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Acute additive effect of montelukast and beclomethasone on AMP induced bronchoconstriction

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Summary
Bronchial hyperresponsiveness to 5-adenosine mono-phosphate (AMP) is a marker of airway inflammation. Inhaled corticosteroids and antileukotrienes are used as anti-inflammatory drugs for the treatment of asthma. To find out if these two drugs exert their protection in an additive fashion, we compared the effects of acute treatment with inhaled beclomethasone (BDP) and montelukast (ML), alone or in combination, on methacholine and AMP induced bronchoconstriction.

15 asthmatic patients undertook methacholine and AMP challenges at baseline and after receiving ML or BDP, alone or in combination, in a randomized, double-blind, double-dummy placebo-controlled, crossover design.

BDP pretreatment significantly increased the AMP PC_{20} value (68.34 ± 15.9 mg/mL) as compared to placebo (22.87 ± 5.7 mg/mL). Combined treatment, BDP plus ML, afforded a further significant increase of AMP PC_{20} (154.57 ± 55.0 mg/mL) as compared to each single treatment. The significant protection exerted by combined treatment as compared to each single active treatment was also demonstrated by the change of AMP PC_{20} doubling dose as compared to placebo and each single active treatment.

Our findings suggest that these two agents exert their acute additive protection against AMP induced bronchoconstriction acting on distinct inflammatory pathways and their combined use might provide greater protection against inflammatory response elicited by AMP than either drug alone.

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Introduction

Bronchial hyperresponsiveness (BHR) and chronic airway inflammation are key features of asthma. Inhaled corticosteroids (ICS), as effective anti-inflammatory drugs, are widely used for the treatment of asthma and have shown to be able to reduce airway inflammation, asthma symptoms and improve lung function.[1] Corticosteroids affect many aspects of the inflammatory process increasing the transcription of genes encoding anti-inflammatory mediators and inhibiting the synthesis and release of proinflammatory mediators, particularly cytokines. More recently, ICS have also demonstrated to possess acute nongenomic anti-inflammatory effects that might have potentially beneficial effects in asthma.[2] However, despite their complex anti-inflammatory activity, ICS do not completely abolish airway inflammation, thus suggesting that other inflammatory mechanisms insensitive to the anti-inflammatory effects of this class of drugs are also present in bronchial asthma.

Among the inflammatory mediators involved in the onset and maintenance of chronic airway inflammation, cys-leukotrienes (cysLTs) are important candidate for contributing to airways dysfunction in asthma.[3] Cys-LTs, through interaction with specific cysteinyl leukotriene -1 receptor (cys-LTR1), exert many biological effects that are relevant to the pathophysiology of this disease, including bronchoconstriction and chemoattraction for inflammatory cells, especially eosinophils.[4,5] Furthermore, there is evidence that leukotriene synthesis and action are relatively resistant to the anti-inflammatory activity played by glucocorticoids,[6,7] suggesting this, that the contemporaneous use of steroids and specific cys-LTR1 receptor-antagonist, such as montelukast, may produce multiple beneficial effects acting on different steps of the inflammatory cascade.

Bronchial responsiveness to 5′-adenosine mono-phosphate (AMP) is closely related to airway inflammation and BHR to AMP has been proposed as a highly specific marker of airway inflammation in asthma.[8] Airway responsiveness to AMP has been demonstrated to be correlated with airway eosinophils,[9] blood eosinophils and serum eosinophil cationic protein levels [10] and modification in AMP responsiveness is related to inflammatory changes in airways.[11,12] On the other hand, response to methacholine, a direct stimulus widely used in clinical practice, reflects mainly airway smooth muscle function, being only moderately correlated with airway inflammation.[13]

Anti-inflammatory therapy, such as ICS, is able to reduce BHR to AMP when used either as regular treatment[14,15] or acutely.[16–18] Of note, the long-term therapy with anti-LTs has also been demonstrated to protect against AMP induced bronchoconstriction,[19,20] although the ability of these drugs to affect AMP response after acute treatment is not well established.[21,22]

Aim of this study was to compare the effects of an acute treatment with an ICS, beclomethasone diproprionate, and an anti-cysLTs, montelukast, alone or in combination, on the bronchoconstriction induced by AMP and methacholine.

