Eur Respir J, 1995, 8, 905–912
DOI: 10.1183/09031936.95.08060905
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European Respiratory Journal
ISSN 0903 - 1936

Inhaled lysine acetylsalicylate (L-ASA) attenuates the bronchoconstrictor response to adenosine 5'-monophosphate (AMP) in asthmatic subjects

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ABSTRACT: When administered by inhalation, adenosine 5'-monophosphate (AMP) provokes dose-related bronchoconstriction in asthmatic subjects by a mechanism believed to involve mast cell mediator release. However, little is known of the change in airway responsiveness to AMP after cyclo-oxygenase blockade.

The aim of this study was to investigate the effect of the potent cyclo-oxygenase inhibitor, lysine acetylsalicylate (L-ASA) administered by inhalation, on AMP-induced bronchoconstriction in a group of nine asthmatic subjects.

The subjects studied attended the laboratory on six separate occasions to receive nebulized L-ASA (solution of 90 mg·ml^{-1}) or matched placebo (glycine solution, 30 mg·ml^{-1}) 15 min prior to bronchoprovocation tests with AMP, histamine and methacholine in a randomized, double-blind order. Changes in airway calibre were followed as forced expiratory volume in one second (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 AMP in all the subjects studied, the geometric mean (range) values for PC_{20} AMP increasing significantly from 36.3 (7.9–250.5) to 101.8 (27.2–1300) mg·ml^{-1} after placebo and L-ASA, respectively. Moreover, nebulized L-ASA induced a small but significant reduction in airway responsiveness to histamine, the geometric mean (range) PC_{20} values for histamine increasing from 2.77 (1.05–5.49) to 4.36 (1.69–11.24) mg·ml^{-1} after placebo and L-ASA, respectively. No significant change in airway responsiveness to methacholine was recorded after L-ASA.

Administration of L-ASA by inhalation protects the asthmatic airways against AMP and, to a lesser extent, histamine-induced bronchoconstriction, thus suggesting that endogenous prostaglandins may play a contributory role in the airways response to AMP in human asthma.

Eur Respir J., 1995, 8, 905–912.

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Keywords: Adenosine
asthma
bronchoconstriction
lysine acetylsalicylate

Received: November 7 1994
Accepted after revision January 31 1995

Adenosine and its related nucleotide, adenosine 5'-monophosphate (AMP), cause bronchoconstriction in atopic [1] and nonatopic [2] asthmatics by a mechanism believed to involve an interaction with cell surface puriniceptors [3].

Adenosine has been shown to potentiate the release of the preformed mediators, β-hexosaminidase and histamine, from immunologically activated rodent [4] and human lung [5] mast cells *in vitro*. *In vivo* sodium cromoglycate and nedocromil sodium, two drugs that inhibit mast cell activation, have been shown to protect against AMP-induced bronchoconstriction in asthmatic subjects [6, 7]. In addition, the potent and selective H_1-histamine receptor antagonists, terfenadine and astemizole, have been shown to inhibit the bronchoconstrictor response after challenge with AMP by >80% in both atopic [8] and nonatopic [2] asthmatic subjects, suggesting that histamine is the predominant mast cell mediator involved in the airway response to this nucleotide. However, the incomplete protection afforded by terfenadine against bronchoconstriction provoked by inhaled AMP could not be improved by increasing the drug dose from 180 to 600 mg [8].

Because the antihistamine-resistant reduction in forced expiratory volume in one second (FEV_1) provoked by AMP was slow in onset and in reaching maximum, an

alternative explanation is that adenosine also augments the release of newly generated bronchoconstrictor mediators, such as prostaglandins (PGs) and thromboxane (Tx) A_2, from immunologically preactivated airways mast cells. Of direct relevance, PEACHELL *et al*. [9] have recently shown that in immunologically stimulated human lung mast cells, adenosine not only enhances histamine release but also potentiates the generation of spasmogenic prostanoids. Using potent inhibitors of cyclo-oxygenase, such as oral indomethacin and flurbiprofen, some evidence for a contribution of contractile prostaglandins to adenosine's response in the asthmatic airways has also been advocated [10, 11].

