

The effects of inhaled budesonide and formoterol in combination and alone when given directly after allergen challenge

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Background: The use of combination inhaled budesonide and formoterol as maintenance and reliever therapy significantly improves the risk and the time to exacerbations in asthma.

Objectives: To explore the mechanisms underlying the effect of the reliever dose on exacerbations by examining the effect of combination therapy on the allergen challenge model when given after allergen exposure.

Methods: In a randomized, double-blind crossover study, single doses of budesonide/formoterol (400/12 µg), formoterol (12 µg), budesonide (400 µg), or placebo were administered during the acute bronchoconstriction response (early airway response) immediately after allergen inhalation in 15 patients with mild asthma. Allergen-induced late airway response (LAR), sputum inflammatory markers, airway hyperresponsiveness, and exhaled nitric oxide were measured.

Results: All active treatments significantly attenuated the LAR, with budesonide/formoterol significantly better than its monocomponents (maximum FEV₁ fall: placebo, [mean ± SEM] 21.2% ± 3.1%; budesonide/formoterol, 4.2% ± 1.4%; formoterol, 7.5% ± 1.7%; budesonide, 10.4% ± 1.6%).

Allergen-induced change in methacholine PC₂₀ was significantly attenuated by budesonide/formoterol, but not by its monocomponents. Sputum cell counts and exhaled nitric oxide increased significantly after all allergen challenges, with no significant attenuation by any of the treatments. Therapy

with combination and formoterol alone, but not budesonide, significantly reduced the early airway response.

Conclusion: A single dose of budesonide/formoterol was superior to its monocomponents in attenuating the allergen-induced LAR and airway hyperresponsiveness. These effects may represent the contribution of the reliever dose to the budesonide/formoterol maintenance and reliever regimen.

Clinical implications: The protective effect against allergic airway responses with a single reliever dose of budesonide/formoterol is predominantly related to greater functional antagonism of airway smooth muscles. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Budesonide/formoterol, allergic asthma, reliever combination therapy use

Maintenance therapy with long-acting β_2 -agonists (LABAs) in combination with inhaled corticosteroids (ICSs) and short-acting β_2 -agonists for as-needed therapy is currently the recommended treatment for moderate-to-severe persistent asthma. Recent evidence, however, has supported a simpler alternative strategy with combination inhaled budesonide and formoterol in a single inhaler as maintenance and reliever therapy. Compared with terbutaline rescue use with regular high-dose ICS or combination therapy, this regimen was superior in reducing the risk and prolonged the time to exacerbations.¹⁻³ This effect was attributed to the early and timely increase in the ICS, which accompanies the increase in reliever medication use during periods of poor asthma control. Furthermore, the combination may have an additive effect on airway function and inflammation that would otherwise not be achieved with either drug alone. To date, little is known of the effects of this combination or its monocomponents on acute airway inflammation and airway function when given as reliever therapy during periods of uncontrolled asthma.

In the current study, the allergen inhalation challenge model was used to investigate the effects of combination budesonide/formoterol (in a single inhaler) and its monocomponents, as reliever therapies, on the allergic airway responses. The aim was to explore the mechanisms by which reliever doses of combination budesonide/formoterol might contribute to the management of uncontrolled asthma and exacerbations. Therefore, in a randomized, double-blind, placebo-controlled crossover study, the

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TABLE I. Subject characteristics at screening

Patient	Age (y)	Sex	FEV ₁ (% predicted)	Methacholine PC ₂₀ (mg/mL)	Inhaled allergen	Allergen dilution
1	23	F	84	14.2	HDM	1:128
2	22	F	117	6.43	HDM	1:256
3	21	F	100	0.52	HDM	1:1024
4	21	F	98	4.22	Ragweed	1:512
5	42	M	96	12.1	HDM	1:128
6	18	F	96	11.6	Grass	1:1024
7	26	F	95	1.52	HDM	1:256
8	20	M	80	1.07	HDM	1:512
9	25	F	96	0.14	HDM	1:2048
10	21	F	90	3.03	HDM	1:512
11	34	M	78	3.48	Grass	1:256
12	24	F	107	3.32	HDM	1:512
13	21	F	103	1.59	HDM	1:512
14	51	F	83	3.82	HDM	1:512
15	20	F	89	2.27	Ragweed	1:256

F, Female; HDM, house dust mite; M, male.

