

A high dose of albuterol does not overcome bronchoprotective subsensitivity in asthmatic subjects receiving regular salmeterol or formoterol

Brian J. Lipworth, MD, FRCPE, and Imran Aziz, MRCP *Dundee, Scotland*

Background: Regular treatment with inhaled, long-acting β_2 -agonists is associated with subsensitivity for bronchoprotective effects. It is not known whether a high dose of short-acting β_2 -agonist could overcome this subsensitivity.

Objectives: The objective of this study was to investigate the acute effects of a high dose of inhaled albuterol on methacholine-induced bronchoconstriction in patients receiving regular treatment with salmeterol or formoterol.

Methods: Ten stable asthmatic subjects (mean age, 34 years; FEV₁, 77% of predicted value), all taking inhaled corticosteroids (methacholine PD₂₀ < 500 μ g), were recruited into a randomized, single-blind, crossover study. After an initial 1-week run-in period, subjects underwent 3 separate treatment periods each of 9 days (separated by a washout of at least 5 days) comprising inhaled placebo twice daily, inhaled salmeterol dry powder 50 μ g twice daily, or inhaled formoterol dry powder 12 μ g twice daily. Methacholine challenge was performed 1 hour after the first dose and after 7 days of treatment. After 9 days of treatment, a third methacholine challenge was performed 1 hour after inhalation of a single 1600 μ g dose of albuterol dry powder.

Results: There was significant ($P < .001$) improvement in geometric mean PD₂₀ after the first dose of active treatment as compared with placebo (78 μ g) versus salmeterol (266 μ g, a 3.4-fold difference [95% CI 1.9 to 6.1]) and versus formoterol (318 μ g, a 4.1-fold difference [95% CI 2.3 to 7.3]). This bronchoprotection diminished with regular treatment, although it remained significant ($P < .01$) compared with placebo (68 μ g) versus salmeterol (144 μ g, a 2.1-fold difference [95% CI 1.2 to 3.8]) and versus formoterol (230 μ g, a 3.4-fold difference [95% CI 1.9 to 6.2]). After 9 days, the protection afforded by a single dose of albuterol after placebo pretreatment (889 μ g) was significantly ($P = .005$) higher in comparison with albuterol protection after salmeterol pretreatment (338 μ g, a 2.7-fold difference [95% CI 1.1 to 6.8]) and after formoterol pretreatment (247 μ g, a 3.6-fold difference [95% CI 1.4 to 9.1]).

Conclusions: Thus in stable asthmatic subjects receiving regular salmeterol or formoterol, bronchoprotective subsensitivity was not overcome by administering a high dose of albuterol. Further studies are required to evaluate the clinical relevance of this pharmacologic phenomenon when albuterol is used in acute asthma. (*J Allergy Clin Immunol* 1999;103:88-92.)

Key words: Asthma, bronchoprotection, downregulation, subsensitivity, methacholine, albuterol, salmeterol, formoterol

Regular treatment with inhaled, long-acting β_2 -agonists has been shown to improve long-term control of asthma¹⁻³ and is now recommended as an additive therapy to inhaled corticosteroids.^{4,5} Regular treatment with long-acting β_2 -agonists leads to the development of bronchoprotective subsensitivity, which occurs despite concomitant inhaled corticosteroid therapy.⁶⁻¹¹ Improvements in asthma control and peak expiratory flow are maintained during treatment with long-acting β_2 -agonists, although bronchoprotective subsensitivity may make the airways more vulnerable to bronchoconstrictor stimuli. In everyday clinical practice patients would use rescue therapy with repeated puffs of short-acting β_2 -agonists to overcome an episode of acute bronchoconstriction, but it is not known whether such treatment would be as effective in patients exposed to continuous long-acting β_2 -agonists.

We have therefore investigated whether bronchoprotective subsensitivity in patients receiving salmeterol or formoterol may be overcome by the acute administration of a high dose of inhaled albuterol in terms of its effects against methacholine-induced bronchoconstriction.

