

15. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;8:307-10.
16. Abraham WM, Perruchoud AP, Sielczak MW, Yerger LD, Stevenson JS. Airway inflammation during antigen-induced late bronchial obstruction. *Prog Respir Res* 1985; 19:48-55.
17. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic response. *Am Rev Respir Dis* 1987;136:740-51.
18. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806-17.
19. Boulet LP, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J ALLERGY CLIN IMMUNOL* 1983;71:399-406.
20. Gonzalez C, Diaz P, Galleguillos F, Ancic P, Cromwell O, Kay AB. Allergen-induced recruitment of bronchoalveolar helper T-cells (OKT4), and suppressor T-cells (OKT8) in asthma: relative increases in OKT8 cells in single early compared with late-phase responses. *Am Rev Respir Dis* 1987;136:600-4.
21. Thorel T, Joseph M, Tsicopoulos A, Tonnel AB, Capron A. Inhibition by nedocromil sodium of IgE-mediated activation of human mononuclear phagocytes and platelets in allergy. *Int Arch Allergy Appl Immunol* 1988;85:232-7.
22. Wells E, Jackson CG, Harper ST, Mann J, Eady RP. Characterization of primate bronchoalveolar mast cells. II. Inhibition of histamine, LTC₄, and PGD₂ release from primate bronchoalveolar mast cells and a comparison with rat peritoneal mast cells. *J Immunol* 1986;137:3941-5.
23. Moqbel R, Cromwell O, Walsh GM, Wardlaw AJ, Kurlak L, Kay AB. Effects of nedocromil sodium (Tilade) on the activation of human eosinophils and neutrophils and the release of histamine from mast cells. *Allergy* 1988;43:268-76.
24. Nagy L, Lee TK, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N Engl J Med* 1982;306:497-501.

A device for overcoming discoordination with metered-dose inhalers

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Background: Despite widespread acceptance of metered-dose inhalers (MDIs) in the treatment of asthma, many patients fail to operate these devices correctly. Inability to properly coordinate activation with onset of inhalation is regarded as the major factor in suboptimal MDI therapy.

Methods: We evaluated Autohaler Inhalation Device (3M Pharmaceuticals, St. Paul, Minn.), a breath-activated MDI that is typically activated at a triggering flow rate of approximately 0.5 L/sec. We compared bronchodilator effect of pirbuterol acetate (Maxair), inhaled from Autohaler and a standard MDI, under conditions that ensured optimal technique in 20 patients with asthma. Spirometric variables (forced expiratory volume in 1 second [FEV₁], forced expiratory flow between 25% and 75% of vital capacity [FEF₂₅₋₇₅], forced vital capacity [FVC]) were measured before and at 15, 30, 60, and 90 minutes after two inhalations of full inspiratory reserve volume for each device.

Results: Both devices produced significant and similar bronchodilation. Mean FEV₁ increased 32% above baseline 60 minutes after use of Autohaler and 31% after use of a standard MDI. Similar changes were noted in FEF₂₅₋₇₅ and FVC for the two devices. Differences between devices for all spirometric variables were not statistically significant.

Conclusion: Autohaler provides a promising alternative to the standard MDI by overcoming breath-hand discoordination. (*J ALLERGY CLIN IMMUNOL* 1993;92:783-9.)

Key words: Metered-dose inhaler, discoordination, Autohaler, breath-activated inhaler, spacers, pirbuterol acetate

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Abbreviations used

FEF ₂₅₋₇₅ :	Forced expiratory flow between 25% and 75% of vital capacity
FEV ₁ :	Forced expiratory volume in 1 second
FVC:	Forced vital capacity
MDI:	Metered-dose inhaler

Inhalation of bronchodilator medications from a metered-dose inhaler (MDI) has become the most popular delivery system in treatment of bronchial asthma. MDIs offer many advantages over systemic and other inhalation routes by combining convenience and portability with smaller dose requirements, minimal side effects, and rapid onset of action.¹⁻⁵ Because appropriate use of MDIs is not intuitive, patients have been taught to use these devices by a variety of means.^{2, 5-8}