Abbreviations used

AHR: Airway hyperresponsiveness
 DD: Doubling dose
 EAR: Early airway response
 ECP: Eosinophil cationic protein
 eNO: Exhaled nitric oxide
 ICS: Inhaled corticosteroid
 LABA: Long-acting β_2 -agonist
 LAR: Late airway response
 MCC: Metachromatic cell
 TCC: Total cell count

effects of single doses of budesonide/formoterol, formoterol, and budesonide administered directly after allergen inhalation on allergen-induced airway responses and airway inflammation were examined.

METHODS

Sixteen atopic adults (≥ 18 years) with stable mild asthma and FEV₁ $\geq 80\%$ predicted were recruited. All were nonsmokers, taking only short-acting β_2 -agonists as needed, and demonstrated a methacholine PC₂₀ of < 16 mg/mL. Subjects also demonstrated a maximum fall in FEV₁ $\geq 20\%$ to inhaled allergen within 0 to 3 hours (early airway response; EAR) and $\geq 15\%$ between 3 and 7 hours (late airway response; LAR) at screening challenge. The same dose of allergen causing the dual response was subsequently used for all allergen challenges. There were 4 randomized, double-blind crossover treatment periods, each composed of 3 consecutive visits separated by 2 to 6 weeks of washout. The latter was to allow methacholine PC₂₀ to return within 1 doubling dose (DD) of screening baseline PC₂₀ before crossing over. Exhaled nitric oxide (eNO) measurements, methacholine test, and sputum induction were performed on days 1 and 3. Allergen challenge was performed on day 2 with eNO and sputum induction repeated at 7 hours postallergen. Single doses of budesonide/formoterol (400/12 μ g), formoterol (12 μ g), budesonide (400 μ g), or placebo were administered during the EAR via identical Turbuhalers

(AstraZeneca, Sweden). The study was approved by McMaster Ethics Board, and informed written consent was obtained.

Spirometry was performed with a Collins water-sealed spirometer (Collins, Braintree, Mass).⁴ Methacholine test was performed with doubling concentrations of methacholine inhaled at tidal breathing for 2 minutes.⁵ The PC₂₀ was calculated from the dose-response curve by linear interpolation on a log scale.

Sputum induction and processing was performed as previously described.⁶ Total cell count was determined by using a Neubauer hemocytometer chamber (Hausser Scientific, Blue Bell, Pa). Differential cells were manually counted out of 400 nonsquamous cells and metachromatic cells out of 1500 cells. Eosinophil cationic protein (ECP) and tryptase levels were measured by UniCAP fluorescence immunoassay (Pharmacia Diagnostics, Uppsala, Sweden; detection limits ≥ 2.0 and ≥ 1.0 μ g/mL, respectively); and fibrinogen by ELISA technique using polyclonal rabbit antihuman antibodies (Dako Canada Inc, Mississauga, Ontario, Canada; detection limit > 194 ng/mL).

Single-flow eNO was measured (Aerocrine, Chicago, Ill) at a flow rate of 0.05 L/s as previously described.⁷

Modified skin prick test was used to identify the administered allergen⁸ and the concentration that caused a 2-mm wheal. The latter, together with screening methacholine PC₂₀, were used to calculate the PC₂₀-allergen.⁹ The starting dose of allergen was 3 DD below the estimated PC₂₀-allergen delivered by a Wright nebulizer (Roxon Medi-Tech, Ltd., Montreal, Quebec, Canada) for 2 minutes at tidal breathing, followed by FEV₁ at 10-minute intervals until the lowest FEV₁ was established.¹⁰ If %FEV₁ fall was $< 20\%$, the next concentration was given, until FEV₁ fell $\geq 20\%$. Then FEV₁ was measured at 10, 20, 30, 45, and 60 minutes, then hourly to 7 hours. The EAR was the maximum %FEV₁ fall between 0 and 3 hours and the LAR between 3 and 7 hours postallergen inhalation. The area under the curve was calculated by using the trapezoidal rule and expressed as % change $\times$ minutes.

