Time-limited protective effect of inhaled frusemide against aspirin-induced bronchoconstriction in aspirin-sensitive asthmatics

P. Sestini*, M.G. Pieroni*, R.M. Refini*, M. Robuschi**, G. Gambaro**,
S. Spagnotto**, A. Vaghi⁺, S. Bianco⁺⁺

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ABSTRACT: Inhaled frusemide effectively prevents the bronchial obstructive response to allergens and to a number of nonallergic stimuli. In most of the experimental models in which it has been tested, the protective effect of frusemide has been evaluated for only a short time after administration. In aspirin-sensitive patients, acetylsalicylic acid causes an asthmatic reaction which typically lasts for 2 h or more after exposure. We investigated the presence and duration of the protective effect of inhaled frusemide against the bronchial response to aspirin in sensitive patients, using a specific inhalation challenge with lysine acetylsalicylate (LASA).

In the first study, eight subjects with aspirin-asthma underwent two bronchial challenges with a single dose of lysine acetylsalicylate administered through a jet nebulizer, after treatment with 40 mg inhaled frusemide or placebo, according to a randomized, double-blind protocol. Forced expiratory volume in one second (FEV₁) was monitored for 120 min after challenge. In the second study in eight patients, the protocol was modified by the use of a dosimeter for delivery of lysine acetylsalicylate, by reducing the dose of lysine acetylsalicylate to avoid intense reactions, and by extending the follow-up to 4 h.

In the first study, after placebo, FEV₁ gradually decreased, reaching a maximum decrement of 39±3% at 120 min. Inhaled frusemide exerted a significant protection at all time-points, although this activity appeared to decrease with time. In the second study, after placebo, inhaled lysine acetylsalicylate caused a gradual decrease in FEV₁, which reached a maximum decrement at 180 min. Frusemide provided a significant protection in the first 90 min of the reaction; thereafter, FEV₁ fell gradually to levels similar to placebo.

We conclude that inhaled frusemide effectively prevents the asthmatic reaction to lysine acetylsalicylate in aspirin sensitive patients, but this protective effect is limited in time.

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*Institute of Respiratory Diseases, University of Siena, Siena, Italy.

**Institute of Respiratory Diseases, University of Milan, Milan, Italy.

⁺Division of Pneumology, Ospedale di Garbagnate, Milan, Italy.

⁺⁺Institute of Respiratory and Cardiovascular Diseases, Ospedale S. Raffaele, University of Milan, Milan, Italy.

Correspondence: P. Sestini
Institute of Respiratory Diseases
Viale Bracci 3
3100 Siena
Italy

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Inhalation of frusemide and, to a lesser extent other loop diuretics, inhibits the bronchoconstriction induced by allergens and by several nonallergic stimuli, such as exercise, hypo- and hypertonic aerosols, cold air, metabisulphite and adenosine [1, 2]. Although frusemide protects against the late asthmatic reaction up to 12 h after allergen exposure [3], the protective effect against ultrasonically-nebulized distilled water and sodium metabisulphite is relatively short-lived, lasting for 1–2 h [4, 5]. Bronchoconstriction induced by inhaled acetylsalicylic acid (ASA) in aspirin-sensitive asthmatics has a relatively long time course, peaking around the second hour and usually lasting more than 4 h after exposure [6, 7]. Previous observations indicating a protective effect of inhaled frusemide on aspirin-induced asthma

have been limited to the initial phase of the response [8, 9]. In this paper, we report our preliminary observations [8], and a subsequent study conducted to verify them and to investigate the duration of the protective effect of frusemide on aspirin-induced asthma.

Materials and methods

Patients

We studied a total of 16 patients, with a clinical history of aspirin-induced asthma confirmed by a preliminary inhalation challenge with lysine acetylsalicylate (LASA) [10]. All the patients were in stable clinical condition, had a baseline forced expiratory volume in one second

Table 1. – Patient characteristics - Study 1

Pat No.	Sex	Age yrs	Nasal polyps	Atopy	FEV ₁ % pred	PD ₃₀ LASA mg	Therapy
1	F	50	Yes	Gr	87	5.2	f, ib, bcl, t
2	M	57	Yes	–	118	5.2	f, ib, bcl
3	F	46	Yes	–	69	5.2	f, ib, bcl, cs
4	F	43	No	–	90	1.3	s
5	F	42	No	–	100	2.6	f, ip, bcl
6	M	72	Yes	–	63	5.2	s
7	M	35	Yes	–	53	3.9	f, ip, t, bcl
8	F	63	No	–	100	5.2	s, bcl

f: inhaled fenoterol; ib: inhaled ipratropium bromide; s: inhaled salbutamol; bcl: inhaled beclomethasone; t: oral theophylline; cs: oral steroids; Gr: grass pollen; FEV₁: forced expiratory volume in one second; F: female; M: male; LASA: lysine acetylsalicylate; PD₃₀: provocative dose producing a 30% fall in FEV₁.

