Dose-related effects of formoterol on airway responsiveness to adenosine 5′-monophosphate and histamine

R.I. Ketchell, M.W. Jensen, D. Spina, B.J. O′Connor

Dose-related effects of formoterol on airway responsiveness to adenosine 5′-monophosphate and histamine. R.I. Ketchell, M.W. Jensen, D. Spina, B.J. O′Connor. ©ERS Journals Ltd 2002.
ABSTRACT: Inhaled short-acting β_2-agonists provide greater protection against airway responsiveness (AR) to the mast-cell stimulus, adenosine 5′-monophosphate (AMP), than to histamine, a direct spasmogen. Both terbutaline and albuterol exhibit this mast-cell stabilizing property in a dose-dependent manner. A single dose of the long-acting β_2-agonist formoterol has also been reported to have a mast cell-stabilizing effect, whereas salmeterol has not. To explore the dose-related actions of the long-acting β_2-agonist formoterol on AR, the authors compared the acute effects of three doses of formoterol and terbutaline on AR to AMP and histamine.

In a double-blind, randomized, placebo-controlled, cross-over study, 25 mild, steroid naive, asthmatic subjects attended on 10 occasions. At each visit, subjects inhaled either a single dose of terbutaline (500 µg), formoterol (6, 12 or 24 µg) or a matched placebo, administered *via* Turbuhaler®, 30 min prior to challenge with both AMP and histamine.

Each dose of β_2-agonist reduced AR to AMP and histamine. The bronchoprotective effects of formoterol (6 µg) and terbutaline (500 µg) were similar in magnitude in reducing AR to histamine (mean±SD: 3.6±0.3 and 3.1±0.3 doubling doses (DD)) and AR to AMP (3.5±0.5 and 3.3±0.4 DD, respectively). Overall, formoterol reduced AR to both spasmogens in a dose-dependent manner. In addition, formoterol (12 and 24 µg) provided a significantly greater protective effect against AMP than against histamine challenge. It decreased AR by 5.7±0.6 and 6.3±0.7 DD against AMP and 4.3±0.4 and 4.8±0.43 DD against histamine, respectively.

The results of this study indirectly demonstrated an *in vivo* dose-dependent mast-cell stabilizing effect of formoterol, in addition to functional antagonism on airway smooth muscle. This property of β_2-agonists may have clinical benefits in asthma management.
Eur Respir J 2002; 19: 611–616.

Dept of Respiratory Medicine and Allergy, GKT School of Medicine, King's College, London, UK.

Correspondence: R.I. Ketchell
Dept of Respiratory Medicine and Allergy
GKT School of Medicine
King's College
Bessemer Road
London
SE5 9PJ
UK
Fax: 44 2073463940
E-mail: ian.ketchell@kcl.ac.uk

The long-acting β_2-agonists formoterol and salmeterol are effective bronchodilators with a much longer duration of action than the short-acting β_2-agonists albuterol and terbutaline [1]. Their addition to low-dose inhaled glucocorticosteroids (GCS) is now recommended as an alternative to the use of high-dose inhaled corticosteroids in asthma management, especially in patients with nocturnal asthma or exercise-induced symptoms [2]. In a similar manner to short-acting β_2-agonists, a single dose of formoterol may have a greater bronchoprotective effect on the indirect spasmogen, adenosine 5′-monophosphate (AMP), than on the direct-acting spasmogen, histamine [3–5]. *In vitro* and *in vivo* evidence of mast-cell mediator release and the attenuation of the bronchoconstrictive effects of AMP by the cromones and antihistamines suggest that AMP acts primarily as a mast-cell stimulus [6–8]. This implies an *in vivo* mast-cell stabilizing effect of formoterol, in addition to functional antagonism on airway smooth muscle, an observation which, to date, has not been demonstrated with salmeterol [9, 10].

To further evaluate the *in vivo* mechanisms of formoterol, the effects of a single dose of formoterol (6, 12 and 24 µg), terbutaline 500 µg (control) and a placebo on both AMP and histamine challenges were investigated. The authors aimed to prove that formoterol produced a dose-related reduction in airway responsiveness (AR). It was hypothesized that formoterol would provide greater, dose-dependent bronchoprotection against the mast-cell stimulus AMP, than against the direct histamine stimulus.

