

Diurnal variation in serum cortisol concentrations in asthmatic subjects after allergen inhalation

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ABSTRACT To assess whether differences in the adrenal response to allergen are important in determining the magnitude of the allergen induced late responses in asthmatic subjects, we measured serum cortisol concentrations after inhalation challenge with allergen or control solution (phosphate buffered saline). The two challenges were performed in random order with an interval of 14 days. A normal diurnal decrease in serum cortisol concentrations was observed on both days. Mean blood cortisol concentrations three hours after inhalation of allergen (before the late response), nine hours afterwards (at the time of the late response), and 24 hours afterwards were virtually identical to those observed after inhalation of phosphate buffered saline. Serum cortisol concentrations before challenge and three, nine, and 24 hours after challenge were not related to the diurnal increase in blood eosinophils on the control day, or to the size of the late asthmatic response or accompanying changes in blood eosinophils after allergen challenge. It is concluded that serum cortisol concentrations show normal diurnal variation after allergen challenge and are unrelated to the size of the late response or associated changes in blood eosinophil counts.

Introduction

Measurements in blood and bronchoalveolar lavage fluid suggest that airway inflammation may be important in the development of the late asthmatic response. The late response in patients has been associated with increased concentrations of mediators¹⁻³ and activation of leucocytes⁴ in peripheral blood, alterations in T lymphocyte subsets^{5,6} and increased eosinophil numbers in blood^{7,8} and bronchoalveolar lavage fluid.^{9,10} The development of a late asthmatic response was shown to be associated with an increase in airway hyperresponsiveness to inhaled histamine.^{11,12} More recent studies indicate that the tissue events and increased bronchial responsiveness may occur within three hours of an inhalation challenge and before the late response is clinically apparent.¹³⁻¹⁵

At present the occurrence or magnitude of a late response cannot be predicted in an individual subject. Possible factors include allergen dose,^{16,17} the severity of clinical asthma,¹⁸ and the subject's allergen sensitivity.¹⁷ Some¹⁷ but not all^{19,20} investigators have found a relation between allergen specific IgE concentrations and the tendency to develop a late response.²¹

Host factors such as the adrenal cortisol response might also limit the development of a late response in some individuals or alter the magnitude of such a response. Small doses of inhaled corticosteroids reduce blood eosinophil counts and are highly effective in inhibiting the late response to allergen in man.²¹ Pretreatment with metyrapone in dogs ("medical adrenalectomy") has been shown to accentuate allergen induced late pulmonary responses.²²

The aim of this study was to assess whether endogenous cortisol concentrations might be important in limiting the magnitude of the late asthmatic response in patients with asthma. We have performed a randomised controlled study in which blood cortisol concentrations, one second forced expiratory volume (FEV₁), and blood eosinophil counts were determined after inhalation challenge with allergen or control solution on separate days.

Methods

PATIENTS

We studied 14 non-smoking atopic adults with asthma (eight of them female) aged 20-36 years. Their clinical features and FEV₁, eosinophil counts, and airway histamine responsiveness measurements after allergen challenge have been reported previously.^{14,15} Subjects were selected on the basis of a clinical history of asthma and allergen sensitivity and a positive

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skinprick test response to the relevant allergen. The subjects' baseline FEV₁ values were normal (80–120% predicted). There was a wide range of baseline airway histamine responsiveness. Values for the concentration of histamine that caused a 20% decrease in FEV₁(PC₂₀) varied from 14 to 0.31 mg/ml. All subjects were inhaling a beta₂ agonist intermittently or regularly and seven were inhaling corticosteroids or sodium cromoglycate regularly. None of the subjects had taken oral corticosteroids in the preceding six months.

STUDY DESIGN

Subjects underwent two inhalation challenges, one with allergen and one with phosphate buffered saline (allergen diluent) in random order with an interval of 14 days. With the exception of inhaled salbutamol, all medication was discontinued for five days before both study days and restarted 48 hours after challenge. Inhaled salbutamol was discontinued at least eight hours before challenge.

