Bronchoprotective Effects of Single Doses of Salmeterol Combined With Montelukast in Thermally Induced Bronchospasm*

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Study objectives: Salmeterol (S) and montelukast (M) individually inhibit the obstructive consequences of thermal stimuli such as exercise and hyperventilation (HV), but there is no information on whether these drugs can interact positively.

Design: Randomized trial.

Setting: University teaching hospital.

Participants: Atopic asthmatic patients with sensitivity to thermal provocations.

Interventions: Eleven asthmatic patients generated stimulus-response curves to isocapnic HV while breathing frigid air without any interventions and then after pretreatment with 42 μg of S, 10 mg of M, and the combination. The order of testing was randomly determined.

Measurements and results: Minute ventilation (V\text{˙}E) was increased in 20-L increments until FEV\textsubscript{1} fell ≥ 15%. Measurements were obtained before and 1 h after drug administration, and then again 5 min after each bout of HV. In the nonintervention trial, the provocation commenced after the patients presented to the laboratory. In the control challenge, the mean (± SEM) FEV\textsubscript{1} decreased 24.6 ± 1.7% from baseline. S and M both increased the mean prechallenge FEV\textsubscript{1} significantly (S, 10.4 ± 1.7% [p < 0.01]; M, 4.1 ± 1.3% [p = 0.02]; S + M, p = 0.01). The combination of S + M produced greater bronchodilatation (mean improvement, 12.4 ± 2.3%) than M alone (p = 0.004), but not greater than S alone (p = 0.80). Both drugs blunted the obstructive response similarly (protection: M, 34.6 ± 15.1%; S, 60 ± 8.7%; p = 0.13). The benefits added arithmetically with the combined regimen (protection with S + M, 84.9 ± 5.5%; p = 0.01 vs S alone; p = 0.003 vs M alone).

Conclusion: These data indicate that the concurrent administration of single standard doses of S and M appears to provide greater protection against thermal stimuli than does either drug alone. Further experimentation will be required to ascertain whether the combination will provide additional clinical benefits to patients over those of the single agents.

Key words: airway hyperresponsiveness; bronchospasm; montelukast; prophylaxis; salmeterol; thermally induced asthma

Abbreviations: EIB = exercise-induced bronchospasm; HV = isocapnic hyperventilation; V\text{˙}E = minute ventilation; M = montelukast; S = salmeterol

The bronchial hyperresponsiveness of asthma is believed to derive from chronic inflammation of the airways and is characterized by hypersensitivity to a variety of stimuli, including thermal agonists such as exercise and voluntary hyperventilation (HV).¹,² Both salmeterol (S) and montelukast (M) are known to attenuate the obstructive response to these provocations,³–¹² but they appear to do so through different mechanisms. S is a potent bron-

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Materials and Methods

Eleven nonsmoking asthmatic volunteers who were recruited from advertisements in the clinics of MetroHealth Medical Center served as our subjects. The study admission criteria included a history of atopy, an improvement in forced expiratory volumes of ≥15% following an antihistamine challenge, sensitivity to thermal provocations, a prechallenge FEV1 ≥60% of predicted, the absence of comorbid pulmonary or systemic illnesses, and an ability to withhold antiasthma medications sufficiently long to undergo bronchial provocation testing. Asthma was defined according to the criteria outlined in the National Asthma Education and Prevention Program guidelines. Sensitivity to thermal provocation was considered to be present if there were symptoms of airway obstruction associated with a decrease in FEV1 of ≥15% after exertion or isocapnic HV. None of the participants smoked tobacco products, had symptoms of an upper respiratory tract infection in the 6 weeks preceding the study, or were receiving oral corticosteroids. To mimic the conditions of daily living, subjects continued receiving their antiasthma medications during the course of the trial and only stopped receiving them temporarily before undergoing a challenge. As in previous studies, therapy with bronchodilators was withheld for ≥24 h, and therapy with long-acting decongestants, specifically H1 antihistamines, and leukotriene-modifying medications was not permitted for 7 days before any investigation. Therapy with inhaled glucocorticoids was continued in patients who were receiving them to prevent deterioration in their disease state. The doses were stable for a minimum of 1 month before the initiation of the protocol and remained constant throughout it. The subjects refrained from receiving medications for 24 h before the study. The recommendations of the Helsinki Declaration guiding physicians in the performance of biomedical research involving human subjects were followed. The institutional review board for human investigation approved the protocol, and all participants gave informed consent.

