

Protective effect of inhaled lysine acetylsalicylate on allergen-induced early and late asthmatic reactions

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Conflicting results have been reported on the effect of non-steroidal antiinflammatory drugs on allergen-induced asthmatic responses. The aim of this study was to investigate the effect of inhaled lysine acetylsalicylate (LASA) on the early and late allergen-induced responses. We studied 16 patients with mild, stable asthma who had an early asthmatic response and 10 patients with a dual (early and late) response. Each patient underwent two challenges with a single dose of allergen assessed in a preliminary test, after inhalation of either 720 mg of LASA in 4 ml of saline solution or placebo, according to a randomized, double-blind protocol. Allergen-induced hyperreactivity to methacholine was measured in six patients from each of the early and the dual response groups 2 hours and 24 hours after the challenge, respectively. In the patients with early response, the maximum fall in FEV₁ after challenge was 24% $\pm$ 1% after inhalation of placebo and 14% $\pm$ 2% after inhalation of LASA ($p < 0.005$). No protection was observed in four patients who received the drug orally instead of by inhalation. In the patients with a dual response, the maximum FEV₁ decrease during the early response was 27% $\pm$ 2% after placebo and 21% $\pm$ 2% after LASA ($p < 0.025$). During the late response (between 3 and 8 hours), the maximum decrease in FEV₁ was 28% $\pm$ 4% after placebo and 16% $\pm$ 4% after LASA ($p < 0.005$). In both groups allergen challenge caused a significant reduction in methacholine PD₂₀ after treatment with placebo but not with LASA. Without allergen challenge, LASA had no effect on methacholine reactivity. We conclude that inhaled LASA significantly reduces both the early and the late asthmatic response to allergen challenge and that it prevents the allergen-induced airway hyperresponsiveness that follows these responses. (*J Allergy Clin Immunol* 1997;100:71-7.)

Key words: Bronchial asthma, allergen challenge, bronchial hyperreactivity, lysine acetylsalicylate, aspirin, nonsteroidal antiinflammatory drugs

Bronchial inflammation is considered a characteristic feature of bronchial asthma,¹ and an antiinflammatory action is considered to be the main mechanism of the therapeutic effect of corticosteroids and chromones.^{2,3} However, disappointing or conflicting results have been

Abbreviations used

AU:	Allergen units
AUC:	Area under the curve
DD:	Doubling doses
LASA:	Lysine acetylsalicylate
NSAIDs:	Nonsteroidal antiinflammatory drugs
PEFR:	Peak expiratory flow rate

observed in several models of asthma in which nonsteroidal antiinflammatory drugs (NSAIDs), a family of powerful antiinflammatory agents whose activity has been attributed to cyclooxygenase inhibition, were used.⁴

With respect to bronchial allergen challenge, oral administration of indomethacin has little or no effect,⁵⁻⁸ and sometimes worsens,⁹ the early response. The same treatment has been initially reported to inhibit the late asthmatic response,^{6,7,10} a finding that has not been confirmed in subsequent controlled studies.^{8,11} Recently, however, a weak protection against the early and the late responses was reported for the more potent cyclooxygenase inhibitor, flurbiprofen,^{12,13} which suggests that arachidonic acid products may participate in allergen-induced bronchoconstriction.

We observed recently that the protective activity of a soluble form of aspirin, lysine acetylsalicylate (LASA), against a physiologic stimulus, ultrasonic mist of distilled water, is greatly enhanced if the drug is given by inhalation,¹⁴ probably because a higher local drug concentration is attained by using this route. We report the results of further studies, which indicate that a single dose of inhaled LASA also attenuates both the early and the late asthmatic response to allergen challenge in patients with allergic asthma.

