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Effects of Inhalation of Acidic Compounds on Pulmonary Function in Allergic Adolescent Subjects

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There is concern about the human health effects of inhalation of acid compounds found in urban air pollution. It was the purpose of this study to investigate three of these acid compounds, sulfur dioxide (SO_2), sulfuric acid (H_2SO_4), and nitric acid (HNO_3) in a group of allergic adolescent subjects. Subjects were exposed during rest and moderate exercise to 0.7 μmole/m^3 (68 μg/m^3) H_2SO_4, 4.0 μmole/m^3 (0.1 ppm) SO_2, or 2.0 μmole/m^3 (0.05 ppm) HNO_3. Pulmonary functions (FEV_1, total respiratory resistance, and maximal flow) were measured before and after exposure. Preliminary analysis based on nine subjects indicates that exposure to 0.7 μmole/m^3 H_2SO_4 alone and in combination with SO_2 caused significant changes in pulmonary function, whereas exposure to air or SO_2 alone did not. FEV_1 decreased an average of 6% after exposure to H_2SO_4 alone and 4% when the aerosol was combined with SO_2. The FEV_1 decrease was 2% after both air and SO_2 exposures. Total respiratory resistance (R_T) increased 15% after the combined H_2SO_4 exposures, 12% after H_2SO_4 alone, and 7% after exposure to air. After exposures to HNO_3 alone, FEV_1 decreased by 4%, and R_T increased by 23%. These results are preliminary; final conclusions must wait for completion of the study.

Introduction

It was the purpose of this study to investigate the pulmonary function effects of inhalation of acidic compounds found in urban air The compounds studied were sulfur dioxide (SO_2), sulfuric acid (H_2SO_4), and nitric acid (HNO_3). These pollutants were studied alone and in combination in asthmatic adolescent subjects, a group shown to serve as an excellent model for individuals who are at risk of reduced respiratory function due to air pollution (*1,2*).

SO_2 and HNO_3 are found in the atmosphere primarily in the gas phase in concentrations that reach 2 μmole/m^3 (~ 50 ppbv, depending on absolute temperature) in urban areas (*3–5*). H_2SO_4 aerosol particles are observed to coexist with these gases in polluted atmospheres at concentrations up to 0.5 μmole/m^3 (49 μg/m^3).

Both SO_2 and HNO_3 have similar properties of solubility and diffusion coefficient and thus should have similar uptake profiles in the upper airways. They are also both acidic in aqueous media, although the anion released is different and will have different physiological reactivity. Another difference between SO_2 and HNO_3 is that HNO_3 reacts readily with ammonia (NH_3) in the vapor phase, whereas SO_2 does not. The resultant ammonium nitrate is nearly neutral and less reactive than HNO_3. Inhalation of H_2SO_4 aerosol has been shown to have effects on pulmonary function and clearance that are presumed to be related to the acidity delivered to the surface of the respiratory tissues (*2,6,7*). It has been shown that the acidity of inhaled H_2SO_4 particles depends on the concentration of endogenous NH_3 in the upper airways (*8,9*). Based on the model of Larson et al., 0.3-μm particles of H_2SO_4 will undergo up to 100% neutralization to ammonium sulfate $(NH_4)_2SO_4$, during inhalation depending on oronasal NH_3 levels. This reaction is highly dependent on particle size and limited by the rate of diffusion of NH_3 to the surface of the particle rather than by the mass of NH_3 available. The presence of a reactive acidic gas, such as HNO_3, uniformly distributed around an H_2SO_4 particle, will efficiently scavenge NH_3 as it mixes from the oral surface into inhaled air stream before it diffuses to the particle surface. Mariglio and co-workers (*10*) showed that the bronchoconstriction induced by H_2SO_4 inhalation by

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adult asthmatics can be increased by lowering oral NH_3 by having the subjects drink lemon juice and presumably reducing neutralization. HNO_3 in a combined HNO_3-H_2SO_4 exposure will have a similar effect by reducing the amount of NH_3 available for neutralization of H_2SO_4, thus allowing increased acid delivery to the respiratory system.

