.

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