

Ex vivo pharmacologic modulation of basophil histamine release in asthmatic patients

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Histamine is one of a range of mediators which play an important role in asthma, and the “releasability” of basophils has been shown to be upregulated in this disease. *In vitro*, β_2 -agonists and to a lesser extent corticosteroids have been shown to reduce histamine release. The *ex vivo* effects of salmeterol and inhaled corticosteroids on histamine release were studied in 78 asthmatic patients with variable disease severity and 20 control subjects. Spontaneous and anti-IgE-induced histamine release was measured in all subjects. Fifteen patients were not receiving any form of treatment, 42 were treated with inhaled corticosteroids, and 21 received inhaled corticosteroids and salmeterol. Seven patients treated with inhaled corticosteroids and seven patients treated with inhaled corticosteroids and salmeterol were tested twice to assess the effect of salmeterol on histamine release. Nine patients treated with inhaled corticosteroids were tested before and after 1 month of salmeterol treatment to determine the possible inhibition by salmeterol. Patients who were treated with inhaled corticosteroids and salmeterol showed significantly lower levels of spontaneous histamine release (median: 2.5%) than untreated (5.2%) and inhaled corticosteroids-treated asthmatics (3.4%). No tachyphylaxis to salmeterol was observed when patients were tested twice at a 3-month interval. This study suggests that salmeterol may have an additive anti-inflammatory effect with inhaled corticosteroids, although this hypothesis must be tested by further studies involving cells obtained by bronchoalveolar lavage and studies with bronchial biopsies.

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Metachromatic cells are found in the bronchi of normal and asthmatic subjects (1, 2). In asthmatic subjects, these cells have been shown to be degranulated by both direct methods such as electron microscopy (3) or immunocytochemistry and indirectly as demonstrated by increased levels of tryptase, histamine, and prostaglandin (PG)D₂ in the bronchoalveolar lavage (BAL) fluid (4–9). Basophils play an important role in the late-phase reaction both after allergen challenge (10, 11) and in acute asthma (12), but their role in chronic asthma remains to be clarified. Some authors have observed an increase in the spontaneous release of histamine from peripheral blood basophils of asthmatic subjects (13–19), an increase which is reduced when asthma symptoms are controlled. In some asthmatics, there is also an increased release of histamine in response to immunologic stimuli (20, 21).

Pharmacologic studies have examined the *in vitro* inhibitory activity of various antiasthma drugs on the immunologic or nonimmunologic release of histamine from blood basophils and mast cells. β_2 -Agonists have been found to be potent inhibitors of histamine release, but differences exist among the cell types studied (22–26). These *in vitro* effects have been confirmed during bronchial allergen challenge (27), exercise challenge (28), or skin tests with allergens (for review, see Bousquet & Michel [29]). However, it is not known whether tachyphylaxis can develop when β_2 -agonists are administered over long periods of time. Corticosteroids can block *in vitro* histamine release (30), but only one study has shown that orally administered steroids can inhibit histamine release (31).

Salmeterol, a long-acting β_2 -agonist (32), may possess some anti-inflammatory properties in

animals and man (33), as suggested by the inhibition of the late-phase reaction after allergen challenge (34), the sustained inhibition of the non-specific airways hyperreactivity, and the reduction of some inflammatory indices of BAL fluid (35) or peripheral blood (36). However, some studies have been inconclusive (37), and tolerance of the non-bronchodilator effects of salmeterol has been observed (38–40). Twenty control subjects and 78 asthmatic patients with variable disease severity were studied to examine 1) whether the spontaneous and anti-IgE release of histamine from blood basophils was correlated with the severity of asthma and its stability, and 2) the *ex vivo* effects of inhaled corticosteroids or salmeterol on histamine release from the basophils of asthmatic patients. The effects of salmeterol were examined in seven patients tested twice to determine whether tachyphylaxis may occur.

Material and methods

Subjects

Seventy-eight asthmatic patients (15–70 years of age) were studied. Asthma was defined as previously described in detail (41). All patients had reversible airways obstruction defined according to the guidelines of the Medical Section of the American Lung Association and characterized by a 12% increase in FEV₁ and an absolute change of 200 ml after the inhalation of 200 µg of albuterol (42). IgE-mediated allergy was defined according to a previous paper (41). Twenty normal nonallergic subjects (22–60 years of age) were used as a control group.