Analysis

Sputum variables and eNO were log-transformed and methacholine PC₂₀ expressed in DD change ($\log_2 \text{PC}_{20}[\text{preallergen}] - \log_2 \text{PC}_{20}[\text{postallergen}]$). Changes from baseline were compared within and between treatments using repeated-measures ANOVA with subjects, treatments, time points, and randomization sequence as factors in the general linear model. A *P* value (2-sided) ≤ 0.05

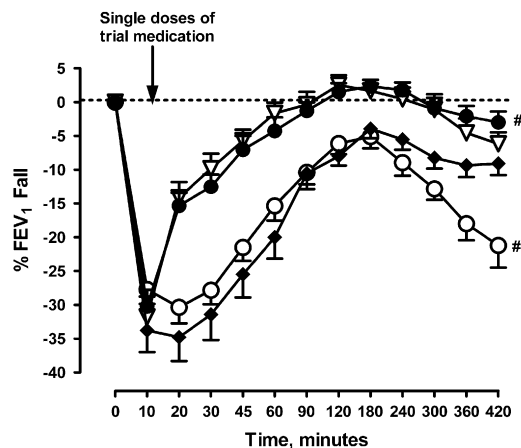
TABLE II. Spirometry and methacholine values for each period*

	Time	Placebo	Budesonide/formoterol	Budesonide	Formoterol
FEV ₁ (%)	Baseline	92.8 ± 2.2	91.5 ± 2.6	92.5 ± 3.1	92.5 ± 2.8
	Preallergen	93.6 ± 2.3	93.1 ± 2.9	93.4 ± 2.6	92.3 ± 2.8
	Postallergen‡	86.3 ± 2.6	91.3 ± 3.1	89.4 ± 3.8	89.2 ± 3.2
FEV ₁ /vital capacity (% predicted)	Baseline	80.6 ± 1.8	79.5 ± 1.5	80.3 ± 1.9	79.8 ± 1.8
	Preallergen	80.9 ± 1.6	80.2 ± 1.9	80.6 ± 1.9	80.5 ± 1.7
	Postallergen‡	77.3 ± 1.7	80.7 ± 2.3	79.4 ± 2.2	80.4 ± 2.5
Methacholine PC ₂₀ (mg/mL)†	Baseline	2.6 ± 1.4	2.1 ± 1.4	2.1 ± 1.4	2.3 ± 1.3
	Postallergen	1.1 ± 1.4	1.8 ± 1.3#	1.2 ± 1.4	1.4 ± 1.3

*Data are means ± SEMs.

†Geometric mean and SEM.

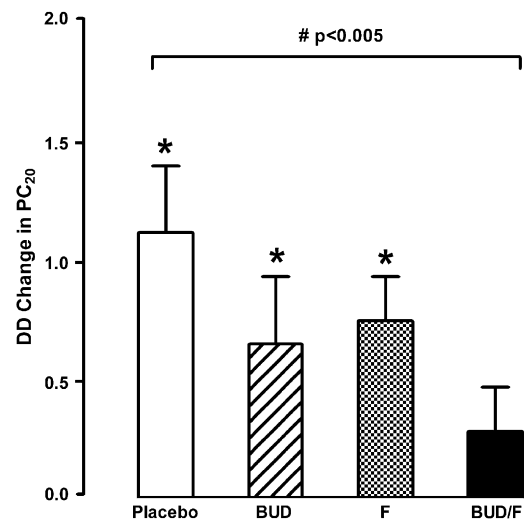
‡Before methacholine test.

#*P* < .05 vs placebo.**FIG 1.** Percent fall in FEV₁ from baseline after allergen inhalation. Data are means ± SEMs. Comparison of the LAR between treatments was significant (#) at *P* < .05. ○, Placebo; ▽, formoterol; ♦, budesonide; ●, budesonide/formoterol.

was accepted as significant. All analyses were performed by using SPSS 12.0 (SPSS, Inc, Chicago, Ill).