A number of studies have shown that although correct MDI usage requires adherence to a seemingly simple set of instructions, a significant proportion of patients find these instructions confusing and fail to use their inhalers adequately.⁵⁻¹¹ Many hours of careful training and retraining plus use of specialized equipment may be required to teach some patients correct inhaler techniques, and even then, failures still frequently occur.^{5, 8-11} Indeed, even physicians' knowledge of the correct techniques of proper MDI usage has been demonstrated to be largely deficient.¹²

Proper use of MDIs has been shown to be dependent on a number of factors including adequate volume of inhalation, rate of inhalation, duration of breath-holding, and coordination between inspiration of aerosol and MDI activation.^{1, 2, 4} If performed incorrectly, MDI technique has been documented to result in a significant loss of efficacy with a correspondingly undesirable loss of bronchodilation.⁸⁻¹⁴

Discoordination has been generally accepted by many to be the dominant factor in MDI misuse.^{2, 5-10, 12} Newman et al.² have shown that release of aerosol during inhalation and not before or afterward is a critical feature in MDI use, and indeed, package inserts and health care professionals instruct patients to use canister inhalers in this fashion. To obviate the problem of discoordination, a number of devices have been introduced, including spacers.¹⁵⁻¹⁷ Larger spacers act as holding chambers, which tend to both minimize the negative effects of discoordination and allow settling out of those large particles that would not

penetrate beyond the larynx and, in the case of steroids, might cause local problems in the throat.¹⁸ Although some spacers largely overcome discoordination problems, these devices create other problems including noncompliance because of inconvenience, size, complexity, and cost.

Activation of MDI by inspiration offers an attractive alternative to one of the functions of spacers/holding chambers. To be useful, breath-activated inhalers should be simple, cost-effective, and nonbulky. Although breath-activated inhalers have been available in the past, earlier breath-activated inhalers have not been well received because of problems with unreliability, excessive noise, bulkiness, and requirement of high flow rates for activation.¹⁸⁻²¹

Autohaler Inhalation Device (3M Pharmaceuticals, St. Paul, Minn.) (Fig. 1) is a breath-activated MDI, developed in Great Britain, which is activated at normal resting inspiratory flow rates (i.e., 0.5 L/sec²²⁻²⁴). Autohaler is approximately the same size as the standard MDI (Fig. 1). Although the Autohaler canister is smaller and its contents are more concentrated than those of a standard MDI canister, it delivers approximately 400 doses of 200 µg of pirbuterol, whereas the standard MDI delivers 300 doses of the same strength.

The design and operation of Autohaler have been described elsewhere²²⁻²⁴ (Fig. 2). Briefly, the device consists of a latching lever at its top, which, when raised, primes the mechanism via compression of a conical spring on the aerosol canister. A precision-molded triggering mechanism resting on a small vane located behind the mouthpiece blocks metering valve actuation until patient inhalation swings the vane upward, releasing the triggering mechanism. With the priming lever placed upward and mouthpiece between closed lips, patients were instructed to inhaled steadily and slowly until activation occurred.

Autohaler use has been studied in Great Britain. Crompton and Duncan¹¹ found that after oral and written instruction 91% of patients were able to efficiently use Autohaler, whereas only 50% could efficiently use an MDI. Newman et al.¹⁹ studied Autohaler and MDI use in good and poor coordinators. They found that lung deposition of albuterol and bronchodilation effect could be improved in poor coordinators either by use of Autohaler or investigator-assisted technique.

