

Inhaled lysine acetylsalicylate (L-ASA) attenuates histamine-induced bronchoconstriction in asthma

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When administered by inhalation, histamine provokes dose-related bronchoconstriction in asthmatic subjects mainly by a direct activation of histamine H_1 -receptors on airway smooth muscle. However, little is known of the change in airway responsiveness to histamine after cyclooxygenase blockade. The aim of the study was to investigate the effect of the potent cyclooxygenase inhibitor, lysine acetylsalicylate (L-ASA), administered by inhalation on histamine-induced bronchoconstriction in a group of 16 asthmatic subjects. The subjects studied attended the laboratory on four separate occasions to receive nebulized L-ASA (solution of 90 mg/ml) or matched placebo (glycine solution of 30 mg/ml) 15 min before bronchoprovocation tests with histamine and methacholine in a randomized, double-blind order.

Changes in airway caliber were followed as forced expiratory volume in 1 s (FEV_1), and agonist responsiveness was expressed as the provocative concentration causing a 20% fall in FEV_1 from baseline (PC_{20}).

Administration of both L-ASA and glycine solution caused a small but significant acute fall in FEV_1 from baseline, which returned to normal within 15 min. When compared to placebo, inhaled L-ASA reduced the airway responsiveness to histamine in 13 of the 16 subjects studied, the geometric mean (range) values for PC_{20} histamine increasing significantly ($P < 0.001$) from 1.72 (0.13–5.49) mg/ml to 3.31 (0.36–12.00) mg/ml after placebo and L-ASA, respectively. No significant change in airway responsiveness to methacholine was recorded after L-ASA. Acute administration of L-ASA by inhalation protects the asthmatic airways against histamine-induced bronchoconstriction, thus suggesting that endogenous prostaglandins may play a contributory role in the airways response to histamine in human asthma.

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In the airways, histamine exerts its wide range of biologic activities via an interaction with specific cell-surface receptors, termed H_1 and H_2 (1). It has long been known that histamine contracts isolated human tracheobronchial smooth muscle (2), and, when administered by inhalation, elicits bronchoconstriction in both asthmatic and normal subjects (3). In asthma, inhaled histamine provokes rapid onset bronchoconstriction which reaches its maximum 3–5 min postinhalation, after which recovery is rapid (4). These contractile responses were subsequently demonstrated to be mediated via activation of the H_1 receptor, whereas H_2 receptor antagonists have no effect (5, 6). Recently, more

potent and selective H_1 -histamine receptor antagonists, such as terfenadine (7) and astemizole (8), have been shown to produce up to 35-fold displacements to the right of the concentration-response curve to inhaled histamine in asthmatic subjects.

There have been controversial reports on the effect of cyclooxygenase blockade on histamine-induced bronchoconstriction (9–13), most of them concluding that the effect was probably not seen because of the poor bioavailability of cyclooxygenase inhibitors given orally at the level of the tracheobronchial tree. Recent work by Bianco et al. (14, 15) and by us (16, 17) has shown that inhaled lysine acetylsalicylate (L-ASA) provides better pro-

tection than oral ASA against the bronchoconstrictor response to various nonspecific stimuli, including fog, adenosine 5'-monophosphate (AMP), and neurokinin A. By this alternative experimental approach, we have extended the previous observations (9–13) on the relative contribution of contractile prostaglandins to the airway response provoked by inhaled histamine in asthma.

We have therefore investigated the effect of prior administration of the potent cyclooxygenase inhibitor, L-ASA, given by inhalation, on histamine-induced bronchoconstriction in asthmatic subjects. Bronchoprovocation challenge with methacholine was included in the study protocol to evaluate more accurately the specificity of the effect of inhaled L-ASA on histamine-induced bronchoconstriction.