Nine allergic adolescent subjects inhaled SO_2 or H_2SO_4 alone and in combination and HNO_3 alone for 40 min of exposure during rest and moderate exercise. Pulmonary function changes were greatest after combined exposure to H_2SO_4 plus SO_2 and after exposure to HNO_3. Thus, the more acidic test atmospheres appeared to produce the greatest changes in pulmonary function.

Methods

Nine allergic adolescent subjects participated in this preliminary study. All had exercise-induced bronchospasm (EIB); five also had allergic asthma and four did not. The subjects' ages ranged from 12 to 18 years, six were male and three were female. Exercise-induced bronchospasm was defined in our study as a greater than 15% drop in forced expiratory volume in one second (FEV_1) after 6 min of exercise on a Quinton treadmill at 85% or greater maximum oxygen consumption (*11*). All subjects also had a positive response to a methacholine challenge test at or below 20 mg/mL. The methacholine, in phosphate buffered saline, was administered via a hand-held Turret #51 nebulizer (Diverse Respiratory Air Products, San Dimas, CA) containing 5 mL of solution, with a airflow rate of 6 L/min at 15 psi. The output of the nebulizer was 0.193 mL/min (SD, 0.007) with an aerosol mass median aerodynamic diameter of 1.75 μm (geometric SD, 1.90). The subjects were instructed to inhale the methacholine by mouth with nose clips in place for 90 sec during tidal breathing. The concentrations used were 0.0, 0.025, 0.25, 2.5, 5.0, 10.0, and 25 mg/mL. FEV_1 was measured 90 and 180 sec after each inhalation. A decrease in baseline FEV_1 of 20% or more at any time during the test and sustained at 180 sec was considered a positive response. The subjects and their parents read and signed a consent form approved by the University of Washington Human Subjects Office.

All subjects inhaled the test atmospheres through a rubber mouthpiece with nose clips in place on different days separated by at least 1 week. The five test atmospheres were: filtered air; 0.69 μmole/m^3 (68 μg/m^3) H_2SO_4; 4.0 μmole/m^3 (0.1 ppm) SO_2; 0.69 μmole/m^3 H_2SO_4 + 4.0 μmole/m^3 SO_2; 2.0 μmole/m^3 (0.05 ppm) HNO_3. All exposures were at approximately 65% relative humidity and 25°C. One percent SO_2 from a cylinder was diluted with the filtered air and the concentration measured by a pulsed fluorescent SO_2 analyzer (Thermo Electron Corp., Waltham, MA). The H_2SO_4 aerosol was generated by bubbling air through a glass frit in a 5% (by weight) aqueous solution of H_2SO_4. The mass median aerodynamic diameter of the H_2SO_4 aerosol was 0.6 μm, with a geometric SD of 1.5 (Royco 220, Menlo Park, CA). An integrating nephelometer (Meteorology Research Inc., Altadena, CA), which measures the light scattering due to aerosol particles, was used as a continuous measure of aerosol mass concentration. Filter sampling and gravimetric analysis were used to check mass concentration for each exposure. The filters subsequently were analyzed for sulfate, nitrate, and sodium ion with an ion chromatograph (Dionex 2020i, Sunnyvale, CA). The complete methods for generating and monitoring the gas-aerosol exposures have been described previously (*12*).