Isocapnic HV of frigid air was employed to assess airway hyperreactivity, and stimulus-response relationships were generated using standard techniques. Minute ventilation (Ve) was progressively increased while the subjects inhaled through a heat exchanger. Hyperpnea started at 20 L/min and progressed in intervals of 20 L/min until the FEV1 fell ≥15%. Each bout of HV lasted 4 min, and recovery took place with room air. Frigid air was inhaled to ensure the maximum degree of obstruction. The water content of the inspire during hyperpnea was <1 mg H2O/L, which, for the purposes of this study, was considered to be zero. The expired air was directed away from the heat exchanger into a reservoir balloon that was being constantly evacuated at a known rate through a calibrated rotameter. The subjects were coached to keep the balloon filled, and, in so doing, their Ve could be controlled at any desired value. The level of ventilation was then verified directly with a dry gas meter. End-tidal concentrations of CO2 were monitored (Nellcor N1000 analyzer; Mallinckrodt; Kansas City, MO), and sufficient CO2 was added to the inspiratory port of the exchanger during hyperpnea to maintain the end-tidal concentrations at eucapnic levels. The provocation was stopped when the FEV1 decreased to the desired threshold. In cases in which the FEV1 did not fall to this level post-drug administration, the value that followed the largest sustainable Ve was employed in the analysis.

Maximum forced exhalations were performed in triplicate using a waterless spirometer. Data were collected when the patient presented to the laboratory, and 1 h after the administration of S and M individually and in combination. This interval was chosen because it allowed us to measure bronchodilator effects and because previous studies had shown that it coincided with the development of substantial prophylaxis. The 1-h post-drug administration data served as the baseline for the subsequent challenges. In the nonmedication trial, HV commenced within 60 min of the patient entering the laboratory. In each experiment, spirometry was repeated 5 min after the cessation of hyperpnea. The curves with the largest FEV1 were chosen for analysis.

The study was performed in four parts. In the control trial, the subjects underwent HV without pretreatment. In the three intervention experiments, they were given either 2 puffs of S (42 µg) [Serevent; GlaxoSmithKline; Research Triangle, NC), 10 mg of oral M (Singulair; Merck; Whitehouse Station, NJ), or the combination. The challenges were undertaken at the same time of day, and the order was randomly determined by drawing one of four unmarked envelopes from a subjects’ file that contained the various investigative arms. The drugs were obtained from the hospital pharmacy and were administered in the laboratory after the initial spirometry. There was no involvement in the study or support provided by industries that either made the test agents or their competitors. Only one experiment was performed per day, and each provocation was separated by a week.

The fall in the FEV1 from baseline in the control experiment and the decreases from the 1-h postintervention values were the primary end points. The corresponding ventilations required to produce these decrements were also compared. The degree of protection offered by each intervention was computed as the difference between the fall in FEV1 in the control and drug challenges divided by the control changes.

The study was powered to detect a ≥50% difference between the results with S administration and administration of the combination of S and M. These regimens were chosen because they were thought to be the ones most apt to provide statistically similar results. The data were analyzed by one-factor analysis of variance and paired t tests. All statistical tests were two-sided, and a p value of <0.05 was considered to be significant.

Results

Our subjects consisted of seven women and four men with a mean (±SEM) age of 34 ± 3 years (Table 1). The mean FEV1 on study entrance was 76.3 ± 3.7% predicted. At enrollment, 10 people were routinely receiving one or more antiasthma medications. Two subjects were receiving S, 10 were receiving albuterol, 5 were receiving inhaled steroids, 3 were receiving antileukotriene agents, and 1 was receiving a selective antihistamine.

The mean values for the prechallenge FEV1 were

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The administration of S, the FEV1 rose a mean of 76.1% (p = 0.01). The degree of bronchodilatation of M was not significantly larger than that with S alone (p = 0.80), but was more than that seen with M alone (p = 0.004).

