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 (SO₂), sulfuric acid (H₂SO₄), and nitric acid (HNO₃) in a group of allergic adolescent subjects. Subjects were exposed during rest and moderate exercise to 0.7 μmole/m³ (68 μg/m³) H₂SO₄, 4.0 μmole/m³ (0.1 ppm) SO₂, or 2.0 μmole/m³ (0.05 ppm) HNO₃. Pulmonary functions (FEV₁, 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/m³ H₂SO₄ alone and in combination with SO₂ caused significant changes in pulmonary function, whereas exposure to air or SO₂ alone did not. FEV₁ decreased an average of 6% after exposure to H₂SO₄ alone and 4% when the aerosol was combined with SO₂. The FEV₁ decrease was 2% after both air and SO₂ exposures. Total respiratory resistance (R₉) increased 15% after the combined H₂SO₄ exposures, 12% after H₂SO₄ alone, and 7% after exposure to air. After exposures to HNO₃ alone, FEV₁ decreased by 4%, and R₉ 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 (SO₂), sulfuric acid (H₂SO₄), and nitric acid (HNO₃). 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).

SO₂ and HNO₃ are found in the atmosphere primarily in the gas phase in concentrations that reach 2 μmole/m³ (~50 ppbv, depending on absolute temperature) in urban areas (3–5). H₂SO₄ aerosol particles are observed to coexist with these gases in polluted atmospheres at concentrations up to 0.5 μmole/m³ (49 μg/m³).

Both SO₂ and HNO₃ 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 SO₂ and HNO₃ is that HNO₃ reacts readily with ammonia (NH₃) in the vapor phase, whereas SO₂ does not. The resultant ammonium nitrate is nearly neutral and less reactive than HNO₃. Inhalation of H₂SO₄ 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 H₂SO₄ particles depends on the concentration of endogenous NH₃ in the upper airways (8,9). Based on the model of Larson et al., 0.3-μm particles of H₂SO₄ will undergo up to 100% neutralization to ammonium sulfate (NH₄)₂SO₄ during inhalation depending on oronasal NH₃ levels. This reaction is highly dependent on particle size and limited by the rate of diffusion of NH₃ to the surface of the particle rather than by the mass of NH₃ available. The presence of a reactive acidic gas, such as HNO₃, uniformly distributed around an H₂SO₄ particle, will efficiently scavenge NH₃ 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 H₂SO₄ inhalation by

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adult asthmatics can be increased by lowering oral NH₃ by having the subjects drink lemon juice and presumably reducing neutralization. HNO₃ in a combined HNO₃-H₂SO₄ exposure will have a similar effect by reducing the amount of NH₃ available for neutralization of H₂SO₄, thus allowing increased acid delivery to the respiratory system.

Nine allergic adolescent subjects inhaled SO₂ or H₂SO₄ alone and in combination and HNO₃ alone for 40 min of exposure during rest and moderate exercise. Pulmonary function changes were greatest after combined exposure to H₂SO₄ plus SO₂ and after exposure to HNO₃. 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 (FEV₁) 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. FEV₁ was measured 90 and 180 sec after each inhalation. A decrease in baseline FEV₁ 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 μmol/m³ (68 μg/m³) H₂SO₄; 4.0 μmol/m³ (0.1 ppm) SO₂; 0.69 μmol/m³ H₂SO₄ + 4.0 μmol/m³ SO₂; 2.0 μmol/m³ (0.05 ppm) HNO₃. All exposures were at approximately 65% relative humidity and 25°C. One percent SO₂ from a cylinder was diluted with the filtered air and the concentration measured by a pulsed fluorescent SO₂ analyzer (Thermo Electron Corp., Waltham, MA). The H₂SO₄ aerosol was generated by bubbling air through a glass frit in a 5% (by weight) aqueous solution of H₂SO₄. The mass median aerodynamic diameter of the H₂SO₄ 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 HNO₃ solution. This concentrated vapor was turbulently mixed into an all glass and polyethylene dilution chamber and delivery system. HNO₃ gas concentrations at the subject mouthpiece were determined with a Thermo Electron NOX chemiluminescence analyzer (Model Series 14) modified to determine HNO₃ 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 HNO₃ gas without disturbing NOX 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 HNO₃ gas. The unit was calibrated with HNO₃ permeation tubes at 1.5 and 3.0 μmol/m³ 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 (Rₚ), 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 (Vmax50 and Vmax75) from a maximal flow volume curve; and FEV₁ calculated from the same flow volume curve. The FRC and flow volume tests were recorded in triplicate. Rₚ was measured for approximately 10 breaths; pressure and flow signals were processed by a microcomputer that calculated total respiratory resistance. Oral NH₃ 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 NH₃ in the air was scrubbed by 10 mL of 0.01 M solution of H₂SO₄, which was then analyzed for ammonium ion (NH₄⁺) by an ion chromatograph (Dionex 2020i, Sunnyvale, CA).