To assess Autohaler effect, we compared bronchodilation produced by inhalation of pirbuterol acetate from Autohaler in patients with asthma

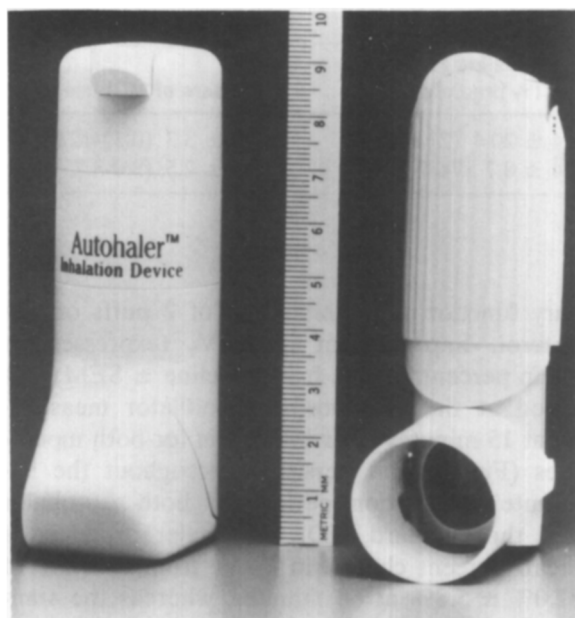


FIG. 1. Comparison of Autohaler Inhalation Device (*left*) with standard Maxair MDI (*right*). Autohaler contains approximately 400 doses, and standard Maxair MDI contains approximately 300 doses of pirbuterol acetate. A scale is included to show similarity of size.

with bronchodilation produced by inhalation of equal doses of pirbuterol acetate from a standard MDI in the same patients using a well-controlled and well-coordinated inhalation technique, carefully monitored in the laboratory.

METHODS

Twenty patients, aged 18 to 72, were studied (mean age, 44 ± 18 years). All were outpatients with stable asthma who had demonstrated at least a 15% improvement in forced expiratory volume in 1 second (FEV_1) after inhalation of β -agonist bronchodilating medication within the 12 months before testing. Other anti-asthma medications were withheld according to the following schedule. All inhaled medications, including β -agonist agents, steroids, cromolyn sodium, and anticholinergics were stopped 12 hours before the study. Theophylline designed to be taken on a 6-, 12-, or 24-hour basis was withheld for 12, 24, and 48 hours, respectively. Antihistamines and nasal steroids were withdrawn at least 24 hours before the study with the exception of hydroxyzine, which was stopped at least 96 hours before testing. Patients who were receiving oral corticosteroids or oral β -adrenergic agents were excluded from the study. No patients with other significant pulmonary or systemic disease were allowed to participate. Written informed consent was obtained from all patients, and the study was approved by the Human Subjects Review Committee of the University of California, Irvine.

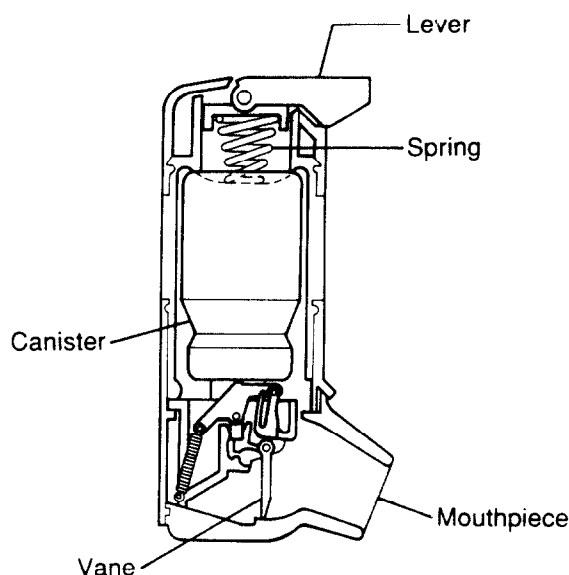


FIG. 2. Schematic representation of Autohaler Inhalation Device. See text for explanation of mechanism of action.

