Anti-inflammatory and Lung Function Effects of Montelukast in Asthmatic Volunteers Exposed to Sulfur Dioxide

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Background: **Sulfur dioxide (SO_2) gas may induce acute asthmatic responses when inhaled by individuals in the setting of community or occupational air pollution during exercise. Some asthma medications mitigate the SO_2 response, which is not fully understood but appears to involve multiple mechanisms.**

Objective: **We tested the hypothesis that pretreatment with the cysteinyl-leukotriene inhibitor montelukast sodium protects against the inflammatory and bronchoconstrictive effects of SO_2 in the airways of asthmatic subjects.**

Methods: **Asthmatic volunteers (enrolled, 12 subjects; completed study, 11 subjects) were exposed to 0.75 ppm SO_2 for 10-min periods during exercise (mean ventilation, 35 L/min) and were exposed similarly to filtered air (control condition) after double-blinded pretreatments with montelukast (10 mg/d for 3 days) and placebo.**

Results: **After montelukast pretreatment, specific airways resistance, FEV_1, symptoms, and eosinophil counts in induced sputum showed statistically and clinically significant improvements in preexposure measurements and/or decreased responses to SO_2 exposure or exercise. The mean FEV_1 immediately after exposure was 95% of baseline FEV_1 with montelukast pretreatment vs 82% with placebo.**

Conclusion: **Montelukast significantly protects against airways eosinophilic inflammation and bronchoconstriction from SO_2 exposure during exercise. This implies a role for leukotrienes in SO_2-induced lung effects.** ***(CHEST 2001; 119:402–408)***

Key words: air pollutants; airway resistance; asthma; leukotrienes; montelukast; spirometry; sputum induction; sulfur dioxide

Abbreviations: IL = interleukin; SO_2 = sulfur dioxide; SRaw = specific airways resistance

Sulfur dioxide (SO_2) is a common irritant pollutant gas in community and workplace air. Many individuals with asthma (even very mild asthma) are highly sensitive to SO_2, experiencing clinically significant airways constriction and symptoms after a few minutes of moderate exercise in SO_2 concentrations as low as 0.25 ppm,[1] which is within the range of community and workplace exposures. The SO_2 response resembles exercise-induced bronchoconstriction in its rapid onset (*ie*, within 1 to 3 min) and spontaneous reversal (*ie*, within 1 to 2 h while resting in clean air). However, exercise is not necessary to induce the response if the inhaled dose rate of SO_2 is sufficiently high (*eg*, at concentrations of several parts per million). Among standard pharmacologic agents, inhaled β_2-agonists are highly (but $< 100\%$) effective in blocking the SO_2-induced bronchoconstrictive response, while ipratropium, cromolyn, and theophylline block the airway response to a lesser degree.[1] The role of airways inflammation and the efficacy of anti-inflammatory medications in the asthmatic response to SO_2 have not been investigated extensively.

The recent development of a new class of pharmacologic agents, cysteinyl-leukotriene receptor an-

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tagonists, offers possibilities for clinical management and for exploring mechanisms of the asthmatic response to SO_2. These medications exhibit both anti-inflammatory and bronchodilator activity, and appear to be generally safe and effective with once-daily or twice-daily oral dosing.[2–5] Lazarus et al[6] investigated the effect of one such agent, zafirlukast, on the SO_2 response by challenging asthmatic volunteers with increasing concentrations of SO_2 during eucapnic hyperventilation at rest. The investigators found that pretreatment with a single 20-mg dose of zafirlukast approximately doubled the SO_2 concentration required to provoke a given degree of bronchoconstriction, and they concluded that SO_2-induced bronchoconstriction involves the release of leukotrienes. Montelukast, another leukotriene receptor antagonist, has been found to reduce airway eosinophilic inflammation in patients with asthma, as measured by cell counts of induced sputum,[7] and to mitigate bronchoconstriction induced by exercise[8,9] or allergen challenge.[10]