Considering that beclomethasone diproprionate (BDP) and montelukast (ML) act on distinct inflammatory pathways we hypothesized that the contemporaneous acute treatment with these two drugs may provide an additive anti-inflammatory effect with a greater protection against AMP induced bronchoconstriction compared to the effect of either drug alone.

Methods

Subjects

Fifteen non-smoking subjects 18–34 yr of age (9 male, 6 female) (Table 1) took part in the study. Each had mild persistent asthma, according to the GINA (Global Initiative for Asthma) guidelines,[1] with FEV1 $\geq$ 80% of predicted. All patients demonstrated a positive skin test in response to common airborne allergens (*dermatophagoides pteronissinus, dermatophagoides farinae, wall pellitory grass, mixed grass pollens, cat fur*), and a documented sensitivity to methacholine and AMP during the previous 4 weeks. Each subject had infrequent symptoms controlled with occasional inhaled short acting inhaled β2-agonists alone and each had not used ICS, oral corticosteroids, theophylline, antihistamines, sodium cromoglycate, CysLTR1 receptor-antagonist, inhaled long acting bronchodilatators within the preceding 8 weeks. None had an exacerbation of asthma or a respiratory tract infection during the preceding 8 weeks. Throughout the study, only short acting inhaled beta2-adrenoreceptor agonists were allowed, but were withheld for at least 8 h prior to each visit to the laboratory. All visits to the laboratory were carried out at the same time of day, in order to avoid change in the response due to diurnal variation, and outside the pollen season. The study was approved by the local Ethic Committee and all subjects gave their written informed consent.

Bronchial provocation test

Changes in airway calibre were measured indirectly using FEV1 with a Turbin Spirometer (μkit, COSMED, Roma, Italy) and the better of two consecutive measurements was used for analysis. AHR was evaluated by means of methacholine and AMP bronchial challenge performed according to recommended guidelines and as previously described.[23–25] In brief, methacholine (Sigma Chemical Co., St Louis, Missouri, USA) and adenosine 5′-monophosphate (Sigma) were made up in phosphate-buffered saline (PBS) and 0.9% sodium chloride to produce a range of increasing doubling concentrations of 0.03–16.00 mg/ml and 3.125–800 mg/ml, respectively. The aqueous solutions were administered as aerosols, generated from a starting volume of 3 ml, in a disposable Inspiron Mininebulizer (C.R. Bard International, Sunderland, UK) driven by compressed air at 8 l/min. Patients inhaled increasing doubling concentrations of agonist in five breaths from functional residual capacity to total lung capacity through a mouthpiece, and FEV1 was measured at 1 and 3 min after each administration. The challenges were stopped when a decrease of 20% in FEV1 had been achieved or when the maximum concentration of agonist had been inhaled. The bronchial responses to the inhaled agonists were expressed as the provocative

Table 1 Individual patient characteristics.

Subjects	Sex	Age (years)	Baseline FEV1% pred	PC_{20} 5-AMP baseline (mg/ml)	PC_{20} Methacholine baseline (mg/ml)	Atopy
1	M	21	95	14.19	0.15	D[a]
2	M	29	96	2.73	0.16	W—D
3	F	18	101	19.30	0.34	W—D
4	M	29	84	5.03	0.28	W—D
5	F	18	92	19.12	0.24	W—D
6	F	32	80	11.24	0.36	D
7	F	28	96	21.90	0.70	W—D
8	M	22	80	20.06	0.90	W[b]
9	F	26	91	8.21	0.70	W
10	M	29	122	29.40	0.17	W
11	M	22	81	21.90	0.79	W—D
12	F	34	83	15.02	0.23	D
13	M	28	90	27.66	0.21	W
14	M	30	85	25.76	0.26	W—D
15	M	20	86	41.68	0.33	W—D
Mean ± SE		25.73 ± 1.33	90.8 ± 2.80	18.88 ± 2.61	0.39 ± 0.06	

[a] Dermatophagoides.
[b] Wall pellitory grass.

concentration causing a 20% decrease in FEV1 (PC_{20}) value, which was calculated by means of linear interpolation from the concentration—response curve constructed on a logarithmic scale.