Assessment of asthma severity and treatment of patients

The severity of asthma was assessed according to the score of Aas (43), a scoring system which we have previously used in several studies (41, 44). This system grades the different forms of chronic asthma on the basis of the events that took place during the previous year. The score ranges from very mild (grade 1) to very severe (grade 5). Asthma instability was defined according to the criteria set by the International Consensus Report on the Diagnosis and Management of Asthma (45). A pulmonary function test was carried out with the same equipment for all patients (Pneumoscreen; E. Jaeger Laboratories, Würzburg, Germany). Predicted values were calculated by the method of Knudson et al. (46).

Patients receiving therapy were always optimally treated according to the guidelines set by the International Consensus Report on the Diagnosis and Management of Asthma (45), and the dose of

inhaled corticosteroids prescribed was adapted to the severity of the disease. Salmeterol was administered only to patients receiving at least 800 µg of budesonide or 1000 µg of beclomethasone and was always added to the inhaled corticosteroids treatment. None of the patients received β₂-agonists in oral, parenteral, or aerosol forms since we have previously found that β₂-agonist aerosols induce a reduction in histamine release (Bousquet et al., unpublished data).

Histamine release

Histamine release from peripheral blood basophils was measured. Venous blood was drawn into heparinized tubes and diluted 1/14 with PBS containing Ca²⁺ (1 mM) and Mg²⁺ (1 mM) at pH 7.4. The challenge was carried out with 200 µl of diluted blood, 70 µl of PBS, and 30 µl of rabbit antihuman IgE (Dako, Versailles, France). Final anti-IgE concentrations were 0 (spontaneous histamine release), 0.1, 1, 10, and 100 µg/ml. After 30-min incubation at 37°C, cells were centrifuged and supernatants were collected and frozen at –20°C until histamine measurements were performed. The total histamine content of the blood cells was measured after cell lysis by a freezing and thawing (three times) technique. Histamine was measured with a highly specific and sensitive enzyme-immunoassay (Immunotech, Luminy, France), a monoclonal antibody against acylated histamine being used (47). The sensitivity of the assay is 0.2 nM.

Calculation methods

Spontaneous (spont) and net histamine release were calculated as follows:

spontaneous release (%)

$$= \frac{\text{spont histamine release (nM)}}{\text{total histamine (nM)}} \times 100$$

net release (%)

$$= \frac{\text{challenge histamine release (nM)} - \text{spont histamine release (nM)}}{\text{total histamine (nM)} - \text{spont histamine release (nM)}} \times 100$$

Design of the study and statistical analysis

All subjects had their spontaneous and IgE-induced histamine release levels determined. To assess whether salmeterol can reduce histamine release *ex vivo*, nine asthmatic subjects who were treated with 800 µg of budesonide or 1000 µg of beclomethasone

were tested at baseline and after 1 month of the same treatment plus salmeterol (100 µg twice daily). To assess the variability of histamine release, seven patients receiving only inhaled corticosteroids were tested twice after at least 1 month of stable dose (1 month) and 7–9 weeks later (3 months), and seven patients receiving salmeterol (100 µg twice daily) and 800 µg of budesonide or 1000 µg of beclomethasone were tested twice after at least 1 month of stable dose (1 month) and 7–9 weeks later (3 months) in order to assess also the possible tachyphylaxis of salmeterol on histamine release. The doses of salmeterol and inhaled corticosteroids administered to the subjects were kept constant during the study periods, although the consumption of β₂-agonists varied from patient to patient.

Statistical analysis was done with nonparametric tests. The Kruskal–Wallis test with Dunn’s multiple comparisons test was used for intergroup analysis. Results are given as medians and range.