On the basis of the above evidence, we hypothesized that montelukast would block inflammatory effects and bronchoconstrictive effects in asthmatic subjects exposed to SO_2 with exercise under conditions representative of outdoor or occupational air pollution exposures. Effective protection by montelukast would strongly imply that leukotrienes play a major causative role in SO_2-induced lung effects, would reveal another therapeutic dimension of leukotriene receptor antagonists in protection against pollution effects, and would motivate further mechanistic studies with other pollutants. To test our hypothesis, we exposed adult asthmatic volunteers, pretreated with montelukast sodium (Singulair; Merck & Co; West Point, PA) or placebo, to filtered air (control condition) and to 0.75 ppm SO_2 in filtered air for 10-min periods with continuous moderate exercise. We measured their responses in terms of symptoms, lung function, and counts of inflammatory cells from induced sputum.

Materials and Methods

Subject Recruitment and Screening

Twelve adult volunteers aged 20 to 48 years with comparatively mild asthma were recruited by word of mouth or advertisements. Each subject gave written informed consent and was paid a participation fee. The protocol was reviewed and approved by the institutional review board for Rancho Los Amigos National Rehabilitation Center. Inclusion criteria were the following: asthma of >1 year duration that had been treated primarily with an inhaled bronchodilator (either regularly or intermittently as needed) and had been clinically stable for >4 weeks prior to entrance into the study; the ability to withhold treatment with respiratory medications prior to each laboratory visit; pretreatment FEV_1 of $\geq 70\%$ of the predicted value; and $\geq 15\%$ FEV_1 decline after a screening SO_2 challenge (see below). Exclusion criteria were the following: the use of a corticosteroid or a leukotriene antagonist medication within 4 weeks of entrance into the study; smoking within 1 year of study; an allergy to the study drug or to inhaled β-agonists; clinically significant cardiopulmonary or metabolic disease other than asthma; an inability to exercise on a cycle ergometer; and pregnancy or nursing. Women with child-bearing potential had negative results for pregnancy tests at the outset and were required to practice effective contraception during the study.

During a screening evaluation, subjects gave medical histories (including medications and allergies) and underwent routine physical examinations, spirometry to determine FVC and FEV_1, 12-lead resting ECGs, urinary pregnancy tests (women only), and 10-min exposures to 0.75 ppm SO_2 in filtered air at 22°C and 60% relative humidity in a previously prepared environmentally controlled chamber.[11] The test gas was metered into the chamber from a cylinder of 5% SO_2 in nitrogen. The SO_2 exposure concentration was monitored by a pulsed fluorescent SO_2 analyzer (Meloy; Springfield, VA), which was calibrated with a permeation-tube apparatus. During the challenge, the subject exercised continuously (for 10 min) on a cycle ergometer to achieve a minute ventilation of 25 to 30 L/min (a work intensity that should not elicit clinically significant exercise-induced bronchoconstriction). A $\geq 15\%$ loss in FEV_1 was required at 5, 10, 15, or 30 min postchallenge to qualify for further participation in the study.

Experimental Protocol

Each subject was scheduled for four laboratory visits, at approximately the same time of day and not <14 days apart, to undergo challenges with the following four pretreatment/exposure conditions: placebo/filtered air; placebo/SO_2; montelukast/filtered air; and montelukast/SO_2. The order of presentation was counterbalanced and randomly assigned. Nominally double-blind conditions were maintained, although we could not rule out the possibility that some subjects distinguished active drug from placebo by their symptom levels, and/or distinguished SO_2 from filtered air by odor, taste, or symptom responses. Tablets of montelukast (10 mg per tablet) and identically appearing placebo, with coded labels, were supplied by Merck & Co. For pretreatment, the subject took one tablet at 8 PM on each of the 3 days immediately preceding each scheduled laboratory visit/exposure. Thus, with ≥ 14 days between visits, the washout interval between successive pretreatments was ≥ 11 days.