Methods

Subjects

Twenty-five nonsmoking subjects (12 males and 13 females) aged 23–42 yrs, took part in this study. All subjects had had mild asthma (forced expiratory volume in one second (FEV_1) >70% predicted), a positive skin-prick test to common airborne allergens (*Dermatophagoides pteronyssinus*, mixed grass pollen,

or cat fur), a documented sensitivity to histamine (geometric provocative concentration causing a 20% fall in FEV_1 (PC_{20}) <2 mg·mL^{-1}) and AMP (geometric PC_{20} <25 mg·mL^{-1}) during the previous 4 weeks. No subjects had an exacerbation of asthma or respiratory infection in the preceding 6 weeks. Each subject had infrequent symptoms, controlled with occasional inhaled short-acting β_2-agonists alone. No subject was taking any regular anti-asthma therapy and none had taken inhaled cortico-steroids for at least 3 months prior to entry. Written informed consent was obtained from each subject and the study was approved by the Ethics Committee of King's College Hospital.

Study design

It was a double-blind, placebo-controlled, cross-over study and consisted of 10 treatment visits 3–7 days apart. Each subject received either terbutaline (500 μg), formoterol (6 μg), formoterol (one inhalation of 12 μg), formoterol (24 μg, two inhalations of 12 μg) or a placebo at each visit, followed by either histamine or AMP challenge. Treatments were randomized using a Latin-square design. The order of the challenge was also randomized and subjects were blinded to the spasmogen delivered. Subjects received each treatment twice, once prior to histamine, the other before AMP challenge. Following baseline FEV_1 (performed by an independent observer), subjects inhaled one actuation from each of three individual Turbuhalers® (Astra Draco AB, Lund, Sweden). At each visit, subjects were instructed in the correct use of the Turbuhaler® by an experienced Asthma Nurse Specialist to ensure they had the correct inhalation technique, flow rate and inspiratory hold time. Two inhalers were identical in appearance and contained 6-μg formoterol, 12-μg formoterol or matched placebo and the other contained 500-μg terbutaline or a matched placebo. Thirty minutes later, spirometry was repeated by a second operator, blinded to initial FEV_1, and subjects were then challenged with either histamine or AMP. Subjects refrained from using rescue medication and caffeinated beverages for ≥8 h prior to each visit and attended the laboratory at the same time each morning.

Bronchial provocation and pulmonary function measurement

Fresh solutions of histamine (Northwick Park and St Marks Hospitals, Harrow, UK) and AMP (Sigma, Poole, UK) were made up in 0.9% saline in concentrations from 0.0625–32 mg·mL^{-1} for histamine and 0.39–800 mg·mL^{-1} for AMP. Each solution was administered from a nebulizer attached to a breath-activated dosimeter (Mefar, Brescia, Italy). The nebulizer delivered particles with an aerodynamic mass median diameter of 3.5–4 μM at a mean output of 9 μL·breath^{-1}.

Pulmonary function was assessed by measurement of FEV_1 using a dry-wedge spirometer (Vitalograph,

Buckingham, UK). A standard challenge protocol was used for all provocation tests. Following a 15-min rest period, subjects performed three measurements of FEV_1, 1 min apart, the best of which was taken as baseline. Subjects then inhaled five breaths of saline as a control, with an inhalation time of 1 s and breath-hold time of 6 s. Providing FEV_1 remained within 10% of the baseline, subjects continued by inhaling five breaths of doubling concentrations of spasmogen at 3-min intervals. FEV_1 was measured at 1.5- and 2.5-min intervals following each challenge, and the highest value was recorded for analysis. Challenges were terminated when a ≥20% fall in FEV_1 from postsaline was recorded or the maximum concentration had been given. A logarithmic concentration/response curve was constructed and the PC_{20} calculated by linear interpolation. If a 20% fall in FEV_1 was not achieved after inhalation of the final concentration of spasmogen, the PC_{20} was estimated by applying the actual fall in FEV_1 to the slope of the log concentration/response curve obtained at screening and a value obtained by linear extrapolation. The assumption that this dose/response curve did not change in the presence of β_2-agonist was made.

Statistical methods

Results are presented as mean±SEM, unless otherwise stated. Serial measurements within groups (baseline FEV_1, change from baseline following treatment) were analysed by repeated-measures analysis of variance (ANOVA) and the difference between means was analysed using a paired t-test with Bonferroni's correction. PC_{20} values following inhalation of active and placebo treatments on each study day were logarithmically transformed for analysis, and the protective effect on each challenge was calculated using the formula:

$$\frac{\text{Log } PC_{20} \text{ active} - PC_{20} \text{ placebo}}{\text{Log} 2} \quad (1)$$

The results are expressed in terms of doubling dose (DD), mean±SEM.