In the control experiment, hyperpnea produced a stimulus-response reduction in pulmonary function (Fig 2). The maximum mean decrement in FEV1 was 24.6 ± 1.7% (p < 0.001). The administration of S shifted the relationship up and to the right, and significantly reduced the severity of the response. After S administration, the maximum mean fall in FEV1 from before to after challenge was 10.1 ± 2.6% (p < 0.001 [vs the control group]). M also attenuated airway responsiveness (maximum mean change in FEV1 from before to after challenge, 15.8 ± 3.4%; p = 0.02 [vs control]). The effect of M was statistically identical to that of S in magnitude (p = 0.08), but was less consistent. Two subjects, (Nos. 3 and 6) were unresponsive, and in two other subjects (Nos. 6 and 10) the impact of M equaled or exceeded that of S. The individual data for therapy with the combination of S and M also showed heterogeneity. In four subjects (Nos. 2, 4, 5, and 7), the effect of combination therapy with S and M was similar to or less than therapy with S. In the other subjects, the effect of combination therapy with S and M was either slightly or markedly better. The mean data indicate that the combination therapy was significantly more effectual than the individual components (maximum mean change in FEV1 from before to after combination therapy with S and M, 4.3 ± 1.6%; vs control group, p < 0.001; vs S group, p = 0.02; vs M group, p = 0.002).

The administration of M reduced bronchial obstruction by 31.2 ± 15.2% from that of the control group. The administration of S blocked 54.2 ± 9.7% of the response, but the difference between the effects of the two drugs was not significant (p = 0.07). Following administration of both agents, the airway narrowing was attenuated by 82.2 ± 6.2% (vs M, p = 0.003; vs S, p = 0.01).

Without treatment, the control decrement in FEV1 developed at a mean V̇e of 53.6 ± 5.8 L/min (Fig 3). The administration of M reduced the severity of the airflow limitation without significantly influencing ventilation (V̇e, 58.6 ± 7.6 L/min; p = 0.25 [vs control group]). In contrast, the administration of S was associated with an increase in mean V̇e to 76.6 ± 6.1 L/min (vs control group, p = 0.005; vs M, p = 0.03). In the combination experiment, the V̇e achieved (76.4 ± 4.8 L/min) did not differ from that found after administration of S alone (p = 0.97), but was significantly larger than that observed with administration of M alone (p = 0.05).

### Table 1—Demographic and Prechallenge Clinical Data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Gender</th>
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<tr>
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<td>M</td>
<td>76.2</td>
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<td>M</td>
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<tr>
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<td>48</td>
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<td>F</td>
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<tr>
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<td>AA</td>
<td>F</td>
<td>65.8</td>
<td>A, IS, S, AL</td>
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<tr>
<td>11</td>
<td>35</td>
<td>AA</td>
<td>F</td>
<td>65.2</td>
<td>A, H</td>
</tr>
</tbody>
</table>

*W = white; AA = African-American; M = male; F = female; A = albuterol; AL = antileukotrienes; IS = inhaled steroids; H = antihistamines.

constant for each trial, and ranged between 2.55 ± 0.27 and 2.62 ± 0.27 L. There were no significant differences between the experimental arms (p = 0.99). The mean inspired temperature remained steady between experiments and varied from −5.5 ± 0.2 to −6.9 ± 0.2°C (p = 0.33).

The impact of the active agents on pulmonary mechanics is shown in Figure 1. One hour after the administration of S, the FEV1 rose a mean of 10.4 ± 1.7% over the corresponding baseline values (p < 0.01). The mean improvement with M was 4.1 ± 1.3% (p = 0.02), and the combination effected a 12.4 ± 2.3% increase (p < 0.001). The degree of bronchodilatation was greater with S than with M (p = 0.01). The degree of bronchodilatation with the combination of S and M was not significantly larger than that with S alone (p = 0.80), but was more than that seen with M alone (p = 0.004).

![Figure 1. Effect of the active agents on FEV1. The heights of the bars represent mean values, and the brackets represent 1 SEM. The solid bars are the pretreatment data, and the open ones indicate the changes that occurred 1 h after administration of the drug. Rx = treatment.](image-url)

Figure 1. Effect of the active agents on FEV1. The heights of the bars represent mean values, and the brackets represent 1 SEM. The solid bars are the pretreatment data, and the open ones indicate the changes that occurred 1 h after administration of the drug. Rx = treatment.
Discussion

The results of the present investigation demonstrate that the use of single standard doses of S and M in combination provide greater protection against the obstructive consequences of isocapnic HV of frigid air more than either drug alone. Within 60 min of administration, M and S individually attenuated between 30% and 54% of the airflow limitation in the control trial, respectively; however, the mixture blunted 82%, indicating an additive effect. Since thermal events, particularly exercise-induced bronchospasm (EIB), are real-world stimuli that can

Figure 2. Individual stimulus-response relationships generated in each of the experimental arms. The ordinate indicates the FEV₁ in liters, and the abscissa indicates the VE in liters per minute. The solid squares and line represent the control observations. The open circles and dotted lines are the S experiments. The open squares and dashed lines present the M data, and the solid circles and solid lines indicate data from the combination trial with S and M.
Adversely influence the lives of virtually all patients with asthma, information of this type, when appropriately validated, could ultimately prove to be of great clinical value.