Nitric acid gas was generated by passing a controlled flow of clean air over reagent grade aqueous HNO_3 solution. This concentrated vapor was turbulently mixed into an all glass and polyethylene dilution chamber and delivery system. HNO_3 gas concentrations at the subject mouthpiece were determined with a Thermo Electron NO_x chemiluminescence analyzer (Model Series 14) modified to determine HNO_3 gas concentrations, a method first described by Joseph and Spicer (*13*). The technique relies on the fact that a nylon filter (Membrana Corp.) will absorb HNO_3 gas without disturbing NO_x gases. Both sides of a split stream sample are fed to a molybdenum converter for reduction to nitrous oxide (NO); one stream is first directed through a nylon filter cartridge, so that the difference in NO concentrations is due to HNO_3 gas. The unit was calibrated with HNO_3 permeation tubes at 1.5 and 3.0 μmole/m^3 and also by comparison with air samples collected on a nylon filter and analyzed by ion chromatography.

All physiologic measurements were recorded with a thermal recorder or X-Y plotter with the subject seated in a pressure-compensated, volume-displacement body plethysmograph. The following were measured: total respiratory resistance (R_T), using the forced-pressure oscillatory technique at 3 Hz (*14*); thoracic gas volume at functional residual capacity (FRC), using the gas compression technique (*15*); maximal flow calculated at 50 and 75% of expired vital capacity ($\dot{V}_{max50}$ and $\dot{V}_{max75}$) from a maximal flow volume curve; and FEV_1 calculated from the same flow volume curve. The FRC and flow volume tests were recorded in triplicate. R_T was measured for approximately 10 breaths; pressure and flow signals were processed by a microcomputer that calculated total respiratory resistance. Oral NH_3 in exhaled air was measured by having subjects breathe for 10 min with a sampling probe inserted between their teeth (*16*). The 1 L/min sample flow was diluted 2:1 with clean, dry air and reduced to a pressure of 70 kPA to prevent water condensation. The NH_3 in the air was scrubbed by 10 mL of 0.01 M solution of H_2SO_4, which was then analyzed for ammonium ion (NH_4) by an ion chromatograph (Dionex 2020i, Sunnyvale, CA).

The protocol, which was identical on each day, was as follows: 10-min NH_4 baseline measurement; pulmonary function baseline (R_T, FRC, maximal flow, and

FEV_1); 40-min exposure to test atmosphere (30 min at rest and 10 min during moderate exercise on a treadmill); pulmonary function postexposure measurements; 10-min NH_4 postexposure measurement. During exposure, minute ventilation ($\dot{V}_E$) was measured with a respiratory integrator (Hewlett-Packard, Palo Alto, CA) and end tidal carbon dioxide (CO_2) tension was measured with a medical gas analyzer (Beckman Instruments, Palo Alto, CA). At the end of each exposure, the subjects were asked to score a symptom rating scale for cough, pain or burning in chest, shortness of breath, fatigue, headache, unusual taste or smell, sore throat, nasal discharge, and wheezing. The subjects took the forms with them to be scored later that day and on the following day.

The mean value was calculated for each pulmonary function parameter at baseline and postexposure. If one value in a set of three was missing, the average of the other two values was substituted. Paired *t*-tests were used to test the difference between matched sets of exposure means.

Results

Fifteen subjects will participate in this study; nine have been exposed to date. The physical characteristics of the nine subjects are given in Table 1. The pulmonary function means and SDs are listed in Table 2. The mean concentration of H_2SO_4 alone was 61.3 ± 4 $\mu g/m^3$; for the H_2SO_4 plus SO_2 exposures, the mean concentration was 66.1 ± 17 $\mu g/m^3$. The mean HNO_3 concentration was 49.4 ± 0.94 ppb. The mean concentration of SO_2 was 0.10 ± 0.01 ppm and 0.11 ± 0.02 ppm for single and combined exposures, respectively. During exposure at rest, the mean $\dot{V}_E$ was 8.4 ± 1.5 L/min; during exercise exposure the mean $\dot{V}_E$ was 31.8 ± 10.2 L/min.