METHODS

Subjects

Ten (6 women, 4 men) subjects with mild-to-moderate asthma (mean $\pm$ SD age, 34 [6.8] years), all taking inhaled corticosteroids (budesonide or beclomethasone; mean dose, 460 μ g/day; Table I), were recruited to take part in a randomized, single-blind, crossover study. All were reactive to methacholine, with a PD₂₀ less than 500 μ g at initial screening. All had stable asthma, according to the American Thoracic Society criteria,¹² for at least 3 months before the study, and none had received a course of oral corticosteroids or antibiotics during this time. All were using inhaled β_2 -agonists for symptomatic relief (<2 puffs/day), 2 were receiving oral theophylline, and 1 was receiving long-acting β_2 -agonist therapy with formoterol. Baseline spirometry at recruitment showed mean ($\pm$ SD) FEV₁ of 2.60 (0.87) L (77% [13.6%] of predicted value) and FEF₂₅₋₇₅ of 2.22 (0.89) L/s (53% [17.3%] of predicted value). All

From the Departments of Clinical Pharmacology and Therapeutics and Respiratory Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee.

Supported by a University of Dundee research grant.

Received for publication July 20, 1998; revised Sept 11, 1998; accepted for publication Sept 15, 1998.

Reprint requests: Brian J. Lipworth, MD, FRCPE, Professor of Allergy & Respiratory Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, Scotland, UK.

Copyright © 1999 by Mosby, Inc.

0091-6749/99 \$8.00 + 0 1/1/95041

TABLE I. Demographic data at recruitment

Patient no	Age (y)	Gender	FEV ₁ (L)	FEV ₁ (% predicted)	FEF ₂₅₋₇₅ (L/s)	FEF ₂₅₋₇₅ (% predicted)	Steroid and dose (μg/day)	Methacholine PD ₂₀ (μg)
1	40	M	2.71	69	1.99	45	1600 BUD	19
2	28	F	2.46	83	2.40	61	400 BDP	60
3	37	F	1.84	67	1.66	45	200 BUD	122
4	34	F	2.25	84	2.79	75	400 BDP	43
5	33	F	2.42	86	1.92	51	800 BUD	161
6	29	M	4.65	107	4.44	90	400 BDP	464
7	36	F	2.02	71	1.72	46	800 BDP	5
8	30	M	3.24	77	2.35	49	200 BDP	28
9	22	M	2.85	68	1.61	33	400 BDP	31
10	46	F	1.60	59	1.33	39	400 BUD	17
Mean	33.5	—	2.60	77.1	2.22	53.4	460	44.8†

BUD, Budesonide; BDP, beclomethasone dipropionate.

†All values are arithmetic means apart from PD₂₀, which are geometric means.

gave written informed consent, and the study was approved by the Tayside Committee on Medical Research Ethics.

Study design

The study had a randomized, single-blind (investigator-blind), placebo-controlled, crossover design. For the screening visit, the subjects attended the laboratory in the morning, having withheld their long acting β_2 -agonist and theophylline therapy for at least 48 hours and their short-acting β_2 -agonist therapy for at least 8 hours. All laboratory visits were performed at the same time of day within a 1-hour window. A methacholine challenge test was then performed, and those subjects with a PD₂₀ less than 500 μg entered an initial 1-week run-in period without β_2 -agonists. From the start of the run-in period until the end of the study, all therapy with short- and long-acting β_2 -agonists was withdrawn and replaced with the study medication. Inhaled ipratropium bromide (Atrovent Forte pMDI, 40 μg per actuation; Boehringer Ingelheim, Bracknell, UK) was substituted as reliever therapy, which was withheld for at least 12 hours before each study visit.