Material and methods

Subjects

Sixteen asthmatic subjects (12 F, 4 M), with a mean ($\pm$ SEM) age of 32.3 ($\pm$ 2.6) years, referred to our chest clinic with stable asthma as defined by the American Thoracic Society (18), participated in the study (Table 1). All subjects had a history of dyspnea with wheezing or chest tightness upon exposure to airborne allergens and were nonsmokers with positive skin prick tests (>3 mm wheal response) to one or more of six common aeroallergens (*Dermatophagoides pteronyssinus*, *D. farinae*, *Parietaria*, mixed grass pollens, cat fur, dog hair). At the beginning of the study, all subjects were asymptomatic with a baseline FEV₁ (forced expiratory volume in 1 s) less than 70% of their predicted

values. None had received oral corticosteroids, theophylline, antihistamines, or sodium cromoglycate within the preceding 4 weeks. Inhaled bronchodilators were discontinued for at least 8 h before each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. None of the subjects studied had a history of aspirin intolerance. Subjects were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma, and all visits to the laboratory were carried out at the same time of day and outside the pollen season. The study was approved by the ethics subcommittee of the Department of Respiratory Diseases (University of Catania, Italy), and all subjects gave their informed consent.

Bronchial provocation

Airway caliber was recorded before and during the provocation as FEV₁ with a dry wedge spirometer (Vitalograph, Buckinghamshire, UK), the better of two consecutive measurements being recorded.

Histamine acid diphosphate and methacholine chloride (Sigma Chemical Co., St Louis, MO, USA) were freshly prepared in 0.9% sodium chloride on each occasion to produce a range of doubling concentrations of 0.03–8 mg/ml and of 0.03–16 mg/ml for histamine and methacholine, respectively.

The aqueous solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron Mini-Nebulizer (C. R. Bard International, Sunderland, UK), driven by com-

Table 1. Demographic details of subjects studied

Subject no.	Sex	Age (yrs)	Baseline FEV ₁ (% predicted)	Baseline FEV ₁ (liters)	PC ₂₀ histamine (mg/ml)	PC ₂₀ methacholine (mg/ml)
1	F	24	92	3.36	1.54	1.54
2	F	46	102	2.73	2.19	0.50
3	F	31	118	3.03	6.55	—
4	M	24	88	3.73	1.63	0.98
5	F	24	94	3.42	1.24	0.70
6	F	45	109	3.08	5.89	—
7	F	23	76	2.72	2.43	3.28
8	F	35	94	2.54	4.12	—
9	F	24	92	2.66	2.38	2.77
10	F	39	91	2.55	0.78	1.70
11	F	40	88	2.36	0.40	1.69
12	M	21	100	4.15	2.83	6.48
13	F	55	82	1.85	4.67	11.90
14	F	25	79	2.53	1.71	0.98
15	M	38	92	2.89	0.50	0.11
16	M	22	72	2.98	1.37	1.59
Mean		32.3	91.8	2.91	1.91*	1.49*
SEM		$\pm$ 2.6	$\pm$ 3.0	$\pm$ 0.14	(0.40–6.55)	(0.11–11.90)

* Geometric mean (range). FEV₁: forced expiratory volume in 1 s; PC₂₀: provocation concentration producing 20% fall in FEV₁.

pressed air at 8 l/min. Under these conditions, the nebulizer had an output of 0.48 ml/min and generated an aerosol with a median particle diameter of 4.7 μ m (19). Subjects inhaled the aerosolized solutions in five breaths from end-tidal volume to full inspiratory capacity via a mouthpiece, as described by Chai et al. (20). Subjects were trained to reach full inspiratory capacity in 3 s.

Study design

The study consisted of two separate phases.

Phase 1. Subjects attended the laboratory on two separate occasions to undertake concentration-response studies with inhaled histamine and methacholine in the absence of drug treatment. On the first occasion, after a 15-min rest, three baseline measurements of FEV₁ were made at 3-min intervals, followed by inhalation of 0.9% sodium chloride and further FEV₁ measurements repeated at 1 and 3 min. If FEV₁ had not fallen by more than 10% of the baseline value, a histamine concentration-response study was carried out. After administration of each histamine concentration, FEV₁ was measured at 1 and 3 min. Increasing doubling concentrations of histamine were inhaled at 5-min intervals until FEV₁ had fallen by more than 20% of the postsaline baseline value and the corresponding PC₂₀ FEV₁ values had been derived. On the following visit, bronchial provocation tests with inhaled methacholine were undertaken in a similar manner to that described for histamine.