METHODS

Patients

We studied patients with mild allergic asthma who were first seen with an early or dual asthmatic reaction in a preliminary bronchial challenge with the relevant allergen. All the patients were untreated except for regular or occasional use of a short-acting inhaled β_2 -agonist. Such treatment was withheld for at least 14 hours before each test. Patients allergic to pollens were studied outside the pollen season. Patients with aspirin-induced asthma were not included in the study. To exclude the possibility of aspirin sensitivity, we performed a bronchial

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TABLE I. Anthropometric data of patients with an early (top) and a late (bottom) response

Patient No.	Sex	Age (yr)	FEV ₁ (% pred)	Allergen	PD ₂₀ (AU)
1	M	18	121	GR	5.31
2	M	23	86	GR	0.75
3	M	25	101	GR	0.8
4	F	26	105	GR	1.4
5	M	26	93	GR	0.53
6	M	22	91	GR	0.65
7	F	23	86	DP	0.3
8	M	32	82	DP	0.4
9	F	29	76	GR	0.2
10	M	18	88	DP	0.8
11	F	24	86	GR	3.2
12	M	22	94	GR	0.55
13	M	23	109	DP	2.2
14	F	22	80	GR	1.0
15	M	18	101	GR	1.8
16	M	14	89	GR	0.3
Mean		23	93		1.3
SEM		1	3		0.3

Patient No.	Sex	Age (yr)	FEV ₁ (% pred)	Allergen	PD ₂₅ (AU)
1	M	25	80	PA	0.3
2	M	19	93	GR	1.2
3	M	23	92	DP	2.6
4	F	26	83	DP	1.2
5	M	25	94	GR	3.6
6	M	18	80	DP	3.2
7	F	28	96	GR	0.4
8	M	17	93	DP	0.7
9	F	36	110	DP	0.6
10	M	18	84	GR	0.8
Mean		24	91		1.5
SEM		2	3		0.4

GR, Grass pollen; DP, *Dermatophagoides pteronyssinus*; PA, pollen of *Parietaria* species (nettles).

challenge with LASA^{15,16} in subjects who could not report having taken aspirin or other known cyclooxygenase inhibitors without side effects in the 12 months preceding the study. All patients underwent a methacholine challenge as part of the preliminary clinical evaluation. The study was conducted according to the guidelines of the ethical committee of our institution, which require informed consent from each patient.

Bronchial challenges

Bronchial challenges with allergen and methacholine were performed with a dosimeter (Mefar; Markos, Brescia, Italy) as previously described.¹⁷ In the allergen challenge, an early reaction was considered to be a decrease of 20% or more in FEV₁ with respect to baseline between 15 and 30 minutes after challenge. The doses causing a 20% and 25% decrease in FEV₁ (PD₂₀ and PD₂₅) were computed by interpolation on the cumulated dose-response curve. The patients with an early response were then monitored for the rest of the day with a Mini Peak Flow Meter, and a late response was considered to be a decrease in FEV₁ or peak expiratory flow rate (PEFR) of 15% or more between 4 and 8 hours after challenge. Patients who were first seen with a late response underwent PEFR monitoring during a control day. If a PEFR variation of more

than 10% was observed, their asthma was considered unstable, and they were excluded from the study. All the experiments were conducted within 3 months of the preliminary test.

Effect of inhaled LASA on the early asthmatic reaction to allergen challenge

We studied 16 patients who were first seen with an early reaction in the preliminary test (Table I). Each patient underwent two additional challenges, at least 3 weeks apart, with a single dose of allergen consisting of the PD₂₀ computed in the preliminary test. The tests were performed 30 minutes after the end of an aerosol spray of 4 ml of 180 mg/ml LASA (Flectadol; Maggioni Winthrop Italia, Milan, Italy), corresponding to 100 mg/ml acetylsalicylic acid, or placebo (lysine 50 mg/ml), administered in a double-blind manner according to a randomized order with a jet nebulizer (Nebula; Markos, Monza, Italy). FEV₁ was recorded before and after treatment and at 5, 15, 30, 45, and 60 minutes.

To investigate whether the protective effect of LASA could be due to a systemic effect of the ingested fraction of the aerosol spray, we tested the effect of oral LASA in four patients with clinical characteristics similar to those of the patients in the

previous study. Each subject underwent two allergen challenges as reported above, 20 minutes after treatment with either 900 mg of oral LASA (corresponding to 500 mg acetylsalicylic acid), or placebo. FEV₁ was recorded at the same time points as with the other treatments.

Effect of inhaled LASA on bronchial hyperreactivity after the early asthmatic reaction

For six of the patients with an early asthmatic reaction (nos. 11 to 16 in Table I), a methacholine challenge was performed 2 hours after allergen administration on the 2 study days. The results were compared with those obtained in the preliminary methacholine challenge.