On arrival at the laboratory on the morning of an exposure, the subject underwent a brief cardiopulmonary examination and gave an interval medical history, and underwent ECG electrode application and preexposure measurements of specific airways resistance (SRaw), FEV_1 (required to be within 10% of screening value), and FVC, as well as respiratory and nonrespiratory symptoms scored on a standardized questionnaire (scoring details are given in Table 2). SRaw measurements employed a constant-volume whole-body plethysmograph (locally manufactured), which was calibrated daily by applying known pressure, volume, and flow signals. Four successive measurements were made at each time of testing, and the result was expressed as the mean. Forced expiratory measurements were made using a pneumotachograph-based spirometer (Vmax System; Sensormedics, Inc; Yorba Linda, CA), which was certified to meet American Thoracic Society standards for accuracy[12] and was calibrated daily with a volumetric syringe according to the manufacturer's procedure. Three blows meeting American Thoracic Society criteria[12] were recorded at each time of testing, and the result was expressed as the largest of the three FEV_1 values. Experimental

exposures employed the same facilities and 10-min protocol used in the screening SO_2 challenges. The individual ergometer workloads that evoked the target ventilation rate of 25 to 30 L/min in the screening examinations were maintained in all exposures; however, exercise ventilation rates during exposures averaged higher (see "Results" section). The subject was continuously monitored by direct observation and ECG telemetry. Breathing was unencumbered throughout exposure, except when ventilation was measured via mouthpiece during the final 2 min. The SRaw measurement was repeated immediately (*ie*, at approximately 1 min) after the completion of exposure and at 1 h and 2 h later. Symptoms were recorded at the time of each SRaw measurement, and FEV_1 was measured immediately afterward (*ie*, approximately 5 min after exposure ended for the initial postexposure measurement). Additional FEV_1 measurements were made 10 min, 15 min, and 30 min after the end of the exposure. No subject required bronchodilator medication to relieve symptoms postexposure.

Sputum induction[13,14] was performed 2 h after completion of the exposure. The subject rested in a filtered-air environment in the interim. After the 2-h postexposure measurements of SRaw and FEV_1, the subject inhaled 360 μg albuterol to avoid possible bronchoconstriction during sputum induction, and the FEV_1 was remeasured. Next, the subject inhaled 3% sterile saline solution for 20 min from an ultrasonic nebulizer (Ultraneb 99; DeVilbiss; Jackson, TN), while actively coughing and expectorating sputum and saliva into separate sterile specimen containers every 2 min. The collected sputum was diluted 1:1 with a 0.1% dithiothreitol solution and was homogenized by gentle vortex mixing and shaking in a water bath at 37°C for 15 min. A 10-μL aliquot of homogenized sputum was used to determine the total cell count using a standard hemocytometer. WBCs, columnar epithelial cells, and squamous epithelial cells were counted under blind conditions, and the results were expressed as thousands of cells per milliliter of sputum. A 250-μL aliquot was diluted in saline solution and cytocentrifuged (Cytospin 3; Shandon; Pittsburgh, PA) onto glass slides, which were air dried and subsequently stained with May-Grünwald-Giemsa stain (Diff-Quik Kit; Hamilton Thorne Research; Beverly, MA) for differential counting. For each subject under each experimental condition, a minimum of 500 nonsquamous cells were read from three or four slides, each with $>50\%$ nonsquamous nucleated cells. Monocytes, lymphocytes, neutrophils, eosinophils, and columnar epithelial cells were counted by a blinded investigator, and the results were expressed as percentages of the total of these five cell types.