Results

Demographic characteristics of the subjects

The demographic characteristics of the subjects are given in Table 1. The median age of subjects was different in the four groups (controls, untreated asthma, asthma treated with inhaled corticosteroids, and asthma treated with inhaled corticosteroids and

salmeterol), ranging from 30 to 50.5 years. The percentages of allergic patients are given in Table 1. Among them, 85% were sensitized to perennial allergens (house-dust mites, molds, and animal danders); 53% of the patients were also sensitized to pollens. However, the studied patients had perennial asthma. The severity of asthma assessed by the Aas score was greater in the inhaled corticosteroids with the salmeterol group than in the other two groups, but median FEV₁ was not significantly different in the three asthma groups.

Total histamine content

Total histamine content was not significantly different in the four groups of subjects (Table 2).

Spontaneous histamine release

Spontaneous histamine release was significantly lower in the group treated with inhaled corticosteroids plus salmeterol (median and range: 2.5; 0.3–7.6%) than in untreated asthmatic patients (5.2; 1.9–18.1%) or asthmatics treated with inhaled corticosteroids (3.4; 0.1–11.4%) (Table 2). There was no significant difference in the spontaneous release of histamine between the control group (1.5; 0.2–6.3%) and the inhaled corticosteroids plus salmeterol asthma group. In asthmatic patients tested twice (Fig. 1), spontaneous histamine release was

Table 1. Demographic characteristics of subjects

	Controls	Asthma			P value*
		Untreated	ICS	ICS + SMR	
No. of subjects	20	15	42	21	
Age (yr)	30 (22–60)	37 (15–64)	45 (15–70)	50.5 (22–60)	0.01
Sex (M/F)	11/9	5/10	17/25	9/12	
Allergy	0	58%	82%	64%	
Severity of asthma					
Aas score		3 (1–4)	3 (1–5)	4 (2–5)	0.035
FEV ₁ (% pred)		88 (58–100)	80 (38–118)	77 (39–105)	NS

* Statistical analysis by Kruskal-Wallis test.
Except for sex ratio, results are expressed in median and range.

Table 2. Results of histamine release

	Controls	Asthma			P value*
		Untreated	ICS	ICS + SMR	
Total histamine (nM)	504 (125–947)	353 (129–706)	562 (216–2009)	451 (264–1760)	NS
Spontaneous HR (%)	1.5 a (0.2–6.3)	5.2 b (1.9–18.1)	3.4 c (0.1–11.4)	2.5 d (0.3–7.6)	P<0.001

Results expressed in medians and range. Statistical analyses were done by Kruskal-Wallis test with Dunn’s multiple comparisons test.
P (b/a) < 0.001, P (b/c) > 0.05 NS, P (b/d) < 0.01.
ICS = inhaled corticosteroids; SMR = salmeterol.