Effect of inhaled LASA on the bronchial response to methacholine

To verify that the results of the previous experiment were not due to a direct effect of LASA on methacholine reactivity, we studied six additional patients who performed two methacholine challenges 2 hours after administration of either LASA or placebo according to a protocol similar to that of the previous study but without allergen challenge (Table II).

Effect of inhaled LASA on the late asthmatic response to allergen and on allergen-induced bronchial hyperreactivity

We studied 10 patients who had a dual asthmatic response in the preliminary test (Table I). The study was conducted according to a protocol similar to that of the early reaction study, except that the PD₂₅ obtained in the preliminary test was used as the allergen dose for further challenges, and FEV₁ was also recorded every 60 minutes for 8 hours. In six patients (nos. 5 to 10 in the bottom part of Table I) a methacholine challenge was repeated 24 hours after each test.

Statistical analysis

The protective activity of LASA was expressed as a percentage of the effect of placebo according to the formula: (placebo – treatment) ÷ placebo × 100, computed by using either the maximum decrease in FEV₁ from the baseline values or the area under the curve (AUC) of the differences from baseline over time. Changes in methacholine reactivity are expressed as doubling doses (DDs) of methacholine PD₂₀ computed as log₂(PD₂₀ control) – log₂(PD₂₀ treatment). FEV₁ values at baseline, after treatment and on different days, were compared by analysis of variance. All other comparisons were performed with paired Student's *t* test. A *p* value less than or equal to 0.05 was considered significant. Unless stated otherwise, data are presented as the mean ± SEM. When the geometric mean is shown, the geometric standard error is presented as a percentage of the mean.¹⁸

RESULTS

Effect of inhaled LASA on the early asthmatic reaction to allergen challenge

Baseline FEV₁ values of the patients were similar on the 2 days of the study. Inhaled LASA was well tolerated and did not cause significant changes in baseline FEV₁ (Table III). After placebo administration, allergen challenge caused a rapid decrease in FEV₁, which was maximal (24% ± 1% of baseline) between 15 and 30 minutes, after which there was a slow recovery. After LASA administration, the early reaction to allergen was

TABLE II. Anthropometric data of patients in the methacholine study

Patient No.	Sex	Age (yr)	FEV ₁ (%pred)	Allergen
1	M	21	87	DP
2	M	16	116	GR
3	F	39	89	GR
4	F	18	94	DP
5	M	47	82	DP
6	M	16	89	DP
Mean		26	93	
SEM		5	5	

DP, *Dermatophagoides pteronyssinus*; GR, grass pollen.

reduced (Fig. 1). There was a maximum decrease in FEV₁ of 14% ± 2% (*p* < 0.005 vs placebo), corresponding to a degree of protection of 38 ± 11 when calculated with the maximum FEV₁ decrease and of 35% ± 13% with the AUC of the reaction. The protection induced by LASA was not homogeneous among the patients: 12 of the 16 subjects showed a degree of protection of 20% or more, whereas in four of them the response to allergen was unaffected or increased after LASA treatment.

Interestingly, the effect of oral treatment with LASA on the response to allergen challenge was not significantly different from that of placebo in any of the four patients in whom it was tested (not shown).

Effect of inhaled LASA on bronchial hyperreactivity after the early asthmatic reaction

The effect of LASA on methacholine reactivity 2 hours after challenge is reported in Table III. Baseline FEV₁ was not significantly different between the preliminary test and the two postchallenge tests. After placebo administration, methacholine PD₂₀ was reduced in all the subjects with respect to the preliminary test (mean 2.3 ± 0.6 DDs, *p* < 0.01). After LASA, however, the methacholine PD₂₀ 2 hours after allergen challenge was not significantly different from the preliminary test (0.1 ± 0.7 DDs). There was no correlation between the protective effect of LASA against allergen-induced bronchoconstriction and its effect on bronchial hyperresponsiveness.

In the six subjects who performed the methacholine test without allergen challenge, there were no significant differences in baseline FEV₁ or in the PD₂₀ values obtained after placebo and LASA administration and in the preliminary test (Table III).