Data Analysis

Response measurements included SRaw, FEV_1, and symptom scores before, immediately after, 1 h after, and 2 h after exposure, as well as airway inflammation indexes (*ie*, total sputum cell counts and differential counts) 2 h after exposure. FEV_1 and SRaw were analyzed in their original units and also as percentages of their baseline values, which were defined as the means of the two preexposure measurements with placebo pretreatment (*ie*, the best estimates of FEV_1 or SRaw in the absence of any experimental intervention). Statistical conclusions were essentially the same either way. For SRaw, FEV_1, and symptom score, we analyzed changes from before exposure to immediately after exposure, when group mean responses were maximal. FEV_1 data were reanalyzed in terms of the time-integrated deficit as determined from the area under the curve of FEV_1 vs time postexposure, plotted from all six measurements during the first 2 h after exposure. Statistical conclusions were essentially the same as in the original analysis. The principal statistical technique was analysis of variance with repeated measures on subjects (each subject as his/her own control), employing commercial statistical software (BMDP Dynamic; SPSS Inc; Chicago, IL). When necessary to stabilize variance, data were log-transformed before analysis. All the aforementioned analyses necessarily excluded subject 2, who missed two treatments. The analyses of total cell counts also excluded subjects 3 and 7, whose data were unsatisfactory on one occasion. As described in the "Results" section, additional analyses were performed to compare the two conditions of most interest (montelukast/SO_2 and placebo/SO_2) for which data were available for all 12 subjects.

Results

Table 1 summarizes demographic and physiologic characteristics of the 12 recruited subjects. Eleven subjects underwent all four pretreatments/expo-

Table 1—*Demographic Characteristics and Physiologic Findings at Screening of Study Subjects

ID No.	Age, yr	Sex	Height, cm	Weight, kg	FEV_1 % Predicted	SRaw†	FEV_1 % Loss‡
1	46	F	155	66	74	4.5	21
2	24	F	168	87	77	11.8	45
3	27	M	188	109	79	7.8	31
5	47	F	155	54	80	8.5	19
6	20	F	163	68	97	6.2	34
7	20	F	147	79	96	3.4	46
9	24	F	173	77	78	11.0	26
10	36	M	183	73	100	3.6	16
11	25	F	165	74	100	3.7	40
13	48	F	175	86	100	6.3	24
14	25	F	168	83	80	5.5	15
15	21	F	170	74	102	4.5	24

*F = Female; M = male; ID = identification.

†Measured near functional residual capacity, as the product of thoracic gas volume in liters and airway resistance in centimeters of H_2O per liter per second.

‡After challenge with 0.75 ppm SO_2 (10-min inhalation during exercise).

sures. Subject 2 withdrew (for personal reasons unrelated to the study) after completing only the montelukast/SO_2 and placebo/SO_2 parts of the study. Apart from the expected postexposure bronchoconstriction, no clinically adverse events were associated with any experimental medications or procedures. The mean (± SD) ventilation rates measured near the end of exposure periods were 35 ± 13 L/min; they did not vary significantly between placebo and montelukast, or between filtered air and SO_2.

Lung Function and Symptom Responses

Tables 2, 3 and Figures 1, 2 show the effects of montelukast pretreatment on lung function and symptom responses to the experimental exposures. With placebo pretreatment, the subjects showed, on average, mild airways constriction after filtered air exposure with exercise (*ie*, a mild increase in SRaw and a mild decrease in FEV_1 without a net increase in symptoms) and a more marked bronchoconstriction after 0.75 ppm SO_2 exposure/exercise (*ie*, much larger decrements in lung function and increases in lower respiratory symptoms). These responses were as expected in a group of subjects with mild-to-moderate asthma who were prescreened for responsiveness to SO_2. With montelukast pretreatment, mean preexposure lung function and symptom scores improved slightly, responses to filtered air/exercise were still slight, and responses to 0.75 ppm SO_2/exercise were mitigated to a clinically and statistically significant extent, relative to placebo pretreatment. Repeated-measures analyses of variance (Table 3) showed that the main effect of montelukast pretreatment vs placebo was significant ($p < 0.05$) in the favorable direction for SRaw, FEV_1, and total