β-adrenergic agonists have been the treatment of choice for EIB for a considerable time. In the last 15 years, the therapeutic benefits of the antileukotriene agents in the treatment of this condition have been recognized. However, there are some subjects who have little response to leukotrienes. The comparative effectiveness of each type of drug was not examined until the past few years. All of the available data to date, including those reported herein, indicate that, on average, both classes of compounds offer statistically similar degrees of prophylaxis. It was not until the work of Coreno and associates, however, that the amounts and duration of treatment required to produce benefits have been addressed. These investigators showed that long-term therapy with leukotriene blockers was not essential and that single doses of either a 5-lipoxygenase inhibitor or a cysteinyl leukotriene 1 receptor antagonist initiated sustained effects. In their study, protection developed within 60 min of ingestion of the available agents, including M, and remained stable for 8 to 12 h. The present findings concur nicely with these results. In addition, Dempsey et al found that single doses of M and S, when administered together to asthmatic individuals, blunted challenges with adenosine monophosphate more than the individual compounds.

The mechanisms by which M and S work together have not been established. One possibility is that S may change the initiation threshold for airflow limitation to begin and M may alter the biochemical elements that follow signal activation. Besides reducing smooth muscle tone, S also dilates the airway microcirculation. Since it appears that bronchial blood flow is important in the regulation of intrathoracic heat exchange, any such elevations can potentially limit the degree of airflow cooling that occurs at a given VE. As a result, after S administration, a far greater VE would be needed to produce the critical thermal gradients that induced airflow limitation pretreatment. Based on BAL studies, there is controversy as to whether the airways release leukotrienes in response to thermal stimuli. Direct measurements in airway surface fluid, however, suggest that they do (E.R. McFadden, Jr., MD; unpublished data). To the extent that there is less cooling, the airways may possibly release fewer cysteinyl leukotrienes. These compounds are generated in the epithelium and mediate heightened mucus production, increased vascular permeability, smooth muscle contraction, and the recruitment of inflammatory cells. Many of these activities are believed to be key elements in the pathophysiology of thermally induced asthma, and urinary concentrations of LTE4 have been found to rise in some studies following thermal challenges. Thus, the blockade of leukotriene receptor activity may interfere with the development of fundamental components of the reaction. The data in Figure 3 indirectly support this reasoning. S provided protection by changing ventilatory thresholds, suggesting an alteration in an initiating or entry phenomenon, whereas the effect of M developed without influencing this parameter implies a reduction in a sustaining component.

The present study was designed to provide pharmacologic proof of concept, and not as a therapeutic trial. Given the heterogeneity of some of the individual responses seen in Figure 2, we appreciate that our relatively small numbers of subjects raise the possibility of unrecognized type 1 and type 2 statistical errors, and that larger comparative investigations will be needed to determine those who will derive maximum benefit. Nonetheless, the current...
pilot data demonstrate promise. Isocapnic HV was employed because it is a well-established means of simulating the hyperpnea of exercise.2,15,19,21 Both forms of ventilation produce identical changes in intrathoracic thermal fluxes and bronchial narrowing while sharing similar therapeutic sensitivities.21 In addition, unlike exercise, HV provides the advantage of being able to examine responsiveness over a range of stimulation without unduly stressing the subjects.19

Again, because this was not a clinical trial, we chose to simplify our protocol by not using placebo or double-dummy techniques. This decision was unlikely to adversely influence our results because the obstructive response to both exercise and HV over time is known to be highly reproducible with minimal within-subject variability when the critical determinants (Ve, and the inspired temperature and water contents) are matched between challenges.3,9,15,19

In summary, the combination of standard single doses of M and S appear to provide additive protection against thermal stimuli. It remains to be determined whether such a mixture will provide additional clinical benefits to patients over the single agents. Further studies are also needed to ascertain whether the combination will increase the duration of the protective benefits and/or will blunt the tachyphylaxis that may occur when EIB is treated with S over time.3,9,32,33

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