

Duration and magnitude of action of 50 and 100 µg of inhaled salmeterol in protecting against bronchoconstriction induced by hyperventilation of dry cold air in subjects with asthma

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Salmeterol is a new long-acting and highly selective β_2 -agonist agent. Clinical studies have shown that it has a very long (at least 12 hours) duration of action in terms of its bronchodilator effect¹ and that its effect on bronchial hyperresponsiveness may even be longer. In a recent study we showed that salmeterol administered in a single dose of 50 µg still had a complete blocking effect on hyperventilation-induced asthma in four of 12 subjects (one third) who were tested up to 12 hours later.² The recommended dose of this preparation varies between 50 and 100 µg every 12 hours.

The aim of this study was to expand our previous work on characterizing the magnitude and duration of action of inhaled salmeterol on hyperventilation-induced bronchoconstriction in a more detailed way, especially on the interval between 12 and 24 hours and later after its administration. We also wanted to compare the efficacy of two different doses, 50 and 100 µg.

METHODS

Subjects

Twelve adult subjects who satisfied the criteria for the diagnosis of asthma³ and were in a clinically stable state (no nocturnal awakenings because of asthma, no

Abbreviations used

FEV₁: Forced expiratory volume in 1 second
PD₂₀: Provocative dose (of cold air) causing a 20% fall in FEV₁
SD_B: Baseline standard deviation

recent modification in their need for a bronchodilator) were included in the study. The project was accepted by a local ethics committee, and a written consent form was obtained from each participant.

Study design

We conducted a three-treatment crossover double-blind study. Subjects came to the hospital for four series of tests, each of which lasted for a maximum period of 40 hours. For the first series of tests (days 1 and 2), subjects arrived at 6:00 PM, and after a 15-minute rest in the laboratory, spirometry was assessed. The baseline within-day (or more precisely, within 36-hour) variability of the response of cold air was first determined by repeating the test several times, once to establish the baseline (time 0) and then 1, 12, 18, 24, and 36 hours later. The mean baseline provocative dose of cold air causing a 20% fall in forced expiratory volume in 1 second (FEV₁) (PD_{20B}) and the baseline SD (SD_B) were assessed in each subject. The subjects did not use any bronchodilators during this 36-hour period. Baseline FEV₁ had to be within $\pm 10\%$ of the first value before each cold air challenge.

For the next three series of tests, the subjects also arrived at 6:00 PM. After a rest of 15 minutes, spirometry was assessed. Baseline FEV₁ had to be within 10% of the first value obtained on day 1 before for first cold air challenge was performed. The result of the test had to be reproducible from one visit to the next (i.e., less than 2 between-day SDs from the mean PD_{20B}). On the 3 treatment days, after functional recovery from the first cold air challenge (FEV₁ within 10% of baseline), the study drug was administered with a standard me-

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Supported by a grant from Glaxo Canada, Inc., Bureau d'affaires du Québec.

J. L. Malo is a research fellow with the Fonds de la Recherche en Santé du Québec and the Université de Montréal School of Medicine.

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J ALLERGY CLIN IMMUNOL 1993;92:488-92.

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0091-6749/93 \$1.00 + .10 1/54/47372

tered-dose inhaler under the direct supervision of the technician. The treatment was assigned in a double-blind and random way. Subjects were given 50 μg of salmeterol (2 puffs of 25 μg + 2 puffs of placebo), 100 μg of salmeterol (2 + 2 puffs of 25 μg) or a placebo (2 + 2 puffs of placebo). Pulse rate, systolic and diastolic blood pressure, and tremor were assessed before and 15 minutes after the drug was inhaled and again with each subsequent cold air challenge. Cold air challenges were repeated 1 and 12 hours after administration of the treatment drug. If there was no evidence of a blockade of the bronchial response to cold air (PD_{20} within 1 SD_B of the standardized baseline PD_{20} value [see below]), the study was terminated. However, if there was a suggestion of a blockade (value greater than standardized baseline $\text{PD}_{20} + 1 \text{SD}_B$), the cold air challenge was repeated after 18 hours. The same criteria were applied to determine whether there was a need to continue the study and repeat the cold air challenge after 24 and 36 hours.

There was an interval of at least 7 days between each drug treatment, and all three treatments were administered within 4 weeks. All subjects were receiving inhaled beclomethasone. They were challenged in a constant relationship to their last dose, and the dose of inhaled corticosteroid was kept constant during the study period.

