

Theophylline mitigates the bronchoconstrictor effects of sulfur dioxide in subjects with asthma

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The objective of the study was to investigate the ability of a sustained-release (SR) theophylline tablet (Uniphyl; Purdue Frederick Co., Norwalk, Conn.) to block or mitigate sulfur dioxide (SO₂)-induced bronchoconstriction in adult subjects with asthma. Eight subjects participated in a double-blind, crossover study with a 400 mg theophylline tablet or placebo once a day for a week before a 10-minute SO₂ challenge. FEV₁ and total respiratory resistance (R_T) were measured before and after the SO₂ challenge and on a different day before and after an air exposure. After exposure to SO₂, average values of FEV₁ dropped 16% after placebo treatment and 7% after theophylline treatment. The corresponding percentages for R_T were a 37% increase after placebo and a 7% increase after theophylline treatment. Analysis of variance demonstrated a significant difference between the SO₂-induced decrease in FEV₁ and increase in R_T after SR theophylline treatment compared with that of placebo treatment. Thus, we conclude that SR theophylline tablets, taken at this concentration for 1 week, mitigate SO₂-induced bronchoconstriction. (J ALLERGY CLIN IMMUNOL 1992;89:789-94.)

Key words: Asthma, air pollution, sulfur dioxide, FEV₁, theophylline, Uniphyl, pulmonary function

SO₂ is one of six common widespread air pollutants regulated by the U.S. EPA (1982). This air pollutant, more than any other pollutant, has been demonstrated to cause bronchoconstrictive responses in subjects with asthma at concentrations that are an order of magnitude lower than concentrations that produce pulmonary responses in healthy subjects (0.5 versus 5.0 ppm).^{1, 2} Allergic subjects who only have exercise-induced bronchospasm also have been demonstrated to be very sensitive to inhaled SO₂.³ It is estimated that 25 million individuals in the United States have underlying bronchial hyperresponsiveness that makes these individuals vulnerable to inhaled irritants, such as SO₂.⁴

The National Ambient Air Quality Standard set by

Abbreviations used

SO ₂ :	Sulfur dioxide
EPA:	Environmental Protection Agency
PD ₂₀ :	Provocative dose of methocholine causing a 20% fall in FEV ₁
R _T :	Total respiratory resistance
SR:	Sustained release

the EPA for SO₂ is 0.14 ppm averaged during a 24-hour period. The EPA has not set a 1-hour standard, even though several states have judged it necessary to protect the public health. The 1-hour SO₂ in Washington state is 0.4 ppm. However, even a 1-hour standard will not protect against brief (10 to 15 minutes) downdrafts of concentrations in the range of 1 ppm of SO₂.⁵

Some commonly used medications for the treatment of asthma, albuterol⁶ and cromolyn,^{7, 8} do block SO₂-induced bronchoconstriction. However, ipratropium bromide did not demonstrate a protective response against SO₂,⁹ and beclomethasone only partially blocked the response.¹⁰

In an earlier study, we demonstrated that there was no significant protection against SO₂-induced bron-

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TABLE I. Clinical characteristics of subjects

Subject No.	Sex	Age (yr)	Baseline FEV ₁ (L)	% Predicted	PD ₂₀ (mg/ml)	Serum levels	
						Placebo (μg/ml)	Uniphyll (μg/ml)
01	M	18	3.26	76	0.075	<1	9.6
02	F	29	3.00	97	5.0	<1	6.2
03	F	19	2.28	108	0.15	<1	9.0
04	F	46	2.77	100	ND	<1	8.4
06	F	21	3.25	90	0.15	<1	7.1
08	M	36	3.52	84	0.15	<1	6.5
09	F	28	3.58	108	ND	<1	8.1
10	F	23	3.03	90	10.0	<1	8.1
Mean		27.5	3.01	94.1	2.59		7.88
SD		9.6	0.42	11.3	4.12		1.19

ND, Not done.

choconstriction from theophylline in a group of subjects with asthma receiving long-term theophylline therapy.¹¹ However, that study was somewhat limited in that subjects were not tested with placebo because of the ethical constraints of removing them from their therapeutic regimen. To extend the results of that study, we performed a double-blind, crossover comparison of the effects of an SR theophylline preparation (Uniphyll) or placebo on the response to 1 ppm SO₂ in a group of adult subjects with asthma. Our data demonstrate that the significant decrease in FEV₁ observed after SO₂ with placebo was not observed after SO₂ with theophylline. This study indicates that SR theophylline affords significant mitigation from the bronchoconstrictor effects of inhaled SO₂.