In line with the observation that administration of bronchoactive drugs by inhalation achieves maximum effect with smaller doses, recent work by BIANCO and co-workers [12, 13] demonstrated that a better protection is afforded by inhalation of acetylsalicylate (ASA) as an aerosol of lysine-acetylsalicylate (L-ASA) solution as opposed to oral ASA against nonspecific stimuli. Using this alternative experimental approach, we have extended our previous observations [10] on the relative contribution of contractile prostaglandins to the airway response provoked by inhaled AMP.

We have, therefore, investigated the effect of prior administration of the potent cyclo-oxygenase inhibitor, L-ASA, given by inhalation, on AMP-induced bronchoconstriction in asthmatic subjects. Bronchoprovocation challenges with histamine and methacholine were included in the present study to evaluate the specificity of inhaled L-ASA on subsequent contractile stimuli.

Methods

Subjects

Nine asthmatic subjects (8 females, 1 male), with a mean (±SEM) age of 30 (±3) yrs, referred to our chest clinic with stable asthma, participated in the study (Table 1). All subjects had a history of dyspnoea with wheezing or chest tightness upon exposure to airborne allergens, and were nonsmokers with positive skin-prick tests (>2 mm weal response) to one or more of six common aeroallergens *(Dermatophagoides pteronyssinus, Dermatophagoides farinae,* wall pellitory grass, mixed grass pollens, cat fur and dog hair). At the beginning of the study, all subjects were asymptomatic, with a baseline FEV1 of >75% 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 prior to each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. On close questioning, none of the subjects studied had a positive history for 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), and all subjects gave their informed consent.

Table 1. – Demographic details of subjects studied

Sub No.	Sex	Age yrs	BL FEV1 % pred	Atopy§	PC20hist mg·mL^{-1}	PC20AMP mg·mL^{-1}
1	F	24	92	D-W	1.54	11.4
2	F	46	102	D-W	2.19	31.2
3	F	31	118	W	6.55	37.8
4	M	24	88	W-G	1.63	51.6
5	F	24	94	W	1.24	5.2
6	F	45	109	G	5.89	79.2
7	F	23	76	W-G	2.43	20.4
8	F	35	94	D	4.12	57.1
9	F	35	92	W	2.38	255.0
Mean		30	96		2.64*	35.3*
SEM		±3	±3		(1.24–6.55)	(5.2–255.0)

*: geometric mean (range); §: atopic, positive immediate skin test to one or more allergens. Sub: subject; D: *Dermatophagoids* sp.; W: wall pellitory grass; G: mixed grass; FEV1: baseline forced expiratory volume in one second; PC20his: provocation concentration of histamine producing a 20% fall in FEV1; AMP: 5'-adenosine monophosphate.

Bronchial provocation

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

Adenosine 5'-monophosphate, histamine, and methacholine (Sigma Chemical Co., St Louis, USA) were freshly prepared in 0.9% sodium chloride on each occasion to produce a range of doubling concentrations of 3.125–800, 0.03–8, and 0.03–16 mg·mL^{-1} for AMP, 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 compressed air at 8 L·min^{-1}. Under these conditions, the nebulizer had an output of 0.48 mL·min^{-1} and generated an aerosol with a mass median particle diameter of 4.7 µm [14]. 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*. [15]. Subjects were trained to take 3 s to reach full inspiratory capacity.

Study design

The study consisted of two separate phases.

Phase 1. Subjects attended the laboratory on two separate occasions at least 48 h apart to undertake concentration-response studies with inhaled histamine and AMP, in the absence of any drug treatment. On the first occasion, after 15 min rest, three baseline measurements of FEV1 were made at intervals of 3 min, followed by inhalation of 0.9% sodium chloride and further FEV1

measurements repeated at 1 and 3 min. Provided FEV_1 had not fallen by >10% of the baseline value, a histamine concentration-response study was carried out. After administration of each histamine concentration, FEV_1 was measured at 1 and 3 min. Increasing doubling concentrations of histamine were inhaled at 5 min intervals until FEV_1 had fallen by >20% of the post-saline baseline value, and the corresponding provocative concentration producing a 20% fall in FEV_1 ($PC_{20}FEV_1$) values derived. On the remaining visit, bronchial provocation tests with inhaled AMP were undertaken in a similar manner to that described for histamine.

