

Respiratory pathophysiologic responses

Salbutamol-induced increased airway responsiveness to allergen and reduced protection versus methacholine: Dose response

Rajesh Bhagat, MD, Veronica A. Swystun, BSc, and
Donald W. Cockcroft, MD, FRCP(C) Saskatoon, Saskatchewan, Canada

Background: Two adverse effects of inhaled β_2 -agonists are increased airway responsiveness to allergen and tolerance to the bronchoprotective effect of β_2 -agonists versus bronchoconstrictors (e.g., methacholine).

Objective: We studied three doses of inhaled salbutamol, 200, 400, and 800 $\mu\text{g/day}$, to determine dose-response curves for these two adverse effects.

Methods: Ten atopic patients with mild, stable asthma free of all asthma medications, allergen exposure, and respiratory tract infection for at least 4 weeks participated in a double-blind, random-order, crossover study. There were four 1-week treatment periods with a 1-week washout period: placebo, salbutamol 200 μg , 400 μg and 800 μg per day. After each treatment, we assessed FEV_D , bronchodilation 10 minutes after administration of 200 μg of salbutamol, methacholine PC_{20} , methacholine dose-shift after administration of 200 μg of salbutamol, and allergen PC_{20} .

Results: There was no significant difference in baseline FEV_D , bronchodilation, or methacholine PC_{20} . The methacholine dose shift was maximum after the placebo (3.4 ± 0.22 doubling doses) and was significantly greater ($p < 0.01$) than all salbutamol regimens (2.2 to 2.6), which were not significantly different from each other ($p > 0.05$). Allergen PC_{20} was significantly lower ($p < 0.02$) after salbutamol 800 $\mu\text{g/day}$ (geometric mean = 288 protein nitrogen units [PNU]/ml) than each of the other treatments (447 to 550 PNU/ml), which were not significantly different from each other ($p > 0.05$).

Conclusion: Significant increase in airway responsiveness to allergen occurred only with the largest dose of inhaled salbutamol (800 $\mu\text{g/d}$); however, tolerance to the acute bronchoprotective effect of salbutamol was observed with all the three salbutamol regimens, even 200 $\mu\text{g/day}$. This suggests different mechanisms may be operative in producing these two effects. (*J ALLERGY CLIN IMMUNOL* 1996;97:47-52.)

Key words: β_2 -agonist tolerance, allergen inhalation test, methacholine inhalation test

Regular use of inhaled β_2 -agonists produces tolerance to their bronchoprotective effect against exercise,^{1,2} histamine,³ methacholine,⁴⁻⁶ and adenosine monophosphate (AMP)-induced⁴ broncho-

From the Division of Respiratory Medicine, Department of Medicine, Royal University Hospital, Saskatoon, Saskatchewan, Canada.

Supported by a grant from the Saskatchewan Lung Association. Received for publication Dec. 29, 1994; revised March 24, 1995; accepted for publication March 30, 1995.

Reprint requests: Donald W. Cockcroft, MD, Division of Respiratory Medicine, Royal University Hospital, 103 Hospital Drive, Ellis Hall, 5th Floor, Saskatoon, Saskatchewan S7N 0W8 Canada.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/1/65253

Abbreviations used

AMP: Adenosine monophosphate
EAR: Early asthmatic response
LAR: Late asthmatic response
PNU: Protein nitrogen units

spasm. We recently demonstrated that salbutamol, 200 μg four times a day for 1 to 2 weeks, reduces the acute protective effect of salbutamol against both methacholine and allergen-induced bronchoconstriction.^{6,7} This also made the airways almost twice as responsive to allergen.⁶ There are no data on the effect of different doses of regularly inhaled

salbutamol on development of tolerance and increased airway hyperresponsiveness in patients with asthma. The present study was designed to determine how frequently the β_2 -agonists could be used without increased airway responsiveness to allergen developing and their protective effect to methacholine challenge being lost. We compared the effect of 1 week each of salbutamol at 200 $\mu\text{g/day}$, 400 $\mu\text{g/day}$, and 800 $\mu\text{g/day}$ with placebo on FEV_1 , bronchodilation, methacholine PC_{20} , allergen PC_{20} , and the acute protective effect of salbutamol on methacholine-induced bronchospasm.