Study design and procedure

Although a double-dummy approach would have allowed double blinding, because this approach would necessarily double the number of measurement occasions and thereby increase the difficulty of achieving similar baseline conditions, the study was conducted as an open-label randomized trial. Patients inhaled 2 puffs of pirbuterol acetate from a standard MDI or from Autohaler on 2 separate days in the laboratory at the same time of day. Order of testing was determined by random computer-generated selection. In order to control standard MDI technique, an MDI (Maxair, 3M Pharmaceuticals) was connected in a series to a pneumotachygraph (Meriam Laboratories, Kansas City, MO.) and transducer (Validyne PM5, Chatsworth, Calif.), and flow rates versus time were displayed on a cathode ray tube (Electronics for Medicine DR12, White Plains, N.Y.). Patients practiced inhaling and exhaling at a steady rate of 0.5 L/sec. Subsequently, a metronome was also used to provide additional feedback and to assist patients in accomplishing constant inspiratory flow rate and breath-holding; in retrospect, the metronome neither assisted nor hindered patients' achievement of constant inspiratory flow. To ensure accurate timing of MDI activation, trained laboratory personnel actuated MDIs at the onset of patients inhalation of a full inspiratory capacity (i.e., functional residual capacity to total lung capacity). Patients wore nose clips and used a closed-lips inhalation technique. After inspiration, breath was held for 10 seconds. Two puffs separated by 1 minute were used. On days of Autohaler use, laboratory personnel demonstrated the technique for patients. Although it has been shown that the open-mouth technique is probably more efficacious

TABLE I. Characteristics of twenty patients

	N	Age (yr)	Baseline FEV ₁ (% predicted)	Years of MDI use
Male	16	45.8 ± 17.1 (21-72)	52.4 ± 20.4 (21.8-87.7)	3.9 ± 3.3 (0.33-12.0)
Female	4	29.0 ± 11.6 (18-44)	87.4 ± 8.7 (74.4-93.0)	5.4 ± 2.5 (3.0-8.75)

Values are expressed as means ± SD. Ranges are in parentheses.

than the closed-mouth technique, we chose the latter method because it is described in the package inserts of all MDIs. When using Autohaler, patients also wore nose clips and held their breath for 10 seconds. Patients waited 1 minute between the 2 puffs.

Pulmonary function studies

Spirometry, including measurement of FEV₁, forced expiratory flow between 25% and 75% of vital capacity (FEF₂₅₋₇₅), and forced vital capacity (FVC), was performed at baseline immediately before inhalation of the drug and at 15, 30, 60, and 90 minutes afterward with a dry rolling seal spirometer (Cardiopulmonary Instruments model 220; Houston, Texas) linked to an XY plotter (Cardiopulmonary Instruments model 750). The best result of three attempts for each variable was recorded in accordance with the current statement on the standardization of spirometry from the American Thoracic Society.²⁵ Baseline FEV₁ values for each patient on the 2 test days did not vary by more than 10%. (Actual mean difference was 0.35%.) Blood pressure and heart rate were also measured at baseline and at 15, 30, 60, and 90 minutes after administration of pirbuterol in accordance with the above schedule.

Data analysis

Absolute change and percent change from baseline of all three spirometric variables were calculated at each time of measurement. Mean percent change from baseline at each time was also calculated and used to compare bronchodilation produced by Autohaler with that produced by standard MDI. All data were statistically analyzed with analysis of variance with repeated measurements (MGLH 5.01, Systat, Evanston, Ill.).

RESULTS

Twenty patients completed the 2 days of testing. Patient characteristics are summarized in Table I. Eighteen of the 20 patients had asthma for more than 5 years, and the remaining two had asthma for 1 to 5 years. All 20 patients were currently using MDIs. Experience with MDIs ranged from 4 months to 12 years. None of the patients reported any side effects, and there were no adverse reactions.