Phase 2. Subjects attended the laboratory on six separate visits, at least 5 days apart, to undertake concentration-response studies with AMP, histamine and methacholine, after receiving nebulized L-ASA (Lirca Synthelabo, Limito, Milano, Italy) or matched nebulized vehicle placebo administered double-blind and in random order 15 min prior to challenge. Both the active and placebo solutions were freshly prepared by an independent investigator on the basis of a randomized code, and then returned to the conducting physician to administer to the attending subject. On each occasion, after 15 min rest, three baseline measurements of FEV_1 were made at intervals of 3 min, followed by inhalation of nebulized L-ASA (90 $mg \cdot mL^{-1}$, 4 ml; 525 $mOsm \cdot L^{-1}$, pH 5.25; a 90 $mg \cdot mL^{-1}$ solution of L-ASA actually contains 50 mg of ASA per mL) or nebulized vehicle placebo consisting of a solution of glycine (30 $mg \cdot mL^{-1}$, 4 ml; 605 $mOsm \cdot L^{-1}$, pH 5.90) in 0.9% sodium chloride adjusted to the same pH and tonicity as the L-ASA. The aerosol solutions were generated from a starting volume of 4 ml in an Inspiron mini-nebulizer driven by compressed air at 6 $L \cdot min^{-1}$, and inhaled to dryness by deep tidal breathing over a 7–9 min time-period. The same nebulizer was used for all studies on all subjects. Further FEV_1 measurements were repeated at 2, 5, 10 and 15 min after drug/placebo inhalation, and dose-response studies with increasing concentrations of AMP, histamine, and methacholine carried out in a similar manner to that described in Phase 1.

Data analysis

Figures refer to the mean±SEM unless otherwise stated, and the $p<0.05$ level of significance was accepted. Pre- and post-treatment baseline values of FEV_1 prior to bronchial challenges were compared between and within study days by two-factor analysis of variance (ANOVA), followed by Neuman-Keuls test where appropriate.

Concentration-response curves were constructed by plotting the percentage change in FEV_1 from the post saline baseline 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 FEV_1 from the postsaline baseline value ($PC_{20}FEV_1$) determined by linear interpolation.

The repeatability of the bronchial challenges were determined according to the method described by ALTMAN and BLAND [16], of plotting the difference against the mean of the logarithmically transformed PC_{20} values obtained on the placebo and open study days. The mean and standard deviation (SD) of the difference between these values were derived and used to calculate their coefficient of repeatability (CoR) between the results of the two study days.

Values of PC_{20} methacholine, histamine, and AMP following treatment with L-ASA and placebo were logarithmically transformed to normalize their distribution and compared by the Student's t-test for paired data. Concentration ratios for the effect of L-ASA against bronchoprovocation with each agonist were calculated by dividing the PC_{20} value obtained after administration of active drug by that obtained after placebo, and compared using the Wilcoxon signed rank test.

Any relationship between the airway responses to methacholine, histamine and AMP was examined by least-squares linear regression analysis of the logarithmically transformed values. Least-squares linear regression analysis, was also used to evaluate: 1) any relationship between the concentration ratio after the drug and the airway responses to methacholine, histamine and AMP; and 2) any relationship between the magnitude of fall in FEV_1 after exposure to L-ASA and baseline airway responsiveness to methacholine, histamine and AMP.