Phase 2. Subjects attended the laboratory on four separate occasions to undertake concentration-response studies with histamine and methacholine after receiving nebulized L-ASA (Lirca Synthelabo, Limite, Milan, Italy) or matched nebulized vehicle placebo administered double-blind and in random order 15 min before challenge. Both the active and placebo solutions were freshly prepared by an independent investigator by a randomized code and then returned to the conducting physician to administer to the attending subject. On each occasion, after a 15-min rest, three baseline measurements of FEV₁ were made at intervals of 3 min, followed by inhalation of nebulized L-ASA (90 mg/ml, 4 ml; 525 mOsm/l, pH 5.25; a 90 mg/ml solution of L-ASA actually contains 50 mg ASA per ml) or nebulized vehicle placebo consisting of a solution of glycine in 0.9% sodium chloride adjusted to similar pH and tonicity to the L-ASA solution (30 mg/ml, 4 ml; 605 mOsm/l, pH 5.90). The aerosol solutions were generated from a starting volume of 4.0 ml in an Inspiron Mini-Nebulizer driven by compressed air at 6 l/min, and inhaled to dryness

by deep tidal breathing over a 7–9 min period. The same nebulizer was used for all studies on all subjects. Further FEV₁ measurements were repeated at 2, 5, 10, and 15 min after drug/placebo inhalation, and agonist challenges were carried out similarly to those described in phase 1.

Data analyses

Figures refer to the mean $\pm$ SEM unless otherwise stated, and the $P < 0.05$ level of significance was accepted. Pre- and post-treatment baseline values of FEV₁ before bronchial challenges were compared between and within study days by two-factor analysis of variance (ANOVA), followed by the Neuman-Keuls test where appropriate.

Concentration-response curves for histamine and methacholine were constructed by plotting the percent change in FEV₁ from the postsaline value against the cumulative concentration of the agonist administered on a logarithmic scale, and the concentration of agonist required to produce a 20% fall in FEV₁ from the postsaline value (PC₂₀ FEV₁) was determined by linear interpolation. Values of PC₂₀ histamine and methacholine after L-ASA and placebo were logarithmically transformed to normalize their distribution and compared by Student's *t*-test for paired data. The possibility of a type II error was assessed where appropriate.

Concentration ratios for the effect of L-ASA against bronchoprovocation with each agonist were calculated by dividing the PC₂₀ value obtained after administration of active drug by that obtained after placebo. The relationship between the airways responses to agonists and the concentration ratios was examined by least-squares linear regression analysis of the log-transformed values. In addition, least-squares linear regression analysis was also used to detect any relationship between the magnitude of fall in FEV₁ after exposure to L-ASA and baseline airway responsiveness.

Results

Effect of inhaled L-ASA on airway caliber

There was no significant difference in baseline values of FEV₁ between any of the study days with mean ($\pm$ SEM) values ranging from 2.82 ± 0.13 to 2.93 ± 0.19 l. After inhaled L-ASA, FEV₁ values were significantly lower than the predrug FEV₁ baseline values. On the histamine study day, this fall in FEV₁ values peaked at 1 min, the mean ($\pm$ SEM) FEV₁ values decreasing by 9.7% from 2.82 ± 0.13 to 2.57 ± 0.15 l ($P < 0.01$). Although 15 min after L-ASA, FEV₁ values were still 5.6% lower than the predrug FEV₁ baseline values, these were significantly higher than those measured at

1 min. When compared at all time points, falls in FEV_1 values after administration of L-ASA were not significantly different from those after placebo (glycine). The mean falls ($\pm$ SEM) FEV_1 obtained at 1 min were 9.7% and 7.5% after L-ASA and placebo, respectively. Similar data were obtained on methacholine study days (data not shown). No significant correlations could be established between the magnitude of fall in FEV_1 postdrug and the concentration ratio after the active drug and the baseline airway responsiveness to histamine.

