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Activity of inhaled lysine acetylsalicylate (L-ASA) on bradykinin-induced bronchoconstriction in asthmatics: evidence of contribution of prostaglandins

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ABSTRACT: When administered by inhalation, bradykinin provokes dose-related bronchoconstriction in asthmatic subjects by a mechanism believed to involve activation of sensory nerve endings. However, little is known of the change in airway responsiveness to bradykinin after cyclo-oxygenase blockade. The aim of the present study was to investigate the effect of the potent cyclo-oxygenase inhibitor, lysine acetylsalicylate (L-ASA), administered by inhalation, on bradykinin-induced bronchoconstriction in a group of 12 asthmatic subjects.

The subjects attended the laboratory on four separate occasions to receive nebulized L-ASA (solution of 90 mg·mL^{-1}) or matched placebo (glycine, solution of 30 mg·mL^{-1}) 15 min prior to bronchoprovocation tests with bradykinin and methacholine in a randomized, double-blind order with at least a 5 day interval. Changes in airway calibre were followed as forced expiratory volume in one second (FEV_1), and responsiveness to agonists was expressed as the provocative concentration causing a 20% fall in FEV_1 from baseline (PC_{20}).

Administration both of L-ASA and glycine solution caused a small but significant acute fall in FEV_1 from baseline, with gradual recovery within 20 min. When compared to placebo, inhaled L-ASA reduced the airway responsiveness to bradykinin in 11 of the 12 subjects studied, the geometric mean (range) values for PC_{20} bradykinin increasing significantly ($p<0.001$) by 1.7 doubling dose from 0.55 (0.11–5.05) to 1.72 (0.26–6.05) mg·mL^{-1} after placebo and L-ASA, respectively. No significant change in airway responsiveness to methacholine was recorded after L-ASA.

It is concluded that administration of lysine acetylsalicylate by inhalation protects the asthmatic airways against bradykinin-induced bronchoconstriction, thus suggesting that endogenous prostaglandins may play a contributory role in the bronchoconstriction to kinins in human asthma.
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Bradykinin is a naturally occurring vasoactive nonapeptide formed *de novo* in body fluids and tissues during inflammatory processes [1]. Evidence that kinin generation may be increased under conditions which prevail in areas of allergic inflammation in the airways has been obtained recently from studies in asthmatic subjects [2, 3]. In addition, bradykinin is reported to possess many pharmacological properties pertinent to the pathogenesis of asthma. These include: vasodilatation and increased microvascular leakage [4, 5]; bronchoconstriction [6, 7]; activation of C fibre nociceptive sensory nerve endings [8, 9]; and induction of bronchial hyperreactivity in animals and man [10, 11].

The precise mechanism by which bradykinin mediates bronchoconstriction in asthmatic subjects is still not clear. The potent H1-histamine receptor antagonist, terfenadine, has been shown to elicit a small but significant inhibition of the bronchoconstrictor response after challenge with bradykinin [12], and the anticholinergic agent, ipratropium bromide, produces only little protection [6]. There have been controversial reports on the effect of cyclo-oxygenase blockade on bradykinin-induced bronchoconstriction [6, 12, 13]; most concluded that the effect was probably not seen because of the poor bioavailability of cyclo-oxygenase inhibitors given orally at the level of the tracheobronchial tree. Recent work by BIANCO and co-workers [14, 15] and by ourselves [16, 17] has shown that inhaled lysine acetylsalicylate (L-ASA) provides better protection than oral cyclo-oxygenase inhibitors against the bronchoconstrictor response to a variety of nonspecific stimuli, including fog, adenosine and neurokinin A (NKA). Using this alternative experimental approach, we have extended the previous observations [6, 12, 13] on the relative contribution of contractile prostaglandins to the airway response provoked by inhaled bradykinin in asthma.

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

Methods

Subjects

Twelve subjects with stable asthma (6 females, 6 males), with a mean (±SEM) age of 31 (±3) yrs participated in the study (table 1). The subjects studied were enrolled from a pool of 23 consecutive patients attending our Asthma Clinic. They were screened on the basis of their baseline value for the provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second (PC_{20} methacholine) (<2 $mg \cdot mL^{-1}$), in order to have subjects sensitive enough to respond to relatively low concentrations of bradykinin. All subjects had a history of dyspnoea with wheezing or chest tightness on exposure to airborne allergens, were nonsmokers, and had positive skin-prick tests (>3 mm weal response) to one or more of six common aero-allergens. At the beginning of the study, all subjects were asymptomatic, with a baseline forced expiratory volume in one second (FEV_1) >70% of their predicted value. 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 reported 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, and all subjects gave their informed consent.

