

Effect of systemic β -agonist therapy on IgE production in allergic subjects in vivo

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Background: Although β 2-adrenoceptor agonists are widely used in the treatment of asthma, a number of studies have suggested that their long-term use may exacerbate the condition. One possible mechanism for this stems from the in vitro observation that β 2-agonists increase IgE synthesis by human blood mononuclear cells.

Objective: We sought to examine the effect of regular β 2-agonist therapy on IgE production in vivo in human volunteers.

Methods: Placebo or salbutamol (8 mg BD) tablets were given in a double-blind, randomized fashion to 25 volunteers allergic to grass pollen throughout a period encompassing the UK grass pollen season (April through September). Levels of serum IgE were measured monthly, and nasal IgE was measured at the height and end of the season. Efficacy was assessed through monthly recordings of symptoms of blocked nose (vascular) and other symptoms of rhinitis (nonvascular). **Results:** For the whole group the geometric mean of serum IgE levels rose from a baseline of 58.7 IU/mL (range, 0 to 1027 IU/mL) to 140 IU/mL (range, 12 to 878 IU/mL) at the height of the pollen season ($P = .0001$). There was no significant difference between the magnitude of the rise in IgE between the groups with a ratio of increase for salbutamol/placebo of 1.17 (confidence interval = 0.78 to 1.75). There was no change in nasal IgE levels. Total and nonvascular symptom scores were reduced by salbutamol, reaching statistical significance at the height of the pollen season ($P < .05$).

Conclusion: An oral dose of the β 2-agonist salbutamol, sufficient to maintain therapeutic levels and provide clinical benefit, does not accentuate the seasonal increase of IgE in human atopic volunteers. (*J Allergy Clin Immunol* 1998;102:727-31.)

Key words: Allergic rhinitis, IgE, salbutamol

The introduction of inhaled isoprenaline in the 1940s revolutionized the treatment of asthma, but concerns soon developed regarding its possible role in the development of severe refractory asthma.^{1,2} Subsequent clinical studies have also suggested that β 2-agonist use may lead to an increase in bronchial responsiveness to aller-

gen³ and nonspecific provoking-stimuli,⁴ deterioration in lung function,^{5,6} and an increase in asthma symptoms.⁷ Partly as a result of these concerns, the International Asthma Consensus Report⁸ recommends that inhaled β 2-agonists are used "as required" for relief of symptoms rather than on a regularly scheduled regimen. Although other studies, including those examining the effects of the long-acting β 2-agonists salmeterol⁹⁻¹¹ and formoterol,¹² have been unable to confirm these findings in relation to asthma control, there is sufficient evidence to warrant the further investigation of the relationship between β -agonist therapy and asthma severity.

A number of mechanisms have been proposed to explain a possible worsening of asthma control with regular β 2-agonist therapy, including an over reliance leading to delayed use of antiinflammatory agents,¹³ the development of tachyphylaxis,¹⁴ and an increase in the inflammatory response through inhibition of mast cell heparin release¹⁵ or increased allergen exposure caused by bronchodilatation.⁷ Two recent in vitro studies have drawn attention to another possible deleterious effect of regular β 2-agonist therapy. Paul-Eugene et al¹⁶ and Coqueret et al¹⁷ demonstrated that the addition of either salbutamol or fenoterol to normal human PBMCs led to a potentiation of IL-4-induced IgE production.

This effect was shown to be due to the β 2-agonist properties of these agents because it was blocked by the β -adrenoreceptor antagonist propranolol, and it was thought to be mediated through inhibition of IFN- γ production. Should this increase in IgE production occur in vivo, there could be increased binding of IgE to high-affinity receptors on the mast cell and perhaps other cells, leading to an increase in mediator release and an exaggeration of the underlying inflammatory response of the airways.

The potential clinical relevance of this effect is suggested by epidemiologic studies that have demonstrated an association between levels of total serum IgE and airway responsiveness to methacholine,¹⁸ prevalence of asthma and rhinitis,¹⁹ and asthma severity.²⁰ The purpose of this study was to test the hypothesis that the use of β 2-agonists in atopic humans leads to an increase in systemic IgE levels thereby providing a mechanism for the proposed worsening of asthma with their use.

METHODS

This was a 6-month, double-blind, randomized, parallel-group study. Volunteers were recruited from the departmental database. All

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volunteers gave their informed written consent, and the study protocol was approved by the Southampton University Joint Ethics Committee.