Cold air inhalation test

The cold air inhalation test was performed with the Airjet Breathing Device (FTS System, Stoneridge, N.Y.) as described elsewhere.⁴

Analysis of results

Dose-response curves to cold air were drawn on a noncumulative semilogarithmic scale by relating the ventilatory equivalent on the abscissa and the percentage change in FEV_1 on the ordinate. PD_{20} was interpolated on the dose-response curve. PD_{20} was extrapolated in the case of changes in FEV_1 from 16 to <20% because the curve is linear for these intervals.⁴ Reference values for FEV_1 and $\text{FEV}_1/\text{forced vital capacity}$ were taken from Knudson et al.⁵ The logarithmic transformation of PD_{20} was used in the analysis of results.

The baseline within-day variability of PD_{20} assessed by the SD_B was determined for each subject by studying the distribution of the PD_{20} results on days 1 and 2 at times 0, 1, 12, 18, 24, and 36 hours. The between-day variability of PD_{20} results was assumed to be similar to the within-day variability of baseline PD_{20} . If the latter was between 0.5 and 2 times the recognized between-day SD of PD_{20} in our laboratory (i.e., 0.28 on a log_e scale),⁴ this value was kept for analysis (which was the case in 11 of 12 instances); if it was less than 0.5 times, the between- and within-day variability of baseline PD_{20} for any individual subject was assumed to be 0.14 on the log_e scale. (This was the case for subject no. 2 only.) The subject would not have been included in the

analysis of results if the value were more than 2 times the SD_B .

To define the change in PD_{20} between time intervals on a treatment day, we used the following formula: $[\text{PD}_{20}(0) + \sqrt{6} (\text{mean baseline } \text{PD}_{20} \text{ at times 0, 1, 12, 18, 24, and 36 hours of baseline days 1 and 2})]/1 + \sqrt{6}$ where $\text{PD}_{20}(0)$ = value of PD_{20} at time "0" on treatment days. A significant blocking effect on the response to cold air was defined as a PD_{20} at time "X" that was greater than standardized baseline $\text{PD}_{20} + 2 \text{SD}_B$. Return to baseline was defined as PD_{20} within standardized baseline $\text{PD}_{20} + 1 \text{SD}_B$. The duration of the blocking effect of each treatment was therefore determined by the interval required to return to baseline, as defined above, provided that a blocking effect had been found at time "X" + 1 hour. If there was no significant effect at that time, the effect of the drug was defined as nil. Any change in PD_{20} on treatment days was therefore corrected for the within-day variability of PD_{20} as observed on days 1 and 2 (standardized change) and was considered significant when it exceeded 2 (i.e., more than 2 SDs over the standardized baseline PD_{20}). A value between 2 and 6 was considered to be a partial blockade. A complete blockade was defined as a change of less than 15% in FEV_1 even after maximal voluntary ventilation was achieved, corresponding to a standardized change greater than 6. Categorical analysis of the results (no blockade, partial blockade, or complete blockade) was performed with logistic regression for ordered categorical variables.

RESULTS

The mean age of the 12 participating subjects was 46 ± 15 years, and the duration of asthma was 19 ± 18 years. All took inhaled beclomethasone at a daily dose varying from 200 to 2000 μg . Mean baseline FEV_1 value was $74\% \pm 15\%$. Eight subjects had FEV_1 values less than 80% of the predicted value, and six had an FEV_1 forced vital capacity ratio of less than 70%. The first PD_{20} value obtained on day 1 varied from 15 to 64 L/min (mean of 32 L/min), a wide range of values.⁴ Baseline FEV_1 and PD_{20} were not significantly different on each study day ($F = 1.9$ and 1.8, respectively; $p > 0.05$). Within-day variability was not different from the between-day variability except in one subject. The duration of the blocking effect was 2.4 ± 5.3 hours for placebo, 19.6 ± 16.1 hours for salmeterol at a dose of 50 μg , and 19.8 ± 15.6 for salmeterol at a dose of 100 μg . The magnitude of the blocking effect in terms of changes in the standardized baseline PD_{20} (see Methods) was 1.2 ± 0.3 , 11.5 ± 4.8 , and 9.9 ± 5.2 , 1 hour after the drug was given for placebo, salmeterol 50 μg and salmeterol 100 μg , respectively. The corresponding figures were 1.2 ± 0.4 , 6.6 ± 6 , and 6.2 ± 6 , respectively 12

TABLE I. Actual PD₂₀ values (in liters per minute) at various intervals according to treatment days