INHALATION TESTS

Allergen²³ and histamine²⁴ inhalation tests were performed with a Wright nebuliser, a tidal breathing method being used. The nebuliser output was 0.13 ml/min and the duration of inhalation was two minutes. Allergen extracts were biologically standardised, partially purified freeze dried preparations (*Dermatophagoides pteronyssinus* or Timothy grass, Pharmacia, Milton Keynes, Bucks). Stepwise half log₁₀ increasing concentrations of allergen solution were given at 10 minute intervals until there was a 20% fall in FEV₁. On the control day phosphate buffered saline alone was inhaled on three occasions at 10 minute intervals. The challenges were started at 1100 h (allergen) or 1130 h (control) so that the last dose of allergen or diluent was given around midday.

BLOOD SAMPLES

Venous blood was withdrawn via a 19 gauge needle inserted into the antecubital fossa. Samples of 15 ml were taken before and at intervals after the inhalation challenge. Blood anticoagulated with EDTA was stored at 4°C for a maximum of 12 hours before eosinophils were counted by an automated cell counter (Technicon H6000, Tarrytown, New York; intra-assay coefficient of variation 7.5%). A further sample was centrifuged and serum separated, and stored at -20°C before measurement of serum cortisol concentrations (double antibody, specific radioimmunoassay; intra-assay coefficient of variation in our laboratory 8.6%). All samples were assayed blind in random order.

ANALYSIS

Blood cortisol concentrations, eosinophil counts, and FEV₁ before allergen challenge and three, nine, and 24 hours after challenge were compared with the corresponding measurements on the control day (Student's paired *t* test). Serum cortisol concentrations and changes in serum cortisol concentrations after challenge on the two days were compared with the corresponding values for FEV₁ and blood eosinophil counts by linear regression analysis. In view of the delayed effects of corticosteroids, associations were sought between change in cortisol concentrations three and nine hours after challenge and change in FEV₁ and eosinophil counts at nine and 24 hours respectively.

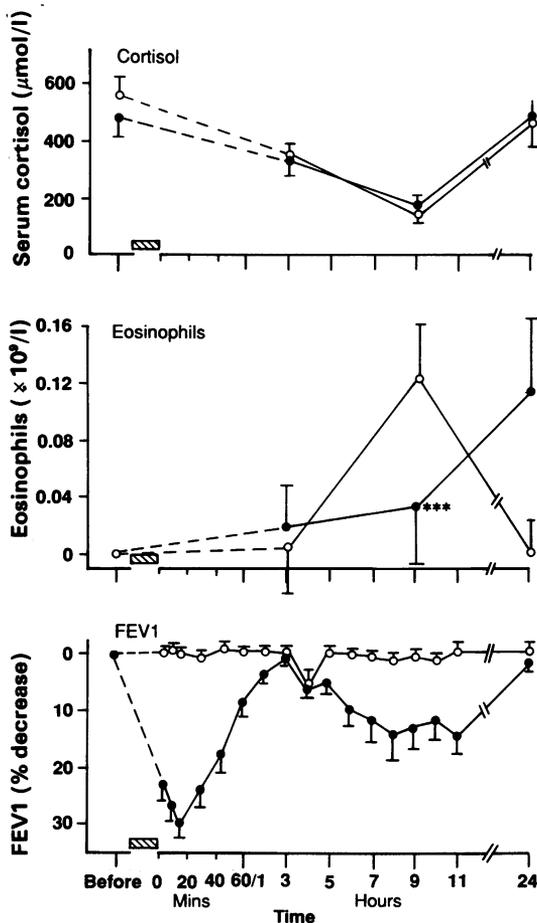
Results

The time course of change in FEV₁, blood eosinophil count and serum cortisol concentration in the 14 asthmatic subjects after inhalation of allergen and control solution are shown in the figure. Mean (SEM) baseline values before allergen and control challenge did not differ significantly for FEV₁ (3.58 (0.59) and 3.61 (0.63) litres) or for blood eosinophils (0.28 (0.04) and 0.39 (0.08) × 10⁹/l), but serum cortisol concentrations were higher before allergen diluent (control) (555 (68) and 481 (66) μmol/l; *p* < 0.05).

After inhalation of allergen diluent (control challenge) FEV₁ varied during the immediate (0–60 min) and late periods (5–11 hours) after challenge by no more than 10% in all but one subject. After allergen inhalation all subjects developed an immediate response with a greater than 20% decrease in FEV₁ and recovery within two hours. There was a wide range of late asthmatic responses, varying from no response (1% decrease in FEV₁) to a 55% decrease in FEV₁ 5–11 hours after challenge.