Results

Effect of inhaled L-ASA on airway calibre

There was no significant difference in baseline values of FEV_1 between any of the study days, with mean (±SEM) values ranging from 2.90±0.16 to 3.17±0.15 L (table 2). After nebulized L-ASA, mean baseline values of FEV_1 were significantly lower than the predrug FEV_1 baseline values, with a peak effect at 1 min, the mean (±SEM) FEV_1 values decreasing 5.6 and 5.8% from 2.90±0.16 to 2.74±0.16 L ($p<0.01$) and from 2.99±0.14 to 2.81±0.14 L ($p<0.01$) on the AMP and histamine study days respectively (table 2a and b). Although 15 min after nebulized L-ASA, mean baseline values of FEV_1 were still 2.3 and 3.7% lower than the predrug FEV_1 baseline values on the AMP and histamine study days, respectively (table 2a and b), these values were significantly higher compared to the values measured at the 1 min time-point. However, the mean values of FEV_1 following administration of L-ASA were not significantly different from those after placebo (glycine) when compared at all time-points. No significant correlations could be established between the magnitude of fall in FEV_1 after L-ASA and baseline airway responsiveness to AMP or histamine.

Effect of inhaled L-ASA on concentration-response curve to AMP

The challenge procedure with AMP was found to be highly repeatable, with a CoR of 0.53 doubling doses.

Table 2. – Baseline FEV_1 values (pre) and post L-ASA/placebo inhalation on a) AMP; b) histamine; and c) methacholine study days

	Placebo					L-ASA				
Subject	Pre	1 min	5 min	10 min	15min	Pre	1 min	5 min	10 min	15 min
a) AMP study days										
1	3.51	3.48	3.44	3.44	3.44	3.31	3.24	3.21	3.19	3.22
2	2.42	2.29	2.36	2.29	2.30	2.36	2.21	2.23	2.38	2.42
3	2.83	2.78	2.78	2.71	2.74	2.89	2.64	2.68	2.65	2.75
4	3.64	3.51	3.44	3.50	3.47	3.64	3.35	3.58	3.55	3.55
5	3.21	2.96	2.93	3.10	3.10	3.49	3.43	3.40	3.37	3.47
6	2.80	2.60	2.86	2.83	2.83	3.00	2.67	2.82	2.83	2.92
7	2.45	2.30	2.30	2.27	2.35	2.45	2.24	2.25	2.42	2.35
8	2.46	2.40	2.26	2.28	2.39	2.31	2.18	2.10	2.18	2.20
9	2.87	2.70	2.73	2.73	2.77	2.63	2.67	2.64	2.63	2.60
Mean	2.91	2.78	2.79	2.80	2.82	2.90	2.74	2.77	2.80	2.83
±SEM	0.14	0.15	0.14	0.15	0.14	0.16	0.16	0.17	0.15	0.16
b) Histamine study days										
1	3.36	3.27	3.27	3.28	3.31	3.42	3.23	3.27	3.19	3.23
2	2.73	2.43	2.58	2.60	2.65	2.74	2.47	2.64	2.50	2.63
3	3.03	3.07	3.13	2.98	3.06	3.16	3.00	2.98	3.02	3.02
4	3.73	3.54	3.65	3.58	3.55	3.81	3.57	3.71	3.63	3.58
5	3.42	3.10	3.22	3.17	3.27	3.23	3.13	3.03	3.08	3.18
6	3.08	2.98	3.00	3.03	3.08	2.81	2.52	2.72	2.75	2.79
7	2.72	2.58	2.61	2.51	2.54	2.56	2.48	2.43	2.40	2.41
8	2.54	2.57	2.62	2.60	2.55	2.40	2.28	2.27	2.30	2.35
9	2.66	2.47	2.55	2.50	2.48	2.73	2.63	6.63	2.66	2.63
Mean	3.03	2.89	2.96	2.92	2.94	2.99	2.81	2.85	2.84	2.87
±SEM	0.13	0.13	0.12	0.12	0.13	0.14	0.14	0.14	0.13	0.13
c) Methacholine study days										
1	3.28	3.26	3.23	3.21	3.28	3.47	3.21	3.19	3.18	3.29
2	2.66	2.58	2.58	2.56	2.61	2.69	2.50	2.47	2.46	2.53
3										
4	3.49	3.37	3.33	3.26	3.42	3.81	3.72	3.71	3.78	3.78
5	3.00	2.80	2.67	2.67	2.77	3.33	3.10	3.13	3.10	3.23
6										
7	2.82	2.70	2.75	2.95	2.90	3.11	2.96	3.09	3.05	3.03
8										
9	2.78	2.61	2.70	2.71	2.73	2.62	2.46	2.48	2.55	2.50
Mean	3.00	2.89	2.88	2.89	2.95	3.17	2.99	3.01	3.02	3.06
±SEM	0.10	0.11	0.10	0.09	0.10	0.15	0.15	0.15	0.15	0.16

AMP: adenosine 5'-monophoshate; L-ASA: lysine acetylsalicylate; FEV_1: forced expiratory volume in one second; sub: subject.