Table 2. – Patient characteristics - Study 2

Pat No.	Sex	Age yrs	Nasal polyps	Atopy	FEV ₁ % pred	PD ₂₀ LASA mg	Therapy
1	F	25	No	–	102	4.0	S, t, bcl
2	F	23	No	Dp	82	16.0	f, ib, bcl
3	F	64	Yes	–	83	1.0	t, s, cs
4	F	18	Yes	Dp	83	16.0	s
5	M	48	No	–	61	1.0	t, ib, f, bcl
6	M	73	No	–	48	2.0	s, ib, bcl
7	F	60	No	–	79	1.0	t, bcl
8	F	25	Yes	Dp	102	8.0	s

Dp: *Dermatophagoides pteronyssinus*. For further abbreviations see legend to table 1.

(FEV₁) above 50% of predicted, and were free of respiratory infections for at least 6 weeks. All the patients were treated with inhaled β_2 -stimulants on demand, and the majority were taking inhaled beclomethasone on a regular basis, in a dose range of 200–2,000 $\mu\text{g}\cdot\text{day}^{-1}$. Two patients also required a low dose of oral prednisone to maintain clinical stability (tables 1 and 2). All treatments were withheld for at least 12 h before the challenge test, except for slow-release theophylline which was withheld for at least 24 h in advance [11]. Patients allergic to pollen were studied outside the pollen season. The study was conducted according to the ethical standards of our institution, requiring informed consent from each patient.

Study design

The study consisted of two parts, both of which were conducted according to randomized, double-blind protocols. Bronchial challenge with LASA was performed using two different techniques, which are progressive modifications of the inhalation challenge originally described [10, 12, 13]. In a first group of patients (Study 1), we used a method of continuous aerosol generation with a jet nebulizer (Nebula, Markos, Monza, Italy) during tidal breathing inhalation for drug delivery. By appropriately combining the concentration of the solution and the time of nebulization, the amounts of LASA (Flectadol, Maggioni Winthrop Italia, Milan, Italy) progressively delivered to the mouth at 1 h intervals were 0.65 mg (0.3 mg if an intense reactivity was

suspected), 1.2, 2.6, 5.6, 10.4, 20.8 and 41.6 mg, until a fall in FEV₁ of 30% or more with respect to baseline was observed within 1 h of exposure.

The patients performed two bronchial challenges, with an interval of 7–10 days, using a single dose of LASA corresponding to the last cumulative dose administered in the preliminary test. Immediately before each challenge, they received 4 ml of an aerosol containing either 40 mg frusemide (Lasix, Hoechst, Frankfurt am Main, Germany) or placebo (the diluent), administered with a jet nebulizer (Nebula, Markos, Brescia, Italy) over a period of 20 min. FEV₁ was recorded before and after treatment and at 30, 60, 90 and 120 min after exposure to LASA. Clinical and anthropometric characteristics of the patients who participated in this part of the study are shown in table 1.

In a second group of patients (Study 2) (table 2) the bronchial challenge was modified [14] using a Mefar dosimeter (Mefar, Bovezza, Italy) set to deliver 5 μl of 180 $\text{mg}\cdot\text{ml}^{-1}$ LASA (corresponding to 500 $\mu\text{g ASA}\cdot\text{puff}^{-1}$) during a deep breath from functional residual capacity (FRC) to total lung capacity (TLC), at intervals of 6 s. FEV₁ was obtained by integration of flows measured with a No. 3 Fleisch pneumotachograph (Fenylves & Gut, Basel, Switzerland) 30 and 60 min after challenge. Doubling doses of LASA were progressively administered at 1 h intervals, starting from 1 mg (0.5 mg if an intense reaction was suspected), until a decrease in FEV₁ of 20% or more was observed, or the final dose of 64 mg was reached. However, if after 1 h FEV₁ decreased by 15–20%, administration of the

subsequent dose of LASA was deferred for a further 30–60 min and was not given if the decrement in FEV_1 reached 20% or more with respect to baseline in this time. FEV_1 was recorded every 30 min for 4 h, thereafter.