The results of the statistical analysis indicated that 0.69 $\mu mole/m^3$ H_2SO_4 alone or in combination with 4.0 $\mu mole/m^3$ SO_2 caused a greater change in pulmonary function from baseline to postexposure than exposure to either SO_2 alone or air. FEV_1 decreased by 6% after exposure to H_2SO_4 alone ($p < 0.05$) and 4% after exposure to H_2SO_4 combined with SO_2 ($p < 0.01$) as compared with a 2% nonsignificant decrease after air exposure. FEV_1 decreased 4% after exposure to HNO_3 alone ($p < 0.05$). The changes in FEV_1 for the nine subjects are displayed in Figure 1. After the combined H_2SO_4 and SO_2 exposure, R_T increased by 15% ($p < 0.001$) as compared with a 7% increase after air and a 4% increase after SO_2 exposure (both nonsignificant). The 12% increase in R_T after H_2SO_4 alone was not significant. R_T increased 23% after HNO_3 exposure ($p < 0.05$). The individual changes in R_T are shown in Figure 2. There was no indication that NH_3 depletion occurred after the H_2SO_4 exposures. After 0.69 $\mu mole/m^3$, six of the eight subjects had higher NH_3 concentrations postexposure as compared with baseline (measurement in one subject was technically flawed). After the combined exposure, seven of nine subjects showed increased postexposure NH_3 values. However, after HNO_3 exposures, the postexposure NH_3 values

Table 1. Physical characteristics of allergic adolescent subjects.

Subject no.	Sex	Age, years	Ht, cm	Baseline FEV_1, L
1	M	15	182.5	3.6
2	M	17	184.7	5.4
3	M	15	171.0	4.0
4	F	14	165.2	3.8
5	F	13	156.5	2.4
6	M	13	148.0	3.0
7	M	14	145.5	2.5
8	F	12	166.5	2.6
9	M	18	181.6	3.4

Table 2. Means and standard deviations for pulmonary function values in allergic adolescent subjects (n = 9).

Functional measurement	Exposure atmosphere	Baseline	PE 1[a]	PE 2
R_T, cm H_2O/L/sec	Air	5.10 ± 0.68	5.48 ± 0.99	
	68 $\mu g/m^3$ H_2SO_4	4.39 ± 1.12	4.93 ± 0.67	
	0.1 ppm SO_2	4.64 ± 1.21	4.81 ± 0.66	
	H_2SO_4 + SO_2	4.30 ± 1.04	4.96 ± 1.15	
	0.05 ppm HNO_3[b]	4.35 ± 0.46	5.33 ± 1.45	
FEV_1, L	Air	3.38 ± 0.83	3.32 ± 0.82	3.28 ± 0.82
	68 $\mu g/m^3$ H_2SO_4	3.39 ± 0.99	3.19 ± 0.97	3.34 ± 1.01
	0.1 ppm SO_2	3.43 ± 0.84	3.35 ± 0.81	3.42 ± 0.86
	H_2SO_4 + SO_2	3.39 ± 1.00	3.27 ± 1.02	3.34 ± 1.01
	0.05 ppm HNO_3[b]	3.38 ± 0.96	3.23 ± 0.96	3.31 ± 0.94

[a] PE, postexposure.
[b] n = 8.

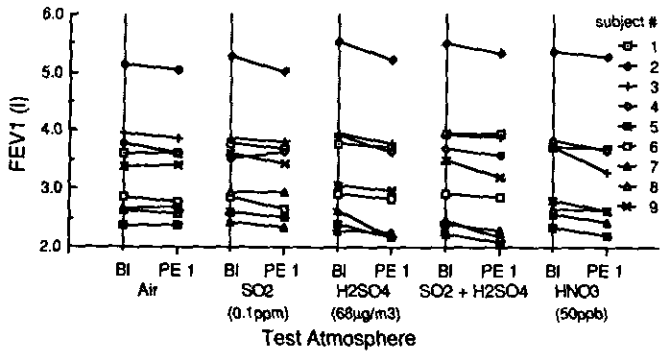

FIGURE 1. Individual changes in FEV_1 in nine asthmatic subjects after H_2SO_4 and SO_2 exposures and eight subjects after exposure to HNO_3.