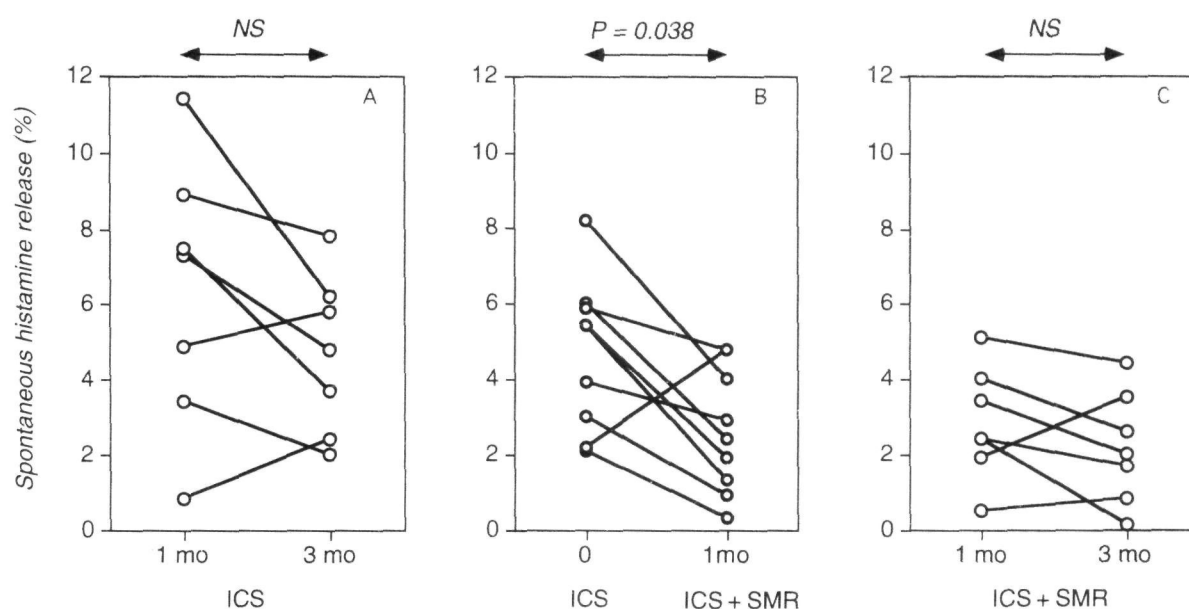


Fig. 1. Spontaneous histamine release (results expressed in median value). First panel: patients receiving inhaled corticosteroid (ICS), tested at 1 and 3 months ($n=7$). Second panel: patients tested before and 1 month after salmeterol (SMR) treatment ($n=9$). Third panel: patients receiving inhaled corticosteroids (ICS) and salmeterol (SMR) tested at 1 and 3 months ($n=7$).

significantly ($P<0.04$ Wilcoxon W test) decreased in those patients receiving inhaled corticosteroids who were subsequently treated with salmeterol. On the other hand, there was no significant difference in inhaled corticosteroids-treated patients or in patients receiving inhaled corticosteroids with salmeterol tested twice.

Anti-IgE-induced histamine release

There was no difference in histamine release between the groups at any concentration of anti-IgE. Patients tested twice presented similar levels of anti-IgE-induced histamine release. Finally, inhaled corticosteroids-treated patients who subsequently received salmeterol demonstrated a nonsignificant decrease in anti-IgE-induced histamine release (Fig. 2).

Discussion

The results of this study indicate that inhaled corticosteroids do not modify the *ex vivo* spontaneous histamine release, whereas salmeterol ($P<0.01$) significantly reduces this release. Neither treatment affected anti-IgE-induced histamine release.

The methods presented in this paper have been widely validated both for the selection of subjects and the measurement of histamine release. The selection and treatment of subjects is always critical in any experiment, and we have attempted to define patients strictly by the score of Aas (43), which has been used consistently in our clinic and validated in many studies. The median age of patients in each of the groups was different because, in all studies dealing with asthma, patients with severe asthma tend to be older than those with mild forms of the

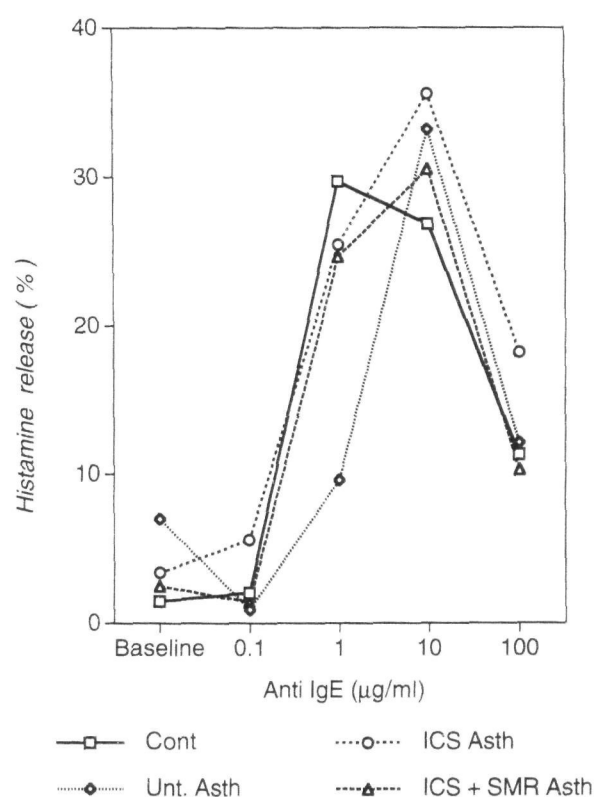


Fig. 2. Spontaneous and anti-IgE-induced histamine release (results expressed in median values). ICS: asthmatics treated with inhaled corticosteroids; ICS + SMR: asthmatics treated with inhaled corticosteroids and salmeterol. Cont: control; Unt. Asth: untreated asthmatics.