The rest of the study was conducted according to a protocol similar to Study 1, except that FEV_1 was recorded every half hour up to 240 min after exposure and a third day of testing was added, in which the patients were treated with placebo and exposed to a placebo (50 mg·ml⁻¹ lysine in saline), to check their clinical stability over the period of the test. The single dose of LASA used for challenge was the dose causing a decrease in FEV_1 of 20% or more during the whole preliminary test, rather than 30% at 1 h used in the first study, to reduce the intensity of the reaction at subsequent times.

Statistical analysis

Analysis of variance was used to compare FEV_1 with baseline at different time points [15]. Student's paired t-test was used for comparison of the two treatment groups at the same time-point [16]. The percentage of protection afforded by frusemide was computed using the formula: $(P-F)/P \times 100$, where P and F are the percentage decrease in FEV_1 with respect to baseline at the same time-point after placebo and frusemide, respectively. A p-value less than or equal to 0.05 was considered significant. Unless stated otherwise, the data are presented as means $\pm$ SEM.

Results

Study 1

Baseline FEV_1 was similar on both days of the study (2.34 ± 0.25 l and 2.41 ± 0.24 l before placebo and frusemide, respectively), and was not modified by placebo or frusemide. After placebo, FEV_1 was already significantly reduced 30 min after LASA, progressively decreasing to a minimum of $-39 \pm 3\%$ at 120 min. After frusemide, FEV_1 was substantially unchanged with respect to baseline 30 min after exposure, gradually decreasing, thereafter, to a significantly lower value than after placebo, and reaching a minimum of $-24 \pm 7\%$ at 120 min (fig. 1). The degree of protection afforded by frusemide was $52 \pm 19\%$ at 30 min, $72 \pm 9\%$ after 60 min, $56 \pm 13\%$ after 90 min and $37 \pm 14\%$ after 120 min.

Study 2

Since, in the previous study, the protection afforded by frusemide seemed to decrease with time, the protocol was modified to include further time-points. A control day was also included to exclude clinical instability during this longer period of observation. As shown in figure 2, the patients remained stable during the control day for the whole period of observation. None showed changes of more than 10% with respect to baseline in this period.

Fig. 1. — Study 1. Effect of inhaled frusemide on the bronchial obstructive response to a single dose of inhaled LASA (PD_{30}) in sensitive asthmatics, monitored for 2 h. LASA: lysine acetylsalicylate; FEV_1 : forced expiratory volume in one second; PD_{30} : provocative dose of LASA causing a 30% fall in FEV_1 . —○—: placebo; —●—: frusemide; †: nonsignificant vs baseline; **: $p < 0.05$ vs frusemide.

After placebo, FEV_1 was significantly reduced 30 min after exposure to LASA and continued to decrease to $-20 \pm 3\%$ with respect to baseline at 3 h. Maximum individual FEV_1 decrease during the test was $26 \pm 2\%$ and occurred between 120 and 240 min after challenge in all the patients except one (table 2 patient No. 7) who had a peak at 30 min (mean 131 ± 20 min).

Fig. 2. — Study 2. Effect of inhaled frusemide on the bronchial obstructive response to a single dose of LASA (PD_{20}), monitored for 4 h. PD_{20} : provocative dose of LASA producing a 20% fall in FEV_1 . —○—: placebo; —●—: frusemide; —▽—: control day. *: not significant vs baseline; **: $p < 0.05$ vs frusemide. For further abbreviations see legend to Figure 1.

After frusemide, FEV₁ 30 and 60 min after LASA was not statistically different from baseline, but was significantly higher than at these time points and 90 min after placebo. Thereafter, however, FEV₁ decreased to levels not significantly different from placebo, dropping by 19±4% with respect to baseline at 3 h. The degree of protection afforded by frusemide was 93±12% at 30 min, 64±14% at 60 min, 45±23% at 90 min, 40±25% at 120 min, 19±16% at 150 min, 10±14% at 180 min decreasing to negative levels, thereafter. The maximum individual decrease in FEV₁ during the test was 23±3%, not significantly different from placebo.