Study design

The study consisted of 5 visits to the clinic. On the screening visit (visit 1) the following were assessed: eligibility, demographic data, medical history, medications and concentration—response studies with inhaled methacholine followed, 3 h apart, by an AMP challenge in the absence of any drug treatment. On this occasion, as well as in the other study days, the 3 h interval between the two challenges warranted a complete recovery of FEV_1 to baseline after the methacholine challenge.

Then, patients attended the laboratory on four separate occasions, at least 7 days apart (visits 2—5) to undertake concentration—response studies with inhaled methacholine and AMP after receiving BDP or ML, alone or in combination, in a randomized, double-blind, double-dummy placebo-controlled, crossover design. Patients were randomized using an appropriate table of random numbers to receive all possible combinations of drug administration (oral ML plus inhaled BDP, oral ML plus inhaled placebo, oral placebo plus inhaled BDP, oral placebo plus inhaled placebo). Montelukast and its matched placebo were kindly supplied by Merck Sharp & Dohme (Rome, West Point, PA, USA). BDP and its matched placebo were kindly supplied by Chiesi Farmaceutici (Parma, Italy). In order to ensure an adequate drug activity, the timing of both drug administrations was established according to previous observations.[16,26—28] Particularly, it has been shown that ML achieves the plasmatic peak approximately 3 h after administration[27] while a single dose of ICS was shown to protect against bronchial provocation tests from 10 min after inhalation.[26] Oral ML (one tablets of 10 mg) or its matched placebo was administered 3 h prior to the challenge with methacholine. Inhaled BDP (20 μg) or its matched placebo were administered 30 min prior to the challenge with methacholine and 30 minutes prior to the challenge with AMP.

For those patients who did not demonstrate a 20% decrease in FEV1 after inhalation of the last concentration of either spasmogen solution, the log PC_{20} was taken as the highest concentration (1600 mg/mL for methacholine, 800 mg/mL for AMP) and included in the analysis as extrapolated data.

Data analyses

Results are expressed as mean ± SE unless otherwise specified and $p < 0.05$ was accepted as the minimum level of statistical significance. Pre and post-treatment baseline values of FEV_1 prior to bronchial challenge were compared between and within study days by two-way analysis of variance (ANOVA). Values of methacholine and AMP PC_{20} following treatment with each combinations of drugs were logarithmically transformed to normalize their distribution and compared by one-way analysis of variance (ANOVA) followed by Neumann—Keuls test, for specific means comparisons, where appropriate.

The protective effect of each drug treatment on responses to provocation of each challenge was also calculated by measuring the change in log PC_{20} from the baseline after all active and placebo treatments in each subject and expressed in terms of doubling doses using the formula:

$$(\log_{10} PC_{20} \text{ active treatment} - \log_{10} PC_{20} \text{ placebo}) \div \log_{10} 2$$

The lower is the value of the doubling dose the higher is the bronchial responsiveness to each spasmogen. One-way analysis of variance (ANOVA) followed by Neumann—Keuls test was used to compare post-drug variations in BHR to methacholine and AMP.

Results

There was no significant difference in baseline values of FEV_1 between any of the study days, and, in each study day, between baseline FEV_1 values before methacholine and AMP challenges.

Administration of each drug (placebo, BDP or ML) did not cause any significant change in FEV_1 from baseline. Inhaled methacholine and AMP in the absence of any drug treatment produced a concentration-related bronchospasm with a geometric mean (M) PC_{20} values of 0.39 ± 0.06 mg/mL and 18.88 ± 2.61 mg/mL, respectively.

Changes of methacholine responsiveness after drug treatment

Placebo administration did not produce any significant change in methacholine responsiveness in comparison to baseline value. BDP or ML alone did not have a significant protective effect against methacholine challenge; pretreatment with BDP plus ML significantly increased methacholine PC_{20} value to 0.93 ± 0.40 mg/mL comparing to baseline and placebo ($p < 0.01$ and $p < 0.05$ respectively) (Fig. 1).

Then, we expressed the protective effect of each treatment on methacholine-induced bronchoconstriction as doubling dose. A mean protection of 1.11 ± 0.21, 0.28 ± 0.17 and 1.70 ± 0.34 doubling doses for BDP, ML and combined BDP plus ML were reported, respectively. Only the protective effect of combined treatment (BDP + ML) as compared to placebo achieved significance ($p < 0.001$).