After the initial run-in period, the subjects attended the laboratory for the first dose (day 1 of treatment) of randomized treatment with the following substances: (1) inhaled formoterol 12 μg twice daily (eformoterol fumarate, dry powder, 12 μg per capsule; Foradil, Novartis, Camberley, UK); (2) inhaled salmeterol 50 μg twice daily (salmeterol xinafoate, dry-powder diskhaler, 50 μg per blister; Allen and Hanbury, Uxbridge, UK); or (3) inhaled placebo 1 puff twice daily (administered by means of placebo Turbuhaler; Astra pharmaceuticals, Kings Langley, UK). FEV₁ was measured before inhalation and again 1 hour after inhalation followed by a methacholine challenge.

The subjects then went home and came back to the laboratory on day 8 after 1 week of treatment, which was taken as 1 puff twice daily at 8 AM for the morning dose and at 8 PM for the evening dose. On day 8 of each treatment, the FEV₁ was measured before and 1 hour after inhalation of the fifteenth dose followed by the second methacholine challenge.

The subjects went home again and took the same treatment for 2 more days and returned to the lab on day 10 of treatment, having completed the 9-day treatment, with the eighteenth and last dose taken on the previous evening of day 9. On this occasion the subjects were given a single dose of 1600 μg albuterol (albuterol sulphate, dry-powder diskhaler, 400 μg per blister). FEV₁ was measured before and 1 hour after inhalation of albuterol, and the third methacholine challenge was then performed. The subjects then entered a washout period of at least 5 days without β_2 -agonists before returning for the next treatment cycle, at which time the same

protocol was repeated for the subsequent randomized treatments. The subjects brought back their respective randomized inhalers at each study visit, and compliance with treatment was checked by a third party by counting the used and unused formoterol capsules and salmeterol blisters. Compliance was required to be in excess of 90% of prescribed doses for the data to be considered evaluable for each treatment arm.

Spirometry

Spirometry was performed according to American Thoracic Society criteria¹³ by using a Morgan Spiroflow bellows spirometer (P.K. Morgan Ltd, Gillingham, UK). The spirometer was calibrated daily by using a Vitalograph (Vitalograph Ltd, Buckinghamshire, UK) 1 L precision syringe.

Methacholine bronchial challenge test

The methacholine bronchial challenge was performed by using a standardized computer-assisted dosimetric method as previously described.¹⁴ In brief, the methacholine was administered in doubling cumulative doses from 3.125 to 1600 μg given at 5-minute intervals until a 20% fall in FEV₁ was recorded. If the FEV₁ did not show a 20% drop when a cumulative dose of 1600 μg had been inhaled, a censored PD₂₀ value of 3200 μg (double the maximum cumulative dose) was used for that test for the purpose of statistical analysis.

Statistical analysis

The data for PD₂₀ were log-transformed to normalize their distribution before analysis. The study was powered at the 80% level to detect a 1.0 doubling dose difference (2.0-fold) in methacholine PD₂₀ between active treatments and placebo by using a crossover design. The change in PD₂₀ was calculated as geometric mean (expressed as micrograms) and as the fold ratio for protection as compared with placebo. Analysis of FEV₁ was performed on absolute values either before or after inhalation. The statistical analysis was performed by multifactorial analysis of variance followed by Bonferroni multiple-range testing by using subject, treatment, period, and visit as factors. A *P* value of < .05 (2-tailed) was considered significant. The analysis was performed with Statgraphics statistical software package (STSC Software Publishing Group, Rockville, Md).

RESULTS

Prechallenge spirometry

FEV₁ values before inhalation of the study medication did not show any significant difference between treat-