Bronchodilator effects

Both Autohaler and standard MDIs produced similar and significant improvements in pulmo-

nary function after inhalation of 2 puffs of pirbuterol. Improvement in FEV₁ (expressed as mean percent change from baseline ± SEM) was noted at the first postbronchodilator measurement 15 minutes after inhalation for both modalities (Fig. 3) and persisted throughout the 90-minute observation period for both Autohaler and the standard MDI. Autohaler produced a mean percent change in FEV₁ from baseline of 32.0% ± 5.8% at 60 minutes, whereas the standard MDI produced a mean percent change from baseline of 31.1% ± 4.8% at 60 minutes. There were no statistically significant differences in the FEV₁ response between these two devices of the entire observation period ($p = 0.905$).

Results of FEF₂₅₋₇₅ and FVC were similar to those for FEV₁ (Fig. 3). Again, mean percent change from baseline over time for each of these variables was used to compare effectiveness of Autohaler with that of a standard MDI used optimally. Significant improvements were obvious by 15 minutes. Autohaler achieved mean percent change from baseline of FEF₂₅₋₇₅ of 45.3% ± 7% at 60 minutes, whereas percent change with use of a standard MDI was 44.6% ± 8.6%. Again, no statistically significant differences between the two techniques were observed at any time throughout the testing period ($p = 0.845$). Mean percent change from baseline of FVC was 23.0% ± 6.1% and 21.8% ± 5.6% for Autohaler and standard MDI, respectively. This difference was also not significant ($p = 0.801$).

Vital sign effects

No significant or consistent change in blood pressure or pulse was observed at any point during the treatment period for either Autohaler or standard MDI technique.

DISCUSSION

We compared bronchodilation produced by inhalation of the β_2 -adrenergic agonist drug pirbuterol from Autohaler, a new breath-activated MDI with that produced by an optimally controlled and properly coordinated inhalation of the same drug from its standard MDI. Both methods effectively increased FVC by more than 20%,

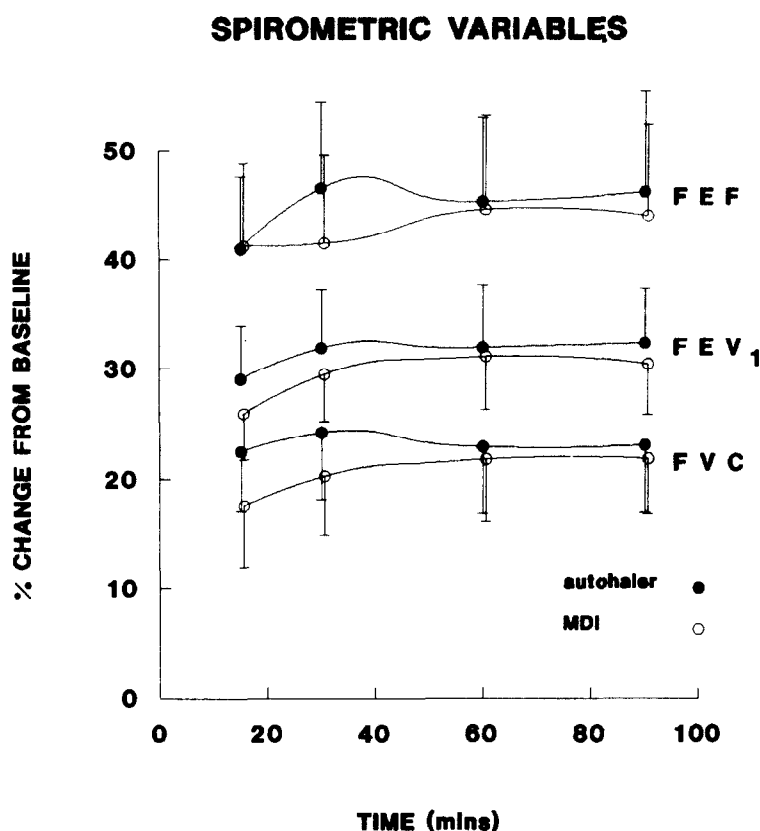


FIG. 3. Mean changes in FEF_{25-75} , FEV_1 , and FVC from baseline for 20 patients with asthma observed for 90 minutes. *Open circles* indicate effect of pirbuterol inhalation from a standard MDI with the use of a well-coordinated inhalation technique. *Closed circles* indicate effects of inhalation of pirbuterol from the Autohaler device. Data show that these two techniques produced no significant differences in bronchodilator response.