Changes of AMP responsiveness after drug treatment

BDP pretreatment significantly ($p < 0.01$) increased the AMP PC_{20} value (58.34 ± 15.90 mg/mL) as compared to placebo (PC_{20} value 22.87 ± 5.70). ML also produced an increase in the AMP PC_{20}, but it did not reach a statistical significance. Combined treatment, BDP plus ML, afforded a significant increase of AMP PC_{20} (PC_{20} value 154.57 ± 55.0 mg/mL) as compared to baseline and each single treatment (Figs. 2 and 3).

Then, changes in the protective effect of each drug treatment on AMP induced bronchoconstriction were expressed as doubling dose. BDP significantly reduced BHR to AMP by 1.75 ± 0.22 doubling doses as compared to placebo. Combined treatment with BDP plus ML significantly reduced airway responsiveness to AMP as compared to the placebo and each single active treatment (Fig. 4).

Discussion

Bronchial hyperresponsiveness measured by bronchial challenge with direct and indirect agonists is a characteristic feature of bronchial asthma. The direct bronchoconstrictors act on airway smooth muscle receptors (e.g., acetylcholine and muscarinic analogues on muscarinic receptors, histamine on H1 receptors) to induce bronchoconstriction. On the other hand, indirect stimuli such as AMP and mannitol are intrinsically dependent on primary airway inflammation to mediate their downstream constrictor effects and are thought to represent a more physiological model of asthmatic airway narrowing than their directly irritant counterparts of methacholine and histamine challenge.[13]

The present study demonstrates that, in asthmatic patients, acute combined treatment with ICS and anti-leukotrienes provides greater protection against induced bronchoconstriction compared to the effect of either drug alone. The protection afforded by combined treatment was greater against bronchoconstriction induced by AMP than methacholine. Because AMP produces bronchoconstriction through the release of inflammatory mediators from mast cells, this suggests that acute combined treatment, acting

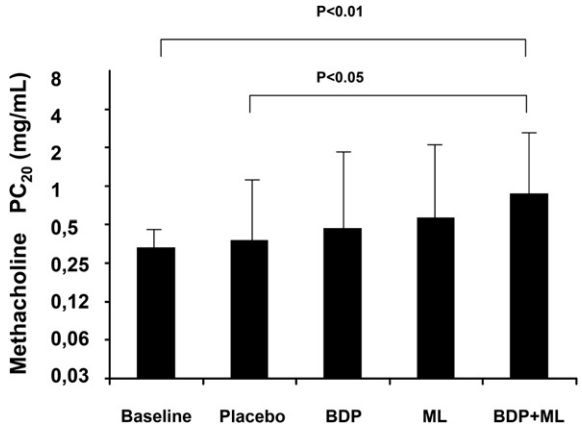

Figure 1 Effect of placebo (P), beclomethasone (BDP) and montelukast (ML), alone and associated, on methacholine-induced bronchoconstriction in asthmatic subjects. The PC_{20} values, after a logarithmic conversion, are expressed as a geometric mean ± SE.

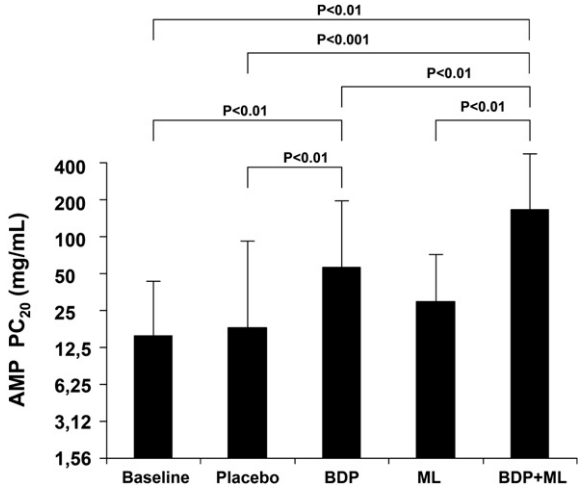

Figure 2 Effect of placebo (P), beclomethasone (BDP) and montelukast (ML), alone and associated, on 5′-AMP-induced bronchoconstriction in asthmatic subjects. The PC_{20} values, after a logarithmic conversion, are expressed as a geometric mean ± SE.

