

Human experimental studies on the effects of inhaled particles

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Human experimental studies on the effects of inhaled particles have mainly dealt with the effects of acid particles and, especially, their effects on lung function. Concerning the effects on lung function, it seems that the more acid the particles are the more severe the effects, and that asthmatic persons are more sensitive than healthy subjects (1, 2). It is therefore understandable that experiments with sulfuric acid aerosols in asthmatics have been given such a high priority.

Acid particles

Effects on lung function

There have been several short-term studies with rather similar designs. The exposure times have been one or several hours. Various groups of asthmatics have been studied using conventional lung function parameters such as forced expiratory volume in 1 second ($FEV_{1.0}$), maximum expiratory flow, and airway resistance (R_{aw}), and in some studies bronchial reactivity has also been measured. During the exposure, both periods with physical exercise and periods with rest have been used. This approach is important as physical exercise has been shown to increase the effect. In spite of the similarities in design, the studies have given markedly different results. Some studies have shown significant but small decreases in lung function for exposure at concentrations around or even below $100 \mu\text{g}/\text{m}^3$ of sulfuric acid. Koenig et al (3) found significant changes in young asthmatics already at $70 \mu\text{g}/\text{m}^3$. The decrease in $FEV_{1.0}$ was, for example, 6% after exposure to sulfuric acid but only 2% after exposure to air. However, the same group found no clear decrease in elderly asthmatics at this concentration (4). Similarly, Utell et al (1) found small effects on the lung function of asthmatic patients at a sulfuric acid concentration of $750 \mu\text{g}/\text{m}^3$. On the other hand, there are studies in which concentrations of $1000 \mu\text{g}/\text{m}^3$ or higher have been necessary to induce significant effects on lung function (5–7).

It seems difficult to explain these remarkably different results by differences in methodology. One important parameter in particle exposure is the size distribution. However, the mass median aerodynamic diameter was $0.6 \mu\text{m}$ in the study by Koenig et al (3), and this figure does not differ much from the mass median aerodynamic diameters in the studies by Avol et al (5) ($0.9 \mu\text{m}$) and Aris et al (7) ($0.4 \mu\text{m}$). As already mentioned, the designs and the method for lung function studies were rather similar. One possible explanation for the

different results may be that, although patients with asthma were used in all the studies, these patients might differ in bronchial reactivity. It is known that, when studied with clinical tests such as the methacholine or histamine test, bronchial reactivity differs markedly. Svarthengren et al (8) found that, in healthy subjects, the concentrations of methacholine needed to cause a 20% reduction in $FEV_{1.0}$ varied by a factor of 100.

Combined effect of acid aerosols and other pollutants

Ozone (O_3) is a common air pollutant that can damage the lungs. Linn et al (9) exposed both 30 asthmatic patients and 15 healthy subjects during a relatively long time, 6.5 hours, to either $100 \mu\text{g}/\text{m}^3$ of sulfuric acid aerosol (mass median aerodynamic diameter $0.5 \mu\text{m}$) or to 0.12 ppm of O_3 or a combination of the two pollutants. The exposure to O_3 with or without sulfuric acid aerosol affected lung function significantly (eg, decreased $FEV_{1.0}$ and increased bronchial reactivity). The exposure to the sulfuric acid aerosol did not induce any significant changes in lung function when compared with exposure to clean air. There were no substantial differences between the effects of O_3 alone and the combination of O_3 and sulfuric acid aerosol. A similar lack of joint effect was also observed by the same group for O_3 and nitric acid on healthy subjects (10).

However, Frampton et al (11) studied the interaction between a sulfuric acid aerosol and O_3 in both healthy subjects and asthmatics using another design. They first exposed 12 subjects during a 3-hour period with both exercise and rest to either a sulfuric acid aerosol (mass median diameter $0.6 \mu\text{m}$) concentration of $100 \mu\text{g}/\text{m}^3$ or a control aerosol of sodium chloride of a similar concentration and particle size distribution. Twenty-four hours later, they exposed the subjects to O_3 at three different concentrations, 80, 120 and 180 ppb, during a 3-hour period with both physical exercise and rest. For the healthy subjects, there was no clear evidence of any effect of the preexposure to the acid aerosol on the response to O_3 . However, for the asthmatic persons, preexposure to the sulfuric acid aerosol but not to the sodium chloride aerosol caused dose-related small decreases (up to about 6%) in forced vital capacity (FVC) and $FEV_{1.0}$ after O_3 exposure and also 4 hours later.