FEV_1 by more than 30%, and FEF_{25-75} by more than 40% (Fig. 3). Although the values obtained after use of Autohaler tended to be slightly higher than those obtained after use of a standard MDI, these differences were neither clinically nor statistically significant; in fact, assuming the same relative variance, study of an additional 75 patients for FVC, 95 more for FEV_{25-75} and 155 more FEV_1 would have been required for a statistically significant difference to be reached. These comparable effects indicate that Autohaler is as effective as a correctly used MDI.

There may be concern that 1 puff of pirbuterol (200 μ g) may be more appropriate than 2 puffs because the latter dose may have been at the top of the dose-response curve, thereby obscuring potential differences between the two devices. Littner et al.²⁶ found that FEV_1 continued to increase up to the highest dose delivered, 800 μ g.

To ensure conditions for reliable comparison between techniques, optimal MDI technique was a prerequisite for this study. We used an experimental setup that assured constant inspiratory flow rates of 0.5 L/sec and breath-holding of 10

seconds. Dolovich et al.⁴ have shown that inspiratory flow rates of 0.5 L/sec produce better results than higher flow rates, and postinhalation breath-holding for 10 seconds has been shown to be superior to shorter periods of breath-holding.^{2, 4, 27} Additionally, by activating the MDI for our patients at the onset of inspiration, we were able to compare Autohaler to a standard MDI under conditions in which discoordination had been eliminated. In a previous study that investigated effects of MDI discoordination on treatment of patients with asthma, Newman et al.² also controlled inhalation flow rate and breath-holding; they compared bronchodilation produced by another β -agonist, terbutaline sulfate, administered from an aerosol canister, which was actuated 3 seconds before, during and after inhalation. Their results clearly demonstrated that maximal bronchodilator effects occur when firing of aerosol canisters is properly coordinated with onset of inspiration²; they emphasized the critical nature of the need for patients to coordinate inhaler activation and inhalation. Indeed, most investigators and package inserts recommend and demon-

strate this technique when instructing patients in the use of inhalers.

Despite the seemingly simplistic appearance of MDI operation, proper technique does require a certain amount of knowledge and coordination skills. Fourteen to seventy-four percent of patients have been shown in previous studies to use MDIs incorrectly,^{6, 7, 28} and so-called hand-lung discoordination still remains the predominant problem in MDI misuse.²⁹

Overcoming inadequate inhalation technique by use of increased number of inhalations has generally been regarded as an unsatisfactory solution to this problem because of the potential for increased side effects, environmental concerns secondary to increased propellant release, increased cost, and increased possibility for development of drug tolerance.^{2, 14, 30-33} Moreover, most patients are neither aware of their inadequate technique nor the resultant losses in bronchodilation that occur.^{2, 14} Although it is possible to improve MDI technique through protracted instruction, one study has shown that 50% of patients who learned proper technique after extensive training had forgotten it when they were retested.⁵

Because Autohaler produces bronchodilation comparable to that produced by an optimally operated MDI, it provides a viable alternative to standard MDIs for those patients who cannot correctly coordinate their inhalation with MDI activation. Patients who would likely benefit from such a device include very young children, older adults, and those who are physically disabled or mentally handicapped. It has been suggested that during the extreme stress of an asthma attack in an anxious patient, the potential for discoordination may be increased,² and if so, then Autohaler may prove to be an asset in this situation as well. Because Autohaler is similar to MDIs in size and makeup, it may improve compliance compared with other devices that have been used to help alleviate discoordination.

Of course, Autohaler use is limited to patients whose peak inspiratory rate exceeds the minimum to activate the device: 0.5 L/sec. Flow rates of resting adults breathing at tidal volume is about 0.5 L/min. Small children, particularly when acutely obstructed, may not achieve the critical flow rate.¹⁰ Under these (or any other) circumstances, Autohaler can be used as a standard MDI.