Inclusion criteria

For inclusion in the study, volunteers had to be between 18 to 65 years of age, have a history of seasonal allergic rhinitis for at least 2 years, have a positive skin test response to mixed grass pollen, and have a normal electrocardiograph.

Exclusion criteria

Volunteers were excluded if they were current smokers, had taken any other investigational drugs within the last 3 months, had a history of asthma requiring medication, or had concomitant heart disease or other significant medical condition.

Pollen levels

Grass pollen levels for the Southampton area were obtained from the local aeroallergen unit, St Mary's Hospital, Isle of Wight.

Visit 1

This study took place in April, before the recognized start of the UK grass pollen season. At entry, a history was taken, and physical examination was performed. A 12-lead electrocardiograph was taken. Skin prick tests to house dust mite, cat, and mixed grass pollen were performed (allergen extract; Miles Inc, Spokane, Wash), and a positive test response was taken as a 3 mm or larger wheal once the negative control had been subtracted. Ten milliliters of blood was taken for baseline IgE measurement. Volunteers were asked to complete a linear analogue scale indicating the severity of their hay fever symptoms over the previous month (runny nose, blocked nose, itchy nose, runny eyes, itchy eyes, and sneezing). Each scale was 10 cm, giving a possible maximum score of 60.

Volunteers were randomized to receive either slow release oral salbutamol 8 mg (Volmax; Allen and Hanburys, Greenford, UK) twice daily or placebo twice daily to be taken throughout the study period. Because the study was undertaken through the pollen season, all volunteers were given astemizole 10 mg (Hismanal; Janssen Pharmaceuticals, Grove, UK) to be taken once daily as required for relief of symptoms. Use of this drug was recorded on a diary card. Use of other medications for allergic rhinitis was not permitted.

Visits 2 through 6

Volunteers returned to the department at monthly intervals for a further 5 months. At each visit they completed the linear analogue scale of severity of symptoms experienced over the previous month and had 10 mL of blood taken. The blood was spun at 3000 rpm for 20 minutes, and the serum was decanted and stored at -20°C for IgE measurements. The volunteers received their medication for the following month.

Nasal lavage

Nasal lavage was performed on visits 2 and 6. Volunteers were instructed to tilt their heads backward at an angle of 30° to the horizontal and to hold their breath and refrain from swallowing during the procedure. Five milliliters of 0.9% prewarmed saline was then instilled into each nostril, and after 10 seconds the volunteers were asked to flex their necks to allow collection of the mucous/saline mixture into a sterile universal container. The samples were then sieved and immediately transported on dry ice to be stored at -20°C for later analysis of IgE.

IgE assay

Total IgE was measured by an ELISA technique. Briefly, ELISA plates (Nunc Immunoplates (Maxisorp)) were coated with coating

proteins in buffer and incubated (200 μL per well) at 37°C for 1 hour. The coating protein was allowed to bind overnight at 4°C , and then the plates were dried and phosphate buffered PBS/BSA was added (200 μL per well). The plates were then washed 3 times with PBS/Tween, and 200 μL of serum was added to each well. The plates were incubated again at 37°C for 1.5 hours and washed 3 times with PBS/Tween. Horseradish peroxidase-conjugated rabbit anti-human IgE (DAKO) was diluted in PBS/Tween (22 mL per plate), and 200 μL was added to each well. The plates were incubated at 37°C for 1.5 hours and washed 3 times in PBS/Tween. Substrate buffer containing H_2O_2 and *o*-phenylenediamine was then added (200 μL per well), and after 3 to 5 minutes, 80 μL of 5N H_2SO_4 was added per well. The absorbance of each well was measured at 450 nm with an MR5000 ELISA plate reader. Concentrations of IgE were calculated from a standard curve. Each sample was assayed in duplicate, and the mean value was taken. The lower limit of detection was 1 IU/mL.

Assay for nasal IgE

Nasal IgE was measured with an automated 2-site chemiluminescent enzyme immunometric assay²¹ (IMMULITE Total IgE, Diagnostic Products Corp) and preproduction research reagents. Controls were prepared at concentrations of 0.15 and 1.5 kU IgE/L, with human cord serum freed from any IgE by charcoal stripping. The lowest concentration of sample that could be distinguished from IgE-free cord serum was 0.1 kU IgE/L.