RESULTS

One subject was withdrawn before randomization because of an asthma exacerbation. Screening baseline characteristics for the 15 completed subjects are presented in Table I. Baseline values for the 4 randomized periods were not significantly different, and no carryover effect was detected. Furthermore, no significant difference in FEV₁ and FEV₁/vital capacity was seen on days 1 to 3 between the periods (Table II). Serial FEV₁ measurements after each allergen challenge (and treatment) up to 7 hours are shown in Fig 1. As expected, the EAR was not significantly different between treatments (placebo, -31.3% ± 2.1%; budesonide/formoterol, -29.5% ± 2.4%; budesonide, -36.4% ± 3.1%; and formoterol, -31.2% ± 2.9%). However, the area under the curve for the fall in FEV₁ between 0 and 3 hours was significantly different for the formoterol (758 ± 144) and budesonide/formoterol (834 ± 156) treatments compared to placebo (2261 ± 218) and

**FIG 2.** Changes in methacholine PC₂₀ at 24 hours postallergen inhalation. Data are means ± SEMs. BUD, Budesonide; F, formoterol. Comparison of DD change from baseline within (*) and between (#) treatments at *P* < .05.

budesonide (2576 ± 328) (budesonide/formoterol or formoterol vs budesonide or placebo, *P* < .05).

All active treatments significantly attenuated the LAR (placebo, -21.2% ± 3.1%; budesonide/formoterol, -4.2% ± 1.4%; budesonide, -10.4% ± 1.6%; formoterol, -7.5% ± 1.7%; placebo vs each active treatment, *P* < .05), with budesonide/formoterol significantly better than its monocomponents (budesonide/formoterol vs formoterol, *P* < .05; and vs budesonide, *P* < .005; Fig 1). Failure to abolish the LAR (>15% fall in FEV₁) was seen in 1, 2, and 4 subjects after budesonide/formoterol, formoterol, and budesonide, respectively.

Allergen-induced changes in methacholine PC₂₀ were significant for placebo (1.2 ± 0.3 DD), formoterol (0.8 ± 0.2 DD), and budesonide (0.7 ± 0.3 DD; compared with baseline, *P* < .05), but not for budesonide/formoterol (0.3 ± 0.2 DD; Fig 2). The change in methacholine PC₂₀ was significantly different for budesonide/formoterol compared with placebo (*P* < .005).

TABLE III. Sputum counts at baseline and postallergen inhalation†

	Time (h)	Placebo	Budesonide/formoterol	Budesonide	Formoterol
TCC (10^6 cells/mL)	Baseline	1.2 ± 0.3	0.8 ± 0.2	0.8 ± 0.1	1.4 ± 0.3
	7*	1.8 ± 0.5	1.1 ± 0.2	1.5 ± 0.3	2.1 ± 0.5
	24*	1.4 ± 0.4	1.3 ± 0.2	1.1 ± 0.3	1.8 ± 0.4
Eosinophils (%)	Baseline	1.9 ± 0.4	2.8 ± 1.2	2.1 ± 0.7	1.9 ± 0.9
	7*	9.7 ± 3.6	14.0 ± 4.8	11.9 ± 3.9	11.7 ± 4.9
	24*	11.5 ± 5.6	15.4 ± 5.6	9.9 ± 3.1	14.6 ± 5.3
Metachromatics (%)	Baseline	0.07 ± 0.02	0.09 ± 0.04	0.04 ± 0.007	0.03 ± 0.008
	7*	0.11 ± 0.03	0.25 ± 0.08	0.08 ± 0.03	0.09 ± 0.02
	24	0.06 ± 0.02	0.20 ± 0.08	0.09 ± 0.03	$0.15 \pm 0.06\#$
Macrophages (%)	Baseline	50.7 ± 7.8	52.6 ± 8.4	51.5 ± 7.2	38.9 ± 7.2
	7*	25.4 ± 5.6	32.2 ± 6.5	29.2 ± 5.1	20.4 ± 5.2
	24*	30.5 ± 5.7	34.4 ± 6.4	32.9 ± 6.8	32.8 ± 5.8

Changes from baseline within (*) and between (# to placebo) treatments was significant at $P < .05$.

†Data are means $\pm$ SEMs.