Table 2—*Physiologic and Symptom Responses

Exposure	Time	Placebo	Montelukast
SRaw preexposure and 1 min postexposure, L(cm H_2O/L/s)			
Filtered air	Pre	6.12	6.57
	Post	8.79	8.37
0.75 ppm SO_2	Pre	6.54	5.49
	Post	19.08	9.12
FEV_1 preexposure and 5 min postexposure			
Filtered air	Pre	2.91	2.96
	Post	2.82	2.93
0.75 ppm SO_2	Pre	2.91	3.00
	Post	2.45	2.76
Total symptom score† preexposure and 1 min postexposure			
Filtered air	Pre	2.9	2.2
	Post	2.8	2.2
0.75 ppm SO_2	Pre	3.7	1.6
	Post	9.2	4.1

*Values given as means for 11 subjects who completed the study. Pre = preexposure; Post = postexposure.

†Symptom scoring uses a modified version of the technique described by Gong et al.[15] Categories scored are cough, sputum, dyspnea, wheeze, chest tightness, substernal irritation, throat irritation, nasal discharge/congestion, eye irritation, fatigue, headache, chills/feverishness, muscle aches, nausea, loss of appetite, and miscellaneous symptoms. Each symptom is scored from 0 (none) to 4 (most severe), with a score change of 1 representing the least perceptible change in severity. Lower respiratory tract symptoms accounted for nearly all the score increase post-SO_2 exposure.

Table 3—*p Values From Analyses of Variance on Lung Function and Symptom Data*

Source of Variation	SRaw (Log Transformed)	FEV_1	Total Symptom Score
Drug*	0.002	0.019	0.043
Exposure†	0.014	0.106	0.018
Drug × exposure‡	0.025	0.335	0.134
Time§	0.001	0.002	0.004
Drug × time‖	0.110	0.023	0.033
Exposure × time¶	0.010	0.001	0.001
Drug × exposure × time**	0.071	0.078	0.030

*Mean of all values with montelukast pretreatment was more favorable than mean of all values with placebo pretreatment, for all three measures.

†The mean of all values on filtered-air study days was more favorable than mean of all values on SO_2 study days, for SRaw and total symptom score.

‡Montelukast pretreatment improved SRaw values on SO_2 exposure days, relative to placebo pretreatment.

§Postexposure values were less favorable than preexposure values for all three measures, considering both filtered-air and SO_2 exposures.

‖Montelukast pretreatment improved postexposure values of FEV_1 and total symptom score, considering both filtered-air and SO_2 exposures.

¶Post-SO_2 exposure values changed more unfavorably than post-filtered-air exposure values, relative to the corresponding preexposure values, for all three measures.

**The change in total symptom score pre-SO_2 to post-SO_2 exposure, relative to that in filtered air, was mitigated significantly ($p < 0.05$) by montelukast pretreatment. For SRaw and FEV_1, the degree of mitigation approached significance ($0.05 < p < 0.10$).

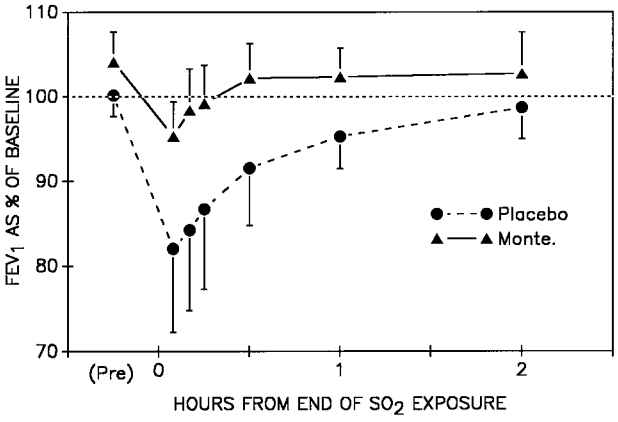

FIGURE 1. FEV_1 (expressed as a percentage of baseline value mean ± 95% confidence limit) before and after exposure to 0.75 ppm SO_2 with exercise following placebo or montelukast (Monte) pretreatment. Pre = pretreatment.