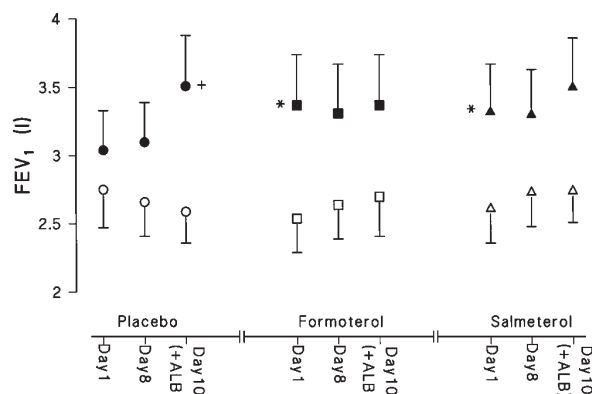


FIG 1. Mean (SE) values for FEV₁ before (open symbols) and 1 hour after (filled symbols) inhalation of study medication with placebo, formoterol, or salmeterol after first dose (Day 1) or fifteenth dose (Day 8) and 1 hour after albuterol (ALB) on day 10 (after eighteenth dose of study medication). Asterisk denotes significant difference between active treatments and placebo for postinhalation absolute FEV₁ values (on Day 1 only), and cross denotes significant difference within treatments among day 1, day 8, and day 10 (for postinhalation values). There were no significant differences in preinhalation values for within- and between-treatment comparisons.

ments (Fig 1). The absolute value for FEV₁ was significantly ($P = .001$) higher 1 hour after the inhalation of formoterol or salmeterol as compared with placebo on day 1. There was a similar numerical trend toward higher FEV₁ values 1 hour after formoterol and salmeterol compared with placebo on day 8, but this was not statistically significant on Bonferroni testing ($P = .054$) for overall comparison.

Methacholine bronchial challenge

For between-treatment comparisons, there was significant improvement in the geometric mean PD₂₀ after the first dose (ie, day 1) of active treatments as compared with placebo (placebo vs salmeterol: 78 μ g vs 266 μ g, a 3.4-fold difference [95% CI 1.9 to 6.1], $P < .001$; placebo vs formoterol: 78 μ g vs 318 μ g, a 4.1-fold difference [95% CI 2.3 to 7.3], $P < .001$). After 1 week of regular treatment (ie, day 8) there was diminution in the bronchoprotective effect, although it remained significant as compared with placebo (placebo vs salmeterol: 68 μ g vs 144 μ g, a 2.1-fold difference [95% CI 1.2 to 3.8], $P = .02$; placebo vs formoterol: 68 μ g vs 230 μ g, a 3.4-fold difference [95% CI 1.9 to 6.2], $P < .001$). After 9 days of treatment (ie, day 10), the protection afforded by a single dose of albuterol after placebo pretreatment was significantly higher as compared with albuterol protection after salmeterol pretreatment (889 μ g vs 338 μ g, a 2.7-fold difference [95% CI 1.1 to 6.8], $P = .002$; albuterol protection after formoterol pretreatment: 889 μ g vs 247 μ g, a 3.6-fold difference [95% CI 1.4 to 9.1], $P = .02$) (Fig 2).

For within-treatment comparisons, in the placebo arm there was a significant increase in PD₂₀ after albuterol treatment (day 10), amounting to an 11.4-fold rise (95%

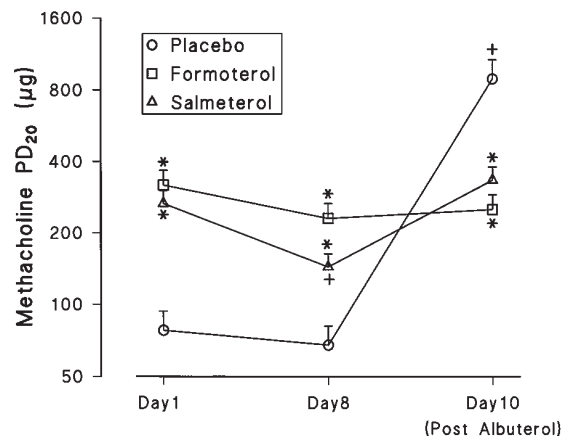


FIG 2. Geometric mean (SE) values for PD₂₀ methacholine after first dose (Day 1), fifteenth dose (Day 8), and eighteenth dose (Day 10 with albuterol) of continuous treatment with placebo, formoterol, or salmeterol. Asterisk denotes significant ($P < .05$) differences between active treatments and placebo. Cross denotes significant ($P < .05$) differences among day 1, day 8, and day 10 of the same treatment. Data for PD₂₀ are plotted on a Log₂ scale to depict doubling doses of methacholine.