Subject No.	Baseline	Time intervals in relation to drug administration				
		1 hr	12 hr	18 hr	24 hr	36 hr
1 Placebo	39	47	47	49	ND	ND
Salmeterol 50 µg	50	99	42	44	ND	ND
Salmeterol 100 µg	52	101	101	59	65	50
2 Placebo	40	81	38	ND	ND	ND
Salmeterol 50 µg	37	78	78	35	ND	ND
Salmeterol 100 µg	39	47	81	81	33	ND
3 Placebo	80	34	NA	72	ND	ND
Salmeterol 50 µg	94	NA	NA	48	ND	ND
Salmeterol 100 µg	53	NA	48	38	ND	ND
4 Placebo	67	58	63	ND	ND	ND
Salmeterol 50 µg	52	NA	NA	NA	101	NA
Salmeterol 100 µg	53	NA	NA	NA	NA	NA
5 Placebo	17	NA	NA	13	ND	ND
Salmeterol 50 µg	22	16	25	18	ND	ND
Salmeterol 100 µg	19	57	13	21	ND	ND
6 Placebo	29	33	32	ND	ND	ND
Salmeterol 50 µg	35	NA	46	24	ND	ND
Salmeterol 100 µg	50	NA	34	44	ND	ND
7 Placebo	42	20	59	40	ND	ND
Salmeterol 50 µg	49	NA	NA	36	ND	ND
Salmeterol 100 µg	37	NA	NA	35	ND	ND
8 Placebo	35	32	30	ND	ND	ND
Salmeterol 50 µg	34	29	49	27	ND	ND
Salmeterol 100 µg	44	60	40	38	ND	ND
9 Placebo	31	35	10	ND	ND	ND
Salmeterol 50 µg	22	NA	72	76	NA	57
Salmeterol 100 µg	43	44	14	49	ND	ND
10 Placebo	47	55	28	39	ND	ND
Salmeterol 50 µg	26	NA	27	23	ND	ND
Salmeterol 100 µg	27	NA	28	32	ND	ND
11 Placebo	46	33	ND	41	ND	ND
Salmeterol 50 µg	54	96	94	94	94	62
Salmeterol 100 µg	30	79	79	79	54	49
12 Placebo	72	82	72	ND	ND	ND
Salmeterol 50 µg	75	NA	106	80	ND	ND
Salmeterol 100 µg	40	73	61	63	61	87

NA, Fall in FEV₁ ≤ 15% achieved at maximum voluntary ventilation; ND, no test done.

hours after the drug was given. The individual PD₂₀ values are shown in Table I. The changes were comparable for the two different doses of salmeterol. The number of subjects who had complete and partial blockades at each of the time intervals is shown in Table II. There was a significant difference in the proportion of subjects who were still experiencing the blocking effect 12 hours after inhaling the two active preparations by comparison with the placebo ($p = 0.03$), but there was no significant difference between the two active preparations ($p > 0.05$). There was also a significant difference in the proportion of

subjects still experiencing the blocking effect at 18 hours when placebo was compared with active preparations but not when the two active drugs were compared. The only subject who showed a complete blockade 36 hours after inhaling both active formulations was rechallenged 42 hours after the 100 µg dose and still had complete blockade. The proportion of subjects who experienced complete and partial blockade was not significantly different in relation to the daily use of beclomethasone (five subjects took ≤ 1000 µg daily as compared with seven who required a larger dose).

TABLE II. Number of subjects who showed complete and partial blockade after inhaling drug

	Hours after administration				
	1	12	18	24	36
Complete blockade					
Placebo	2	2	0	0	0
Salmeterol 50 µg	10	5	2	1	1
Salmeterol 100 µg	8	5	3	1	1
Partial + complete blockade					
Placebo	2	2	0	0	0
Salmeterol 50 µg	10	8	3	3	2
Salmeterol 100 µg	11	6	5	3	2

Any change in PD_{20} on treatment days was corrected for the within-day variability of PD_{20} as observed on days 1 and 2 (standardized change) and was considered significant when it exceeded 2 (i.e., more than 2 SDs over the standardized baseline PD_{20}). A value between 2 and 6 was considered to be a partial blockade. A complete blockade was defined as a $<15\%$ change in FEV_1 even after maximal voluntary ventilation was achieved, corresponding to a standardized change >6 . The study had 80% power to detect a difference of 7 subjects between any two groups for both complete and partial + complete blockades at 1 and 12 hours.