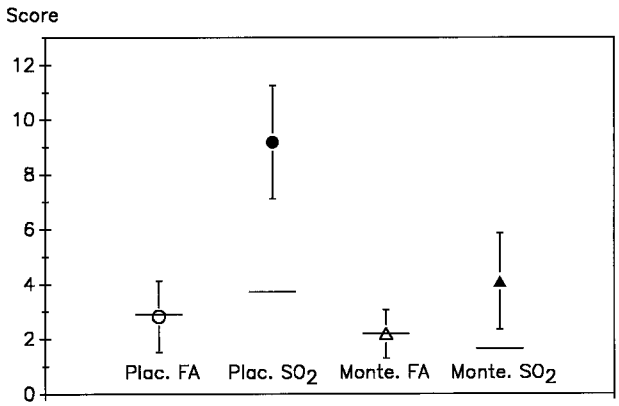

FIGURE 2. Symptom score change under each pretreatment/exposure condition. Horizontal line indicates the mean score preexposure, symbols indicate the mean score immediately postexposure, and the vertical error flags indicate 95% confidence limits of change preexposure to postexposure. The scoring procedure is described in Table 2. Plac = placebo. See Figure 1 for other abbreviations not used in the text.

symptom score; one or more interactions of drug with exposure and time effects were also significant for each of the three response measures, reflecting the mitigation of SO_2 and/or exercise effects by montelukast. As Table 2 indicates, the improvement of preexposure FEV_1 or SRaw attributable to montelukast pretreatment was small; thus, mitigation of SO_2/exercise effects was the more important effect of montelukast in maintaining airway patency.

For the 11 subjects who completed the study, the mean FEV_1 immediately after exposure, relative to baseline (*ie*, the mean of placebo preexposure measurements) was 97% with placebo/filtered air, 101% with montelukast/filtered air, 85% with placebo/SO_2, and 95% with montelukast/SO_2. For all 12 subjects, the means were 95% with montelukast/SO_2 and 82% with placebo/SO_2; this difference was significant ($p < 0.05$), as measured by paired *t* test. The analogous paired comparisons were also significant for SRaw and total symptom score. In terms of SO_2/exercise effects on FEV_1, the protective index (*ie*, one minus the ratio of the percentage loss after montelukast administration to the percentage loss after placebo administration) was 54%. This understates the beneficial effect of montelukast, in that mean FEV_1 preexposure/postmontelukast administration was increased relative to the mean FEV_1 preexposure/postplacebo administration, thus yielding a larger percentage loss for a given level of FEV_1 postexposure/postmontelukast administration. Calculated in terms of the mean 5% loss from baseline FEV_1 postmontelukast administration, relative to the mean 18% loss from baseline postplacebo administration, the protective index was 72%. With montelukast pretreatment, 9 of 12 subjects retained at least 90% of baseline FEV_1 after SO_2 exposure/exercise, and no subject fell to < 80% of baseline FEV_1. With placebo pretreatment, only five subjects retained at least 90% of their baseline FEV_1, while five subjects fell to < 80% of baseline. These differences were significant ($p < 0.05$ by McNemar's test for paired measurements of categorical variables). Even with placebo pretreatment, unfavorable responses to SO_2/exercise reversed promptly; FEV_1 averaged 95% of baseline after 1 h, and 98% after 2 h (Fig 1).

Inflammatory Cells in Induced Sputum

Table 4 presents results of total cell counts for the nine subjects with complete data. None of the total cell counts varied significantly according to pretreatment and/or exposure atmosphere ($p > 0.10$ for main effects and interactions), although they tended to be lower after montelukast pretreatment than after placebo, and higher after SO_2 exposure than after filtered air exposure. Table 5 presents results from differential counts of sputum cells for the 11 subjects who completed the study. Variations in the percentages of monocytes, lymphocytes, and columnar epithelial cells were nonsignificant. Neutrophil percentages were lower with montelukast pretreatment than with placebo, and the main effect of the drug approached significance ($p = 0.053$). Significant variation occurred with the measurement of sputum eosinophils. In the analysis of log-transformed data, the main effect of the drug (*ie*, the decreased percentage of eosinophils after montelukast pretreatment) was significant ($p = 0.008$), as was the main effect of atmosphere (*ie*, the increased percentage after SO_2 exposure) ($p = 0.039$). The interaction was nonsignificant. A paired *t* test comparing montelukast/SO_2 with placebo/SO_2 showed a significant ($p = 0.03$) difference for all 12 subjects. Individual sputum eosinophil data are shown in Figure 3.