Statistical analysis

Serum IgE levels underwent logarithmic transformation and were expressed as a geometric mean for the active and control groups. Changes in IgE between April and the last 3 time points (July, August, and September) were analyzed by using analysis of covariance with adjustment for baseline measurements. Results were then antilogged and presented in ratio form as changes in IgE in salbutamol group/placebo group. Changes in IgE from April to August, irrespective of treatment group, were analyzed by using a paired *t* test.

Symptom scores and astemizole use could not be transformed to parametric data. A Mann-Whitney test was therefore used to compare these measures in the salbutamol and placebo groups during the height of the pollen season after first ensuring that there was no statistical difference between the groups at baseline.

RESULTS

Twenty-nine volunteers were randomized into control or salbutamol groups. One volunteer withdrew after 2 days because of side effects of the medication (tremor), 1 was lost to follow-up after the first visit, and 2 were lost to follow-up after 2 visits. This left 11 volunteers randomized to receive salbutamol.

Baseline characteristics

There was no significant difference in terms of age (mean age: salbutamol group, 32.5 years; placebo group, 30.1 years), sex distribution (M:F, 11:3 for both groups), or previous smoking habits (all life-long nonsmokers). Baseline IgE values were not significantly different between the 2 groups (geometric means and ranges: placebo group, 112 IU/mL [range, 54 to 1027 IU/mL]; salbutamol group, 39.2 IU/mL [range, 0 to 225 IU/mL]). There was no significant difference between baseline symptom scores between the 2 groups (placebo group, 10.1; salbutamol group, 6.97).

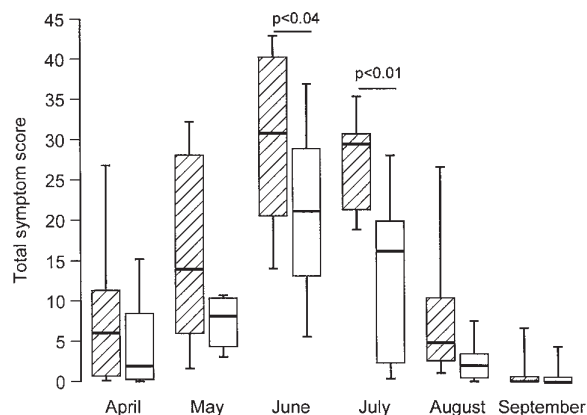


FIG 1. Box and whisker plot showing median, interquartile range, and range for total symptom scores for placebo- (hatched squares) and salbutamol-treated groups. Significant differences between placebo- and salbutamol-treated groups are marked.

Pollen levels

Mean monthly pollen levels increased from 0.6 grains/m³/24 h in April to a maximum monthly mean of 154 grains/m³/24 h in June, with a peak level during June of 622 grains/m³/24 h. Levels then fell to a monthly mean of 0 grains/m³/h in September.

Symptom scores

Median total symptom scores increased in both groups to reach a maximum of 18.2 in the salbutamol group and 29.9 in the placebo group (Fig 1). Symptom scores were significantly lower in the salbutamol group than in the placebo group (Mann-Whitney) for both the months of June ($P = .04$) and July ($P = .01$). There was no significant difference between the 2 groups for the number of antihistamines taken during the study period, although there was a trend for fewer antihistamines to be taken in the salbutamol group (Fig 2).

Serum IgE

Mean levels of serum IgE reached their maximum in August for the placebo group and September for the salbutamol group. Geometric means for serum IgE in August were 192.5 IU/mL for the placebo group compared with 108.9 IU/mL for the salbutamol group, and in September these values were 183.1 IU/mL and 126.5 IU/mL, respectively. Values of IgE were logged, and to allow for differences in the baseline values, IgE levels were analyzed in terms of adjusted ratios for the rise in IgE in the salbutamol group/placebo group by using an analysis of covariance. In August the ratio was 1.17 (0.78 to 1.75), and in September it was 1.43 (0.82 to 1.7), with neither of these differences being statistically significant (Fig 3).