Anderson et al (12) exposed 15 healthy and 15 asthmatic volunteers to carbon black particles both alone and in combination with sulfuric acid particles. Both the carbon black particles and the sulfuric acid particles had a diameter of $0.5 \mu\text{m}$. In the exposure to carbon

particles alone, the mean concentration was 200 $\mu\text{g}/\text{m}^3$ for the healthy subjects and 145 $\mu\text{g}/\text{m}^3$ for the asthmatics. In the combined exposure, the concentration of carbon black was 230 $\mu\text{g}/\text{m}^3$ for the healthy subjects and 205 $\mu\text{g}/\text{m}^3$ for the asthmatics, and the concentration of sulfuric acid was around 100 $\mu\text{g}/\text{m}^3$. All the exposures lasted 60 minutes with alternating 10-minute periods of physical exercise and rest. Specific airway resistance (SR_{aw}), FVC and $\text{FEV}_{1.0}$ were measured before, during, and at the end of the exposure. In addition, bronchial reactivity was assessed after the exposure. Neither the exposure to carbon particles alone nor to the combination of carbon black and sulfuric acid particles induced any clear effect on lung function.

Effects on mucociliary clearance

When the concentration of a pollutant increases until it produces an effect on mucociliary clearance, the effect is usually increased mucociliary transport. A further increase in the concentration or a longer exposure may cause impaired transport. An increased mucociliary clearance, caused by acute inhalation of a pollutant, can therefore be considered to be an undesirable effect for the same reason that bronchoconstriction is generally considered to be undesirable (bronchoconstriction can be considered a positive defense mechanism as it results in a more central deposition of the particles and thereby faster elimination). For example, short-term cigarette smoking results in increased mucociliary transport (13, 14), but it is well known that long-term cigarette smoking results in impaired mucociliary transport.

There have been several studies in which persons have been exposed to aerosols of sulfuric acid with a mass median aerodynamic diameter of around 0.5 μm , and tracheobronchial clearance using radiolabeled test aerosols have been studied. Newhouse et al (15) exposed healthy subjects to 1 mg/m^3 of a sulfuric acid aerosol for 2 hours. Clearance was studied with a test aerosol of radiolabeled particles with a mass median aerodynamic diameter of 3 μm , and it was significantly increased by the sulfuric acid exposure. Leikauf et al (16) exposed healthy subjects to sulfuric acid particles at concentrations of 0, 110, 330, and 980 $\mu\text{g}/\text{m}^3$ for 1 hour. Clearance was studied using 7.5 μm particles. The lowest concentration of sulfuric acid caused a significant increase in tracheobronchial clearance, from a half-time of 80 minutes to one of 50 minutes, and the highest concentration caused a significant reduction in clearance. Leikauf et al (17) also exposed healthy subjects to the same concentrations of sulfuric acid and studied clearance using somewhat smaller particles, 4.2 μm . In this investigation, clearance was significantly reduced at both 100 (from a half-time of 80 minutes to 110 minutes) and 1000 $\mu\text{g}/\text{m}^3$ (half-time 142 minutes) and tend-

ed to be reduced at 300 $\mu\text{g}/\text{m}^3$ (half-time 106 minutes). The same group also exposed asthmatics to the same concentrations of sulfuric acid particles and used radiolabeled 3.9 μm particles for clearance studies (18). Of the 10 asthmatics, six suffered from asymptomatic mild asthma and four from more severe asthma. The group with the mild asthma had a slower clearance in the exposures to all three concentrations (100, 300 and 1000 $\mu\text{g}/\text{m}^3$) of sulfuric acid than in the control exposure without sulfuric acid, but only for the highest concentration was clearance significantly slower. The group with the severe asthma showed no effect on clearance after exposure to sulfuric acid particles. Spektor et al (19) also exposed a group of healthy subjects to 100 $\mu\text{g}/\text{m}^3$ of sulfuric acid particles, once for 1 hour and a second time for 2 hours. Clearance was studied using 5.2 μm particles. Clearance was reduced in both sulfuric acid exposures, but more markedly after the 2-hour than after the 1-hour exposure.