Bronchial provocation

Airway calibre was recorded before and during the provocation as the FEV_1, using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK). The better of two consecutive measurements was recorded.

Bradykinin and methacholine (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 0.00375–4 and 0.03–16 $mg \cdot mL^{-1}$ for bradykinin 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 $L \cdot min^{-1}$. Under these conditions, the nebulizer had an output of 0.48 $mL \cdot min^{-1}$ and generated an aerosol with a mass median particle diameter of 4.7 μm [18]. 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.* [19]. 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 methacholine and bradykinin. On the first occasion, after 15 min rest, three baseline measurements of FEV_1 were made at intervals of 3 min, followed by inhalation of 0.9% sodium chloride and further FEV_1 measurements repeated at 1 and 3 min. Provided FEV_1 had not fallen by >10% of the baseline value, a methacholine concentration-response study was carried out. After administration

Table 1. – Demographic details of subjects studied

Ss No.	Sex	Age yrs	Baseline FEV_1 % pred	Atopy§	PC_{20} methacholine $mg \cdot mL^{-1}$	PC_{20} bradykinin $mg \cdot mL^{-1}$
1	F	42	96	P	0.45	0.39
2	F	36	75	P	1.42	0.49
3	M	35	96	P–G	2.35	0.39
4	F	25	85	P	0.36	0.50
5	M	57	92	P–G	0.52	0.36
6	M	38	102	P	0.89	3.09
7	M	23	90	D	0.32	1.70
8	F	20	83	P–D	0.88	0.84
9	M	19	110	P	1.46	1.17
10	M	30	72	P	0.50	0.72
11	F	22	89	P	0.09	0.10
12	F	29	78	P–D	0.74	1.32
Mean		31	89		0.62+	0.66+
SEM		±3	±3		(0.09–2.35)	(0.10–3.09)

+: geometric mean, and range in parenthesis; §: atopic, positive immediate skin test to one or more allergens. Ss: subjects; M: male; F: female; D: Dermatophagoides; P: Parietaria; G: grass pollens; FEV_1: forced expiratory volume in one second; PC_{20}: provocative concentration producing a 20% fall in FEV_1.

of each methacholine concentration, FEV_1 was measured at 1 and 3 min. Increasing doubling concentrations of methacholine were inhaled at 5 min intervals until FEV_1 had fallen by >20% of the postsaline baseline value, and the corresponding PC_{20} values were derived. On the remaining visit, bronchial provocation tests with inhaled bradykinin were undertaken in a similar manner to that described for methacholine.

Phase 2. Subjects attended the laboratory on four separate visits, at least 5 days apart, to undertake concentration-response studies with bradykinin 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 (4 mL at 90 $mg \cdot mL^{-1}$; 525 $mOsm \cdot L^{-1}$, pH 5.25), or nebulized vehicle placebo consisting of a solution of glycine (4 mL at 30 $mg \cdot mL^{-1}$; 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 8 $L \cdot min^{-1}$, 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 1, 5, 10 and 20 min after drug or placebo inhalation, and dose-response studies with increasing concentrations of bradykinin and methacholine were carried out in a similar manner to that described in Phase 1.

Data analyses

Values refer to the mean±SEM unless otherwise stated, and a p-value of less than 0.05 was considered significant. 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 postsaline 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}) determined by linear interpolation.

The coefficient of repeatability (CR) for the bradykinin challenge was calculated according to the method described by ALTMAN and BLAND [20], using the formula: $CR=2\sqrt{([\sum d^2]/n)}$, where d = ln($PC_{20}$ baseline) - ln(PC_{20} placebo) for the subjects studied, and n = number of subjects. In brief, the standard deviation of the differences between the logarithmically transformed PC_{20} values obtained on the placebo and open study days was derived; the CR is twice this standard deviation.