Effect of inhaled LASA on the late asthmatic response

In the patients with a dual asthmatic response, inhaled LASA had little effect on the early response but consistently reduced the late asthmatic reaction (Fig. 2). The maximum FEV₁ decrease within the first hour after challenge (early response) was 27% ± 2% after placebo administration and 21% ± 2% after LASA administra-

TABLE III. Effect of LASA on methacholine reactivity 2 hours after allergen challenge

	Preliminary	Placebo	LASA
Allergen challenge			
Baseline FEV ₁ (L)			
Time 0 (before treatment)	3.45 ± 0.36	3.45 ± 0.42	3.36 ± 0.39
15 min (after treatment)	ND	3.46 ± 0.44	3.57 ± 0.33
2 hr (before methacholine)	ND	3.35 ± 0.42	3.35 ± 0.39
Max. FEV ₁ decrease during allergen challenge (%)	ND	25 ± 4	15 ± 2
Methacholine PD ₂₀ * (%)	1474 ± 56	295 ± 43†	1334 ± 28
No allergen challenge			
Baseline FEV ₁ (L)			
Time 0 (before treatment)	2.83 ± 0.34	2.83 ± 0.30	2.88 ± 0.34
15 min (after treatment)	ND	2.83 ± 0.31	2.83 ± 0.33
2 hrs (before methacholine)	ND	2.83 ± 0.32	2.83 ± 0.31
Max. FEV ₁ decrease during allergen challenge (%)	ND	ND	ND
Methacholine PD ₂₀ (%)	140 ± 31	84 ± 19	106 ± 17

ND, Not done.

*Geometric mean in micrograms.

† $p < 0.025$ versus LASA, $p < 0.01$ versus preliminary.

tion, resulting in a small but significant protection of $17\% \pm 6\%$ when computed on the maximum FEV₁ decrease ($p < 0.025$) and protection of $11\% \pm 9\%$ (not significant) with the AUC. A protective effect on the early response of more than 20% was observed in only four subjects. After returning to values similar to baseline at 2 hours, FEV₁ declined again in both groups, but this decrease was smaller after LASA administration than after placebo administration. A mean FEV₁ value significantly lower than baseline was observed after 4 hours with placebo and after 5 hours with LASA, and values remained significantly below baseline for the remainder of the experiment in both trials. However, FEV₁ values after LASA remained significantly higher with respect to placebo at all the time points after 4 hours (Fig. 2). The maximum FEV₁ decrease between the third and the eighth hours (late response) was $28\% \pm 4\%$ after placebo and $16\% \pm 4\%$ after LASA, resulting in a degree of protection of $43\% \pm 10\%$ ($p < 0.005$). A degree of protection on the late response greater than 20% was observed in eight subjects. When computed with the AUC, the protection between the third and eighth hours was $56\% \pm 16\%$.

Effect of inhaled LASA on bronchial hyperreactivity after the late asthmatic reaction

In the six subjects who underwent the methacholine test 24 hours after allergen challenge, treatment with LASA significantly inhibited the allergen-induced increase in bronchial hyperresponsiveness (Fig. 3). No significant differences were observed in the baseline FEV₁ values before the three challenges. After placebo, methacholine PD₂₀ significantly decreased by 2.4 ± 0.6 DDs with respect to the preliminary test ($p < 0.01$), whereas the change after LASA was not significant (0.9 ± 0.7 DDs). The difference between the methacholine PD₂₀ values after LASA and placebo administration was 2.5 ± 0.4 DDs ($p < 0.025$).

DISCUSSION

Our results indicate that inhaled LASA significantly attenuates both the early response (at least in some of the patients) and the late asthmatic reaction after allergen challenge. These data extend our previous observations on the protective effect of inhaled LASA on bronchoconstriction induced by ultrasonically nebulized water¹⁴ and confirm that this drug affects the bronchial response to bronchoconstrictor stimuli clinically relevant in asthma. The effect on the early response is in agreement with findings previously reported on the effect of topically applied aspirin on the immediate allergic response in the nose¹⁹ and in the eye.²⁰ The uneven distribution of the protection against the early reaction, as observed in our patients, has also been reported by others.^{7, 13} This could be due to the heterogeneity of the individual response to NSAIDs, also reported in patients with rheumatic diseases.²¹ Nevertheless, this effect is still remarkable because it is known that a single dose of either systemic or inhaled corticosteroids does not affect this part of the asthmatic reaction. Because steroids become more effective after long-term administration,²² it would be interesting to verify whether patients, in whom LASA does not provide protection against allergen, would be protected after sustained treatment with the drug. The protective effect of inhaled LASA in this study is in the same range as that reported for oral leukotriene synthesis inhibitors, although some of the more potent leukotriene receptor antagonists have shown a better protection against the early response.²³