Effects on the mucociliary clearance of larger particles containing sulfuric acid have also been studied. Laube et al (20) exposed healthy subjects during 1 hour (rest and physical exercise) to an acid or saline fog concentration of 500 mg/m^3 . The acid fog contained sulfuric acid and had a pH of 2 and a mass median diameter of 10.3 μm . The saline fog had a mass median aerodynamic diameter of 10.9 μm . Mucociliary clearance was studied using 3.4 μm of a radiolabeled test aerosol. Clearance was significantly increased after the acid fog exposure when compared with that after the saline fog exposure. Ventilatory function and bronchial reactivity were also studied, but they were unaffected by both fogs.

Effects on bronchoalveolar lavage

Frampton et al (21) exposed healthy subjects to a 1000 $\mu\text{g}/\text{m}^3$ concentration of sulfuric acid or sodium chloride (mass median aerodynamic diameter 0.9 μm) and studied bronchoalveolar lavage, which was made 18 hours later. The volunteers performed intermittent physical exercise during the 2-hour exposure period. There were no significant changes between the two exposures in the distribution of alveolar macrophages, lymphocytes, mononuclear leukocytes, mast cells, or the total number of cells obtained by lavage.

Diesel and other vehicle exhaust

Rudell et al (22) exposed healthy nonsmokers to clean air and filtered and unfiltered diesel exhaust. The exposure time was 60 minutes with alternated 10-minute periods of rest and light exercise. The number of particles was $2.6 \times 10^6/\text{cm}^3$ (estimated to correspond with about 300 $\mu\text{g}/\text{m}^3$) in the unfiltered diesel exhaust and

$1.4 \times 10^6/\text{cm}^3$ in the filtered exhaust. The concentrations of nitrogen oxide, nitrogen dioxide (NO_2), carbon monoxide (CO), total hydrocarbons, and formaldehyde were highly similar in the two diesel exhaust exposures. The NO_2 concentration was, for example, 1.9 ppm in the unfiltered exhaust and 1.7 ppm in the filtered exhaust. Both diesel exhausts caused similar and significant increases in R_{aw} and SR_{aw} (around 15%). In a later study with 10 healthy never smokers, the same group used the same exposure design with filtered and unfiltered diesel exhaust and studied the effects on the lung lavage fluid (23). In this study, the concentration of particles was 2.6×10^6 particles/ cm^3 for the unfiltered exhaust and 1.3×10^6 particles/ cm^3 for the filtered exhaust. The same gases were measured as in the former study, and also in this study the concentrations were similar in both exhausts, for example, the concentration of NO_2 was 1.3 ppm in the unfiltered exhaust and 1.2 ppm in the filtered exhaust. In the lavage investigation, the first recovered 20 ml of the first instilled 60 ml was analyzed separately and defined as the bronchial portion. The remaining recovered fluid of the totally instilled 4×60 ml was defined as the bronchoalveolar portion. The number geometric mean was $0.041 \mu\text{m}$ for the unfiltered exhaust and $0.044 \mu\text{m}$ for the filtered exhaust, and the volume mean diameters were 0.11 and $0.13 \mu\text{m}$, respectively. In the bronchial portion of the lavage fluid, the total number of cells and the number of macrophages were significantly increased only after the exposure to the unfiltered diesel exhaust, whereas neutrophils increased by a factor of 3–4 after exposure to both filtered and unfiltered exhaust. In the bronchoalveolar portion, which should mainly reflect the alveolar region, there were only significant effects on the numbers of lymphocyte subsets. The percentage of CD3+ lymphocytes was increased to about the same extent after filtered and unfiltered exhaust exposure. Phagocytic activity of alveolar macrophages was studied *in vitro* after diesel exposure and was found to be significantly reduced after both types of diesel exposures.