METHODS

Subjects

Ten atopic volunteers with stable asthma and an $\text{FEV}_1 \geq 70\%$ of that predicted were included in the study. Subjects did not take asthma medications for at least 4 weeks and had no allergen exposure or respiratory tract infection for at least 4 weeks and during the study period. All had at least one allergen to which there were both historical and skin test evidence of high degree of type I sensitivity. This study was approved by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation, and signed informed consent was obtained.

Methacholine inhalation test

Methacholine inhalation tests were done by a modification⁶ of our previously described method.^{8,9} Aerosols were generated by a Puritan Bennett Twin jet nebulizer (Puritan-Bennett Corp., Carlsbad, Calif.) containing 3 ml of fluid and powered by an airflow of 4.5 to 6 L/min to give an output of 0.13 ml/min. The same nebulizer was used for an individual subject on each occasion. Subjects had their noses clipped and inhaled the aerosols through a loose-fitting face mask for 2 minutes of tidal breathing. Sterile isotonic saline, the diluent for the methacholine solutions, was inhaled initially and at 5-minute intervals by doubling concentrations of methacholine from 0.03 mg/ml to 16 mg/ml. The FEV_1 was initially measured in triplicate and was measured 30 and 90 seconds after each inhalation. The change in FEV_1 was calculated from the lowest postsaline value to the lowest post-methacholine value. The methacholine PC_{20} was then calculated by formula from the log dose versus response curve.¹⁰

Allergen inhalation test

Allergen inhalation test was done as described.^{11,12} On the allergen challenge control day, after the baseline FEV_1 was recorded in triplicate, the patient inhaled the diluent, isotonic saline, 0.04% phenol, 0.03% human serum albumin (Hollister Stier, Miles Canada Inc., Etobicoke, Ontario, Canada), which was nebulized with a Wright nebulizer (output 0.13 ml/min) through a Hans

Rudolf valve, and the exhalate and uninhaled nebulized aerosol were collected by two breathing circuit filters (BB-50T, Pall Biomedical Inc., Fajardo, P.R.) attached on the expiratory line. The same nebulizer was used in an individual patient on all occasions. The diluent was inhaled for 2 minutes of tidal breathing three times at intervals of 12 minutes. Ten minutes after each inhalation, the FEV_1 was recorded in duplicate; the largest value at each time was retained. After the third inhalation, the FEV_1 was recorded in duplicate at 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300, 360, and 420 minutes. Duplicate skin prick tests with the allergen (in doubling dilutions 1:8 to $\geq 1:1024$) to be used for inhalation were done on the control day. The least concentration causing a 2×2 mm mean wheal diameter at 10 minutes was taken as skin test end point. The allergen PC_{20} was predicted by a formula using the skin test end point and methacholine PC_{20} .¹¹

Multiple batches of 3 ml aliquots of the allergen dilutions were prepared in Wright's nebulizer-compatible vials and stored in the freezer (-17°C). On the allergen challenge day, allergen inhalation was done after recording the FEV_1 in triplicate and in duplicate 10 minutes after inhalation of diluent. Allergen inhalation was done with the same setup as on control day, starting from 4 to 5 concentrations below the predicted allergen PC_{20} . Inhalation in doubling concentrations was continued until the FEV_1 at 10 minutes showed a fall of 15% or more. Then the FEV_1 was monitored for 7 hours as on the control day, to look for late responses. Allergen PC_{20} (protein nitrogen units [PNU] per milliliter) was calculated from the log dose-response curve by interpolation and extrapolation, as for methacholine PC_{20} .¹⁰ Subjects were enrolled in this study if they showed an isolated early asthmatic response (EAR).

Study design

A double-blind, random-order, cross-over study comparing four 1-week treatment periods each separated by a 1-week washout was done. The treatments compared were:

1. Salbutamol (Ventolin, Glaxo Canada Inc., Mississauga, Ontario, Canada) 100 μg , two puffs once a day (200 $\mu\text{g/day}$).
2. Salbutamol 100 μg , two puffs twice a day (400 $\mu\text{g/day}$).
3. Placebo two puffs three times a day.
4. Salbutamol 100 μg , two puffs four times a day (800 $\mu\text{g/day}$).