METHODS

Eight subjects with asthma, six of whom had bronchial hyperresponsiveness documented by a standardized methacholine challenge test, were recruited for this study. For each subject, a PD₂₀ was calculated (Table I). From results of the subject's response to the methacholine test, their asthma ranged from severe (PD₂₀ of 0.075 mg/ml) to moderate (PD₂₀ of 10 mg/ml).¹² Their ages ranged from 18 to 46 years. Only two subjects were male. Subjects withheld all antiasthma medications other than the study formulations for the duration of the study. All subjects read and signed an informed consent form approved by the University of Washington Human Subjects' Office. Two additional subjects experienced adverse effects from the theophylline and did not complete the study.

The study protocol was a double-blind, crossover design (Fig. 1). Each subject took medication A or B once a day at bedtime for approximately 1 week before exposures; medications were continued until exposures were complete (Fig. 1). Subjects were exposed in an air-pollution laboratory, as previously described,¹³ to air or 1 ppm of SO₂ and returned 1 to 2 days later for exposure to the other test atmosphere.

Each subject then changed to the other medication, and the process was repeated. Serum theophylline determinations were made approximately 1 hour before visits 1 and 3 to the air-pollution laboratory. Subjects were instructed to continue taking the medication through visits 2 and 4. Since the SO₂ and air challenges were determined by random, there should have been no bias related to compliance. The theophylline serum concentration was determined by reverse-phase, high-performance liquid chromatography.^{14, 15}

All physiologic measurements were made while the subject was seated in a pressure-compensated, volume-displacement body plethysmograph. R_T with the forced oscillatory technique at 3 Hz¹⁶ and FEV₁ were recorded. R_T was measured from mouth pressure, and flow signals were monitored for approximately 10 breaths by a microprocessor that calculated an average value. Thoracic gas volume, with the gas-compression technique,¹⁷ was determined to verify that lung volume did not change between baseline and postexposure measurements. FEV₁ was measured in triplicate. The mean of three acceptable tracings was used. FEV₁ and R_T were measured before (baseline) and immediately after exposure (postexposure). Postexposure FEV₁ was measured between 1 and 3 minutes after exposure; R_T was measured between 4 and 6 minutes after exposure.

Each exposure consisted of inhalation of clean air or 1 ppm of SO₂ on a mouthpiece for 10 minutes during moderate exercise on a treadmill. The SO₂ was supplied from a tank of 1% SO₂ and mixed with filtered, humidified air before inhalation by the subject, as described in detail previously.¹³ The concentration of SO₂ was monitored continuously with a pulsed fluorescence SO₂ monitor (Thermo Electron Corp., Waltham, Mass.). All exposures were at ~65% relative humidity and 22° C. While the subject was exercising, minute ventilation (Hewlett-Packard, Cupertino, Calif., respiratory integrator) and end tidal CO₂ tension (Beckman Instruments, Inc., Fullerton, Calif., gas analyzer) were measured.

Analysis of variance with Statview 512+ software package (BrainPower, Inc., Calabasas, Calif.) was used to determine whether there was a significant treatment effect.

Fisher's test for contrasts was used to determine which comparisons were significant at or below the 5% level.

RESULTS

The clinical characteristics of the subjects are presented in Table I. Serum theophylline levels ranged from 6.5 to 9.6 µg/ml with a mean of 7.88. The average SO₂ concentration was 1.02 ± 0.03 ppm. The exercise level was light, as demonstrated by the minute ventilatory rates in Table I. The mean and standard deviations for FEV₁ and R_T values for each subject before and after exposure to air or SO₂ for both placebo and active drug treatments are presented in Table II.

There were significant treatment effects for both FEV₁ ($p = 0.005$) and for R_T ($p = 0.03$) by analysis of variance. Fisher's tests for contrasts demonstrated that the difference in FEV₁ between air and SO₂ exposure after placebo treatment was significant ($p < 0.05$), whereas the same comparison was not significant after theophylline treatment ($p > 0.40$). However, the two SO₂ treatments (placebo versus theophylline) were not quite significant ($p = 0.052$). Large differences in FEV₁ changes after SO₂ exposure, depending on medication treatment, are depicted in Fig. 2. SO₂ induced an average 16% decrease in FEV₁ after placebo treatment but only an average 7% decrease after theophylline treatment.