Discussion

Our results indicate that inhaled frusemide significantly inhibits aspirin-induced asthma, but this effect is limited to the initial part of the reaction. The trend towards a decreasing protective effect of frusemide with time was already evident in the first study, but it was underestimated because of the short period of observation. The protective effect was probably more evident in that study because the higher dose of LASA used for challenge caused a stronger reaction during placebo, increasing the difference with respect to the reaction modified by frusemide. The short duration of the protective effect of frusemide was completely missed in another study, conducted without randomization and with a period of observation limited to 30 min [9]. Although, in practice, the bronchial response to LASA is normally evaluated 60 min after challenge, often terminating the reaction with an inhaled bronchodilator [6, 7, 10, 12, 13, 17], the time course of the reaction is known to be longer [6, 7], and such a short observation period is unsuitable for evaluating the effect of pharmacological agents on the bronchial response to LASA.

Although limited in time, frusemide nevertheless provided very effective protection in the first hour after challenge, suggesting that it has a powerful interaction with the pathogenic mechanism of aspirin-induced asthma. These mechanisms are not yet fully understood, but it has long been suggested that they involve the cyclooxygenase inhibitory activity of aspirin and other non-steroidal anti-inflammatory drugs [18, 19], which might cause a shift in the metabolism of arachidonic acid towards the production of bronchoconstrictor leukotrienes, possibly by removing a prostaglandin-mediated inhibitory mechanism [20–22]. This hypothesis was recently supported by the observation of increased urinary excretion of leukotrienes during aspirin-induced asthma [23–26], and by studies indicating that aspirin-induced asthma is effectively prevented by leukotriene-receptor inhibitors [27, 28], reviewed in [29]. Interestingly, recent studies indicate that frusemide may interact with leukotriene production in the bronchial mucosa in several experimental models *in vitro* and *in vivo*. *In vitro*, frusemide has been shown to inhibit the release of leukotriene-like activity from passively sensitized human lung [30], and to inhibit allergen-induced human airway smooth muscle contractions in a leukotriene dependent model [31] (and A. Sala, personal communication). *In vivo*, we have recently

shown that inhaled frusemide inhibits the urinary excretion of leukotriene E₄ (LTE₄), which accompanies the early asthmatic reaction after allergen challenge [32]. Taken together, these data suggest that an inhibitory effect on the biosynthesis of leukotrienes might contribute to the protective effect of frusemide in aspirin-induced asthma.

An additional and possibly related hypothesis, is that inhaled frusemide might be acting by causing a short-lived enhancement of the local production of prostaglandins, as suggested in other situations *in vitro* [31] and *in vivo* [33, 34]. Inhaled prostaglandin E (PGE) has previously been reported to efficiently protect against aspirin-induced asthma [12], and might act by inhibiting mediator releasing cells [35], affecting leukotriene metabolism [22, 26], or simply counteracting the inhibitory effect of LASA.

The protective effect could be limited in time either because a different, frusemide-resistant mechanism of bronchoconstriction is operating during the second part of the response to aspirin, or because frusemide has fast local kinetics in the airways. The latter hypothesis is supported by the observation that the protective effect of inhaled frusemide on bronchoconstriction induced by ultrasonic mist of distilled water [4], and by sodium metabisulphite [5], also decreases rapidly in the first 2 h after treatment, and is no longer operating at 3 h. These observations apparently contrast with the protective effect against the late allergic asthma reaction, which occurs beyond the expected time of activity of inhaled frusemide. However, the release of leukotrienes, as assessed by urinary excretion, has a different time course in allergen-induced reactions than in ASA-induced asthma. In allergic reactions, it is limited to the first period after allergen exposure, and there is no evidence of leukotriene release during the late response [25, 36]. The release of leukotrienes during the early asthmatic response to allergen may cause both transient bronchoconstriction and the development of the subsequent late reaction, possibly by increasing bronchial hyperreactivity [37], and by promoting the influx of inflammatory cells into the airways [38]. Frusemide may block both these event, by inhibiting the release of leukotrienes and other mediators occurring whilst it is still active in the airways. In aspirin-induced asthma, the increased production of leukotrienes lasts much longer, and closely parallels the asthmatic reaction [23, 25, 39]. Since a late reaction does not occur in this condition [7], the limited duration of the effect of frusemide may be explained by the simple fact that leukotriene release in this case lasts longer than the activity of the drug.

In conclusion, our results indicate that inhaled frusemide inhibits aspirin-induced asthma, and that its effectiveness is limited by the relatively short duration of its local effect. The hypothesis that this protective effect is mediated by the inhibition of leukotriene production in the bronchi requires experimental verification in further studies.

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