The protective effects ($\log_{10}$ active-$\log_{10}$ placebo) of the different β_2-agonist doses were analysed using two-way factorial analysis with repeated measures. Differences between means were analysed using an unpaired t-test with a Bonferroni's correction.

FEV_1 values, following inhalation of the final four concentrations of spasmogen for each treatment (where a PC_{20} was achieved), were calculated as a percentage of the postsaline value (expressed as mean±SEM) and concentration/response curves were constructed and compared using one-way repeated ANOVA.

Using individual subject data, linear regression analysis of the log active–log placebo for each β_2-agonist dose was used to determine the dose-dependency of the bronchoprotective response. A p-value <0.05 was considered to be statistically significant.

Table 1. – Airway calibre measured as forced expiratory volume in one second at baseline and 30 min after treatment prior to challenge with histamine and adenosine 5′-monophosphate (AMP)

	Prehistamine challenge			Pre-AMP challenge		
	Before	After	% change	Before	After	% change
Placebo	3.53±0.13	3.57±0.14	1.1±1.0	3.56±0.18	3.58±0.13	0.26±0.7
Formoterol 6 μg	3.51±0.13	3.82±0.15[#]	9.0±1.2	3.47±0.11	3.74±0.13[#]	7.61±1.5
Formoterol 12 μg	3.53±0.13	3.87±0.15[#]	9.6±1.5	3.52±0.13	3.86±0.15[#]	9.66±1.5
Formoterol 24 μg	3.40±0.10	3.81±0.13[#]	12.1±1.8	3.51±0.14	3.84±0.15[#]	9.71±1.5
Terbutaline 500 μg	3.54±0.13	3.86±0.16[#]	8.9±1.5	3.56±0.14	3.88±0.15[#]	9.34±1.6

Data are presented as mean±SEM, unless otherwise stated. [#]: $p < 0.0002$ *versus* placebo.

Results

After screening, all 25 randomized subjects (mean age 26.6±0.7 yrs), completed the study. The mean screening PC_{20} of spasmogen was 0.3±1.1 mg·mL^{-1} and 3.55±1.2 mg·mL^{-1} for histamine and AMP, respectively.

Bronchodilation

Mean baseline FEV_1 was 91±2% pred, with no significant differences between visits. There was a significant increase in baseline FEV_1 30-min post-inhalation of a single dose of formoterol (6, 12 and 24 μg) and terbutaline (500 μg) relative to placebo ($p < 0.0002$), prior to histamine and AMP challenges on each study day. Although there was a trend towards a dose-dependent increase in FEV_1 following formoterol, this did not achieve significance (table 1).

Bronchoprotection against histamine and adenosine 5′-monophosphate

Following formoterol (24 μg), 11 subjects failed to achieve PC_{20} after inhalation of the final concentration of AMP (800 mg·mL^{-1}). Following 12-μg formoterol, nine subjects failed to achieve PC_{20}, whereas one and two subjects failed to achieve PC_{20} after 6-μg formoterol and 500-μg terbutaline, respectively. Following challenge with the highest concentration of histamine (32 mg·mL^{-1}), only one subject failed to achieve PC_{20} and this was after inhalation of 12-μg formoterol.

The log PC_{20} for histamine and AMP challenge following placebo and β_2-agonist are presented in table 2. Formoterol induced dose-dependent broncho-protection against both AMP (p=0.001) and histamine (p=0.033).

Analysis of the log active-log placebo data revealed that the β_2-agonist provided significant broncho-protection against spasmogen ($F_{(3,72)}=34.9$, $p < 0.01$). However, the bronchoprotection provided by the β_2-agonist was dependent upon the stimulus used, as reflected by a significant interaction ($F_{(3,72)}=4.53$, $p \leqslant 0.01$). The bronchoprotective effect of 12-μg formoterol was significantly greater against AMP (5.7±0.6 DD) than against histamine (4.3±0.4 DD) ($p < 0.01$). Bronchoprotection following 24-μg formoterol was also significantly greater against AMP (6.3±0.7 DD) than against histamine (4.8±0.43 DD) ($p < 0.01$). In contrast, there was no significant difference between the bronchoprotective effect of inhaled formoterol at a dose of 6 μg against AMP (3.5± 0.5 DD) and histamine (3.6±0.3 DD) or following inhaled terbutaline 500 μg against AMP (3.3±0.4 DD) and histamine (3.1±0.3 DD) (fig. 1).