Despite the potential difference between these two devices in metering chamber size (25 μ l for MDI used in Autohaler and 50 μ l for standard

pirbuterol MDI), they were otherwise similar with respect to dose released at the orifice (200 μ g) and particle size distribution (mass media aerodynamic diameter and geometric standard deviation were 2.92 and 1.58 for Autohaler vs 3.03 and 1.61 for standard pirbuterol MDI; 51% of Autohaler particles were in the respirable fraction [5 μ m or less] vs 45.5% for standard pirbuterol MDI).

Autohaler provides a simple-to-use, convenient, and reliable alternative to standard MDIs in patients with actual or potential discoordination problems. As a vehicle for maximizing delivery of aerosol bronchodilators, Autohaler should lead to more successful therapeutic outcomes in this patient population.

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REFERENCES

1. Newman SP, Clarke SW. Therapeutic aerosols. 1. Physical and practical considerations. *Thorax* 1983;38:881-6.
2. Newman SP, Clarke SW. How should a pressurized beta-adrenergic bronchodilator be inhaled? *Eur J Respir Dis* 1981;62:3-20.
3. Larsson S, Svedmyr N. Bronchodilatory effect and side effects of beta2-adrenoreceptor stimulants by different modes of administration (tablets, metered aerosol, and combination thereof): a study with salbutamol in asthmatics. *Am Rev Respir Dis* 1977;116:861-9.
4. Dolovich M, Ruffin RE, Roberts R, Newhouse MT. Optimal delivery of aerosols from metered dose inhalers. *Chest* 1981;80(suppl):911-5.
5. Shim C, Williams MH. The adequacy of inhalation of aerosol from canister nebulizers. *Am J Med* 1980;69:891-4.
6. Paterson IC, Crompton GK. Use of pressurized aerosols by asthmatic patients. *Br Med J* 1976;1:76-7.
7. Self TH, Brooks JB. Necessity of teaching patients correct bronchodilator inhalation technique. *Immunol Allergy Pract* 1982;4:40-4.
8. Orehek J, Gayard P, Grimand C, Charpin J. Patient error in use of bronchodilator metered aerosols. *Br Med J* 1976;1:76.
9. Crompton GK. Problems patients have using pressurized aerosols inhalers. *Eur J Respir Dis* 1982;63(suppl 119):101-4.
10. Pedersen S, Frost L, Arnfred T. Errors in inhalation technique and efficiency in inhaler use in asthmatic children. *Allergy* 1986;41:118-24.
11. Crompton G, Duncan J. Clinical assessment of a new breath-actuated inhaler. *Practitioner* 1989;233:268-9.
12. Kelly JS, Strohl KP, Smith RL, Altose MD. Physician knowledge in the use of canister nebulizers. *Chest* 1983; 83:612-4.
13. Lindgren S, Bake B, Larsson S. Clinical consequences of inadequate inhalation technique in asthma therapy. *Eur J Respir Dis* 1987;70:93-8.
14. Saunders KB. Misuse of inhaled bronchodilator agents. *Br Med J* 1965;1:1037-8.
15. Hiding KG, Perk J. Clinical trial of a modified inhaler for pressurized areosols. *Eur J Clin Pharmacol* 1981;20: 109-11.