CI 5.4 to 24.1; $P < .001$) as compared with day 1 and a 13.1-fold rise (95% CI 6.2 to 27.8; $P < .001$) as compared with day 8. There were no significant differences between the 3 days during the formoterol arm. Treatment with salmeterol showed a significant ($P = .001$) fall in PD₂₀ after 1 week (day 8) compared with the first dose (day 1), amounting to a 1.9-fold drop (95% CI 1.1 to 3.1). After 9 days of salmeterol treatment (day 10) in comparison with day 8, there was significant ($P = .001$) increase in PD₂₀ after albuterol, amounting to a 2.3-fold rise (95% CI 1.4 to 3.8) (Fig 2). Individual data for the protective effect of albuterol on day 10 are shown in Fig 3. This illustrates the wide individual variability in the degree of protection loss.

DISCUSSION

The results of this study showed that bronchoprotective subsensitivity develops in patients taking regular formoterol or salmeterol, which cannot be overcome by administering a high dose of inhaled albuterol. Nonetheless, both salmeterol and formoterol exhibited a significant residual degree of bronchoprotection after regular treatment. Our findings are in keeping with a previous study in which regular treatment with salmeterol 50 μ g twice daily was associated with downregulation of lymphocyte β_2 -adrenoceptors along with subsensitivity of airway β_2 -adrenoceptors in terms of a rightward shift in the albuterol dose-response curve.¹⁵

We accept that there are limitations to our study. We were unable to use the different formoterol and salmeterol dry-powder inhalers in a double-blind manner, and we did not have access to corresponding identical "placebos" to make the study double-dummy. We did, however, ensure that patients were able to use the dry-powder

inhalers, which were easy to use and were both given on a twice daily basis. Also, we were able to check on compliance by counting the blisters or capsules with each inhaler, which in each was greater than 90%. Although we used a small sample size, we were able to show a reduction in albuterol protection greater than 1 doubling dose of methacholine, the latter representing the degree of biologic variability of this test. However, there was a considerable degree of individual variability in the propensity for protection loss, and therefore certain patients may be more susceptible than others in terms of subsensitivity to albuterol.

What is the possible explanation for our findings? It has been shown that regular exposure to long-acting β_2 -agonists results in receptor downregulation and attenuation of the maximal cyclic adenosine monophosphate response to isoproterenol stimulation, as measured *ex vivo* in lymphocyte β_2 -adrenoceptors.^{15,16} Also, it is possible that persistent receptor occupancy by formoterol or salmeterol (at 12 hours after the last dose at day 10) might result in noncompetitive antagonism of β_2 -adrenoceptors. This is supported by an *in vitro* study in which prior incubation with formoterol and salmeterol attenuated the maximal bronchorelaxant effect of albuterol in carbachol-contracted human bronchial smooth muscle.^{17,18} Indeed, we have recently demonstrated that at 12 hours after administration of a single dose of formoterol (12 μ g) or salmeterol (50 μ g) there was significant attenuation of the relaxant effect of albuterol (1600 μ g) against methacholine bronchoconstriction.¹⁹

We had expected that a 1600 μ g dose of albuterol would overcome the subsensitivity or prolonged receptor occupancy induced by long-acting β_2 -agonists on the assumption that the albuterol dose-response curve would be shifted in a competitive manner. That the effect of high-dose albuterol was significantly blunted raised the possibility that the shift might be noncompetitive, with a consequent reduction in maximal achievable response. In this respect we administered albuterol in a dose that is 8-fold higher than the usual recommended dose of 200 μ g. It is of course possible that much higher doses (eg, 32-fold higher) would have overcome the airway β_2 -adrenoceptor subsensitivity, although in reality patients would be most unlikely to self-administer such high doses.