One individual in the placebo group had a particularly high baseline level of serum IgE (1027 IU/mL), which accounts for the wide range of baseline values in the placebo group. Serum IgE levels of this individual fell

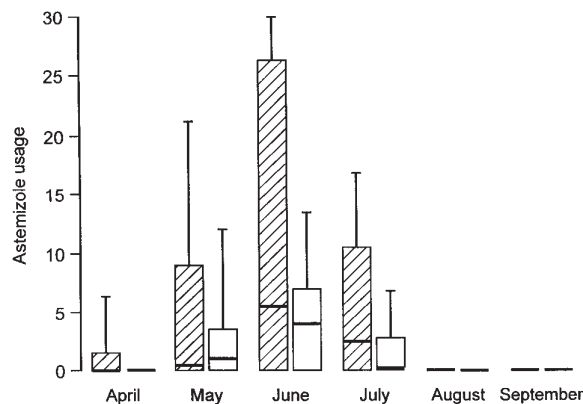


FIG 2. Box and whisker plot showing median, interquartile range, and range for number of astemizole tablets taken in placebo- (hatched squares) and salbutamol-treated groups.

during the study to a minimum of 566 IU/mL in June and then rose to 820 IU/mL in August. This individual may have been sensitive to tree pollen as well as grass pollen, which would account for the initial fall in serum IgE. Repeating the analysis with this individual excluded did not change the comparison between groups and strengthened the statistical significance of the rise in serum IgE for the group as a whole.

Nasal IgE

Nasal IgE was measured in June (in season) and September (out of season). Geometric means for levels of nasal IgE were 0.2 IU/mL (range, 0 to 1.44 IU/mL) in season compared with 0.18 IU/mL (range, 0 to 2.22 IU/mL) out of season. A 2-sample t test showed no significant difference between levels in and out of season and no differences between the salbutamol and placebo groups.

DISCUSSION

In this study we have confirmed the results of previous studies that have shown a rise in serum IgE levels in association with the development of symptoms in seasonal rhinitis, but we were unable to identify any alteration in the local nasal production of IgE. The seasonal changes we have identified in serum IgE are comparable to those demonstrated by Lichtenstein et al²² and Naclerio et al²³ in ragweed-sensitive subjects with seasonal rhinitis and confirm the validity of this naturally occurring model of allergen-driven IgE production. This therefore provides an *in vivo* model with which to investigate the *in vitro* findings of Paul-Eugene et al¹⁶ and Coqueret et al¹⁷ who found that β_2 -agonists potentiated the IL-4-induced B cell-IgE production. We did not verify their *in vitro* findings *in vivo* and showed no enhancement of the seasonal increase in IgE in subjects taking regular salbutamol compared with those taking placebo. This cannot be attributed to insufficient subject numbers because the power of the study was such that there was a 90% power to detect a 3-fold difference in IgE levels between the 2

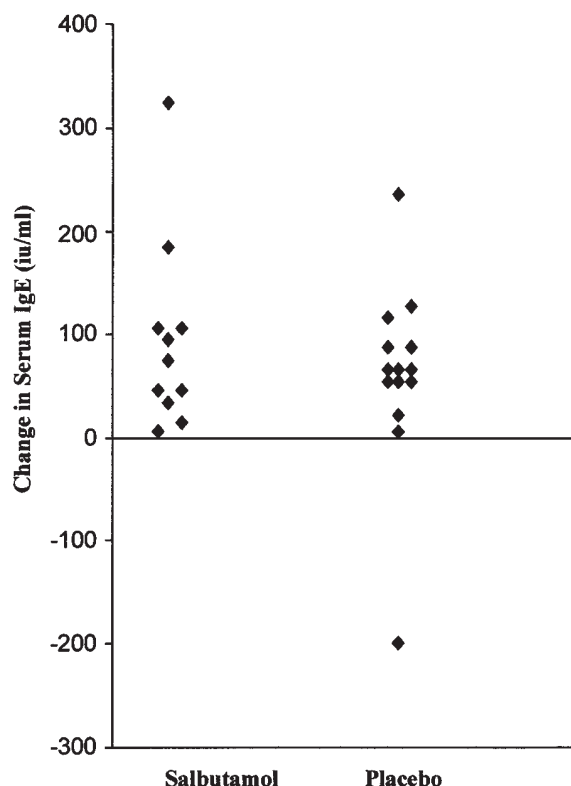


FIG 3. Changes in serum IgE, expressed as serum IgE in August to serum IgE in April, for salbutamol- and placebo-treated groups.

groups, far less than the 28-fold difference reported in vitro. Furthermore, with the oral dose of salbutamol used, plasma levels of 3.4 nmol/L would be anticipated,²⁴ which approximates the salbutamol concentration producing the maximum potentiating effect in vitro (10 nmol/L) when a concentration range of 1 pmol/L to 1 μ mol/L was investigated.¹⁶ Thus the treatment would have been anticipated to result in an effect in vivo should such an effect occur.