16. Pederson S. Aerosol treatment of bronchoconstriction in children with or without a tube spacer. *N Engl J Med* 1983;308:1328-30.
17. Bloomfield P, Crompton GK, Winsey NJP. A tube spacer to improve inhalation of drugs from pressurized aerosols. *Br Med J* 1979;2:1479.
18. Wilson AF. Aerosol delivery systems. In: Weiss EB, Stein M, eds. *Bronchial asthma: mechanisms and therapeutics*. 3rd ed. Boston: Little Brown, 1993.
19. Newman SP, Weisz AWB, Talaei N, Clarke SW. Improvement of drug delivery with a breath actuated pressurized aerosol for patients with poor inhaler technique. *Thorax* 1991;44:712-6.
20. Coady TJ, Davies HJ, Barnes P. Evaluation of a breath actuated pressurized aerosol. *Clin Allergy* 1976;6:1-6.
21. D'Arcy PF, Kirf WF. Development of a new device for inhalation therapy. *Pharm J* 1971;206:306-7.
22. Baum EA. Design, development and testing of a new breath actuated inhaler. *Proceedings of the Medicine Publishing Foundation Symposium Series 26. "Inspiration: Development in Inspiratory Therapy"*; York, England. Oxford: Medicine Publishing Foundation; 1989:20-30.
23. Baum EA, Bryant AM. The development and laboratory testing of a novel breath actuated pressurized inhaler. *J Aerosol Med* 1988;3:219-20.
24. Baum EA, Bryant AM. Design, development and testing of a new breath actuated aerosol inhaler. *Proceedings of the Aerosol Society Third Annual Conference: Aerosols, Their Generation, Behavior and Application*; West Bromwich, England. London: Aerosol Society, 1989:235-40.
25. Gardner RM, Baker CD, Broennle AM, Jr, et al. ATS statement Snowbird workshop on standardization of spirometry. *Am Rev Respir Dis* 1979;119:831-8.
26. Littner MR, Tashkin DP, Calvarese B, Bautistia M. Acute bronchial and cardiovascular effects of increasing doses of pirbuterol acetate in asthma. *Ann Allergy* 1982;48:14-20.
27. Newman SP, Clarke SW. Inhalation technique with aerosol bronchodilators: does it matter? *Practical Cardiol* 1983;9:157-64.
28. Epstein SW, Manning CPR, Ashley MJ, Corey PN. Survey of the clinical use of pressurized aerosol inhalers. *Can Med Assoc J* 1979;120:813-6.
29. Munt PW. Aerosol bronchodilators and hand lung discoordination. *Can Med Assoc J* 1979;120:781.
30. Newman SP. Aerosol deposition considerations in inhalation therapy. *Chest* 1985;88(suppl):1525-1605.
31. Molina MJ, Rowlands FS. Stratospheric sink for chlorofluoromethanes: chlorine atom catalyzed destruction of ozone. *Nature* 1974;249:1810-2.
32. Holgate ST, Stubbs W, Alberti KGMM, Tattersfield AE. Bronchial and metabolic resistance to beta adrenergic bronchodilators. *Thorax* 1977;32:643.
33. Nelson HS, Raine D, Doner HC, Posey WC. Subsensitivity to the bronchodilator action of albuterol produced by chronic administration. *Am Rev Respir Dis* 1977;116: 871-8.

Characterization of grass group I allergens in timothy grass pollen

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Using Phl p V-depleted timothy grass pollen extract (Phleum pratense) as immunogen, we obtained a monoclonal antibody, QG 4, which recognized proteins of 33, 35, and 37 kd as determined by Western blotting. The antibody cross-reacted with pollen proteins of other grass species in the molecular weight range of 30 to 37 kd. By means of two-dimensional polyacrylamide gel electrophoresis blot of timothy grass pollen extract, we demonstrated at least seven protein spots: two of 37 kd with isoelectric points of 6.4 and 6.6; four of 35 kd with isoelectric points of 6.5, 6.8, 7.1, and 7.3; and one of 33 kd with an isoelectric point of 8.5. These protein spots were also detected by patients' pooled serum. Microsequencing of the 20 N-terminal amino acid residues revealed structures with sequence identities up to 90% to the well-established allergen, Lol p I of ryegrass (Lolium perenne). Therefore we assume that the monoclonal antibody QG 4 recognized the corresponding allergen Phl p I in timothy grass pollen. (J ALLERGY CLIN IMMUNOL 1993;92:789-96.)

Key words: Grass pollen allergens, immunoblotting, microsequencing, two-dimensional gel electrophoresis

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