The R_T change between air and SO₂ exposure for theophylline treatment also was significant ($p < 0.025$). There was a 37% increase in R_T after SO₂ and placebo and only a 7% increase after SO₂ and theophylline treatment.

DISCUSSION

Our data demonstrate that treatment with SR theophylline tablets for approximately 1 week before a standard SO₂ challenge test significantly mitigated the bronchoconstrictor effects of the air pollutant. However, as depicted in Fig. 2, theophylline does not completely block SO₂-induced bronchoconstriction. These data are in contrast to our earlier observation that subjects receiving long-term theophylline therapy experience significant SO₂-induced bronchoconstriction. In that study, 12 patients with asthma demonstrated a 14% decrease in FEV₁ after SO₂.¹¹ In the present study, subjects taking theophylline demonstrated a 7% decrease in FEV₁ after SO₂. A similar pattern was observed for R_T values; a 15% increase in R_T was observed after SO₂ in the previous study and a 7% increase with theophylline treatment in the present study. This finding could be explained by differences in the severity of asthma between the two groups. Patients in the earlier study may have had

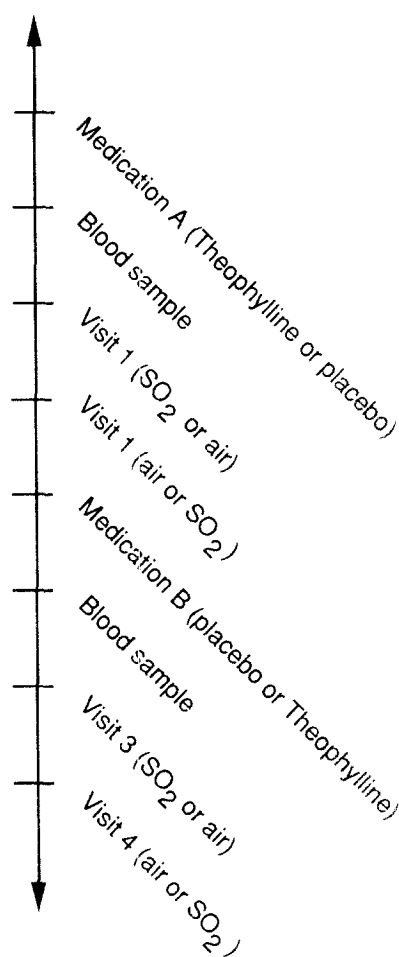


FIG. 1. Chronologic representation of the protocol for the double-blind crossover study.

more chronic disease because they were receiving long-term theophylline therapy for their asthma.

The mechanism of SO₂-induced airway obstruction is unclear, but this study would suggest that bronchial smooth muscle constriction is involved because theophylline is known to relax bronchial smooth muscle, although the precise mechanism for this finding has not been described.¹⁸

Theophylline has been demonstrated to prevent bronchoconstrictor effects of other environmental challenges, namely, exercise-induced bronchospasm.¹⁹ Since our study investigated the effects of SO₂ during moderate exercise, some confounding could be from the effect of theophylline on exercise. However, our data demonstrate very little or no effect of theophylline on the air exposure compared with that of placebo and air exposure. In fact, there was a slightly smaller average decrease in FEV₁ after placebo treatment (3% compared with 4% after theophylline treatment).