The same group also studied acute inflammatory responses in the airways and peripheral blood after unfiltered diesel exposure (24). Fifteen healthy nonsmokers were exposed for 1 hour to air or diesel exhaust on two occasions. The particle concentration was $300 \mu\text{g}/\text{m}^3$ (4.3×10^6 particles/ cm^3), and the concentration was 1.6 ppm for NO_2 , 4.5 ppm for nitrogen oxide, 7.5 ppm for CO, 4.3 ppm for total hydrocarbon, and $0.26 \text{ mg}/\text{m}^3$ for formaldehyde. Standard lung function parameters, such as peak expiratory flow rate (PEFR), FVC, $\text{FEV}_{1.0}$ and forced expiratory volume at 0.25–0.75 seconds ($\text{FEF}_{25-75}\%$), were measured, and no significant changes were seen immediately after the exposure. Six hours after the exposure, there was a threefold increase in the number of neutrophils but no significant change in the

number of macrophages in the bronchial portion of the lavage. In the bronchoalveolar lavage, there was an increase in B lymphocytes. Bronchial biopsies were taken after the diesel exhaust exposure, and immunostaining for inflammatory cells was performed. There was a substantial increase in the number of neutrophils in the epithelium and submucosa. Mast cells were significantly increased in the submucosa and also lymphocytes with differentiation clusters (CD) of specific surface molecules (CD3+, CD4+ and CD8+ cells) were increased. There was also an increased expression of endothelial and leucocyte adhesion molecules, for example, of intercellular adhesion molecule-1 (ICAM-1), in the bronchial tissue after the exposure to diesel exhaust. Six hours after the diesel exhaust exposure, there was a significant increase in neutrophils and platelets in the peripheral blood and a significant reduction in lymphocytes with human leucocytes antigen related to the D-region (HLA-DR +).

The group also studied the level of antioxidants after the diesel exhaust inhalation (25). These levels were studied in the bronchial portion of the lavage, the bronchoalveolar lavage, and the nasal lavage. There was a marked increase in the concentration of ascorbic acid in the nasal lavage after 60 minutes of exposure to the diesel exhaust, but no effects on antioxidants in the bronchial or bronchoalveolar lavage.

Svartengren et al (26) have studied the effects of 30 minutes of exposure to the atmosphere in a road tunnel in Stockholm on 20 volunteers. The volunteers had mild asthma (allergic to pollen) and were inside a car during the exposure. As a control, 30-minute exposure to fairly low levels of air pollutants in a hotel was studied. Both exposures took place during the winter and in random order. Inside the car in the tunnel, the median concentration of NO_2 was $313 \mu\text{g}/\text{m}^3$ and the PM_{10} and $\text{PM}_{2.5}$ (particulate matter with an aerodynamic diameter of $10 \mu\text{m}$ and $2.5 \mu\text{m}$, respectively) were $170 \mu\text{g}/\text{m}^3$ and $95 \mu\text{g}/\text{m}^3$, respectively. The median concentration of the hotel air during rush hour was $14 \mu\text{g}/\text{m}^3$ for NO_2 , $7 \mu\text{g}/\text{m}^3$ for PM_{10} and $5 \mu\text{g}/\text{m}^3$ for $\text{PM}_{2.5}$. The symptoms during the exposures were studied using a questionnaire. Symptoms such as cough, irritation, chest tightness, and breathing discomfort were significantly higher in the tunnel than in the control exposure, although the magnitude of these symptoms was rather small. There was no clear effect on lung function (eg, the $\text{FEV}_{1.0}$ or the SR_{aw} of the subjects exposed in the tunnel). Four hours after the end of the tunnel and control exposures, the volunteers inhaled pollen allergens. After the tunnel exposure, the SR_{aw} increased from 5.0 to $7.2 \text{ cm water} \times \text{l}^{-1} \times \text{s}^{-1}$ and the thoracic gas volume (TGV) increased from 3.5 to 3.7 l. After the control exposure, the SR_{aw} increased from 5.1 to $6.7 \text{ cm water} \times \text{l}^{-1} \times \text{s}^{-1}$, and the TGV decreased from 3.4 to 3.2 l. The increases in

SR_{aw} and TGV were significantly larger after the tunnel exposure than after the control exposure. In the evening, the volunteers had more asthma symptoms after the tunnel exposure than after the control exposure. The asthma symptoms in the evening, night, and morning after the allergen inhalation correlated both with the NO_2 exposure and the $PM_{2.5}$ exposure. The relationship between the asthmatic symptoms and the NO_2 concentration seemed to be clearer than the relationship between the asthmatic symptoms and the particle concentration.