Effect of inhaled L-ASA on concentration-response curve to histamine and methacholine

On the open study days, inhaled histamine and methacholine produced concentration-related falls in FEV_1 . The geometric mean (range) of PC_{20} values obtained in the absence of treatment were 1.91 (0.40–6.55; $n=16$) mg/ml and 1.49 (0.11–11.90; $n=13$) mg/ml for histamine and methacholine, respectively (Table 1). Inhaled L-ASA had a significant protective effect against the fall in FEV_1 produced by histamine (Fig. 1, Table 2). The geometric mean (range) PC_{20} histamine values increased 1.9-fold from 1.72 (0.13–5.49) mg/ml after placebo to 3.31 (0.36–12.00) mg/ml after L-ASA ($P<0.01$) (Table 2).

Inhaled L-ASA failed to alter the airway response to a subsequent inhalation with methacholine, the geometric mean (range) PC_{20} value of 1.56 (0.17–9.59) mg/ml after placebo not being significantly different from that of 1.50 (0.09–14.01) mg/ml obtained after L-ASA (Fig. 2, Table 2). No correlation could be found between baseline airway reactivity and the protection of airway response to histamine after L-ASA exposure. We have calculated that for a significance level of 5%

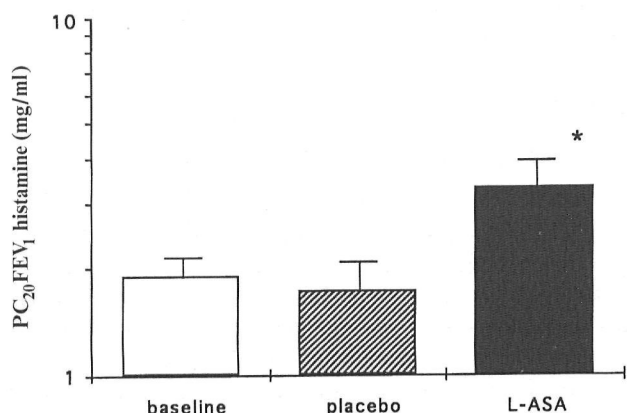


Fig. 1. Changes (mean $\pm$ SEM) in provocation concentrations of histamine required to provoke 20% decrease in FEV_1 (PC_{20} FEV_1) on baseline (open bar), placebo (hatched bar), and L-ASA (closed bar) study days in 16 asthmatics. *Significant difference at $P<0.01$ vs placebo.

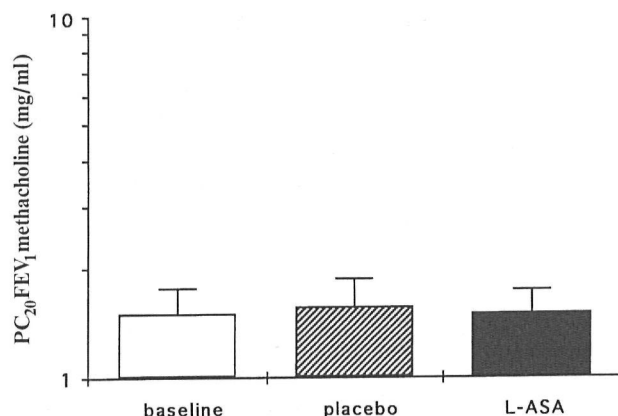


Fig. 2. Changes (mean $\pm$ SEM) in provocation concentrations of methacholine required to provoke 20% decrease in FEV_1 (PC_{20} FEV_1) on baseline (open bar), placebo (hatched bar), and L-ASA (closed bar) study days in 13 asthmatics.

(two-tailed), and assuming as a significant change in the mean PC_{20} methacholine value a rightward shift of at least one doubling dose, the corresponding power is about 0.90. Consequently, the possible occurrence of a type II error is excluded.