The four treatments were supplied in identical boxes with enclosed written instructions as to once a day (at night), twice a day (morning and night), three times a day, or four times a day. Subjects were aware that we were studying various doses of salbutamol and were instructed not to reveal the dosage schedule to the investigators.

After the 7-day treatment period, the subjects came to

TABLE I. Anthropometric and baseline lung function data

Subject No.	AGE (years)	Sex	Height (cm)	Weight (kg)	FEV ₁		Methacholine PC ₂₀ (mg/ml)	Allergen and Concentration	
					(L)	% Predicted			
1	25	F	170	52	2.56	75	0.67	Cat†	1:32
2	47	M	170	64	2.38	70	0.25	Grass†	1:1024
3	19	F	155	45	2.51	83	0.16	Horse*	1:256
4	18	F	160	52	3.58	111	0.39	Horse*	1:16
5	23	F	157	50	2.51	83	0.25	Dog*	1:16
6	26	M	183	88	4.48	99	1.11	Horse*	1:8
7	23	F	165	61	2.85	87	1.53	Birch*	1:64
8	20	M	174	70	3.90	89	1.81	Grass†	1:16
9	16	F	170	50	3.18	88	0.27	Horse*	1:16
10	21	F	173	55	3.66	107	4.0	Horse*	1:128

*Hollister Stier Laboratories (Miles Canada), Etobicoke, Ontario, Canada.

†Bencard Allergy Laboratory, Mississauga, Ontario, Canada.

TABLE II. Results

	Placebo	Salbutamol 200 µg/day	Salbutamol 400 µg/day	Salbutamol 800 µg/day	ANOVA <i>p</i>
FEV ₁ (L)	3.09 (0.21)	3.22 (0.21)	3.23 (0.26)	3.14 (0.21)	0.22
Change in FEV ₁ (%)	8.6 (2.1)	7.7 (1.6)	6.6 (2.2)	5.9 (1.7)	0.30
LogMeth PC ₂₀ (mg/ml)	-0.19 (0.13)	0.05 (0.18)	-0.12 (0.19)	-0.03 (0.24)	0.13
GM	0.65	1.1	0.76	0.93	
Dose shift	3.4 (0.28)	2.6* (0.3)	2.6* (0.28)	2.2* (0.36)	0.0021
LogAllergen PC ₂₀ (PNU/ml)	2.74 (.22)	2.68 (.23)	2.65 (.22)	2.46† (.22)	0.0035
GM	550	479	447	288	

ANOVA, Analysis of variance; GM, geometric mean.

**p* < 0.01 versus placebo but not significant (*p* > 0.05) versus each other.

†*p* < 0.02 versus each of the other three treatments that were not significant (*p* > 0.05) versus each other.

the laboratory, having taken the last dose of blinded medication 8 to 10 hours earlier and underwent a methacholine challenge. After resting for 50 minutes, the subjects took two puffs (200 µg) of salbutamol, and another methacholine challenge was done 10 minutes later. During that day, the subject continued the instructed number of doses. The morning of the next day (day 8, 8 to 10 hours after the last dose) an allergen challenge was done, the allergen PC₂₀ determined, and the FEV₁ followed only until the EAR had resolved. Subjects were advised to pay particular attention to any possible late symptoms and were given a phone number if there were any problems. They were supplied with "rescue" salbutamol. This was followed by at least 1 week of washout period before starting the next treatment.

Statistical analysis

Analysis of variance using a computer program was done on the four treatments with the following five end points:

1. FEV₁ (day 7 baseline).
2. Bronchodilation (% Δ FEV₁ from before first methacholine test to 10 minutes postsalbutamol on day 7).
3. Methacholine PC₂₀ (log transformed) on day 7.

4. Methacholine PC₂₀ dose shift after two puffs (200 µg) of salbutamol ($\Delta \log PC_{20} / \log 2 =$ number of doubling doses⁴).
5. Allergen PC₂₀ (log transformed) on day 8.