Allergen inhalation induced a significant increase in the total cell counts (TCCs), eosinophil %, and metachromatic cells (MCCs) % at 7 hours after all treatments, which remained significantly elevated for TCC and eosinophils at 24 hours (Table III). In contrast, the MCC% change from baseline at 24 hours was not consistently elevated for all treatments. A significant reduction in the macrophage % and no significant change for neutrophils and lymphocytes were seen. Compared with placebo, only the change in MCC% at 24 hours for formoterol was significant. Analysis of the absolute cell counts demonstrated similar results.

All subjects had measurable sputum ECP levels. Undetectable baseline levels of sputum fibrinogen and tryptase were found in 1 and 5 subjects, respectively. For the purpose of the analysis, these were replaced with the lower limit of detection values for the respective diagnostic kits (ie, 194 ng/mL and 1.0 ug/mL, respectively). Significant increases in sputum ECP levels were observed at 7 hours and 24 hours postallergen for all treatments. Fibrinogen levels were significantly elevated from baseline only at 7 hours but not at 24 hours, and no significant change from baseline was seen for sputum tryptase levels for all treatments. Significant changes from baseline in eNO at 7 hours and 24 hours were seen for all treatments. There was no significant difference between treatments for any of these changes (Fig 3, A-D).

DISCUSSION

This study demonstrated that single doses of budesonide/formoterol, formoterol, and budesonide administered after allergen inhalation significantly attenuated the LAR. Budesonide/formoterol was superior to its monocomponents in this regard and provided protection against allergen-induced airway hyperresponsiveness (AHR), whereas treatment with the monocomponents did not achieve this benefit. Treatments with formoterol and budesonide/formoterol were equally effective in alleviating the EAR. However, when given after inhaled allergen, none of these therapies provided any apparent

suppressive effect on allergen-induced increases in eNO levels and in the recruitment and activation of inflammatory cells.

Similar to the findings from other allergen challenge studies,¹¹⁻¹³ we found that a single dose of budesonide had no effect on the EAR but significantly attenuated the LAR, even when given after an allergen exposure. This lack of effect on the early-phase response agrees with previous data demonstrating the failure of corticosteroids to inhibit human mast cell activation and degranulation.^{14,15} These cells and their mediators are responsible for the allergic early bronchoconstrictive response and are implicated in the processes leading to the late-phase inflammatory response and AHR.¹⁶ However, pretreatment with corticosteroids significantly inhibits the production and release of T_H2 cytokines by steroid-sensitive cells with consequent reduction in the late-phase inflammatory and physiological response.¹⁷ By contrast, we found that single doses of ICS given after allergen exposure significantly attenuated the LAR, but had no discernible effect on airway inflammation. This suggests that the immunologic events important in the generation of the allergic inflammatory response occur at the time of allergen inhalation, and that inhaled anti-inflammatory therapies given after allergen exposure are unlikely to attenuate the recruitment and activation of inflammatory cells. Nonetheless, the benefit observed on the LAR could still be related to other anti-inflammatory mechanisms, such as the effects of corticosteroids on airway mucosal vasculature. These effects range from acute alteration of mucosal vascular tone to the delayed inhibition of the generation and activity of vasoactive mediators. All of these effects may contribute to a reduction in asthma-associated vascular hyperpermeability, airway edema, and airflow obstruction.¹⁸ Although the response of sputum fibrinogen in the current study was not significantly different between treatments, a trend for lower levels with budesonide therapies was noted that might lend some support to the effect of ICS on microvascular leakage and plasma exudation.