What are the possible clinical implications of our findings? We used methacholine as our bronchoconstrictor stimulus under controlled experimental conditions in stable patients. It may not be possible to extrapolate these findings to what happens in unstable acute asthma in patients in whom there is also an increased level of bronchomotor tone. In the domiciliary setting of an acute episode of bronchoconstriction, patients would normally take reliever therapy with repeated puffs of a short-acting β_2 -agonist in addition to their usual regular long-acting β_2 -agonist medication.²⁰ We designed our study to closely mimic this clinical scenario in that the patients continued to use their long-acting β_2 -agonist on a regular basis until 12 hours before administration of a bolus dose of

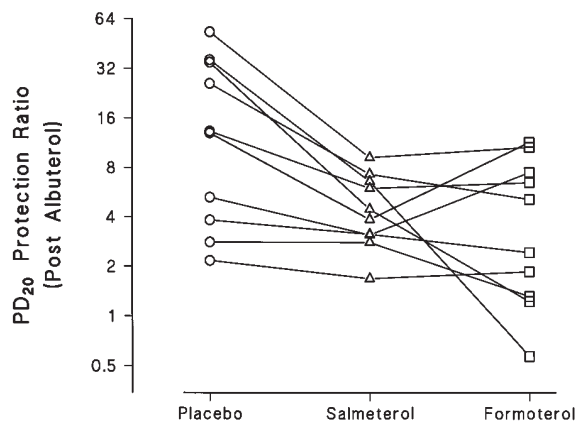


FIG 3. Individual protection ratios for effect of albuterol on methacholine challenge after the eighteenth dose of continuous treatment with placebo (circles), formoterol (squares), or salmeterol (triangles) (on day 10) for the 3 treatments. Protection ratio is shown as fold protection compared with day 1 protection with placebo. The geometric mean fold-protection ratios (not shown in the figure) for the 3 treatments were as follows: placebo, 11.4; formoterol, 3.2; and salmeterol, 4.2. Data points for individual patients are joined by a solid line.

albuterol and the subsequent methacholine challenge. In a real-life situation rescue medication with albuterol would be taken after, rather than before, the episode of bronchoconstriction. It should be pointed out that methacholine acts directly on bronchial smooth muscle, whereas bronchoconstrictor stimuli in everyday life act indirectly by means of inflammatory mediators or neuronal mechanisms.

The observed adverse effects of regular, long-acting β_2 -agonist therapy on bronchoprotective subsensitivity should be considered in the perspective of their long-term beneficial effects on disease control when used as additive therapy to inhaled corticosteroids.¹⁻³ However, it is conceivable that improvements in peak expiratory flow with a long-acting β_2 -agonist might lull patients into a false sense of security in that their airways might be more vulnerable to bronchoconstrictor stimuli as a consequence of β_2 -adrenoceptor subsensitivity.

It is important not to confuse the current results with previous studies that have specifically looked at effects of long-acting β_2 -agonists on the bronchodilator response to albuterol in the presence of resting bronchomotor tone.^{15,21-24} Several of these studies have found no evidence of airway β_2 -adrenoceptor subsensitivity to albuterol,²¹⁻²⁴ although they have been confounded by an insufficient washout period of 12 hours or less before the albuterol dose-response curve and hence a carryover effect of long-acting β_2 -agonist on the albuterol response. It is known, however, that a high dose of systemic corticosteroid rapidly reverses bronchodilator subsensitivity and lymphocyte β_2 -adrenoceptor downregulation in patients undergoing regular long-acting β_2 -agonist therapy.²⁵ This is reassuring in that systemic corticosteroids and high-dose inhaled albuterol are usually given together in patients with an acute asthma exacerbation.

In conclusion, we have shown that in patients with stable asthma, regular treatment with long-acting β_2 -agonists induces bronchoprotective subsensitivity, which results in reduced efficacy of a high dose of albuterol against bronchoconstriction caused by methacholine. Further prospective studies are required to assess the relevance of this phenomenon in the setting of acute asthma in which there is an increased level of bronchomotor tone.