RESULTS

The anthropometric data, details of lung function, and allergen challenge are given in Table I. There was one protocol violation. Subject 6, with an initial screening allergen PC₂₀ of 1:8, did not respond by more than 10% to allergen at 1:4 dilution after the first treatment. No further allergen challenges were done with this individual; however, the remainder of the study was completed and data were analyzed for FEV₁, Δ FEV₁, methacholine PC₂₀, and dose shift.

The four baseline FEV₁ values after the four treatment periods were found to be similar, with no statistically significant treatment effect (FEV₁ = 3.09 to 3.23 L, *p* = 0.22, Table II). There was also no treatment effect on bronchodilation (Δ FEV₁ = 5.9% to 8.6%, *p* = 0.30, Table II).

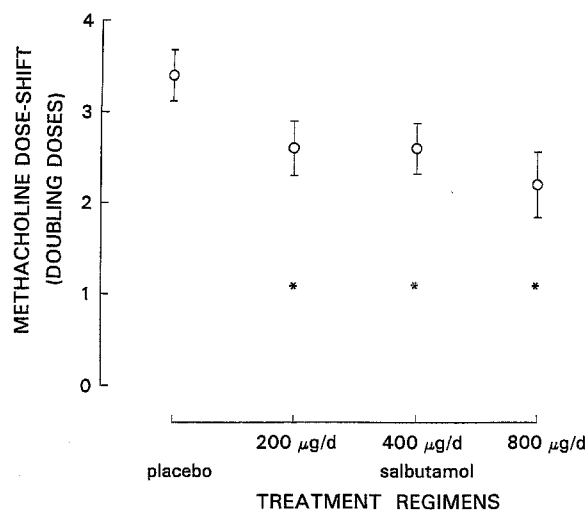


FIG. 1. Effect of three different doses of inhaled salbutamol (1 week) on protective effect of salbutamol versus methacholine. The methacholine dose shift (doubling doses) is on the vertical axis (mean $\pm$ SEM) plotted versus placebo, 200, 400, and 800 μ g/day salbutamol. The dose shift is calculated by the formula:

$$\frac{(\log_{10} \text{methacholine PC}_{20} \text{ postsalbutamol} - \log_{10} \text{PC}_{20} \text{ presalbutamol})}{\log_{10} 2}$$

*Significantly lower than placebo ($p < 0.01$); there were no other significant between mean differences ($p > 0.05$).

There was no statistically significant difference ($p = 0.13$) in $\log_{10}$ methacholine PC_{20} during the four treatment periods (Table II). The methacholine dose shift, the acute bronchoprotective effect of two puffs (200 μ g) of salbutamol against methacholine-induced bronchospasm, showed a highly significant treatment effect ($p = 0.0021$, Table II, Fig. 1). The dose shift was maximum after the placebo period (3.4 ± 0.23 doubling doses), and was statistically significantly greater ($p < 0.01$) than after 1 week each of salbutamol 200 μ g/day, 400 μ g/day, and 800 μ g/day (2.6 ± 0.30 , 2.6 ± 0.28 , 2.46 ± 0.22 doubling doses respectively); these three were not significantly different from each other ($p > 0.05$).

The allergen PC_{20} ($n = 9$) showed a highly significant treatment effect ($p = 0.0035$, Table II, Fig. 2). Allergen PC_{20} was the lowest after 1 week of 800 μ g/day of salbutamol (geometric mean = 288 PNU/ml) and was statistically significantly lower ($p < 0.02$) than any of the other three regimens: placebo, 200 μ g/day, and 400 μ g/day (550 PNU/ml, 479 PNU/ml, 447 PNU/ml, respectively); the latter two were not different than the placebo period ($p > 0.05$). There were no reported late responses and no requirements for rescue salbutamol.