In patients who were first seen with a dual response, LASA provided a lower degree of protection against the early response than in patients with only an isolated early response. This might be due to the higher allergen dose used in this group to elicit the late response. On the other hand, these patients showed a much more homogeneous pattern of protection against the late response. The late asthmatic reaction reflects more closely the

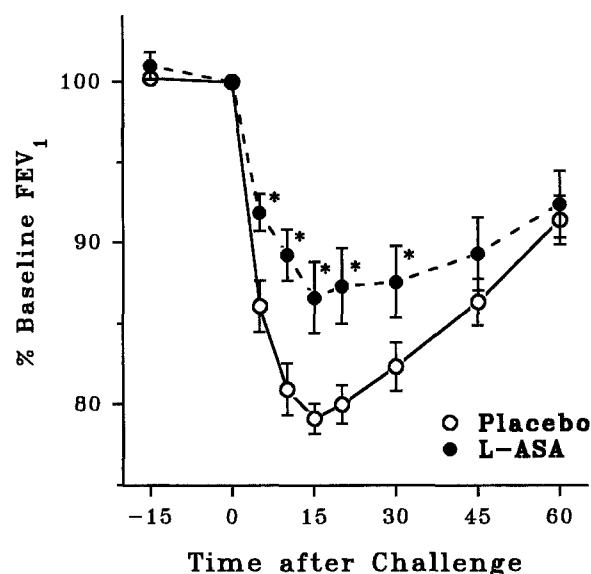


FIG. 1. Effect of inhaled LASA (dashed line) and placebo (solid line) on the early asthmatic response. Bars represent mean $\pm$ SEM. *Statistically significant versus placebo were observed at 5 minutes ($p < 0.01$); 10, 15, and 20 minutes ($p < 0.005$); and 30 minutes ($p < 0.025$).

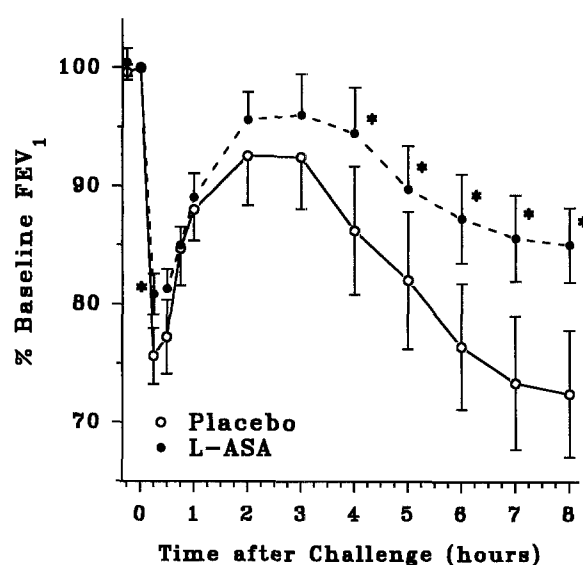


FIG. 2. Effect of inhaled LASA on the asthmatic response to allergen challenge in 10 patients with both early and late asthmatic responses. Bars represent mean $\pm$ SEM. *Statistically significant differences versus placebo were observed at 15 minutes ($p < 0.025$), 4 hours ($p < 0.005$), 5 hours ($p < 0.025$), 6 hours, 7 hours, and 8 hours ($p < 0.005$).

pathogenetic mechanisms of clinical asthma than does the early reaction, particularly the inflammatory events associated with the chronicity of the disease.^{24,25} Although we did not directly measure the local inflammatory response in our patients, the observation that LASA also inhibits the allergen-induced increase in airway reactivity after both the early and late reactions, which has been related to the inflammatory response that follows allergen exposure,²⁶ supports the hypothesis that these effects are mediated by the well-known antiinflammatory activity of this drug. However, recent experimental studies suggest that allergen-induced bronchoconstriction and bronchial hyperresponsiveness are the results of different mechanisms, the latter possibly due to local release of endothelin-1.²⁷ Interestingly, in some species the bronchoconstrictor effect of endothelin-1 is mediated by cyclooxygenase products,²⁸ and cyclooxygenase blockade with oral indomethacin was found to inhibit allergen-induced bronchial hyperresponsiveness but not the late asthmatic response.⁸ Thus it is possible that inhibition of endothelin release or activity contributes to the effect of LASA on this phenomenon. The effect of LASA on bronchial reactivity is not due to a direct effect on methacholine reactivity, because in this study, as in a previous one,²⁹ inhaled LASA had no effect on the bronchial response to methacholine when the test was not preceded by allergen challenge.