There were no significant changes in systolic and diastolic blood pressures or in pulse rate at any of the time intervals for those receiving the active medication in comparison with those receiving placebo. Tremor was present in one subject who was receiving 50 µg of salmeterol and in three after they were given the 100 µg dose. Other side effects included difficulty in sleeping in two subjects.

DISCUSSION

We previously showed that a single inhalation of 50 µg of salmeterol could block the bronchoconstriction induced by hyperventilation of cold dry air for an interval of 16 hours.² The present study extends these findings in two ways. First, by repeating the hyperventilation test on several occasions after 12 hours, which was not done in the previous study, we were able to show that the mean duration of the blocking effect is almost 20 hours. Second, by giving two doses of salmeterol (50 and 100 µg) instead of only one (50 µg) as in our previous study, we showed that the magnitude and duration of effect were comparable. The majority of previous studies on salmeterol used only one single dose (generally 50 µg) to evaluate its acute effect in terms of either bronchodilation or of its blocking effect on bronchial hyperresponsiveness, another key feature of asthma. These results are relevant because it is important to set the ideal dose of a β_2 -adrenergic agent in regard to its bronchodilator and bronchoprotector effects, since questions have been raised about possible deleterious effects of short-acting β_2 -adrenergic agents in the treatment of asthma.

Derom et al.⁶ recently assessed the blocking effect of 50 and 1000 µg of salmeterol in 12 subjects with asthma who were followed up for up to 12 hours after methacholine testing. The magnitude of the protective effect was greater with the 100 µg dose as compared with the 50 µg dose at 1, 4, 8, and 12 hours after administration of the drug. No test was carried out at later intervals. These results are different from our findings: we did not detect any significant difference in either the magnitude or duration of the blocking effect between 50 and 100 µg. There is no explanation for these discrepancies other than that the stimuli were different and there was no correction for the within-day variability of methacholine responsiveness in the study by Derom et al.⁶

We used a study design in which we first assessed the within-subject variability of responsiveness to hyperventilation of unconditioned air. This allows for a proper estimate of the magnitude and the duration of the effect, since any change that occurs after administering a medication can occur by chance. The within-subject variability was comparable to that obtained in a previous study.⁴

In conclusion, our findings would suggest that a dose of 50 µg has a blocking effect similar to that of 100 µg on bronchial responsiveness assessed by a natural stimulus, hyperventilation of unconditioned air, as well as a lower occurrence of tremor. Furthermore, even if the administration modality of every 12 hours is usually suggested, the duration of the blocking effect was longer than this interval in at least half of the subjects included in the study. It would be interesting to

compare the duration of the bronchodilator and blocking agents on bronchial responsiveness in subjects with asthma assessed on two occasions because it can be postulated that the duration of the blocking effect can be longer than the bronchodilator effect. Discrepancies between the bronchodilator and the bronchoprotective effects can be related to the number of available β_2 -receptors.

We thank Réal Laliberté, PhD, from Glaxo Canada, Inc., Bureau d'affaires du Québec for invaluable and sustained collaboration, and Katherine Tallman for reviewing the manuscript.

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Papain-induced allergic rhinoconjunctivitis in a cosmetologist

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Papain is a proteolytic enzyme extracted from the sap of the papaya tree and fruits (*Carica papaya*). Papain has been widely used in medicine as a digestive, in treatment of insect and jelly-fish stings, in intradiscal therapy of lumbar disc protrusions, and in debridement of ulcers and necrotic tissue. Papain can be found in digestive aids, douche powders, meat tenderizers, and in beer chill-proofing agents. Additionally, papain

Abbreviation used
SPT: Skin prick test

can be used in tanning of skins and hides, in chewing gum manufacture, and in caroid tooth powder.¹

Papain is highly immunogenic in both human beings and animals. Papain has caused allergic asthma, rhinitis, and even a case of lethal anaphylaxis.¹⁻⁴ Here we report the first case of papain-induced hypersensitivity associated with cosmetics in a beauty salon worker after use of abrasive cream containing papain on her clients.

CASE REPORT

The patient, a 30-year-old nonatopic woman, had been the owner of a beauty salon for 5 years. The patient had used an abrasive cream, which was prepared by mixing two powders into a moisturizing cream (Piana Cosmetici, Bologna, Italy). One powder in-

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Supported in part by the Finnish Work Environment Fund, Helsinki, Finland.

J ALLERGY CLIN IMMUNOL 1993;92:492-3

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