REFERENCES

- Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1994;344:219-24.
- Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996;153:1481-8.
- Pauwels RA, Lofdahl C-G, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N Engl J Med* 1997;337:1405-11.
- National Asthma Education and Prevention Programme. Expert Panel Report II: Guidelines for the diagnosis and management of asthma. Bethesda (MD): National Institute of Health; 1997; Publication No 97-4051.
- British Thoracic Society. Guidelines on the management of asthma. *Thorax* 1997;52(suppl 1):S1-21.
- Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long term effects of long-acting β_2 -adrenoceptor agonist salmeterol on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;327:1198-203.
- Ramage L, Lipworth BJ, Ingram CG, Cree IA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Respir Med* 1994;88:363-8.
- Lipworth B, Tan S, Devlin M, Aiken T, Baker R, Hendrick D. Effects of treatment with formoterol on bronchoprotection against methacholine. *Am J Med* 1998;104:431-8.
- Booth H, Bish R, Walters J, Whitehead F, Walters EH. Salmeterol tachyphylaxis in steroid treated asthmatic subjects. *Thorax* 1996;51:1100-4.
- Kalra S, Swystun VA, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1996;109:953-6.
- Yates DH, Kharitonov SA, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled β_2 -agonist. *Am J Respir Crit Care Med* 1996;154:1603-7.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
- American Thoracic Society. Standardization of Spirometry-update. *Am Rev Respir Dis* 1987;136:1285-98.
- Beach JR, Jung CL, Avery HA, Stenton SC, Dennis JH, Walters EH, et al. Measurement of airway responsiveness to methacholine: relative importance of the precision of drug delivery and method of assessing response. *Thorax* 1993;48:239-43.
- Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. *Lancet* 1995;346:201-6.
- Newnham DM, McDevitt DG, Lipworth BJ. Bronchodilator subsensitivity after chronic dosing with formoterol in patients with asthma. *Am J Med* 1994;97:29-37.
- Kallstrom BL, Sjoberg J, Waldeck B. The interaction between salmeterol and β_2 -adrenoceptor agonists with higher efficacy in guinea-pig trachea and human bronchus in vitro. *Br J Pharmacol* 1994;43:687-92.
- Molimard M, Naline E, Zhang Y, Le Gross V, Begaud B, Advenier C. Long- and short-acting β_2 adrenoceptor agonists: interactions in human contracted bronchi. *Eur Respir J* 1998;11:583-8.
- Aziz I, Lipworth BJ. In-vivo effect of salbutamol on methacholine contracted bronchi in the presence of salmeterol and formoterol [abstract]. *Thorax* 1998;53(Suppl 4):A74.
- Lipworth BJ. Treatment of acute asthma. *Lancet* 1997;350(suppl II):18-23.
- Wilding P, Clark M, Coon JT, Lewis S, Rushton L, Bennett J, et al. Effect of long term treatment with salmeterol on asthma control: a double-blind, randomised crossover study. *BMJ* 1997;314:1441-6.
- Ullman A, Hedner J, Svedmyr N. Inhaled salmeterol and salbutamol in asthmatic patients: evaluation of asthma symptoms and the possible development of tachyphylaxis. *Am Rev Respir Dis* 1990;142:571-5.
- Langley SJ, Masterson CM, Batty EP, Woodcock A. Bronchodilator response to salbutamol after chronic dosing with salmeterol or placebo. *Eur Respir J* 1998;11:1081-5.
- Arvidsson P, Larsson S, Lofdahl C-G, Melander B, Svedmyr N, Wahlander L. Inhaled formoterol during one year in asthma: a comparison with salbutamol. *Eur Respir J* 1991;4:1168-73.
- Tan KS, Grove A, McLean A, Gnosspeliuss Y, Hall IP, Lipworth BJ. Systemic corticosteroid rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. *Am J Respir Crit Care Med* 1997;156:28-35.