Table 4—*Total Cell Counts From Induced Sputum

Exposure	Cell Type	Placebo	Montelukast
Filtered air	WBC	261	224
	Columnar epithelial	32	19
	Squamous epithelial	56	52
	All the above	348	296
0.75 ppm SO_2	WBC	289	255
	Columnar epithelial	21	20
	Squamous epithelial	49	59
	All the above	360	334

*Values given as thousands of cells per milliliter and are means for nine subjects with complete data. No differences between placebo and montelukast pretreatment were statistically significant.

Table 5—*Differential Cell Counts From Induced Sputum

Exposure	Cell Type	Placebo	Montelukast
Filtered air	Monocytes	56.01	61.58
	Lymphocytes	0.11	0.10
	Neutrophils	37.42	32.31
	Eosinophils	0.59	0.12
	Columnar epithelial cells	5.86	5.88
0.75 ppm SO_2	Monocytes	52.40	60.10
	Lymphocytes	0.12	0.03
	Neutrophils	41.35	35.24
	Eosinophils	1.30	0.55
	Columnar epithelial cells	4.83	4.09

*Values given the percentage of all nonsquamous cells and are means for 11 subjects with complete data. Only eosinophil percentages varied significantly by pretreatment and exposure condition (see text and Fig 3).

Discussion

A variety of therapeutic agents, including β-adrenergic agonists, theophylline, atropine, ipratropium bromide, cromolyn, nedocromil, and H_1-antihistamine, can partially block SO_2-induced bronchoconstriction in asthmatic subjects.[1,15] β-Adrenergic agonists appear to be the most effective, but none of these drugs completely blocks the response, suggesting that more than one mechanism is involved. Proposed mechanisms of SO_2-induced bronchoconstriction include a reflex involving both vagal afferent nerves and cholinergic efferent nerves to airway smooth muscle, as well as stimulation of mast cells or sensory receptors. Our finding that montelukast can mitigate bronchoconstrictive responses to SO_2 is consistent with the previous physiologic findings reported by Lazarus et al[6] concerning another leukotriene receptor antagonist, zafirlukast. Quantitative comparisons of the SO_2/lekotriene-modifier studies are difficult because the study designs and SO_2 exposures differed substantially. However, both studies support the concept that leukotrienes are important mediators of the bronchoconstrictive response to SO_2. Both show only partial blocking of the response, again suggesting that more than one mechanism is involved. Nevertheless, our findings indicate that montelukast has relatively high effectiveness in preventing SO_2 response, similar to that of β-agonists. In our previous study of medication effects on SO_2/exercise response,[15] the immediate decrease in FEV_1 averaged about 7% after pretreatment with inhaled salmeterol. In the present study, with a similar exposure protocol and subject selection criteria, the immediate FEV_1 decrease averaged about 8%.