Dose/response curves

The dose/response curves for AMP and histamine were similar in slope after inhalation of placebo and were not significantly altered by either terbutaline (500 μg) or formoterol (6, 12 or 24 μg) (fig. 2).

Discussion

The results of this study demonstrate the bronchodilator and bronchoprotective effects of acute

Table 2. – Bronchoprotective effect of formoterol and terbutaline on airway responsiveness to histamine and adenosine 5′-monophosphate (AMP)

	Histamine challenge		AMP challenge	
	Log PC_{20}	PC_{20} (mg·mL^{-1})	Log PC_{20}	PC_{20} (mg·mL^{-1})
Placebo	-0.55±0.08	0.28	0.77±0.11	5.84
Formoterol 6 μg	0.53±0.07	3.38	1.81±0.13	64.42
Formoterol 12 μg	0.75±0.10	5.58	2.49±0.19	310.46
Formoterol 24 μg	0.89±0.08	7.8	2.67±0.18	464.52
Terbutaline 500 μg	0.38±0.08	2.42	1.75±0.14	56.36

Data are presented as mean±SEM, unless otherwise stated. PC_{20}: Provocation concentration of spasmogen causing a 20% fall in the forced expiratory volume in one second.
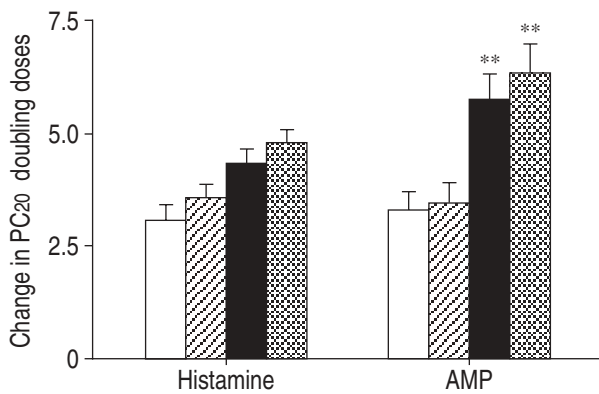
Fig. 1. – Doubling dose bronchoprotection of formoterol (▨: 6 µg; ■: 12 µg; ▨: 24 µg) and terbutaline (□: 500 µg) relative to the placebo on airway responsiveness, 30 min before challenge with histamine and adenosine 5′-monophosphate (AMP). PC20: Provocative concentration of spasmogen causing a 20% fall in the forced expiratory volume in one second. Results are presented as means±SEM. **: p<0.01, AMP *versus* histamine.

administration of both the short-acting β2-agonist terbutaline and the long-acting β2-agonist formoterol. A significant improvement in FEV1 30 min after each
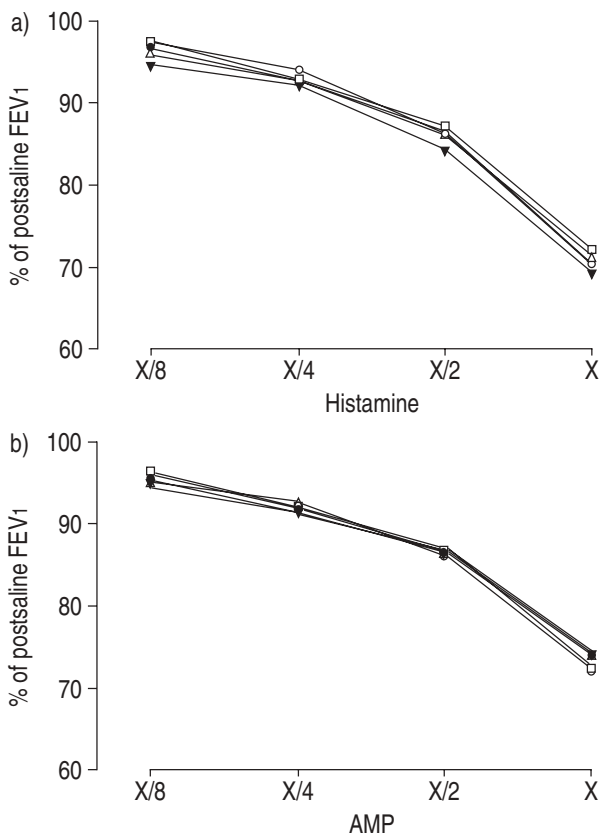