The protocol, which was identical on each day, was as follows: 10-min NH₄ baseline measurement; pulmonary function baseline (Rₚ, FRC, maximal flow, and
FEV1): 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 NH4 postexposure measurement. During exposure, minute ventilation (VE) was measured with a respiratory integrator (Hewlett-Packard, Palo Alto, CA) and end tidal carbon dioxide (CO2) 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 H2SO4 alone was 61.3 ± 4 μg/m3; for the H2SO4 plus SO2 exposures, the mean concentration was 66.1 ± 17 μg/m3. The mean HNO3 concentration was 49.4 ± 0.94 ppb. The mean concentration of SO2 was 0.10 ± 0.01 ppm and 0.11 ± 0.02 ppm for single and combined exposures, respectively. During exposure at rest, the mean V̇E was 8.4 ± 1.5 L/min; during exercise exposure the mean V̇E was 31.8 ± 10.2 L/min.

The results of the statistical analysis indicated that 0.69 μmole/m3 H2SO4 alone or in combination with 4.0 μmole/m3 SO2 caused a greater change in pulmonary function from baseline to postexposure than exposure to either SO2 alone or air. FEV1 decreased by 6% after exposure to H2SO4 alone (p < 0.05) and 4% after exposure to H2SO4 combined with SO2 (p < 0.01) as compared with a 2% nonsignificant decrease after air exposure. FEV1 decreased 4% after exposure to HNO3 alone (p < 0.05). The changes in FEV1 for the nine subjects are displayed in Figure 1. After the combined H2SO4 and SO2 exposure, RT increased by 15% (p < 0.001) as compared with a 7% increase after air and a 4% increase after SO2 exposure (both nonsignificant). The 12% increase in RT after H2SO4 alone was not significant. RT increased 23% after HNO3 exposure (p < 0.05). The individual changes in RT are shown in Figure 2. There was no indication that NH3 depletion occurred after the H2SO4 exposures. After 0.69 μmole/m3, six of the eight subjects had higher NH3 concentrations postexposure as compared with baseline (measurement in one subject was technically flawed). After the combined exposure, seven of nine subjects showed increased postexposure NH3 values. However, after HNO3 exposures, the postexposure NH3 values

Table 1. Physical characteristics of allergic adolescent subjects.

| Subject no. | Sex | Age, years | Ht, cm | Baseline FEV1, 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         | 165.5  | 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* | PE 2 |
|------------------------|---------------------|----------|-------|------|
| ṘF, cm H2O/L/sec       | Air                 | 5.10 ± 0.68 | 5.28 ± 0.99 |
| 68 μg/m3 H2SO4         | 4.39 ± 1.12         | 4.93 ± 0.67 |
| 0.1 ppm SO2            | 4.64 ± 1.21         | 4.61 ± 0.66 |
| H2SO4 + SO2            | 4.30 ± 1.04         | 4.96 ± 1.15 |
| 0.05 ppm HNO3b         | 4.35 ± 0.46         | 5.33 ± 1.45 |
| FEV1, L                | Air                 | 3.38 ± 0.83 | 3.32 ± 0.82 |
| 68 μg/m3 H2SO4         | 3.39 ± 0.99         | 3.19 ± 0.97 |
| 0.1 ppm SO2            | 3.43 ± 0.84         | 3.35 ± 0.81 |
| H2SO4 + SO2            | 3.39 ± 1.00         | 3.27 ± 1.02 |
| 0.05 ppm HNO3b         | 3.38 ± 0.96         | 3.23 ± 0.96 |

