

Short communication

Allergic bronchial eosinophilia: a therapeutic approach for the selection of potential bronchial anti-inflammatory drugs

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Aeroallergen-induced infiltration of eosinophils in the bronchoalveolar lavage fluid (BALF) in guinea pigs was used as a marker of bronchial inflammation. Drugs were administered orally 4 h after aeroallergen challenge. Allergic bronchial eosinophilia in guinea pigs was inhibited by orally administered dexamethasone and methylprednisolone. Terfenadine (a newer H_1 -receptor antagonist), theophylline (a nonspecific phosphodiesterase inhibitor), and salbutamol (a β_2 -agonist) did not influence allergic eosinophilic infiltration. Many of these agents, administered prophylactically, have been reported to suppress allergic eosinophilic infiltration in the BALF of guinea pigs. Methylprednisolone, a steroid, inhibits allergic bronchial eosinophilia regardless of the time of administration; that is, 2 h before or 4 h after aeroallergen challenge. The therapeutic approach used in this study may facilitate drug discovery for bronchial inflammation/asthma.

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Bronchial inflammation and hyperresponsiveness are the characteristic features of asthma. T cells and eosinophils have been reported to play an important role in the pathogenesis of asthma (6, 11). The products of T-cell activation, cytokines, and eosinophil activation, including MBP, ECP, O_2^- , LTB_4 , EPO, EDN, IL-5, etc., have been reported to cause bronchial hyperreactivity (7, 11). Therefore, drugs which could inhibit either infiltration of eosinophils or their activation may be beneficial in the management and treatment of clinical asthma.

Steroids (3, 9), an LTB_4 antagonist (U-75302) (13), or theophylline (3) given before aeroallergen challenge in guinea pigs suppresses bronchial eosinophilic infiltration in the BALF. Aeroallergen challenge in actively sensitized guinea pigs produces bronchial eosinophilia which persists for several days (5, 9). It allows the opportunity to test potential bronchial anti-inflammatory drugs after bronchial inflammation has already been initiated. The

purpose of this study was to investigate the modulation of developing allergic bronchial eosinophilia by antiallergic/asthma drugs through a therapeutic approach; that is, drug administration after aeroallergen challenge.

Material and methods

Adult, male, Hartley-strain guinea pigs (Charles River Laboratories, Kingston, MA, USA) weighing 268-614 g were sensitized by i.p. injection of 0.5 ml saline containing 10 μ g ovalbumin (OA) and 100 mg aluminum hydroxide, $Al(OH)_3$. This procedure of sensitization produces both IgG and IgE antibodies (1). After 2-3 weeks, the animals were used for the aeroallergen challenge (2, 3).

Individual guinea pigs were placed in a 2-l glass chamber and exposed to a fine aerosol of OA solution (0.5 mg/ml in saline containing 0.02% antifoam emulsion for 30 s). The aerosol was produced by

forcing compressed air at a pressure of 103 kPa through a Wright's nebulizer containing 10 ml of OA solution. The animals were immediately removed from the exposure chamber after OA challenge to prevent death (2, 3).

Four hours after aeroallergen challenge, the guinea pigs that recovered from immediate allergic responses received either vehicle (1% acacia in distilled water, 2 ml/kg) or drugs suspended in 1% acacia by oral gavage in a randomized, blind fashion.

Soon after aeroallergen challenge, the inflammatory processes (adhesion, diapedesis, migration, etc.) are initiated in the lung (5). Four hours after challenge, no detectable eosinophilic infiltration was seen in the BALF of challenged guinea pigs (unpublished observations). We allowed 4 h to give the animals time to recover from the early bronchoconstrictor responses, and to allow time for the initiation of inflammation in the lungs (5). Therefore, this situation simulates a clinical situation.

Twenty to 24 h after aeroallergen challenge, the animals were killed with an i.p. overdose of pentobarbital sodium (100 mg/kg). The abdominal cavity was opened, and each animal was exsanguinated by cutting the abdominal aorta. A midline incision was made through the rib cage, exposing the heart and lungs. The trachea was cannulated with an 18-gauge stainless steel feeding tube; 20 ml/kg of prewarmed (37°C) physiologic saline (0.9% NaCl) was instilled and the BALF was gently withdrawn. The recovered BALF (amounting to 60–80% of the saline infused) was transferred to a 10-ml vacutainer containing 0.10 ml of 15% EDTA solution and BALF centrifuged at 2400 rpm for 10 min. The supernatant was poured off, and the cell pellet was suspended in physiologic saline equal to the ratio of BALF recovered (ml) and physiologic saline instilled in the lungs (ml), multiplied by 2. This step was taken to normalize the volume of BALF regardless of the recovery of BALF and body weight of the animals.

The eosinophils were counted in BALF obtained

Table 2. Pharmacologic modulation of aeroallergen (ovalbumin 0.5 mg/ml, 30 s, 103 kPa)-induced infiltration of eosinophils in bronchoalveolar lavage fluid (BALF) of actively sensitized guinea pigs

Group	n	Dose mg/kg peroral + 4 h	Eosinophil $\times 10^3$ cells/ml BALF	% inhibition
Control	28	0	1859 $\pm$ 176	
Methylprednisolone	14	30	729 $\pm$ 102*	61
Dexamethasone	8	30	405 $\pm$ 86*	78
Salbutamol	6	30	1869 $\pm$ 354	0
Terfenadine	8	30	1654 $\pm$ 277	11
Theophylline	8	30	1383 $\pm$ 234	28

Values are means $\pm$ SEM.