Our study provides very few data with which to evaluate the possible mechanism of action of LASA. It has been believed that the main mechanism of action of acetylsalicylic acid and of the other NSAIDs was the inhibition of cyclooxygenase activity and thus suppression of the production of proinflammatory prostaglan-

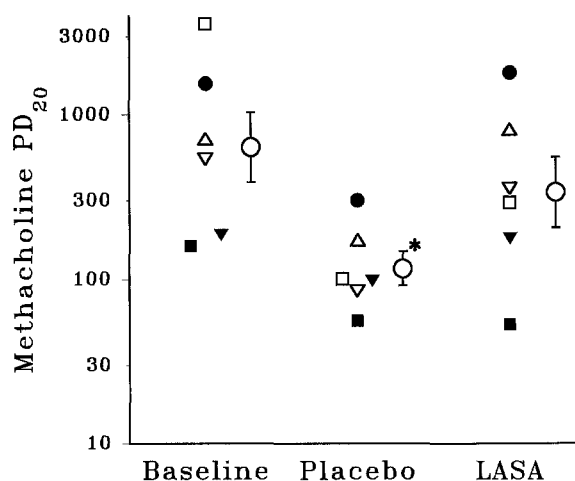


FIG. 3. Methacholine PD₂₀ (in micrograms) measured before challenge and measured again 24 hours after two specific challenges preceded by placebo and LASA administration. Each symbol represents a patient reported in Table II. Each of the six patients is indicated by the same symbol in the three columns. (The large symbol with bars represents the mean.) The geometric mean and SEM are shown to the right of each group of values. * $p < 0.025$ versus LASA.

dins.³⁰ More recently, this picture has been further complicated by the discovery of different cyclooxygenases, variously expressed in different cells and conditions, showing different sensitivity to inhibition by various NSAIDs.³¹ However, lung cyclooxygenase appears to be relatively resistant to inhibition by aspirin or indomethacin.^{32,33} Furthermore, prostaglandins do not

appear to have a pathogenetic role in allergic asthma, because prostaglandins E_1 and E_2 rather effectively inhibit both the early and the late asthmatic reaction,^{34,35} and cyclooxygenase blockade with indomethacin actually increases allergen-induced contractions of human bronchi in vitro.³⁶

Although we have preliminary evidence that inhaled LASA has systemic effects on arachidonic acid metabolism,³⁷ we did not directly address any of the issues related to the role of cyclooxygenase products in asthma. Indeed, the doses used in this study probably result in relatively high concentrations of the drug in the airways. At these concentrations mechanisms of action other than cyclooxygenase inhibition,^{21,38} including inhibition of phospholipase³⁹ or downregulation of adhesion molecules and inflammatory cytokines,⁴⁰ may occur.

Whatever the mechanism of the protective effect of LASA against the early asthmatic response might be, the effect that we observed is probably due to the small amount of the drug that reached the airways, because oral administration of the drug at a dose higher than could be expected to be the ingested fraction of the aerosol was ineffective in all patients in whom it was tested. This is in agreement with previous observations that oral indomethacin and flurbiprofen have little or no effect on the bronchial response to allergen challenge^{8,13} but is in contrast to what has been observed in the nose, when oral intake of aspirin has been reported to inhibit the immediate response to local allergen challenge.¹⁹ However, the number of subjects in this experiment was too limited to rule out that oral aspirin, possibly at higher doses or with a different time schedule, may affect the bronchial allergic response.

In previous studies, treatment with inhaled LASA, in association with furosemide, has proven clinically effective in reducing the dose of systemic and inhaled steroids in steroid-dependent asthma.^{41,42} The present observation that it attenuates the bronchial response to allergen in patients with mild, stable asthma is not sufficient to recommend this treatment for allergic asthma. However, it suggests that LASA could prove clinically useful in the treatment of this disease and indicates the need for specific clinical studies to investigate this topic more thoroughly.

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