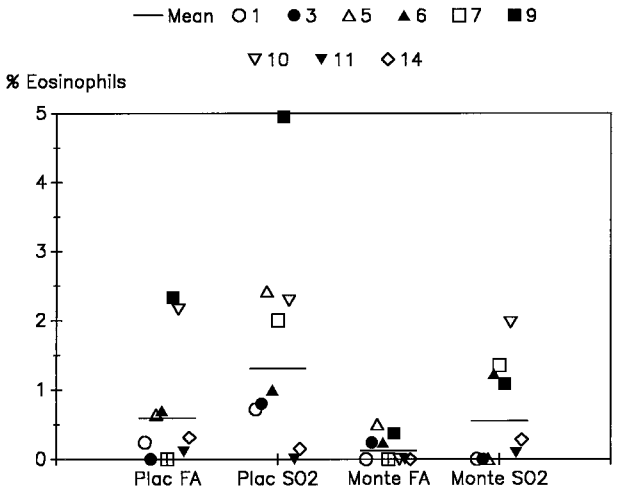

Figure 3. Individuals' percentages of eosinophils among sputum cells induced 2 h after exposure to 0.75 ppm SO_2 or filtered air (FA), after placebo or montelukast pretreatment. Symbols represent individual subjects, and horizontal lines represent means. Two subjects showed no eosinophils in any pretreatment/exposure condition. See Figures 1, 2 for abbreviations not used in the text.

We also detected a significant SO_2-induced increase in sputum eosinophilia within several hours after exposure, indicating that sufficient exposure to SO_2 in asthmatic subjects can acutely elicit allergic (eosinophilic) airways inflammation. Such a cellular response in the proximal airways has not been previously reported with exposure to these levels of SO_2, to our knowledge, although inflammatory cell influx has been observed by BAL after exposure to 8 ppm SO_2.[16] The increased airways responsiveness of atopic/asthmatic individuals to the effects of air pollutants such as SO_2 may result from increased susceptibility to cell-membrane injury by pollutants and the propensity of airway epithelial cells to release increased amounts of specific proinflammatory mediators following interaction with inhaled irritants.[17] The release of interleukin (IL)-5, IL-8, and cysteinyl leukotrienes, as well as other mediators, may cause chemoattraction, activation, and recruitment of eosinophils into the airways.[17–19]

A short course of montelukast pretreatment had a significant anti-inflammatory effect by reducing the number of sputum eosinophils with both filtered air and SO_2 exposures. The findings with filtered air are qualitatively consistent with the previous observation by Pizzichini et al[7] of a 3.6 percentage point decrease in sputum eosinophils after 4 weeks of montelukast treatment in asthmatic subjects. A direct comparison between their study and ours is problematic, in that we did not recruit asthmatic subjects with $>5\%$ sputum eosinophils and did not conduct sputum induction weeks after the montelukast therapy was initiated. However, Diamant et al[10] reported that montelukast pretreatment did not significantly affect sputum eosinophils or other markers of airway in-

flammation in nine asthmatic volunteers who were challenged with house dust mite extract. However, sputum analysis was not a main focus of their study, and their subjects' eosinophil counts were somewhat unstable and typically higher than those of our subjects, so no firm conclusions can be drawn from the comparison.

This study was limited by several factors. The number of subjects was relatively small, but the group still demonstrated both significant SO_2 exposure and drug (montelukast) effects, including a rapid onset of drug protection. Measurements of drug levels in blood and of mediators in sputum (*eg*, IL-5, IL-8, and eosinophil cationic protein), as well as measurements of response at more than one concentration of SO_2, would have been helpful for mechanistic reasons but were not feasible in this study. The cellular profiles in the induced sputum might have been different if sputum induction had been performed 18 h or 24 h after exposure, allowing more time for effects of the same or different mediators to be manifested. If anything, greater or more highly significant effects of montelukast and SO_2 would be expected 18 to 24 h postexposure.

In summary, in our mildly asthmatic, SO_2-responsive volunteer group, pretreatment with montelukast (10 mg/d for 3 days) had unequivocally favorable effects on the following different outcome measures: airways inflammation (as reflected by sputum eosinophil counts); airway physiology (as reflected by SRaw and FEV_1 levels); and respiratory symptomatology (as reflected by symptom scores) after exposure to 0.75 ppm SO_2 during moderate exercise. Montelukast both improved airway status preexposure and mitigated responses to exposure. On average, improvement preexposure was more important with respect to sputum eosinophil counts, while the mitigation of the SO_2/exercise response was more important with respect to lung function and symptom measures.

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