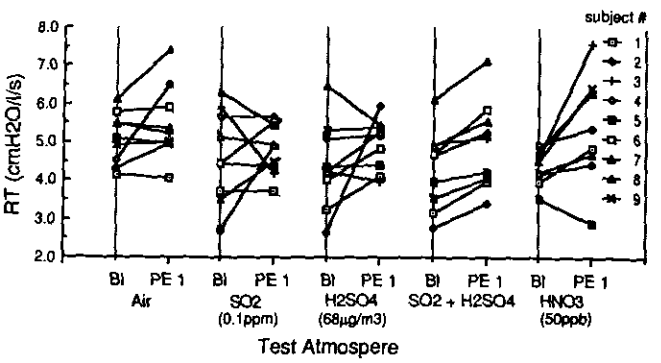

FIGURE 2. Individual changes in R_T in nine asthmatic subjects after H_2SO_4 and SO_2 exposure and eight subjects after exposure to HNO_3.

were lower than the baseline values in four of six subjects. One subject's measurements were technically flawed. The mean values for NH_3 on HNO_3 exposure days were 342 ppb at baseline and 313 ppb at post-exposure. These values were 306 ppb and 516 ppb, respectively, on the air exposure days. There were no apparent trends in the answers to the symptom rating scale dependent upon specific pollutant exposure.

Discussion

This preliminary study has shown marginal changes in pulmonary function in allergic subjects after exposure to 0.69 µmole/m^3 H_2SO_4 alone and slightly greater effects when the H_2SO_4 was combined with 4.0 µmole/m^3 SO_2. The subjects in this study are similar to those in an earlier study of the inhaled effects of 100 µg/m^3 (1.0 µmole/m^3) H_2SO_4, which elicited significant changes in all the pulmonary function parameters measured (*2*). After a 40-min H_2SO_4 exposure (including 10 min of moderate exercise), 10 asthmatic adolescent subjects showed an average 5% decrease in FEV_1, an average 40% increase in R_T, and 21 and 29% decreases in $\dot{V}_{max50}$ and $\dot{V}_{max75}$, respectively.

A second group of asthmatic adolescent subjects was exposed to 1.0 µmole/m^3 H_2SO_4 during an investigation of the effects of sulfur oxides on nasal function (*17*). The pulmonary function changes seen in this group of subjects confirmed the earlier findings. Based on the marginal changes found in pulmonary function after exposure to 0.69 µmole/m^3 (68 µg/m^3) H_2SO_4, it is concluded that 1.0 µmole/m^3 (100 µg/m^3) is very close to the threshold concentration of H_2SO_4 that produces pulmonary function changes in this population of adolescents with asthma.

The results of the present study also suggest that a combination of H_2SO_4 and SO_2 produced a slightly greater change in pulmonary function than did exposure to these pollutants alone. Both R_T and FEV_1 showed a significant change after the combined exposure. It is apparent from Figure 2 that R_T increased in all subjects after the combined exposures and not after the single exposures. Although pollutant combination exposures have not been reported in adolescent subjects, others have studied combined exposures in adult subjects. None of these studies demonstrated convincing additive or synergistic effects of the pollutant combinations.

Studies of SO_2 in combination with other gases were conducted by Linn and co-workers (*18*) and Kagawa (*19*). Studies of SO_2 and aerosols have been reported by Kleinman and associates (*20*), Stacy et al. (*21*), and Kulle and co-workers (*22*). Kulle and co-workers (*23*) also studied pulmonary function and bronchial reactivity following exposure to H_2SO_4 and O_3 but found no significant changes. Kleinman did report that a mixture of SO_2, O_3, and H_2SO_4 may have produced a slightly greater effect than the exposure to O_3 alone (*20*), and the Kulle study noted more respiratory symptoms after combined exposure to SO_2 and $(NH_4)_2SO_4$ (*22*).