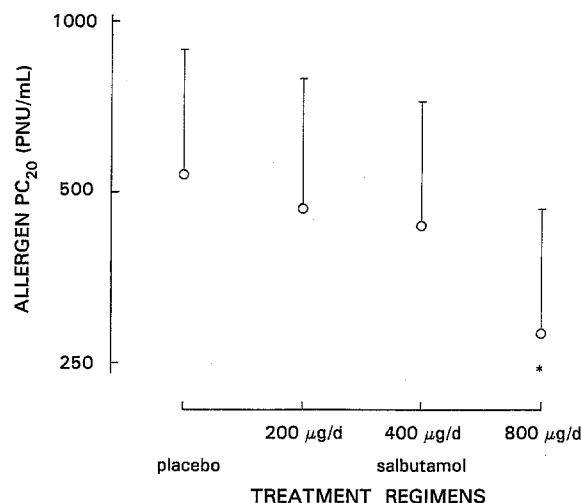


FIG. 2. Effect of the three different doses of salbutamol (1 week) on allergen PC_{20} . The geometric mean ($\pm$ SEM) allergen PC_{20} (PNU/ml) is on the vertical axis plotted versus placebo and the three doses of salbutamol.

*Significantly lower than each of the other three means ($p < 0.02$); there were no other significant between mean differences ($p > 0.05$).

DISCUSSION

The present study confirms our earlier finding of increase, almost doubling, in airway responsiveness to allergen after regular use of inhaled salbutamol for 1 week. This increase in responsiveness was significant only after use of inhaled salbutamol 800 μ g per day. Although there was a slight increase (less than one quarter of a doubling concentration) after salbutamol 200 and 400 μ g/day, it failed to achieve statistical significance compared with the placebo period. Seven of the 10 had not participated in our earlier study; this is important because it confirms our findings in new subjects and because there were no subject biases from the previous investigation. However, the loss of bronchoprotective effect of salbutamol against methacholine-induced bronchospasm was observed even with use of 200 μ g/day of salbutamol for 1 week. The difference in the dose-response relationships of salbutamol on allergen and methacholine challenges suggests different mechanisms may be involved.

Allergen causes an EAR by releasing mediators from mast cells, which then act on smooth muscle, while methacholine causes bronchospasm by direct action on the smooth muscle. O'Connor et al.⁴ demonstrated different effects of inhaled β_2 -agonist on a mast cell mediator-releasing stimulus (adenosine monophosphate [AMP]) and a direct smooth muscle bronchoconstrictor (methacholine). They demonstrated that terbutaline provided

greater protection versus AMP than versus methacholine. This suggested that stimulation of β_2 -receptors on mast cells is important and provides an additive effect to the stimulation of smooth muscle β_2 -receptors in blocking AMP, whereas only the latter is relevant in blocking methacholine.⁴ They further demonstrated a much greater tolerance to the AMP protective effect (compared with the tolerance vs methacholine) after 1 week of regular terbutaline; this suggested a differential sensitivity of mast cell β_2 -receptors and smooth muscle β_2 -receptors.⁴ Our initial study showed a similar pattern using allergen as the mast cell stimulus rather than AMP.⁶ We also showed a substantial increase in airway responsiveness to allergen after 14 days of conventional dose-inhaled salbutamol.⁶ Because methacholine PC₂₀ did not change, and because allergen-specific IgE is not likely to have changed, an increased EAR with the same dose of allergen implies a greater discharge of mediators from mast cells. This hypothesis has not been tested; however, salbutamol 800 μ g/day for 7 days increases both the allergen-induced late asthmatic response (LAR)^{13, 14} and allergen-induced inflammation¹⁴ after inhalation of the same dose of allergen. This also suggests greater mast cell mediator release. One possibility is that regular use of inhaled β_2 -agonists might predispose mast cells to release mediators with greater ease, in fact becoming "leaky," a recognized feature of asthma.¹⁵

Thus it is likely that the increased airway responsiveness to allergen is due to mast cell β_2 -receptor disfunction, while the reduced protective effect against methacholine relates to smooth muscle β_2 -receptors. The differential dose-response effect seen for these two adverse effects may relate to differential susceptibility of the two different β_2 -receptors, differential accessibility of the drug (salbutamol) to the receptors, or some other as-yet-unidentified reason.