Values of PC_{20} methacholine and PC_{20} bradykinin 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 and bradykinin 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 and bradykinin; and 2) any relationship between the magnitude of fall in FEV_1 after exposure to L-ASA and baseline airway responsiveness to methacholine and bradykinin.

Results

There was no significant difference in baseline values of FEV_1 between any of the six study days, with mean (±SEM) values ranging from 3.05±0.24 to 3.19±0.23 L. One minute after nebulized L-ASA, the mean FEV_1 values decreased 12 and 9% from baseline on the bradykinin (fig. 1) and methacholine (data not shown) study days, respectively. However, 20 min later, the mean FEV_1 values were only 4 and 3% lower compared to baseline on the bradykinin (fig. 1) and methacholine (data not shown) study days, respectively. On the bradykinin study day, 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 (fig. 1). No significant correlations could be established between the magnitude of fall in FEV_1 after the active drug and baseline airway responsiveness to bradykinin or methacholine.

The challenge procedure with bradykinin in this group of subjects was found to be repeatable, with a CR of

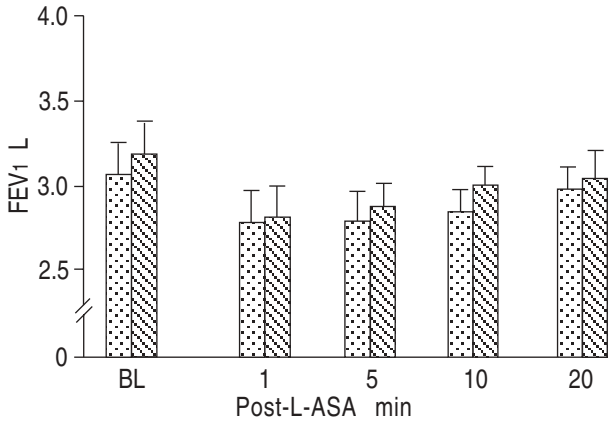

Fig. 1. – Time-dependent changes of FEV_1 after administration of inhaled L-ASA (▭) and placebo (□) in 12 asthmatic subjects on the bradykinin study day. Values are presented as mean±SEM. FEV_1 values post-L-ASA were not significantly different to those post-placebo (glycine) when compared at all time-points. FEV_1: forced expiratory volume in one second; L-ASA: lysine acetylsalicylate; BL: baseline.

1.5 doubling dilutions. The PC_{20} bradykinin values were within a single doubling dilution in 10 of the 12 subjects receiving bradykinin.

In Phase 1, inhaled methacholine and bradykinin produced concentration-related falls in FEV_1. The geometric mean (range) of PC_{20} values obtained were 0.62 (0.09–2.35) and 0.66 (0.10–3.09) $mg \cdot mL^{-1}$ for methacholine and bradykinin, respectively (table 1). No significant correlation was observed between PC_{20} values for methacholine and bradykinin (r=0.45; p=NS).

In Phase 2, when compared to placebo, inhaled L-ASA had a significant protective effect against the fall in FEV_1 produced by bradykinin. L-ASA produced a displacement of the bradykinin concentration-response curve to the right in 11 of the 12 subjects studied. For these subjects the geometric mean (range) PC_{20} bradykinin values increased 1.7 doubling dilutions from 0.55 (0.11–5.05) to 1.72 (0.26–6.05) $mg \cdot mL^{-1}$ after placebo and L-ASA, respectively ($p < 0.001$) (fig. 2a). No correlation could be found between baseline PC_{20} values and the protection of airway response to bradykinin after L-ASA. Inhaled L-ASA, despite being effective in inducing significant changes in baseline airway calibre, failed to alter the airway response to methacholine. The geometric mean (range) PC_{20} methacholine value of 0.65 (0.09–1.59) $mg \cdot mL^{-1}$ after placebo was not significantly different from that of 0.50 (0.12–1.47) $mg \cdot mL^{-1}$ after L-ASA (fig. 2b).