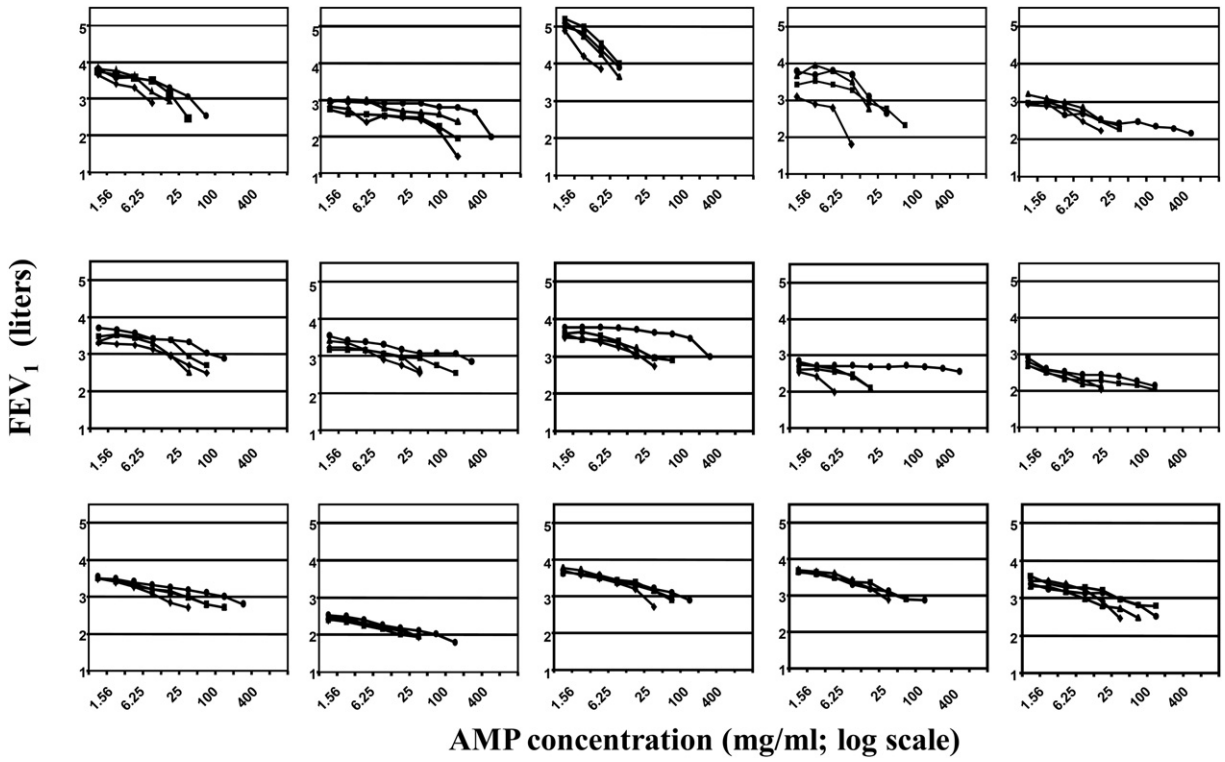

Figure 3 Effect of placebo (◆), BDP (■), ML (▲) and BDP plus ML (●) on the concentration—response curves to inhaled 5-AMP in 15 subjects with asthma.

in an independent fashion, confers additive anti-inflammatory benefits as compared to the single drug treatment.

Several lines of evidence suggest that endogenous adenosine contributes to the pathophysiology of asthma,[8] primarily through the stimulation of adenosine A2B receptors on "primed" mast cells[29] so inducing the generation and the release of inflammatory mediators such as histamine, lypoxygenase products, tryptase, interleukins and other cytokines. Inhalation of exogenous AMP is able to induce a rapid inflammatory response with increase of eosinophils and cys-LTs in the airway of asthmatic subjects.[30,31] AMP induced bronchoconstrictive effects are proportional to the degree of inflammatory cell airway infiltration, and AMP challenge, better than other stimuli such as methacholine, has been suggested as a highly sensitive marker of disease activity for monitoring asthma control and to identify success of treatment.[11,12]