TABLE II. Individual pulmonary function values

Subject No.	FEV ₁				R _T		V̇ _E
	BSL mean	SD	PE mean	SD	BSL mean	PE mean	
Placebo-air							
1	3.20	0.03	3.12	0.13	2.49	2.77	24.7 ± 1.00
2	2.97	0.03	2.97	0.03	3.67	3.00	21.1 ± 1.18
3	2.25	0.00	2.05	0.05	2.41	3.93	24.5 ± 1.15
4	2.67	0.03	2.62	0.12	3.16	4.66	19.6 ± 1.27
6	3.03	0.01	2.98	0.03	4.18	3.99	31.3 ± 1.95
8	3.57	0.10	3.50	0.09	2.33	2.74	21.4 ± 2.77
9	3.65	0.03	3.37	0.03	3.72	4.17	26.1 ± 0.81
10	3.00	0.06	3.07	0.08	3.22	3.59	13.4 ± 0.56
Mean ± SD	3.04	0.45	2.96	0.45	3.15	3.61	22.8 ± 5.30
Placebo-SO ₂							
1	3.45	0.03	2.30	0.20	2.56	4.31	25.2 ± 2.62
2	3.07	0.05	3.02	0.03	2.78	3.98	23.6 ± 2.45
3	2.50	0.00	1.95	0.09	2.54	3.24	23.3 ± 1.11
4	2.65	0.06	2.32	0.08	4.44	6.11	20.2 ± 0.32
6	3.13	0.05	1.80	0.00	3.70	7.90	21.9 ± 2.20
8	2.68	0.08	2.55	0.26	3.79	3.65	19.2 ± 3.12
9	3.50	0.03	2.48	0.08	4.63	5.65	23.7 ± 1.64
10	3.05	0.05	3.05	0.05	3.10	2.71	15.9 ± 1.96
Mean ± SD	3.00	0.37	2.43	0.45	3.32	4.69	21.6 ± 3.04
Theo-air							
1	3.33	0.08	3.45	0.00	2.07	1.82	26.1 ± 1.14
2	3.03	0.02	2.88	0.08	3.81	2.96	ND
3	2.50	0.06	2.30	0.10	2.18	2.42	29.0 ± 2.07
4	2.87	0.05	2.78	0.03	2.77	3.59	17.1 ± 1.35
6	3.47	0.01	3.05	0.09	4.23	4.62	ND
8	2.45	0.03	2.42	0.03	4.02	4.62	26.4 ± 0.57
9	3.57	0.05	3.48	0.06	3.01	3.41	27.7 ± 1.15
10	3.12	0.10	3.03	0.03	3.13	2.63	21.3 ± 2.71
Mean ± SD	3.04	0.42	2.93	0.43	3.15	3.26	24.6 ± 4.50
Theo-SO ₂							
1	3.18	0.02	2.58	0.12	2.74	3.10	22.8 ± 1.86
2	2.85	0.21	2.93	0.03	3.30	2.57	26.7 ± 2.37
3	2.65	0.00	2.43	0.06	2.06	2.75	28.0 ± 1.58
4	2.80	0.00	2.62	0.10	3.27	5.11	21.4 ± 2.04
6	3.62	0.03	3.43	0.03	3.65	3.51	23.7 ± 3.15
8	2.68	0.03	2.47	0.12	4.90	4.98	24.6 ± 1.17
9	3.48	0.04	3.08	0.03	3.67	4.22	21.4 ± 0.89
10	3.02	0.03	3.08	0.03	3.72	3.05	15.7 ± 1.97
Mean ± SD	3.04	0.36	2.83	0.36	3.41	3.66	23.0 ± 3.80

BSL, Baseline; PE, postexposure; ND, Not done.

The dosage of Uniphyll tablets used in this study was low, as evidenced by the relatively low serum theophylline levels (an average of 7.88 $\mu\text{g}/\text{ml}$). A higher dosage might provide more blockade of the SO₂-induced effects, depending on individual metabolism. However, since two subjects did not tolerate the 400 mg dose, the side effects would need to be balanced against the therapeutic effects. This study does demonstrate that theophylline is effective at levels lower than the so-called "therapeutic range."

There are several limitations to this study. Since only one concentration of SO₂ was used, dose-response curves could not be presented. However, our intent was to compare the ability of theophylline to prevent SO₂-induced bronchoconstriction with that of albuterol,⁶ cromolyn,⁸ and ipratropium bromide,⁹ as studied by our laboratory with a single concentration of SO₂. Thus, theophylline appears to be not as effective against SO₂-induced bronchoconstriction as albuterol or cromolyn, but more protective than ip-