The inhalation test was repeatable to within a single doubling dilution in all subjects receiving AMP.

In Phase 1, inhaled histamine and AMP produced concentration-related falls in FEV_1. The geometric mean (range) of PC_{20} values obtained were 2.64 (1.24–6.55) and 35.3 (5.2–255.0) $mg \cdot mL^{-1}$ for histamine and AMP, respectively (table 1). No significant correlation was observed between PC_{20} values for histamine and AMP.

In phase 2, when compared to placebo, inhaled L-ASA had a significant protective effect against the fall in FEV_1 produced by AMP. L-ASA produced a displacement of the AMP concentration response curve to the right in all subjects studied (fig. 1 and table 3). For these subjects the geometric mean (range) PC_{20} AMP values increased 2.8 fold from 36.3 (7.9–250.5) to 101.8 (27.2– 1300) $mg \cdot mL^{-1}$ after placebo and L-ASA, respectively ($p<0.01$;

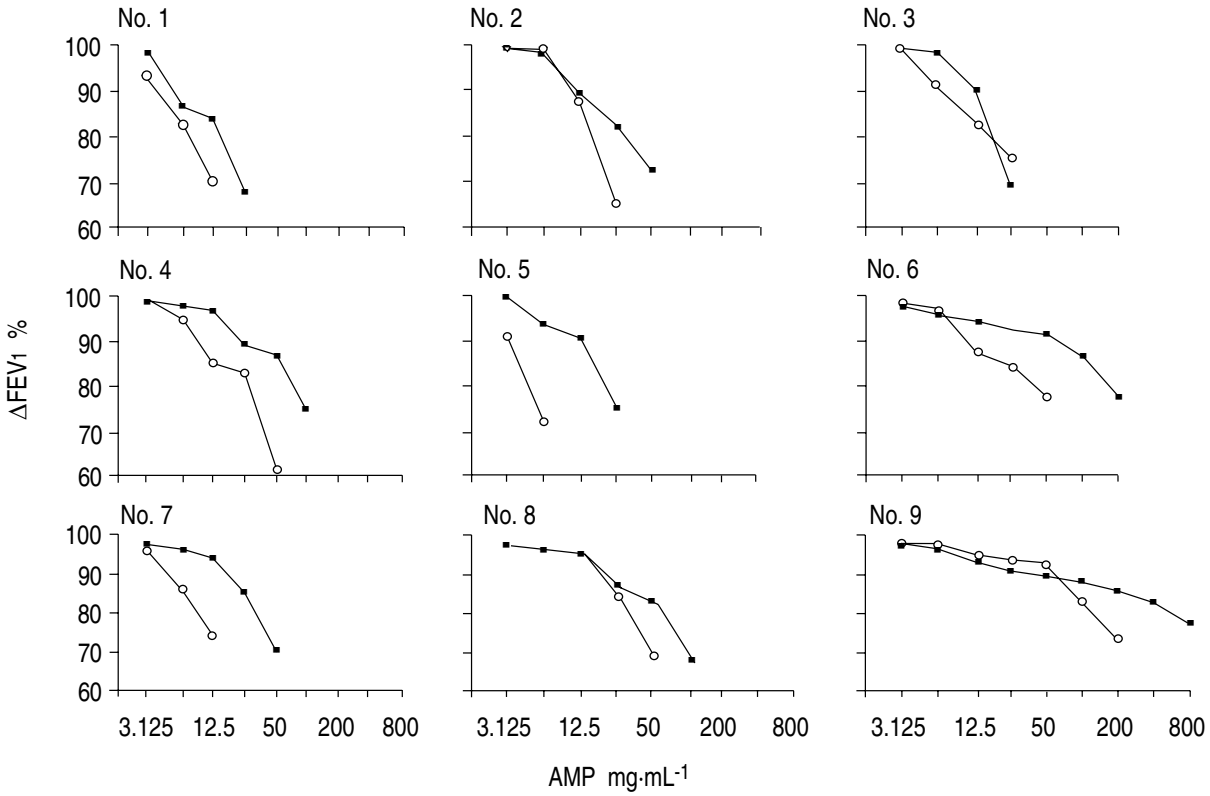

Fig. 1. – Effect of placebo (—○—) and L-ASA (—■—) on the concentration-related falls in forced expiratory volume in one second (FEV_1) from baseline produced by inhaled AMP in nine subjects with asthma. L-ASA: lysine acetylsalicylate; AMP: adenosine 5'-monophosphate.

Table 3. – Effects of pretreatment with inhaled L-ASA and placebo on airway AMP, histamine and methacholine responsiveness

Subject No.	PC_{20} AMP		PC_{20} histamine		PC_{20} methacholine	
	Placebo	L-ASA	Placebo	L-ASA	Placebo	L-ASA
1	11.9	27.2	1.05	1.69	0.20	1.34
2	29.2	50.8	3.25	5.50	0.32	0.42
3	36.1	45.2	5.49	11.42		