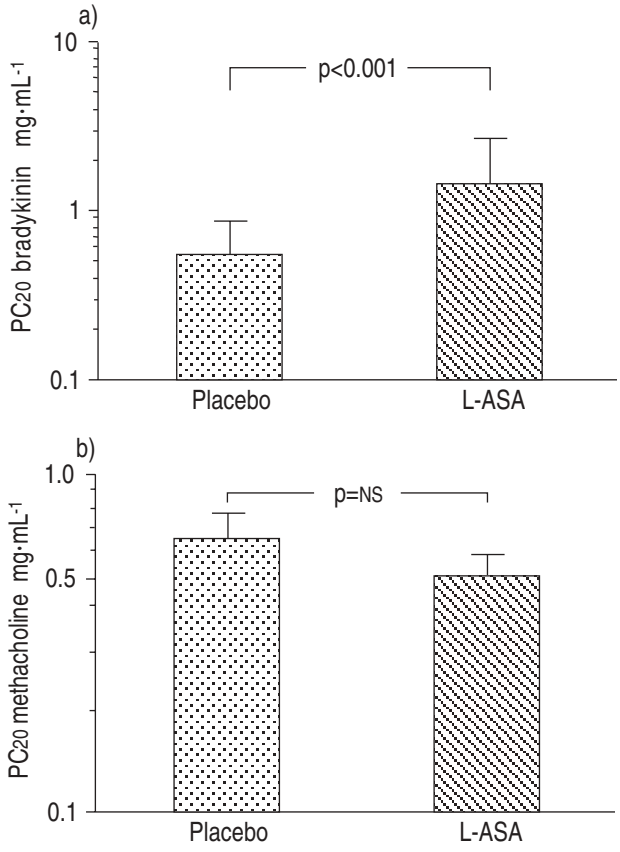

Fig. 2. – a) Changes in provocative concentrations of: a) bradykinin; and b) methacholine required to provoke a 20% decrease in FEV_1 (PC_{20}) after administration of placebo (☐) and L-ASA (▨) in 12 asthmatic subjects. Values are presented as mean±SEM. FEV_1: forced expiratory volume in one second; L-ASA: lysine acetylsalicylate; NS: nonsignificant.

Discussion

In this study, we have demonstrated that administration of L-ASA by inhalation elicits a small but significant protection of the asthmatic airways against bradykinin-induced bronchoconstriction. The protection afforded by L-ASA occurred in 11 of the 12 subjects studied, and amounted to 1.7 doubling dilutions, which is similar to that reported with other bronchoprovocants [14, 15, 17]. In addition, following L-ASA exposure, we have failed to show a significant change in airway responsiveness 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 a variety of nonspecific stimuli in asthmatic subjects [14, 15, 17].

We have shown that inhaled L-ASA increased the PC_{20} bradykinin values by 1.7 doubling dilutions. Although not directly comparable with the results obtained in similar studies with oral cyclo-oxygenase blockers, the observed increase of 1.7 doubling dilutions in the present study is significantly better compared to the small increase in PC_{20} bradykinin values of 1.0 doubling dilutions after oral flurbiprofen [12]. Therefore, 90 mg inhaled L-ASA was more effective in inhibiting bradykinin-induced bronchoconstriction than the doses of oral flurbiprofen that have been given. Although flurbiprofen is approximately 5,000 times more potent than aspirin in inhibiting cyclo-oxygenase in human rheumatoid synovial microsomes [21], the present data suggest that the route of administration may have contributed to a better protection against the effect of contractile prostaglandins to the airway response to bradykinin as opposed to the oral dosing of more potent cyclo-oxygenase inhibitors.

The mechanism by which L-ASA attenuates the bronchoconstrictor response to bradykinin requires some consideration. In the present study, no bronchodilator effect of this drug was shown, but rather an immediate bronchoconstriction, which was probably the result of a irritation due to the high osmolality of the solution. This was confirmed by the observation that the hyperosmolar control solution of glycine elicited a similar fall in FEV_1. In the subjects studied, it was not possible to demonstrate any correlation between the magnitude of fall in FEV_1 after hyperosmolar solutions and baseline airway responsiveness. That cyclo-oxygenase blockade has a specific effect on airway responsiveness to bradykinin is supported by the lack of change in methacholine responsiveness after inhaled L-ASA. This is in agreement with previous data with inhaled L-ASA [17] and with oral cyclo-oxygenase blockers [22, 23], which have repeatedly failed to show an effect on methacholine-induced bronchoconstriction.