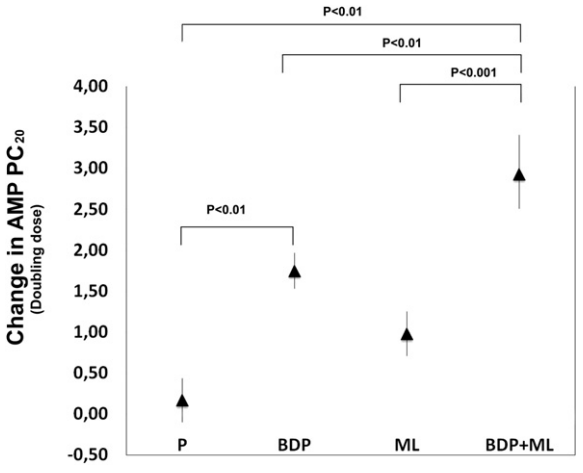

Figure 4 Doubling dose difference from baseline values for each treatment: placebo (P), beclomethasone (BDP) and montelukast (ML), alone and associated. Combined treatment caused a significant double dose difference as compared to each single active treatment. Results are expressed as mean ± SE.

ICS and antileukotrienes are used as maintenance therapy in persistent bronchial asthma,[1] and it has been suggested that these drugs may act on the different steps of the inflammatory cascade. It has also been observed that the addition of antileukotrienes to inhaled corticosteroids is able to induce a reduction of surrogate airway inflammatory markers,[32] including exhaled nitric oxide and blood eosinophils, and better asthma control.[33]

A number of studies have demonstrated that long-term therapy with ICS and antileukotrienes either alone or in combination protects against AMP induced bronchoconstriction, mainly reducing airway inflammatory cells, including mast cells and eosinophils.[14,15,19,20,34,35] However, the effect of acute treatment of both drugs on AMP induced bronchoconstriction has not been fully investigated. In a limited number of previous studies, ICS acute treatment demonstrated a short-term protective effect against AMP bronchial hyperresponsiveness.[16—18] The protective capacity of a single dose of inhaled corticosteroid against AMP induced bronchoconstriction was also

observed in our asthmatic patients. Of interest, in our study the acute protective effect of a single glucocorticoid inhalation was obtained by using a lower dose of ICS as compared to those used in most of previous studies. These findings underline the high sensitivity to the effect of ICS of the airway response to inhaled AMP that has been recently pointed out.[11]

As regards to the protective efficacy of acute treatment with antileukotrienes against AMP induced bronchocostriction in asthmatic patients, previous reports suggested that montelukast possesses a protective effect.[21,22] In a recent study, a two-day course of therapy with oral montelukast produced a small but significant protection against AMP induced bronchoconstriction,[21] whereas in another study the administration of a single dose of oral montelukast, although it did not produce significant improvement in airway sensitivity to AMP, induced a significantly more rapid recovery of FEV_1 after the AMP challenge.[22] In the present study, a single dose of montelukast exerts a small protective effect against AMP induced bronchoconstriction, but it did not reach statistic significance. However, interestingly, the addition of montelukast to a single dose of inhaled BDP produced a protective effect on AMP induced bronchoconstriction significantly higher as compared to single treatment with BDP. Thus, our results support the hypothesis that contemporaneous administration of ICS and antileukotrienes act acutely on different inflammatory pathways within the inflammatory cascade that is activated during AMP induced bronchospasm.

In our study, acute combined treatment also provides protection against bronchoconstriction induced by methacholine, while each single active treatment (BDP or montelukast) did not. However, the protection afforded by combined treatment (BDP plus montelukast) against methacholine was less relevant than that observed against AMP. This might be strictly correlated to the different mechanisms of bronchoconstriction elicited by methacholine in respect of those by AMP. Methacholine acts directly on acetylcholine receptors on smooth muscle causing contraction and airway narrowing. As a consequence, BHR to methacholine in asthmatic patients correlates mainly to structural changes following airway remodelling due to increased airway smooth muscle contractile properties, sub-epithelial reticular basement thickness and, only moderately, with airway inflammation.[13] Following this, in asthmatic patients hyperresponsiveness to methacholine has been found to be mainly related to FEV_1, while the level of AMP PC_{20} was predominantly predicted by the percentage of sputum eosinophils.[9] In the present study, we demonstrated that the combined anti-inflammatory protection afforded by contemporaneous acute treatment with BDP and ML had a greater effect on responsiveness to AMP than methacholine. Our findings well correspond to previous studies demonstrating that indirect airway responsiveness to AMP is more closely linked with airway inflammation than does direct airway methacholine responsiveness.[9]