The subjects in the Stacy study had a 50% greater change in specific airway resistance after exposure to O_3 and H_2SO_4 than after O_3 alone; however, this change was not significant (*21*). Thus, there has been a suggestion from studies of pollutant combinations that the combined exposures enhance pulmonary effects. None of the subjects in the cited studies had asthma, which may explain the significant changes following combined H_2SO_4 and SO_2 seen in the present study. However, many variables differ among laboratories studying controlled air pollutant exposures in human volunteers, and comparison of results is difficult.

To the best of our knowledge, there are no published reports of controlled human exposures to HNO_3. The present study has demonstrated significant changes in pulmonary function after 40 min inhalation of HNO_3. These changes are similar to those seen after the combined exposures to H_2SO_4 and SO_2 and slightly greater than those seen after either 0.69 µmole/m^3 H_2SO_4 alone or 4.0 µmole/m^3 SO_2 alone. Thus, there is

a suggestion that the more acidic compounds investigated in this study produced the greater changes in pulmonary function.

The fact that little depletion of oral NH_3 was seen in this study is not surprising. Larson and co-workers (*16*) found mean levels of 28 μmole/m^3 (700 ppbv) of NH_3 in expired air, indicating a large flux of NH_3 from the mucous lining of the upper airways, primarily the mouth and nose. On a stoichiometric basis this level of NH_3 could neutralize 14 μmole H_2SO_4 to $(NH_4)_2SO_4$. However, during normal inspiration, neutralization to equilibrium cannot occur in the upper airways due to the short residence times, except for particles that are large and dilute (*9*). Thus, although inspired H_2SO_4 particles may be neutralized to a varying degree depending on their size, the amount of H_2SO_4 inhaled and deposited on the surface of the mouth, nose, and upper airways in this study will not be enough to reduce the NH_3 levels in the mucous layer or in the air in contact with that layer. In fact, in our study, the NH_3 tended to increase from baseline to postexposure. It is possible that oral breathing on a mouthpiece dries the mouth and tends to concentrate the oral NH_3 levels, causing postexposure values to be high. There was a trend toward lower NH_3 levels after HNO_3 exposures. Even though the differences were small, the fact that increases in NH_3 were seen after air exposure may mean that the small differences (in spite of airway drying) were true depletions of oral NH_3.

The subjects in this study represent a group with mild to moderate asthma. Four of the subjects only develop asthmatic symptoms upon exertion; that is, they only have EIB. The current definition of asthma has been redefined as bronchial hyperresponsiveness (*24,25*). This definition takes into account patients with normal airway function who demonstrate bronchial hyperresponsiveness to known provocative agents such as methacholine, histamine, exercise and, in some cases, SO_2. All the subjects in the present study demonstrated such hyperresponsiveness by their positive responses to methacholine challenge and exercise challenge tests.

The effects of inhalation of acid compounds seen in adolescent asthmatic subjects can be related to the general population. Approximately 15% of the general population has atopy or allergic hypersensitivity, of these, 3 to 4% are asthmatic (*26*). One-third of the remaining 11 to 12% percent of atopic individuals have EIB. This total number of persons equals about 3% of the general population. Some studies indicate that 4 to 5% of nonallergic people have EIB (*27*). Altogether, it is estimated that at least 10% of the general population has asthma or EIB as a manifestation of bronchial hyperresponsiveness. Thus, in the U.S., approximately 25 million people are likely to have bronchial hyperresponsiveness to inhaled irritants or provocative challenges. Experience with the U.S. Olympic committee confirms this estimate. Of 597 members of the Olympic team, 67, or 11% had documented EIB when given an exercise treadmill test (*28*).

In summary, it is concluded that adolescent subjects with asthma and EIB show significant pulmonary function changes following short-term exposures during moderate exercise to a combination of H_2SO_4 and SO_2, and also to a low concentration of HNO_3. It is suggested that the acidity of the inhaled compounds determines the degree of pulmonary function change.

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