Thus, the protective effect of inhaled L-ASA may be ascribed to prostaglandin synthetase inhibition. In support of this view, cyclo-oxygenase blockers were found to inhibit anti-immunoglobulin E (IgE)-provoked release of prostaglandin D_2 (PGD_2) and thromboxane B_2 ($Tx\text{-}B_2$) from passively sensitized human dispersed lung cells *in vitro* [24]. In a variety of animal studies, it has been shown that bradykinin has the capacity to generate the

release of spasmogenic prostanoids [25]. In the guinea-pig, bronchoconstriction provoked by bradykinin is mediated largely through the formation of thromboxane A_2 ($Tx-A_2$), and as a consequence could be abolished by prior treatment with a cyclo-oxygenase inhibitor [26]. In the guinea-pig trachea, the production of prostanoids was increased by bradykinin and largely prevented by indomethacin [27]. Bradykinin may also augment the release of newly generated bronchoconstrictor mediators, such as prostaglandins (PGs) and $Tx-A_2$ from rodent mast cells [28]. Further support for the involvement of prostanoids in bradykinin-induced responses is gained from the finding of two recent investigations in human isolated peripheral airways [29, 30]. In particular, an elegant study by HULSMANN *et al.* [30] has shown that indomethacin, 5×10^{-7} M, abolished the response of human airways to bradykinin, and prevented the associated bradykinin-induced release of prostaglandins. However, involvement of prostaglandins in bradykinin-induced bronchoconstriction in man remains controversial. Indeed cyclo-oxygenase inhibitors administered orally are relatively ineffective in relieving bradykinin-induced bronchoconstriction in human asthma [6, 12, 13], and the findings of the present study are no substantial exception.

The contribution of additional mechanisms in bradykinin-induced bronchoconstriction must be considered. Evidence is now accumulating that local neural reflexes may contribute to the contractile airway response to inhaled kinins in asthma. Ipratropium bromide produces an approximately fivefold protection against bronchoconstriction provoked by bradykinin [6], indicating that cholinergic vagal reflexes may, in part, contribute to this response. Additional support for neuronal involvement in bradykinin-induced bronchoconstriction is gained from the finding that pretreatment with sodium cromoglycate and nedocromil sodium, both considered to have the ability to inhibit peptidergic neural reflexes in addition to their effect on mast cells [31], is very effective in attenuating the airways response to bradykinin challenge in asthmatic subjects [6, 32]. It is also possible that at least some of the reported protection afforded against the airways effect of bradykinin by the loop diuretic, frusemide, is due to the ability of this drug to inhibit peptidergic neural reflexes when administered by inhalation in asthmatics [33]. Thus, the effect of L-ASA on bradykinin-induced bronchoconstriction could also be viewed as an inhibition of the modulating effect of cyclo-oxygenase products on presynaptic neural mechanisms and on neurogenic inflammation. Capsaicin and bradykinin are thought to stimulate the release and subsequent depletion of sensory neuropeptides from unmyelinated afferent fibres in a variety of animals [34], and in human skin *in vivo* [35], thus indicating that release of neuropeptides may also be involved in the kinin-induced responses. In support of this view, it must be noted that the potent neurokinin-2 (NK_2) receptor antagonist, FK-224, is effective in inhibiting bronchoconstriction induced by bradykinin [36]. There is evidence *in vitro* that part of the biological effect of exogenously applied neuropeptides may result from the local release of bioactive prostaglandins [37–39]. Moreover, a recent study in asthmatic subjects has shown a significant decrease in airway responsiveness to NKA after administration of L-ASA [16]. Therefore, part of the airway response to bradykinin may also be mediated by the prostanoid-dependent component of the bronchoconstriction produced by NKA in asthma.

In conclusion, our results confirm that cyclo-oxygenase blockade with inhaled lysine acetylsalicylate produces significant protection against bradykinin-provoked bronchoconstriction in asthmatic subjects, implying a role for endogenous prostanoids in this response. Thus, part of the airway response to bradykinin in asthma may be directly and indirectly mediated by endogenous prostaglandins. However, conclusive evidence of the role of spasmogenic prostaglandins in bradykinin-induced bronchoconstriction will have to await the results of studies with selective prostanoid receptor antagonists.

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