disease. However, these differences do not appear to have a significant effect on histamine release since in patients tested twice who were treated with inhaled corticosteroids alone on the first occasion and received salmeterol before the second test, we observed a decrease in the spontaneous histamine release. All patients who were given salmeterol were also taking inhaled corticosteroids since this is a prerequisite for the use of salmeterol in France. However, the patients were not treated with oral corticosteroids as this could influence histamine release. In one study, these drugs were found to

reduce histamine release *ex vivo* (31), while other authors found no effect of dexamethasone on histamine release from basophils of patients receiving long-term steroid therapy (48). The release of histamine was anti-IgE-concentration dependent. We found a higher spontaneous histamine release in nontreated asthmatic patients than in normal subjects; this difference was significant. An elevation in histamine release in asthmatic subjects has been reported by some other authors; however, in most of these reports, the increase in releasability was evident only when the basophils were "primed" with D₂O (14, 16–19, 49, 50). This study was not carried out with D₂O priming.

Inhaled steroids did not reduce the increased histamine releasability of basophils from asthmatic subjects. We used two groups of patients to study the effects of inhaled corticosteroids: a small group of seven patients tested twice at a 3-month interval to test the reproducibility of histamine release and a larger group of 42 patients receiving long-term inhaled corticosteroids. Corticosteroids may affect the numbers of basophils (51), but the lack of significant statistical difference in total histamine between groups strongly suggests that the number of basophils is unaltered.

To the best of our knowledge, the lack of effect of inhaled corticosteroids on anti-IgE-induced histamine release has not been previously reported. It is possible that this lack of effect is due to the poor compliance of patients with inhaled drugs (52), but we attempted to control this factor as far as possible by questionnaire. Prednisolone, dexamethasone, and deflazacort can inhibit anti-IgE histamine release *in vitro* (30, 53, 54), and oral corticosteroids have been shown to have a similar *ex vivo* effect (31), suggesting that the dose of inhaled corticosteroids received by the patients was insufficient to induce a reduction in basophil activation. On the other hand, salmeterol was found to reduce spontaneous histamine release. We used three patient groups to study the effects of treatment: a small group of nine inhaled corticosteroids-treated patients tested twice, before and after 1 month of salmeterol treatment, in order to show that salmeterol decreased histamine release; a second group of seven patients treated with inhaled corticosteroids and salmeterol tested at a 3-month interval to examine whether tachyphylaxis might occur, as had been observed for the nonbronchodilating activities of salmeterol (38–40); and a larger group of 21 patients receiving long-term treatment with inhaled corticosteroids and salmeterol. First, salmeterol was shown to reduce spontaneous but not anti-IgE-induced histamine release, confirming that salmeterol, a long-acting β_2 -agonist, has similar *in vitro* and *ex vivo* properties. Moreover, the effect of

salmeterol was prolonged over time, and no tachyphylaxis was seen after a 3-month treatment period. The clinical significance of these data is not completely clear since basophils are peripheral blood cells, the importance of which needs elucidation in asthma despite the significant correlation between basophil histamine release and bronchial hyperreactivity in this disease (21). Allergen challenge leads to increased numbers of basophils in peripheral blood (10) and increased histamine release from blood basophils (55). Basophils have been found in BAL fluid during the late-phase allergic reaction (56), and basophil counts are increased during acute exacerbation of asthma. Salmeterol adds anti-inflammatory effects to those of inhaled corticosteroids, which were unable to reduce histamine release, but differential effects of this drug may occur in mast cells, which are thought to be important in the early asthmatic reaction, unlike basophils, which are generally associated with the late-phase reaction (13). Therefore, the effects of salmeterol should be investigated using bronchial biopsies and BAL in patients receiving high doses of inhaled corticosteroids and whose asthma is not fully controlled, to assess whether salmeterol has additional clinical and anti-inflammatory effects. This study may help to clarify why patients with unstable asthma receiving inhaled corticosteroids apparently benefit more from salmeterol than from an additive dose of inhaled corticosteroids (57).

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