Fig. 2. – Dose/response curves for each spasmogen, a) histamine and b) adenosine 5′-monophosphate (AMP), following inhalation of placebo (□), formoterol (●: 6 µg; △: 12 µg; ▲: 24 µg) and terbutaline (○: 500 µg). Measured by forced expiratory volume in one second (FEV1) values (expressed as a percentage of the postsaline value), following the final four doubling doses of spasmogen in those subjects achieving a provocative concentration of spasmogen causing a 20% fall in FEV1.

active treatment and a dose-dependent reduction in AR to both AMP and histamine following formoterol was observed. The two higher doses of formoterol produced a greater bronchoprotective effect against AMP compared to that against histamine, whereas 6-µg formoterol and 500-µg terbutaline reduced AR to both spasmogens to a similar extent.

The similarity between AMP and histamine dose/response curves following placebo and each active treatment allows direct comparison of the bronchoprotection against each spasmogen. Thus, to explain the findings, it is likely that there are additional mechanisms to β2-agonist-mediated functional antagonism against induced airway smooth muscle contraction. The greater degree of bronchoprotection against AMP afforded by formoterol compared with histamine, is consistent with previous studies on the differential effects of short-acting β2-agonists on AR to these spasmogens [3]. This effect has been previously reported 30 min after a single dose of formoterol (12 µg) [5] but not 2 or 14 h after a single dose of salmeterol (50 µg) [9, 10].

Both short- and long-acting β2-agonists inhibit mast mediator release *in vitro* [11–13]. As the major bronchoconstrictive effect of AMP results from mast-cell degranulation and mediator release [6–8], the additional bronchoprotection seen with the higher doses of formoterol is likely to be due to its mast-cell stabilizing properties. The difference between the *in vivo* effect of formoterol and salmeterol is surprising as they have similar effects on mast cells *in vitro* [11–13]. The differences in the pharmacological properties of salmeterol and formoterol may provide an explanation for the lack of an additional effect of a single dose of salmeterol (50 µg), against AMP in previous studies [9, 10]. Salmeterol, a partial agonist, may simply not achieve adequate levels of receptor occupancy on mast cells at this dose. Indeed, salmeterol is two-fold less potent than formoterol in relaxing human smooth muscle *in vitro* and is ⩾20-fold less active than formoterol at inhibiting mast-cell degranulation [11].

An alternative explanation for the additional bronchoprotective effect of formoterol must be considered. In addition to its effect on mast-cell degranulation and mediator release, adenosine, the active metabolite of AMP, may have direct effects on mucosal blood flow, microvascular leakage and contractile neuropeptide release from sensory nerve endings *via* A2b receptors [14–16]. Since formoterol inhibits plasma exudation on postcapillary venule cells and neuropeptide release from sensory nerves [17] *via* β2-receptors, it is plausible that some of its nonsmooth muscle effects involve pathways that are independent of its mast-cell stabilizing properties.

As the authors had shown an additional bronchoprotective effect of 500-µg terbutaline against AMP in two previous studies [3, 18], they were surprised that there was no significant difference between the bronchoprotective effect of the same doses of terbutaline against histamine and AMP in the present study. The possibility that the bronchoprotective effect of terbutaline had worn off at the time of completion of AMP challenge was considered. The maximum

possible time between inhalation of treatment and the completion of each challenge was 63 min for histamine and 69 min for AMP (30 min before the start of challenge and $\leqslant$39 min to administer all doses of spasmogen). Mean times (95% confidence intervals) for completion of histamine and AMP challenges were 53.6 min (50.3–54.9) and 58.9 min (56.1–61.7), respectively. Short-acting β_2-agonists protected against histamine for $\leqslant$4 h with a peak of between 1–2 h [19, 20] and 500-μg terbutaline protected against exercise-induced asthma for $\leqslant$2 h [21]. It is unlikely, therefore, that the timing of the challenges could explain the results.