The clinical significance of the modest reduction in bronchoprotection is uncertain. However, the increased airway responsiveness to allergen may be very important. Increase in allergen-induced EAR,^{6, 7} LAR,^{13, 14} and inflammation¹⁴—particularly the latter two—may cause worsening asthma in atopic subjects. This is a possible explanation for β_2 -agonist-induced airway hyperresponsiveness in some studies,^{3, 16, 17} and also for β_2 -agonist-induced airway inflammation observed by Davies et al.¹⁸ The possibility that allergen exposure and regular β_2 -agonist use might cause more airway inflammation than allergen exposure alone is

clearly important. It is at least partially reassuring that this effect appears to require a larger dose of β_2 -agonist.

The epidemiologic data from Saskatchewan show an association between overuse of inhaled β_2 -agonists and asthma mortality.^{19, 20} However, there have also been concerns about regular use of conventional doses of inhaled β_2 -agonists and worsened control of asthma.²¹ Our findings may help explain this more subtle insidious worsening of asthma reported with chronic use of β_2 -agonists in conventional doses. The clinical relevance of differing dose-response effects for allergen- and methacholine-induced bronchospasm is, at present, not clear. However, from the number of studies demonstrating preservation of the bronchodilating effect with regular use of inhaled β_2 -agonists,⁵⁻⁷ it seems that different mechanisms are involved in the bronchodilating effect, the bronchoprotective effect against various challenges, and increase in airway responsiveness to allergen.

We thank Jacquie Bramley for assistance in the preparation of the manuscript.

REFERENCES

1. Gibson GJ, Greenacre JK, Konig P, Conolly ME, Pride NB. Use of exercise challenge to investigate possible tolerance to beta-adrenoceptor stimulation in asthma. *Br J Dis Chest* 1978;72:199-206.
2. Ramage L, Lipworth BJ, Ingram CG, Cree JA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Resp Med* 1994;88:363-8.
3. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988;1:554-7.
4. O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled β_2 -agonists in asthma. *N Engl J Med* 1992;327:1204-8.
5. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting β_2 -adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;327:1198-203.
6. Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet* 1993;342:833-7.
7. Cockcroft DW, Swystun VA, Bhagat R. Interaction of inhaled β_2 agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. *Am J Respir Crit Care Med* 1995 (in press).
8. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
9. Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978;33:705-10.

10. Cockcroft DW, Murdock KY, Mink JT. Determination of histamine PC₂₀: comparison of linear and logarithmic interpolation. *Chest* 1983;84:505-6.
11. 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.
12. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:734-40.
13. Cockcroft DW, O'Byrne PM, Swystun VA, Bhagat R. Regular use of inhaled albuterol and the allergen-induced late asthmatic response. *J ALLERGY CLIN IMMUNOL* 1995;96:44-9.
14. Gauvreau GM, Watson RM, Jordana M, Cockcroft D, O'Byrne PM. The effect of regular inhaled salbutamol on allergen-induced airway responses and inflammatory cells in blood and induced sputum. *Am J Respir Crit Care Med* 1995;151:A39.
15. Flint KC, Leung KBP, Hudspith BN, Brostoff J, Pearce FL, Johnson NM. Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *BMJ* 1985;291:923-6.
16. Kraan J, Koeter GH, Mark ThWvd, Sluiter HJ, deVries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J ALLERGY CLIN IMMUNOL* 1985;76:628-36.
17. Kerrebijn KF, van Essen-Zandvliet EEM, Neijens HJ. Effects of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. *J ALLERGY CLIN IMMUNOL* 1987;79:653-9.
18. Davies RJ, Trigg CJ, Wang JH, et al. Regular inhaled salbutamol may exacerbate bronchial inflammation in patients with mild asthma. *Thorax* 1993;48:1060.
19. Spitzer WO, Suissa S, Ernst P, et al. The use of beta-agonist and the risk of death and near death from asthma. *N Engl J Med* 1992;326:501-6.
20. Suissa S, Ernst P, Boivin J-F, et al. A cohort analysis of excess mortality in asthma and the use of inhaled β -agonists. *Am J Respir Crit Care Med* 1994;149:604-10.
21. Sears MR, Taylor DR, Print CG, et al. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;336:1391-6.