Long acting β_2 -agonists exhibit *in vitro*¹⁹ and potentially *in vivo* mast cell stabilizing effects²⁰ that may

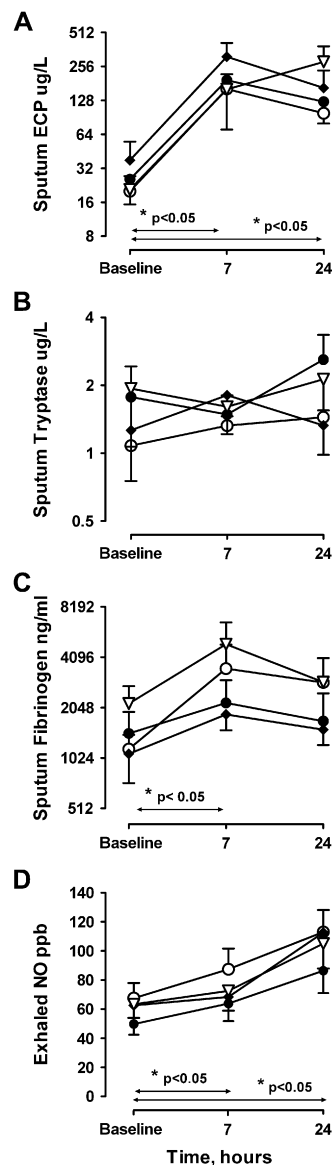


FIG 3. Changes in ECP (A), tryptase (B), fibrinogen (C), and eNO (D) levels after allergen inhalation. Data are means $\pm$ SEMs. Comparison of the changes from baseline within (*) and between treatments. Arrows indicate the effect was seen for all treatments. ○, Placebo; ▽, formoterol; ◆, budesonide; ●, budesonide/formoterol.

contribute to their effects on the EAR and LAR. Single-dose studies with LABA given before inhaled allergen have demonstrated complete inhibition of the EAR and LAR.^{12,13,21-23} When given after allergen exposure, as in the current study, allergic airway responses were significantly attenuated but not completely averted. We speculate that the effect of LABA in this case was dominated by its functional antagonism of airway smooth muscle because mast cells have already undergone activation. Furthermore, we observed no effect on the late-phase inflammatory response with formoterol therapies, consistent with the expectation that mast cell release of proinflammatory T_H2 cytokines was minimally affected. In contrast,

the bronchoprotection afforded by single doses of LABA given before allergen challenge demonstrated by previous studies suggests that there may be some contribution of an anti-inflammatory effect,^{21,23} in addition to its bronchodilator activity.

We found no difference between formoterol and budesonide on the LAR, but their combination provided a benefit on the LAR that was superior to either drug alone. This is in agreement with the one other study that has compared combination therapy to ICS alone in the allergen model. Treatment with a single dose of fluticasone/salmeterol (100/50 μ g) combination given before allergen inhalation was found to be better than fluticasone (100 μ g) alone in attenuating the EAR and LAR.²⁴ Because there was no comparative LABA arm in the aforementioned study, it was difficult to determine whether the superiority observed was a result of the LABA component only or an additive effect of the combination. Similarly, we found that neither ICS nor LABA provided protection against allergen-induced AHR, but the combination did, albeit this effect was not statistically different when compared with the monocomponents. In this regard, our study may have been underpowered to detect a difference between the active treatments,²⁴ because our sample size was based on the expected differences in sputum parameters between treatments.²⁵ However, the nonsignificant treatment effect of formoterol and budesonide alone against allergen-induced AHR was a somewhat surprising finding. The doses of formoterol (12 μ g) and budesonide (400 μ g) may have been too small in this case to be effective, particularly in the presence of established inflammation. Previous studies have used larger single doses of LABA (salmeterol 100 μ g, formoterol 24 μ g) given before inhaled allergen and have demonstrated protection against allergen-induced increase in AHR.^{12,13,23,26} This effect was not consistent at a lower dose.²⁷ Indeed, a dose-response effect has been demonstrated for the bronchodilator action of formoterol²⁸ and recently for its anti-inflammatory activity.²⁹ Likewise, higher doses of ICS are more likely to offer protection^{11,30} than lower doses^{12,24} even when given after allergen inhalation.^{31,32} However, the addition of LABA to low-dose ICS significantly improves the protection against allergen-induced AHR.²⁴ Furthermore, our findings indicate that this protection remains evident even when therapy is administered after allergen exposure. The mechanisms underlying the superior bronchoprotective effect (against LAR and AHR) are as yet unknown. At least with a single dose of budesonide/formoterol reliever therapy, it would seem that this protective effect is predominantly related to greater functional antagonism of airway smooth muscle rather than an additive anti-inflammatory effect. However, this is unlikely to be the case in the clinical setting of repeat dosing of add-on reliever therapy with budesonide/formoterol to maintenance combination treatment. In this scenario, the clinical evidence supports a greater anti-inflammatory effect derived from this therapeutic approach because better rates of improvement in asthma exacerbations have been observed.¹⁻³