Discussion

In this study, we have used the potent cyclooxygenase inhibitor L-ASA, administered by inhalation, to explore the relative contribution of prostanoids in the airway response to histamine and have shown that pretreatment with inhaled L-ASA led to a small but significant reduction of the airway response to histamine, but not to methacholine.

The dosage of inhaled L-ASA used in the present study and the timing of administration before bronchial challenge were chosen on the basis of previous studies which have shown L-ASA to be effective in reducing asthma caused by fog, AMP, and neurokinin A (14–17).

It has been previously suggested that a major component of the bronchoconstriction provoked by histamine occurs through the activation of specific receptors on smooth-muscle cells (1). However, the present findings might be interpreted to mean that histamine produces some of its bronchoconstrictor effect in asthma by releasing products of the cyclooxygenase pathway.

Researchers using oral cyclooxygenase inhibitors produced inhibition of the airway response to histamine (9, 13), while others failed to show any significant change (10–12). Although the cause of these discrepancies remains unclear, one possible explanation is that insufficient concentrations of drug were available at the surface of the airways to inhibit effectively the cyclooxygenase activity of mediator-secreting cells. Indeed, in the studies by

Table 2. Effects of pretreatment with inhaled L-ASA and placebo on airway histamine and methacholine responsiveness

Subjects	Histamine (mg/ml)		Methacholine (mg/ml)	
	Placebo	L-ASA	Placebo	L-ASA
1	1.05	1.69	2.02	1.34
2	3.25	5.48	0.32	0.42
3	5.49	11.24		
4	1.66	4.89	1.62	2.16
5	2.39	5.48	0.72	0.90
6	4.97	7.98		
7	2.24	2.53	3.14	2.94
8	4.44	4.31		
9	2.56	2.33	2.62	1.62
10	0.64	2.00	1.83	1.77
11	0.35	0.96	1.81	1.68
12	5.43	10.39	6.27	5.69
13	4.30	12.00	9.59	14.01
14	0.39	1.71	0.88	1.06
15	0.13	0.36	0.17	0.09
16	2.26	2.45	1.71	1.58
Geometric mean	1.72	3.31	1.56	1.50
(range)	(0.13–5.49)	(0.36–12.00)	(0.17–9.59)	(0.09–14.01)

Walters (13) and Curzen et al. (9), cyclooxygenase inhibitors were administered regularly for several days, whereas Fish et al. (10), Phillips & Holgate (11), and Polosa et al. (12) used a single-dose regimen in their investigations. In the present study, inhaled L-ASA inhibited the airway response to histamine by approximately twofold. Although aspirin is approximately 5000 times less potent than flurbiprofen in inhibiting prostaglandin synthetase *in vitro* (21), these data suggest that the route of administration may have contributed to better protection against the effect of contractile prostaglandins on the airway response to histamine than that achieved by oral dosing of the drug, in agreement with the recent observations by Bianco et al. (14, 15) that inhaled L-ASA provides better protection than oral ASA in experimental models of bronchoconstriction.

The subjects studied in the present investigation were a heterogeneous group of asthmatic subjects, showing a wide range of baseline bronchial hyperresponsiveness and use of inhaled corticosteroids. Comparison between subjects showed no significant difference in terms of effect of L-ASA on their histamine PC₂₀.

The mechanism by which L-ASA attenuates the bronchoconstrictor response to histamine is unknown. We have shown no bronchodilator effect of this drug, but rather a short-lived bronchoconstriction, in agreement with previously reported data on inhaled L-ASA by our group (12). The reason for this is not known, but differences in subjects' characteristics are a possibility. Most of the asthmatic subjects studied in the present investigation showed bronchial reactivity in the severe to