The 3.1 DD bronchoprotection against histamine was greater than the 2.1 and 2.7 previously reported by the authors and the DD protection following the same dose of terbutaline against methacholine [3, 18]. The geometric mean PC_{20} to histamine of 0.28 mg·mL^{-1} after placebo was much lower than in a previous study by the authors, in which the values after placebo were 1.04 and 0.91 mg·mL^{-1}. It is possible that the acute effect of terbutaline on histamine is greater in subjects with more severe AR. There was no other difference in subject characteristics and the same inhaler device was used in each of the studies.

Higher doses of the short-acting partial agonist albuterol (400 μg via Diskhaler) [9] and albuterol (2.5 mg nebulized) [4] provided greater bronchoprotection to AMP than histamine, a finding not seen at the lower dose of albuterol, 200 μg via Turbuhaler® [5]. The authors have also previously demonstrated dose-dependent additional bronchoprotection of terbutaline against AMP relative to methacholine [3]. Therefore, the lack of additional bronchoprotection following 6-μg formoterol or 500-μg terbutaline in this study provides further evidence that the presumed mast-cell stabilizing effects of β_2-agonists are dose-dependent.

The failure of a number of subjects to achieve a PC_{20} to AMP following treatment with formoterol may have led to an underestimation of the bronchoprotective effect against AMP. The highest dose of AMP available for inhalation was 800 mg·mL^{-1}, and a PC_{20} was not achieved in 11 of the 25 subjects after 24-μg formoterol, in nine subjects after 12-μg formoterol and in one subject after 6-μg formoterol. To overcome this and to obtain an estimate of the true PC_{20} for these subjects, the slope of the screening AMP concentration/ response curve was utilized. By fitting the maximum per cent FEV_1 fall achieved following 800 mg·mL^{-1} of AMP to that slope, the log PC_{20} by linear extrapolation was determined. This assumed that β_2-agonist treatment did not alter the slope of the AMP dose/response curve. The parallel shift in dose/response curves after each treatment in subjects achieving a PC_{20} suggested that the authors' assumption was correct (fig. 2). Therefore, the failure of a number of subjects to achieve PC_{20} at the highest concentration of AMP was unlikely to have significantly influenced the true bronchoprotective effect of formoterol (12 and 24 μg).

The clinical relevance of the additional bronchoprotective effect of a single dose of formoterol against AMP compared to histamine remains uncertain.

Long-acting β_2-agonists are not used as monotherapy but only in conjunction with inhaled GCS [2]. Therefore, any mast-cell effects of long-acting β_2-agonists would be in addition to the known ability of inhaled corticosteroids to deplete airway mast-cell numbers [22, 23]. However, inhaled corticosteroids do not fully protect against allergen- or exercise-induced bronchospasm, which are both regarded as mast-cell mediated events [23, 24]. This suggests that even in the presence of inhaled corticosteroids there are potentially active mast cells residing in the airways and it is possible that β_2-agonists would confer additional benefit. The addition of formoterol to inhaled budesonide improves symptoms and lung function and reduces asthma exacerbations over a 12-month period [25]. Furthermore, recent evidence from TATTERSFIELD et al. [26] suggested that as-needed treatment with formoterol provided better asthma control than terbutaline in patients requiring moderate doses of relief medication, despite inhaled GCS treatment. Although this additional benefit of formoterol requires further explanation, it is probably due to a nonbronchodilator action that may involve modulation of the inflammatory process.

This study clearly shows a nonsmooth muscle action of formoterol, which the authors believe is due to an effect on the mast cells. This dose-dependent mast-cell stabilizing effect occurs 30-min after a single dose of formoterol. The rapid onset of both the bronchodilator and bronchoprotective effects of therapeutic doses of formoterol may have a beneficial effect against mast-cell stimuli, such as inhalation of allergen and exercise, in addition to functional antagonism on bronchial smooth muscle. These benefits may account for the improvement seen in asthma control with the combination of long-acting β_2-agonist and inhaled glucocorticosteroids. Further study of the mechanisms governing the action of formoterol and related molecules is required.

References

1. Palmqvist M, Persson G, Lazer L, Rosenborg J, Larsson P, Lotvall J. Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. *Eur Respir J* 1997; 10: 2484–2489.

2. British Thoracic Society. The British guidelines on asthma management 1995 review and position statement - Introduction. *Thorax* 1997; 52: S1–S21.

3. O'Connor BJ, Fuller RW, Barnes PJ. Nonbronchodilator effects of inhaled beta2 agonists: Greater protection against adenosine monophosphate- than methacholine-induced bronchoconstriction in asthma. *Am J Respir Crit Care Med* 1994; 150: 381–387.