* PE, postexposure.

b n = 8.
were lower than the baseline values in four of six subjects. One subject's measurements were technically flawed. The mean values for NH₃ on HNO₃ 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 umole/m³ H₂SO₄ alone and slightly greater effects when the H₂SO₄ was combined with 4.0 umole/m³ SO₂. The subjects in this study are similar to those in an earlier study of the inhaled effects of 100 μg/m³ (1.0 umole/m³) H₂SO₄, which elicited significant changes in all the pulmonary function parameters measured (2). After a 40-min H₂SO₄ exposure (including 10 min of moderate exercise), 10 asthmatic adolescent subjects showed an average 5% decrease in FEV₁, an average 40% increase in RT, and 21 and 29% decreases in V₉₅₀ and V₉₅₇ respectively.

A second group of asthmatic adolescent subjects was exposed to 1.0 umole/m³ H₂SO₄ 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 umole/m³ (68 μg/m³) H₂SO₄, it is concluded that 1.0 umole/m³ (100 μg/m³) is very close to the threshold concentration of H₂SO₄ that produces pulmonary function changes in this population of adolescents with asthma.

The results of the present study also suggest that a combination of H₂SO₄ and SO₂ produced a slightly greater change in pulmonary function than did exposure to these pollutants alone. Both RT and FEV₁ showed a significant change after the combined exposure. It is apparent from Figure 2 that RT 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 convincingly additive or synergistic effects of the pollutant combinations.

Studies of SO₂ in combination with other gases were conducted by Linn and co-workers (18) and Kagawa (19). Studies of SO₂ 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 H₂SO₄ and O₃ but found no significant changes. Kleinman did report that a mixture of SO₂, O₃, and H₂SO₄ may have produced a slightly greater effect than the exposure to O₃ alone (20), and the Kulle study noted more respiratory symptoms after combined exposure to SO₂ and (NH₄)₂SO₄ (22).

The subjects in the Stacy study had a 50% greater change in specific airway resistance after exposure to O₃ and H₂SO₄ than after O₃ 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 H₂SO₄ and SO₂ 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 HNO₃. The present study has demonstrated significant changes in pulmonary function after 40 min inhalation of HNO₃. These changes are similar to those seen after the combined exposures to H₂SO₄ and SO₂ and slightly greater than those seen after either 0.69 umole/m³ H₂SO₄ alone or 4.0 umole/m³ SO₂ 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 NH₃ was seen in this study is not surprising. Larson and co-workers (16) found mean levels of 28 μmole/m³ (700 ppbv) of NH₃ in expired air, indicating a large flux of NH₃ from the mucous lining of the upper airways, primarily the mouth and nose. On a stoichiometric basis this level of NH₃ could neutralize 14 μmole H₂SO₄ to (NH₄)₂SO₄. 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 H₂SO₄ particles may be neutralized to a varying degree depending on their size, the amount of H₂SO₄ inhaled and deposited on the surface of the mouth, nose, and upper airways in this study will not be enough to reduce the NH₃ levels in the mucous layer or in the air in contact with that layer. In fact, in our study, the NH₃ tended to increase from baseline to post-exposure. It is possible that oral breathing on a mouthpiece dries the mucous and tends to concentrate the airway NH₃ levels, causing postexposure values to be high. There was a trend toward lower NH₃ levels after HNO₃ exposures. Even though the differences were small, the fact that increases in NH₃ were seen after air exposure may mean that the small differences (in spite of airway drying) were true depletions of oral NH₃.

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, SO₂. 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 H₂SO₄ and SO₂, and also to a low concentration of HNO₃. It is suggested that the acidity of the inhaled compounds determines the degree of pulmonary function change.

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