4	53.4	154.0	1.66	4.90	1.62	2.16
5	7.9	34.5	2.39	5.40	0.72	0.90
6	75.9	334.0	4.90	7.98		
7	16.2	64.2	2.24	2.50	3.14	2.94
8	67.3	116.0	4.49	4.30		
9	250.5	1300.0	2.30	2.56	2.62	1.62
G. mean (range)	36.3 (7.9–250.5)	101.8 (27.2–1300)	2.77 (1.05–5.49)	4.36 (1.69–11.24)	1.36 (0.32–3.14)	1.32 (0.42–2.49)

G. mean: geometric mean. For abbreviations see legends to tables 1 and 2.

n=9) (table 3). No correlation could be found between baseline airway reactivity and the protection of airway response to AMP after L-ASA exposure.

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

The bronchoprovocation test with histamine showed a good repeatability, with a CoR of 0.91 doubling doses. This test was repeatable to within a single doubling dilution in all the subjects receiving histamine. Inhaled L-ASA, was effective in reducing the airway response to a subsequent inhalation with histamine but not methacholine. L-ASA produced a displacement of the histamine concentration-response curve to the right in 6 out of 9 subjects (fig. 2 and table 3). The geometric mean (range) PC_{20} histamine value after L-ASA 4.36 (1.69–11.24) $mg \cdot mL^{-1}$ was significantly higher than that after placebo 2.77 (1.05–5.49 $mg \cdot mL^{-1}$) ($p<0.01$ n=9). When expressed as concentration ratio, L-ASA afforded a 1.6 fold protection of the airways response against histamine. No correlation could be found between baseline airway reactivity and the protection of airway response to histamine after L-ASA exposure.