As expected by the time points measured, no allergen-induced changes in sputum tryptase levels were found, consistent with the notion that mast cell activation is an acute event in the allergic response. Conversely, we detected a significant increase in MCC with formoterol therapy after allergen inhalation compared with placebo. This probably reflects a predominant change in basophils rather than mast cell numbers, because this was not accompanied by an increase in tryptase levels. Furthermore, we have previously shown that allergen-induced increase in basophil numbers greatly exceeds mast cells by the order of 20-fold to 70-fold.³³ We do not have an obvious explanation for this observation.

In conclusion, a single dose of budesonide/formoterol administered after allergen exposure was superior to its monocomponents by significantly attenuating the allergen-induced EAR and LAR and protecting against AHR. However, no apparent effects on sputum inflammatory markers were demonstrated. For future studies, the effects of repeated and higher dosing may be useful.

REFERENCES

- O'Byrne PM, Bisgaard H, Godard PP, Pistolesi M, Palmqvist M, Zhu Y, et al. Budesonide/formoterol combination therapy as both maintenance and reliever medication in asthma. *Am J Respir Crit Care Med* 2005; 171:129-36.
- Vogelmeier C, D'Urzo A, Pauwels DR, Merino JM, Jaspal M, Boutet S, et al. Budesonide/formoterol maintenance and reliever therapy: an effective asthma treatment option. *Eur Respir J* 2005;26:819-28.
- Scicchitano R, Aalbers R, Ukena D, Manjra A, Fouquert L, Centanni S, et al. Efficacy and safety of budesonide/formoterol single inhaler therapy versus a higher dose of budesonide in moderate to severe asthma. *Curr Med Res Opin* 2004;20:1403-18.
- ATS 1994 update: standardization of spirometry: the official statement of the American Thoracic Society was adopted by the ATS Board of Directors. *Am J Respir Crit Care Med* 1994;152:1107-36.
- Juniper EF, Cockcroft DW, Hargreave FE. Histamine and methacholine inhalation tests: a laboratory tidal breathing tidal protocol. Lund, Sweden: Astra Draco AB; 1994.
- Pizzichini E, Pizzichini MMM, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid phase measurements. *Am J Respir Crit Care Med* 1996;154:308-17.
- The official statement of the American Thoracic Society was adopted by the ATS Board of Directors: recommendations for standardized procedures for on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. *Am J Respir Crit Care Med* 1999;160:2104-17.
- Pepys J. Skin tests in diagnosis. In: Gell P, Coombs R, Lachmann PJ, editors. *Clinical aspects of immunology*. Oxford: Blackwell Scientific Publications; 1975. p. 55-80.
- Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;135:264-7.
- O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic response. *Am Rev Respir Dis* 1987;136:740-56.
- Kidney JC, Boulet LP, Hargreave FE, Deschesnes F, Swystun VA, O'Byrne PM, et al. Evaluation of single-dose inhaled corticosteroid activity with an allergen challenge model. *J Allergy Clin Immunol* 1997; 100:65-70.
- Pizzichini MMM, Kidney JC, Wong BJO, Morris MM, Efthimiadis A, Dolovich J, et al. Effect of salmeterol compared with beclomethasone on allergen-induced asthmatic and inflammatory responses. *Eur Respir J* 1996;9:449-55.
- Wong BJO, Dolovich J, Ramsdale EH, O'Byrne PM, Gontovnick L, Denburg JA, et al. Formoterol compared to beclomethasone and placebo on allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992;146: 1156-60.
- Cohan VL, Undem BJ, Fox CC, Adkinson NFJ, Lichtenstein LM, Schleimer RP. Dexamethasone does not inhibit the release of mediators from human mast cells residing in airway, intestine or skin. *Am Rev Respir Dis* 1989;140:951-4.