4. Phillips GD, Finnerty JP, Holgate ST. Comparative protective effect of the inhaled beta2-agonist salbutamol (albuterol) on bronchoconstriction provoked by histamine, methacholine, and adenosine 5′-monophosphate in asthma. *J Allergy Clin Immunol* 1990; 85: 755–762.

5. 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: 1786–1790.

6. Forsythe P, McGarvey LPA, Heaney LG, Macmahon J, Ennis M. Adenosine induces histamine release from human bronchoalveolar lavage mast cells. *Clin Science* 1999; 96: 349–355.

7. Polosa R, Ng WH, Crimi N, *et al.* Release of mast-cell-derived mediators after endobronchial adenosine challenge in asthma. *Am J Respir Crit Care Med* 1995; 51: 624–629.

8. Polosa R, Holgate ST. Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma? *Thorax* 1997; 52: 919–923.

9. Taylor DA, Jensen MW, Aikman SL, Harris JG, Barnes PJ, O′Connor BJ. Comparison of salmeterol and albuterol-induced bronchoprotection against adenosine monophosphate and histamine in mild asthma. *Am J Respir Crit Care Med* 1997; 156: 1731–1737.

10. Soler M, Joos L, Bolliger CT, Elsasser S, Perruchoud AP. Bronchoprotection by salmeterol-cell stabilization or functional antagonism-comparative effects on histamine-induced and AMP-induced broncho-constriction. *Eur Respir J* 1994; 7: 1973–1977.

11. Nials AT, Ball DI, Butchers PR, *et al.* Formoterol on airway smooth muscle and human lung mast-cells - a comparison with salbutamol and salmeterol. *Eur J Pharmacol* 1994; 251: 127–135.

12. Butchers PR, Vardey CJ, Johnson M. Salmeterol - a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Brit J Pharmacol* 1991; 104: 672–676.

13. Chong LK, Cooper E, Vardey CJ, Peachell PT. Salmeterol inhibition of mediator release from human lung mast cells by beta-adrenoceptor-dependent and independent mechanisms. *Br J Pharmacol* 1998; 123: 1009–1015.

14. Haynes J, Obiako B, Thompson WJ, Downey J. Adenosine-induced vasodilation: receptor characterization in pulmonary circulation. *Am J Physiol* 1995; 268: H1862–H1868.

15. Gawlowski DM, Duran WN. Dose-related effects of adenosine and bradykinin on microvascular perms-electivity to macromolecules in the hamster-cheek pouch. *Circ Res* 1986; 58: 348–355.

16. Feoktistov I, Biaggioni I. Adenosine A(2B) receptors. *Pharmacol Rev* 1997; 49: 381–402.

17. Barnes PJ. Effect of beta-agonists on inflammatory cells. *J Allergy Clin Immunol* 1999; 104: S10–S17.

18. O′Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled beta-2-agonists in asthma. *N Eng J Med* 1992; 327: 1204–1208.

19. Gongora HC, Wisniewski AFZ, Tattersfield AE. A single-dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991; 144: 626–629.

20. Ahrens RC, Harris JB, Milavetz G. Use of bronchial provocation with histamine to compare the pharmaco-dynamics of inhaled albuterol and metaproterenol in patients with asthma. *J Allergy Clin Immunol* 1987; 79: 876–882.

21. Woolley M, Anderson SD, Quigley BM. Duration of protective effect of terbutaline sulfate and cromolyn sodium alone and in combination on exercise-induced asthma. *Chest* 1990; 97: 39–45.

22. Laitinen LA, Laitinen A, Haahtela T. A comparative-study of the effects of an inhaled corticosteroid, budesonide, and a beta-2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma - a randomized, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992; 90: 32–42.

23. Barnes PJ. Effect of corticosteroids on airway hyperresponsiveness. *Am Rev Respir Dis* 1990; 141: S70–S76.

24. Gauvreau GM, Doctor J, Watson RM, Jordana M, Obyrne PM. Effects of inhaled budesonide on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* 1996; 154: 1267–1271.

25. Pauwels RA, Löfdahl CG, Postma DS, *et al.* Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. *N Eng J Med* 1997; 337: 1405–1411.

26. Tattersfield AE, Löfdahl CG, Postma DS, *et al.* Comparison of formoterol and terbutaline for as-needed treatment of asthma: a randomised trial. *Lancet* 2001; 357: 257–261.