* $P < 0.05$, as compared with number of eosinophils in the BALF of control guinea pigs.

from the sensitized animals which were unchallenged and the sensitized animals which were challenged with antigen (OA 0.5 mg/ml, 30 s, 103 kPa). The BALF from aeroallergen-challenged guinea pigs was collected at 24, 48, 72, 96, 168, and 336 h postchallenge time intervals. The number of eosinophils in sensitized, unchallenged, and postchallenged time intervals was compared by analysis of variance.

Eosinophils in BALF were counted by Unopette Test 5877 (Becton-Dickinson, Division of Becton-Dickinson and Co, Rutherford, NJ, USA) (4, 12).

The following drugs and chemicals were used in this study: α -methylprednisolone, theophylline, dexamethasone, salbutamol, ovalbumin (OA; grade VI), and antifoam B emulsion, Sigma Chemical Co; aluminum hydroxide, Fisher Scientific Co; 0.9% sodium chloride, Abbott Laboratories; acacia, J. T. Baker Chemical Co; and terfenadine, Merrill-Dow.

The mean number of eosinophils $\pm$ SEM in the BALF of drug and vehicle-treated animals was compared by analysis of variance and Fisher's LSD test. A P value of 0.05 or less was considered to be significant.

Results

A 10-fold increase in the number of eosinophils in BALF collected 24 h after aeroallergen challenge of guinea pigs was observed (as compared with the unchallenged group). The eosinophil count remained significantly elevated for 7 d (Table 1).

The oral administration of dexamethasone and methylprednisolone (30 mg/kg) 4 h after aeroallergen challenge significantly inhibited allergic eosinophilic infiltration. Salbutamol, terfenadine, and theophylline (30 mg/kg, peroral, + 4 h) did not influence bronchial eosinophilia (Table 2). The oral administration of theophylline (100 mg/kg) killed 50% (4/8) of guinea pigs. Therefore, its effect was not determined.

Table 1. Aeroallergen (ovalbumin 0.5 mg/ml, 30 s, 103 kPa)-induced infiltration of eosinophils in bronchoalveolar lavage fluid (BALF) of actively sensitized guinea pigs

Group	n	Eosinophil $\times 10^3$ cells/ml BALF
Unchallenged	18	124 $\pm$ 51
Challenged		
24 h (1 d)	22	1226 $\pm$ 166*
48 h (2 d)	8	1483 $\pm$ 246*
72 h (3 d)	8	922 $\pm$ 119*
96 h (4 d)	8	1195 $\pm$ 178*
168 h (7 d)	18	624 $\pm$ 110*
336 h (14 d)	17	363 $\pm$ 62

Values are means $\pm$ SEM.

* $P < 0.05$, as compared with number of eosinophils in BALF of unchallenged guinea pigs.

Discussion

The success of drug discovery for asthma depends upon the nature, reproducibility, and reliability of the experimental models used. A variety of antiallergic/antiasthmatic drugs given before allergen challenge suppress bronchial inflammation (the influx of the inflammatory eosinophils into the lung of guinea pigs), perhaps by inhibiting the synthesis and release of mediators of early asthmatic responses; that is, initial allergic insult in the airways. The data obtained in this study suggest that once inflammatory processes have been initiated (e.g., expression of adhesion molecules or gene expression for interleukins on the endothelial, epithelial, and T cells and other inflammatory cells), the nonsteroidal antiallergic/antiasthmatic drugs have limited or no effect on the ongoing inflammatory processes. Obviously, more definitive studies are needed to support this concept in animals and man.

The use of aeroallergen-induced bronchial infiltration of eosinophils in the BALF of guinea pigs as a model of allergic late-phase bronchial inflammation offers several advantages. These include very small doses of antigen and short duration of aerosol provocation, quantitative enumeration of eosinophils in BALF, persistence of bronchial eosinophilia for several days (chronic model of bronchial inflammation) (5, 9, this study), and its inhibition by oral steroids (3, 5, 9, 10, this study) and anti-IL-5 antibodies (2, 8). Furthermore, the inhibition of T-cell-mediated allergic bronchial inflammation by methylprednisolone in the prophylactic (3) and the therapeutic approaches (this study) demonstrates that the therapeutic model may be used to select potential bronchial anti-inflammatory compounds. This model can also be used to evaluate the effect of sustained release formulations (miniosmotic pumps, or slow-release tablets).

Steroids are generally effective in the management and treatment of asthma. These agents selectively inhibit T-cell-dependent allergic eosinophilic infiltration in the BALF of guinea pigs. The time of administration does not seem to influence their effects. For other nonsteroidal antiallergic/antiasthma compounds, the time of administration is very critical. A variety of compounds, including theophylline, β_2 -bronchodilators, and terfenadine, given before aller-

gen challenge suppress eosinophilic infiltration (unpublished observations). However, these agents were not effective when administered after the inflammatory processes had been initiated. Therefore, this method may help distinguish between true bronchial anti-inflammatory drugs (steroids) and other agents (β -agonists, theophylline, terfenadine, etc.).

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