- Schleimer RP, Shulman ES, MacGlashan DWJ, Peters SP, Hayes EC, Adams GKI, et al. Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J Clin Invest* 1983;71:1830-5.
- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma from bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161:1720-45.
- Liu MC, Proud D, Lichtenstein LM, Hubbard WC, Bochner BS, Stealey BA, et al. Effects of prednisone on the cellular responses and release of cytokines and mediators after segmental allergen challenge of asthmatic subjects. *J Allergy Clin Immunol* 2001;108:29-38.
- Horvath G, Wanner A. Inhaled corticosteroids: effects on the airway vasculature in bronchial asthma. *Eur Respir J* 2006;27:172-87.
- Kay LJ, Peachell PT. Mast cell beta 2-adrenoceptors. *Chem Immunol Allergy* 2005;87:145-53.
- Nightingale JA, Rogers DF, Barnes PJ. Differential effect of formoterol on adenosine monophosphate and histamine reactivity in asthma. *Am J Respir Crit Care Med* 1999;159:1786-90.
- Dente FL, Bancalari L, Bacci E, Bartoli ML, Carnevali S, Cianchetti S, et al. Effect of a single dose of salmeterol on the increase in airway eosinophils induced by allergen challenge in asthmatic subjects. *Thorax* 1999;54:622-4.
- Palmqvist M, Balder B, Lowhagen O, Melander B, Svedmyr N, Wahlander L. Late asthmatic reaction decreased after pretreatment with salbutamol and formoterol, a new long-acting beta 2-agonist. *J Allergy Clin Immunol* 1992;89:844-9.
- Pedersen B, Dahl R, Larsen BB, Venge P. The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X. *Allergy* 1993;48:377-82.
- Koopmans JG, Lutter R, Jansen HM, van der Zee JS. Adding salmeterol to an inhaled corticosteroid reduces allergen-induced serum IL5 and peripheral blood eosinophils. *J Allergy Clin Immunol* 2005;116:1007-13.
- Gauvreau GM, Watson RM, Rerecich T, Baswick E, Inman MD, O'Byrne PM. Repeatability of allergen-induced airway inflammation. *J Allergy Clin Immunol* 1999;104:66-71.
- Brusasco V, Crimi E, Gherson G, Nardelli R, Oldani V, Francucci B, et al. Actions other than smooth muscle relaxation may play a role in the protective effects of formoterol on the allergen-induced late asthmatic reaction. *Pulm Pharmacol Ther* 2002;15:399-406.
- Weersink EJM, Aalbers R, Koeter GH, Kauffman HF, De Monchy JGR, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of salmeterol. *Am J Respir Crit Care Med* 1994;150: 1262-7.
- Lotvall J. Combination therapy in asthma: fixed or variable dosing in different patients? *Curr Med Res Opin* 2004;20:1711-27.
- Ketchell RI, Jensen MW, Spina D, O'Connor BJ. Dose-related effects of formoterol on airway responsiveness to adenosine 5'-monophosphate and histamine. *Eur Respir J* 2002;19:611-6.
- Gauvreau GM, Sulakvelidze I, Watson RM, Inman MD, Rerecich T, O'Byrne PM. Effects of once daily dosing with inhaled budesonide on airway hyperresponsiveness and airway inflammation following repeated low-dose allergen challenge in atopic asthmatics. *Clin Exp Allergy* 2000; 30:1235-43.
- Cockcroft DW, McParland CP, O'Byrne PM, Manning P, Friend JL, Rutherford BC, et al. Beclomethasone given after the early asthmatic response inhibits the late response and the increase methacholine responsiveness and cromolyn does not. *J Allergy Clin Immunol* 1993;91:163-8.
- Paggiaro PL, Dente FL, Morelli MC, Bancalari L, Di Franco A, Giannini D, et al. Postallergen inhaled budesonide reduces late asthmatic response and inhibits the associated increase of airway responsiveness to methacholine in asthmatics. *Am J Respir Crit Care Med* 1994;149:1447-51.
- Gauvreau GM, Jong ML, Watson RM, Irani AA, Schwartz LB, O'Byrne PM. Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatic. *Am J Respir Crit Care Med* 2000;161:1473-8.