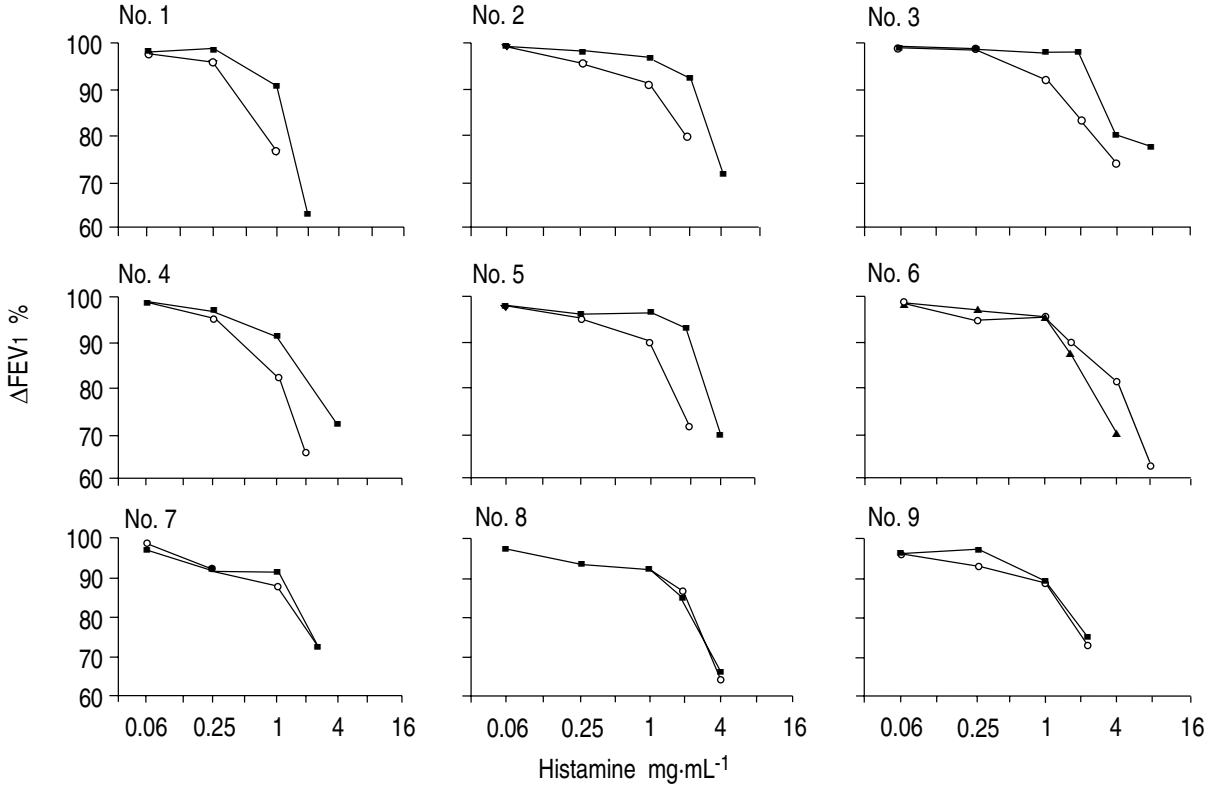

Fig. 2. – Effect of placebo (—○—) and L-ASA (—■—) on the concentration-related falls in forced expiratory volume in one second (FEV_1) from baseline produced by inhaled histamine in nine subjects with asthma.

Inhaled L-ASA, despite being effective in inducing significant changes in baseline airway calibre, failed to alter the airway response to a subsequent inhalation with methacholine. The geometric mean (range) PC_{20} value of 1.36 (0.32–3.14) $mg \cdot mL^{-1}$ after placebo not being significantly different from that of 1.32 (0.42–2.94) $mg \cdot mL^{-1}$ obtained after L-ASA (table 3).

Discussion

In this study, we have demonstrated that administration of L-ASA by inhalation protects the asthmatic airways against AMP-induced bronchoconstriction. Following L-ASA exposure, we have also found a small but significant change in airway responsiveness 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 been shown to effectively reduce the bronchospastic response to nonspecific stimuli in asthmatic subjects [12, 13].

Although not directly comparable with the results obtained in similar studies with oral cyclo-oxygenase blockers, our findings indicate that inhaled L-ASA is more potent in inhibiting AMP-induced bronchoconstriction than oral indomethacin or flurbiprofen. We have shown that inhaled L-ASA inhibits the airway response to AMP by approximately three fold. Although flurbiprofen is approximately 5,000 times more potent than aspirin in inhibiting cyclo-oxygenase [17], the data of the present study suggests that the route of administration may have contributed to a better protection against the effect of contractile prostaglandins to the airway response to AMP as opposed to the oral dosing of more potent cyclo-oxygenase inhibitors.