Acute anti-inflammatory effects of ICS have been reported in previous studies.[16–18,36–38] A single dose of ICS showed to protect against bronchoconstriction induced by indirect stimuli such as AMP,[16–18] hypertonic solution[36] and exercise[38] and inhibited nasal output of IL-5 and GM-CSF following nasal allergen challenge.[37] This rapid onset of the action of ICS is thought to be mainly related to different mechanisms than the conventional activation of nuclear glucocorticoid receptors. These nongenomic effects occurring acutely (within minutes) may include airway vascular smooth muscle contraction, modulation of secretory response of airway epithelium, and inhibition of mast cell activation. Previous studies demonstrated that ICS produce a transient decrease of airway blood flow and that ICS induced acute vasoconstriction might have potentially beneficial effects in asthma and could be considered as an anti-inflammatory effect of ICS.[2,39] Another possibility is that ICS may acutely modulate the fluid balance in the airway wall acting on the secretory response of the airway epithelium.[40] Finally, a recent study[26] demonstrated that a single dose of BDP had a rapid effect on reducing the airway reactivity to hyperpnea and the urinary excretion of LTE4 and the bronchoconstrictive mediators 9α, 11β-PGF (considered a sensitive marker of mast cell activation). These findings suggest that ICS may acutely regulate mast cell release of mediators, probably through a reduction in intracellular calcium.[41]

According to this data, it is conceivable that the acute ICS protective effect on induced bronchoconstriction which we observed in our patients 30 min after BDP inhalation was mainly related to the nongenomic anti-inflammatory effects of BDP. As regards to the protective effect observed on AMP BHR, it cannot be excluded that the administration of BDP before methacholine challenge could have produced a genomic anti-inflammatory effect contributing to the protective effect observed at the time of AMP challenge, 3 h later. However, considering the low dose of inhaled steroid used, it can be surmised that the observed protective effect on AMP challenge is mainly related to the acute nongenomic anti-inflammatory effects of BDP inhaled 30 min prior to the AMP challenge.

In patients with asthma the CysLTs are known to be either potent inducers of bronchoconstriction and mediators of airway inflammation.[4,5] Leukotriene receptor-antagonists attenuate the proinflammatory effects of leukotrienes, such as increased microvascular permeability, eosinophil chemotaxis, mucus secretion, as well as blocking leukotriene-induced smooth muscle constriction and proliferation.[42] It is also well recognized that leukotriene synthesis and action is relatively resistant to the glucocorticoid activity.[6,7,43] Mechanisms by which antileukotriene adding exerts an acute additional protective effect on AMP induced bronchoconstriction as compared to BDP treatment may be mainly related to the lack of effectiveness of corticosteroid on leukotrienes pathway. The early generation and the release of lypoxygenase inflammatory products occurring soon after AMP challenge,[31] and the following bronchoconstrictor response could be poorly controlled by corticosteroid treatment while it is effectively counteracted by previous administration of a specific CysLTR1 receptor-antagonist, such as montelukast which rapidly attenuates the proinflammatory effects of leukotreines, as well as blocks leukotriene-induced smooth cell muscle constriction. These findings are consistent with previous clinical studies where acute or regular treatment with montelukast conferred additive beneficial effects on AMP BHR in patients who were suboptimally controlled with ICS monotherapy.[34,44]

Of interest, results from this study support previous clinical reports showing that adding montelukast to steroids in acute treatment of asthma exacerbation produces additive clinical benefits and improvement in lung function.[45–47]

In conclusion, although it is not possible to provide a conclusive explanation for the mechanism by which ICS and antileukotrienes exert an additional protective effect on AMP induced bronchoconstriction, a selective and complementary therapeutic activity of these two kinds of drugs occurs. Considering the importance of AMP as a local mediator involved in airway pathophysiology of bronchial asthma, we believe that these findings may have clinical relevance, suggesting that ICS and antileukotrienes exert their activities in airways acting on distinct inflammatory pathways and their acute combined use might provide greater protection against inflammatory responses and additional asthma control than either drug alone.

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Conflict of interest

The authors have no conflict of interest.

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