On the control day there was a decrease in serum cortisol concentration in all but one of the 14 subjects three hours after challenge and in all subjects at nine hours (*p* < 0.0001; figure). After allergen challenges there were similar decreases in serum cortisol concentrations in all but two subjects, (*p* < 0.0001). Serum cortisol concentrations were slightly higher at baseline on the control day than on the allergen day but did not differ significantly between the two days three, nine, or 24 hours after challenge (figure). In all but two subjects serum cortisol concentrations were within our normal reference range (0800 h: 280–296 μmol/l; 2400 h: 80–280 μmol/l).

On the control day there was no significant correlation between the fall in serum cortisol from baseline at three hours (*r* = -0.48, *p* = 0.096) or at nine hours (*r* = -0.45, *p* = 0.13) and the diurnal increase in the blood eosinophil count nine hours after challenge.



The time course of change in FEV₁ (% change), blood eosinophil count (absolute change $\times 10^9/\text{litre}$), and serum cortisol concentration ($\mu\text{mol/l}$) in 14 asthmatic subjects after inhalation challenge with allergen (closed circles) and allergen diluent (open circles).

* $p < 0.05$; *** $p < 0.005$.

After allergen inhalation there was no correlation between the fall in serum cortisol concentrations from baseline to values at three hours ($r = -0.21$, $p = 0.46$) or at nine hours ($r = -0.04$, $p = 0.90$) and change in blood eosinophil count at nine hours. There was also no correlation between the fall in serum cortisol nine hours after allergen and the increase in blood eosinophils above baseline at 24 hours ($r = -0.33$, $p = 0.225$). Serum cortisol concentrations (absolute values or change in serum cortisol concentrations after challenge) did not relate to the magnitude of the late asthmatic response (maximal fall in FEV₁ at 5–11 hours).

Discussion

In this study the change in serum cortisol concentrations in asthmatic subjects after allergen inhalation challenge was virtually identical to that observed on a separate control day. A highly significant diurnal decrease in cortisol concentrations was observed on both days. The decrease in serum cortisol concentrations observed on the control day did not correlate significantly with the diurnal increase in blood eosinophil count. The decrease in blood cortisol concentration after allergen inhalation was unrelated to the size of the late asthmatic response or the accompanying changes in blood eosinophil count.

We have previously reported that the late response in these subjects was accompanied by a significant increase in histamine airway responsiveness three and 24 hours after allergen challenge.¹⁴ By comparison with the control day there was a reduction in the blood eosinophil count at nine hours, which correlated strongly with the size of the late response and with the magnitude of the accompanying changes in airway histamine responsiveness.¹⁵ This was a controlled, randomised, single blind study, in which measurements were performed at the same time of day after the two challenges. The reason for the difference in baseline (0930 hours) serum cortisol concentration between the control and allergen days is not clear as the measurements were performed at the same time. The differences could not be explained by an "order" effect in view of the randomised study design. The differences, though statistically significant, were small and unlikely to be physiologically significant. More frequent cortisol estimations between the early and the late response and during the late response are unlikely to have detected differences between the two study days as the changes in all but one of the 14 subjects were very similar after both allergen and control challenges.

Diurnal increases in blood eosinophil counts have been reported in both asthmatic and normal subjects.^{8,25} Exogenously administered corticosteroids are known to depress eosinophil counts²⁶ and eosinophilia is a feature of Addison's disease. It was perhaps surprising that the diurnal fall in serum cortisol concentrations on the control day was not related to the increase in blood eosinophil counts. This suggests that the diurnal changes in eosinophil counts in asthmatic subjects might depend on factors other than circadian adrenal cortisol responses. Although cortisol concentrations are lowest at night, when peak expiratory flow rates are lowest, preventing the nocturnal fall in plasma cortisol by intravenous cortisol infusion did not prevent the fall in peak flow rates in one study.²⁷ The circadian rhythm of corticosteroid secretion does not appear to be the main cause of nocturnal and early morning asthma.

In our study we thought that the immediate response to allergen might be associated with an adrenal stress response and an increase in cortisol secretion, and that the magnitude of the increase in serum cortisol concentrations might prevent or modify the late asthmatic response. We found no increase in serum cortisol concentrations after the immediate response or during the late response. These results suggest that variation in endogenous cortisol concentrations are not a major influence on the outcome of allergen inhalation tests.

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