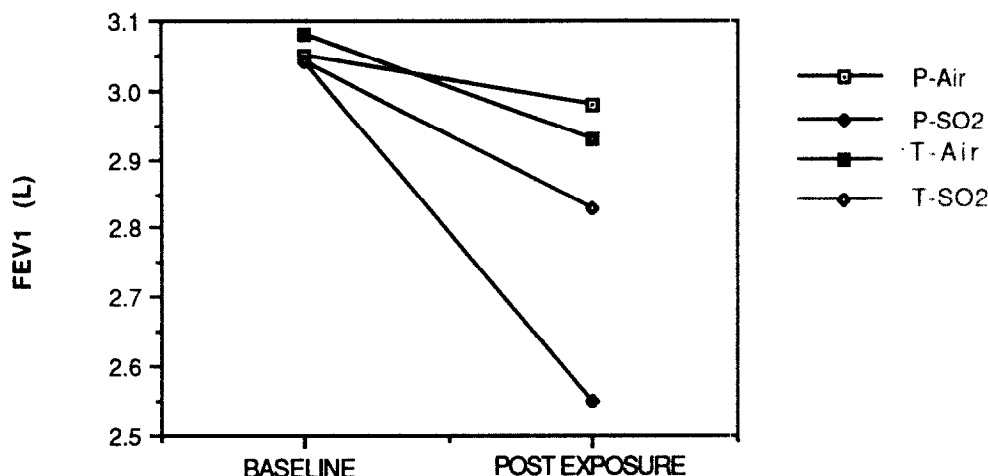


FIG. 2. FEV₁ values for air or SO₂ exposure after placebo or theophylline treatment; P, placebo treatment; T, theophylline treatment.

ratropium bromide. It also should be noted that the sample size in this study was small and that the subjects had mild asthma, as judged by the fact that their percent of predicted normal FEV₁ was 76% to 108%. The two subjects with the highest PD₂₀s did demonstrate minimal or no response to the SO₂ challenge.

A general conclusion from this study is that theophylline mitigates but does not completely block the effects of inhaled SO₂. The SO₂ challenge of 1 ppm was realistic for a 10-minute period when down drafts occur around SO₂ sources. Air-quality policy that assumes that asthma medications will protect individuals from short-term SO₂ episodes may not be wise. However, such asthma medications may be helpful to susceptible patients. These data support adoption of a short (10 to 15 minute) National Ambient Air Quality standard for SO₂.

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Mononuclear cell subsets in the nickel-allergic reaction in vitro and in vivo

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*Nickel is the major cause of metal-induced contact allergy. To understand the mechanism of its immune reaction, we studied changes in lymphocyte surface markers during nickel challenge in both allergic and healthy subjects using an in vitro nickel reaction in which the lymphocytes of allergic subjects divide when they are stimulated with nickel sulfate. The lymphocytes were labeled with monoclonal antibodies (MAbs) to cell-surface antigens and studied by flow cytometry. Mononuclear cells from the nickel reaction in vivo were studied from skin biopsy specimens using MAbs and avidin-biotin immunohistochemistry. Nickel-induced lymphoblast transformation occurred in vitro only in cells from nickel-allergic subjects. CD4⁺ cells and CD45RO⁺ cells were overrepresented among the lymphoblasts of nickel-sensitive subjects, whereas CD8⁺ and CD8⁺CD11b⁺ and CD4⁺CD45R⁺ cells were underrepresented. The lymphoblasts contained T cells with the following activation markers: CD25, HLA-DR, CD26, CD71, Ki-67, and activation-associated antigen detected by the MAb, M21C5, but they were CD30⁻. CD16⁺ cells were overrepresented among the lymphoblasts. Nickel-reacting T cells used predominantly the T cell receptor, $\alpha\beta$ -heterodimer, but no preferential selection of either V β 5, V β 6, or V β 8 was observed. The phenotypes of nickel-reacting cells from cutaneous biopsy specimens were in agreement with the in vitro results. (*J ALLERGY CLIN IMMUNOL* 1992;89:794-800.)*

Key words: Nickel sensitivity, lymphocyte phenotypes, in vitro stimulation, skin reaction

Nickel is one of the leading causes of contact sensitivity. The prevalence of the disease is 10% in Finnish female subjects¹ and 16.7% in female subjects in

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Abbreviations used

TCR: T cell receptor
CD: cluster of differentiation
MNC: Mononuclear cell
PBS: Phosphate-buffered saline
FITC: Fluorescein isothiocyanate

Toronto, Canada.² Nickel allergy manifests itself in the form of contact dermatitis.

Nickel dermatitis is due to delayed-type hypersensitivity to nickel bound as a hapten on serum or cellular proteins. The reacting lymphocytes can be studied