There are a number of reasons why the potentiating effects of salbutamol demonstrated in vitro are poorly reflective of the in vivo situation. First, the in vivo control of IgE production results from a complex interaction that cannot be reproduced in vitro. This process involves allergen penetration of the upper airway mucosa; uptake, processing, and presentation of allergen by antigen-presenting cells; and an interaction between T lymphocytes, B lymphocytes, and mast cells. It is recognized that β 2-agonists can stabilize mast cells, and this may lead to inhibition of the synthesis and release of IL-4 and IL-13, cytokines that are involved in the B-cell switch to IgE production. An in vitro model with PBMCs and IL-4 will not take these interactions into account. Furthermore, a proposed mechanism of the in vitro effect is stimulation of T-lymphocyte β 2-adrenoreceptors leading to inhibition of T-lymphocyte IFN- γ production. Downregulation of the T-lymphocyte β 2-agonist receptor has been clear-

ly demonstrated to occur within 7 days of salbutamol administration²⁵ thus reducing any effect of chronic salbutamol administration. Another possibility is that the effect of salbutamol is limited to activated B lymphocytes, and by measuring changes in total IgE levels, we are missing an effect of salbutamol on grass-specific IgE production. However, it is likely that most, if not all, of the 2½-fold rise in total IgE levels was due to increases in grass pollen-specific IgE. This is because the study was performed in grass pollen-sensitive individuals, the rise coincided with increases in symptoms of allergic rhinitis and increases in environmental grass pollen levels, and there is no reason to believe that there were significant changes in the levels of other allergens. Thus although it would have been helpful to measure grass pollen-specific IgE, it is likely that any effect of salbutamol on grass pollen-specific IgE would be reflected in changes in total IgE levels.

Our in vivo model also differs from the previous in vitro work in that in the latter PBMCs were taken from normal, as opposed to atopic, individuals. We chose to study patients with seasonal rhinitis because serum IgE levels are often unmeasurable in nonatopic individuals. We specifically excluded asthmatic subjects because it would be difficult to control for additional β 2-agonist use and because allergen-induced changes in IgE are often less marked than in subjects with seasonal rhinitis. Patients with seasonal rhinitis have detectable levels of serum IgE, exhibit a seasonal increase, and do not use β 2-agonists for symptom control. Subjects in this study took astemizole for symptom control, and we are not aware of any in vitro or in vivo work showing any effect of antihistamines on IgE production.

This study has also allowed the opportunity to assess changes in local IgE production, reflected by nasal IgE levels. IgE synthesis is predominantly thought to occur in regional draining lymph nodes, but there has been a suggestion that local synthesis of IgE may occur within the nasal mucosa.²⁶ Our study, although not directly assessing tissue IgE synthesis within the nose, does suggest that there is not a significant increase in local IgE production.

The implication of the previous in vitro work^{16,17} was that regular administration of β 2-agonists could lead to a worsening of asthma control through potentiation of IgE production. There is a close correlation between asthma severity and serum IgE levels,¹⁹ and serum levels of IgE are important determinants of the late response, suggesting a role for IgE in the pathogenesis of the underlying bronchial hyperreactivity. Our study suggests, however, that potentiation of IgE production cannot be used to explain any proposed link between β 2-agonist use and worsening of asthma control.

In this study we recorded symptom scores and antihistamine use as a check on possible confounding factors. An unexpected finding was that the administration of an oral β 2-agonist significantly reduced the symptoms of seasonal rhinitis, with a trend toward reduced use of antihistamines in the salbutamol-treated group. However, this data must be interpreted with caution because volun-

teers were only asked to record symptoms on a monthly rather than on a daily basis. Some previous studies have shown an effect of salbutamol on the symptoms of allergic rhinitis,²⁷ and this study suggests that further evaluation of its use in allergic rhinitis may be worthwhile.

In conclusion, using an *in vivo* model of allergen-induced IgE production, we have failed to show any effect of regular oral salbutamol on IgE production at doses that would generate serum levels equivalent to those that produced maximal effects *in vitro*. These findings indicate that potentiation of IgE production by β_2 -agonists cannot be invoked as a mechanism for possible β_2 -agonist-induced worsening of allergic inflammation.

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