The mechanism by which L-ASA attenuates the bronchoconstrictor response to AMP is subject to some speculation. We have shown no bronchodilator effect of this drug, but rather an immediate bronchoconstriction, which is probably the result of an irritative effect due to the high osmolality of the solution. This is confirmed by the notion that the hyperosmolar control solution of glycine elicited a similar fall in FEV_1. In the subjects studied, we were unable to demonstrate any correlation between the magnitude of fall in FEV_1 after hyperosmolar solutions and baseline airway responsiveness. 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 in asthmatic subjects.

Thus, the protective effect of inhaled L-ASA may be ascribed to the prostaglandin synthetase inhibition. In support of this view, cyclo-oxygenase blockers inhibit anti-immunoglobulin E (IgE)-provoked release of PGD_2 and TxB_2 from passively sensitized human dispersed lung cells *in vitro* [18]. In immunologically stimulated human lung mast cells, adenosine has been shown to potentiate the release of spasmogenic prostanoids [9].

These observations, together with the reported inhibitory effects of oral indomethacin and flurbiprofen [10, 11], and the evidence that inhaled L-ASA protects against AMP-induced bronchoconstriction (this study), provide some evidence for a contribution of contractile prostaglandins to adenosine's response in the asthmatic airways. More direct evidence that newly generated mediators may contribute to adenosine-induced bronchoconstriction stems from a study in which a significant rise in plasma levels of TxB_2 [19] was reported after adenosine challenge. In addition, mediator release from activated mast cells is likely to take place in the airways *in vivo* during adenosine-induced bronchoconstriction. We have recently demonstrated that asthmatic airways respond to endobronchial instillation with AMP with bronchial narrowing, and this is paralleled by a significant rise in bronchoalveolar lavage (BAL) fluid levels of PGD_2 when compared to endobronchial sham challenge with saline [20].

We have also found a small but significant change in airway responsiveness to histamine, but not to methacholine, after L-ASA. The presence of attenuation of the histamine response also argues in favour of L-ASA inhibiting production of cyclo-oxygenase products by exogenous histamine. WALTERS [21] reported decreased sensitivity to inhalation of histamine in asthmatics pretreated for 3 days with the cyclo-oxygenase inhibitor, indomethacin, an effect similar to the three fold protection against inhaled histamine, that CURZEN *et al*. [22] have previously shown with oral flurbiprofen. In addition, several authors have demonstrated that preinhalation of PGD_2 [23] and $PGF_{2\alpha}$ [24, 25] increases sensitivity to subsequent challenge with histamine. PLATSHON and KALINER [26] have found that exogenous histamine induces release of $PGF_{2\alpha}$ from human lung fragments *in vitro* by an H_1-histamine receptor mediated mechanism. Similar results have been obtained by ADKINSON *et al*. [27], who found that histamine provoked release of prostanoids from passively sensitized human bronchial tissue *in vitro*.

Hence, our data might also be interpreted as histamine released endogenously from mast cells by AMP, producing some of its bronchoconstrictor effect by releasing contractile cyclo-oxygenase products.

The contribution of alternative mechanisms other than mast cell mediator release in AMP-induced bronchoconstriction must be considered. Adenosine has been reported to enhance the contractile response to transmural nerve stimulation in isolated rabbit bronchial smooth muscle [28]. Despite initial negative results [29], there is some evidence that both cholinergic [30] and peptidergic [31] neural pathways may also contribute to the airway effects of adenosine in asthma. Thus, the effect of L-ASA on AMP-induced bronchoconstriction could also be viewed as an inhibition of the modulating effect of cyclo-oxygenase products on presynaptic neural mechanisms [32].

In conclusion, our results confirm that cyclo-oxygenase blockade with inhaled L-ASA produces significant protection against AMP-provoked bronchoconstriction in asthmatic subjects, implying a role for endogenous prostanoids in this response. Moreover, the partial protection of the airways against histamine seen after the drug, implies that prostanoids are also involved in the airway response to this amine, suggesting that part of the airway response to histamine released from immunologically preactivated airway mast cells by adenosine in asthma may be mediated by prostaglandins. However, conclusive evidence of the role of spasmogenic prostaglandins in adenosine-induced bronchoconstriction will have to await the results of studies with selective prostanoid receptor antagonists.

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