moderate range (PC₂₀ methacholine <4 mg/ml). Since none of the patients studied had a history of aspirin intolerance, we may speculate that in these subjects with grossly inflamed airways exposure to a high-osmolality solution such as that of L-ASA may elicit a transient fall in FEV₁ as the result of an irritant effect. This is confirmed by the observation that the hyperosmolar control solution of glycine elicited a similar fall in FEV₁. Unfortunately, we were unable to demonstrate any correlation between the magnitude of fall in FEV₁ after hyperosmolar solutions and baseline airway responsiveness in the subject studied. In addition, although inhaled L-ASA had a significant protective effect against the airway response to inhaled histamine, a reduction in nonspecific airway responsiveness is unlikely since we have also shown that inhaled L-ASA had no effect on responsiveness to methacholine. This agrees with previous studies with cyclooxygenase blockers, which have repeatedly failed to show an effect on methacholine-induced bronchoconstriction (10, 22).

The findings of the present study are in line with the observations of Platshon & Kaliner (23) that exogenous histamine is capable of releasing newly generated spasmogenic mediators, such as prostaglandin (PG)F_{2α}, from human lung tissue *in vitro* by an H₁-histamine receptor-mediated mechanism. Similar results have been obtained by Adkinson et al. (24), who found that histamine caused release of PGE₂ and PGF_{2α} from passively sensitized human bronchial tissue *in vitro*.

Of direct relevance to the results of the present study is the notion that preinhalation with PGD₂ (25) and PGF_{2α} (26) increases sensitivity to sub-

sequent challenge with histamine in man. Moreover, cyclooxygenase blockers inhibit immunologic and nonimmunologic release of PGD_2 and thromboxane (Tx) B_2 from passively sensitized human dispersed lung cells *in vitro* (27, 28). Thus, our data might also be interpreted to mean that histamine given by inhalation produces some of its bronchoconstrictor effect by releasing contractile cyclooxygenase products such as TxB_2 , PGD_2 , and $\text{PGF}_{2\alpha}$, removal of which by inhaled L-ASA is expected to attenuate the airways response to histamine.

Other possible explanations of L-ASA's alteration of histamine responsiveness in asthma include inhibition of the reflex component of bronchoconstriction provoked by histamine, downregulation of histamine receptors, and modulation of postreceptor events coupled to histamine-mediated contraction of smooth muscle.

Since histamine (not methacholine, which was diluted in PBS) was dissolved in 0.9% saline, it is possible that part of the positive effect of L-ASA on histamine airway responsiveness, as opposed to methacholine, might be due to a pH-induced component of bronchoconstriction. However, the evidence of a pH-induced component of bronchoconstriction in asthma remains controversial. First of all, one must appreciate the considerable buffering capacity of airway surface fluid and that the volume delivered with each breath is very small (approx. 25–30 μl) and unlikely to change the pH. In addition, Fine et al. (29) have shown that the bronchoconstrictor potency of acidic aerosols is related to their total available hydrogen ion concentration (titratable acidity) rather than to their free hydrogen ion concentration (pH), since, at a constant pH (pH 2.0), increasing amounts of titratable acidity caused increasing severity of bronchoconstriction for the two chemically distinct acid aerosols studied (HCl and H_2SO_4). Moreover, the free hydrogen ion concentration (pH) per se is a weak stimulus of bronchoconstriction in asthmatic subjects with a significant effect at pH value as low as 2 (29). Thus, if we consider that in our study the range of pH values measured in histamine solutions made up in 0.9% saline (pH 5.4) varied from at lowest 4.3 (histamine 8 mg/ml) to at highest 5.2 (histamine 0.03 mg/ml), it is unlikely that a pH-induced component had any effect.

In conclusion, our results demonstrate that cyclooxygenase blockade with inhaled L-ASA produces a small but significant protective effect against histamine-provoked bronchoconstriction in asthmatic subjects, implying a role for released prostanoids in mediating this response. This, together with the evidence of a lack of effect on methacholine-induced bronchoconstriction, leads us to suggest that cyclo-

oxygenase products directly contribute to the airway effect of histamine in asthma. However, further definition of the potential role of spasmogenic cyclooxygenase products in histamine-induced bronchoconstriction must await the availability of specific prostanoid receptor antagonists.

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