Number or mass as a dose measure

It is clear that aerosol particles that have the same chemical composition with a different size distribution can give different toxic effects due to different deposition in the respiratory tract. Another and more difficult question to answer is whether or not the effects on a certain region in the respiratory tract are dependent on the size of particles deposited in this region, that is, if the effect is dependent on the number, the surface area, or the mass of the particles or a combination of these parameters. For the answers to these questions, both data on effects and dose in a certain region of the respiratory tract are needed.

Anderson et al (27) exposed healthy subjects to two aerosols of methacholine with mass median aerodynamic diameters of 3 and 7 μm . The concentrations of the two aerosols were chosen for each subject so that they induced a $FEV_{1.0}$ decrease of 20%, and they also induced the same increase in R_{aw} . The dose to the tracheobronchial region (ie, the region responsible for changes in the measured R_{aw}) was calculated from data from human experimental studies with well-defined test particles. Although the increase in R_{aw} was the same for the two aerosols, the calculated dose (mass) to the tracheobronchial tract was three to four times higher for the aerosol with the larger particles than for that with the smaller particles. It can therefore be concluded that, for cholinergic aerosols, a certain dose (mass) to the tracheobronchial tract apparently gives a markedly greater effect if the mass is spread over many small particles than over a few larger ones. This result may be relevant also for at least some ambient air particles, as Utell et al (28) found a significant relationship between the effect of a cholinergic aerosol and sulfuric acid aerosol on airway conductance in asthmatics.

Summary and concluding remarks

Most experimental studies have dealt with sulfuric acid aerosols and asthmatic persons who are more sensitive to these particles with respect to lung function than

healthy subjects are. The studies on ventilatory function, design, and methods for lung function measurements have been similar. Still, the results have differed remarkably. Significant changes in young asthmatics have, for example, been found already at 70 $\mu g/m^3$, whereas concentrations of 1000 $\mu g/m^3$ or higher have been necessary to induce significant changes in asthmatics in other studies. An explanation for the different results may be that the groups of asthmatics differed in bronchial reactivity. In combined exposures of sulfuric acid aerosol and O_3 , there was no indication of any effect larger than an additive one. Short-time exposure of healthy subjects and asthmatics to carbon black particles (0.5 μm , 200 $\mu g/m^3$) together with sulfuric acid particles (6.5 μm , 100 $\mu g/m^3$) gave no effect on lung function, nor did the carbon black or the sulfuric acid particles alone.

Despite the fact that the methods for studying bronchial clearance are more complicated and less standardized than those for lung function tests, the results of the clearance studies seem more consistent. Apparently, there is an effect of sulfuric acid particles on mucociliary clearance at least down to 100 $\mu g/m^3$ in short-term exposures. The effect seems to increase with increasing exposure time. In contrast to the results of the studies on ventilatory function, asthmatics do not seem more sensitive to exposure to sulfuric acid particles than healthy subjects are.

Healthy volunteers exposed to both filtered and unfiltered diesel exhaust showed only small differences in effects. The filtered exhaust had about half the number of particles as the unfiltered one (1.4×10^6 and 2.6×10^6 particles/cm³), but the NO_2 concentration was similar in the two exposures (1.7 and 1.9 ppm). In a later study 4.3×10^6 particles/cm³ was reported to correspond to 300 $\mu g/m^3$. There was an increase in R_{aw} (SR_{aw} and R_{aw}) (around 15%) for both exposures. In the bronchial proportion of the lavage fluid, the number of macrophages was significantly increased only after the exposure to the unfiltered diesel exhaust, whereas the neutrophils increased by a factor of 3–4 after both exposures. The phagocytic activity of alveolar macrophages was significantly reduced after both exposures. In these studies, it was difficult to evaluate whether the effects were caused by particles or by gases, for example, NO_2 , or a combination of particles and gases, because only half the number of particles was removed by the filter.

In another study with realistic but complex exposures, volunteers with mild asthma were exposed to the atmosphere in a road tunnel. Compared with subjects exposed in rather clean ambient air, they showed more symptoms, such as coughing, irritation, and chest tightness, but no clear effect on lung function. However, when the volunteers inhaled pollen antigens 4 hours after the end of the exposures, the SR_{aw} and TGV increased significantly more after the tunnel exposure than

after the control exposure, and later in the evening more asthma symptoms were noted after the tunnel exposure. The asthma symptoms correlated with both the NO₂ and PM_{2.5} levels, but the relationship seemed to be stronger for NO₂. The late-phase reaction tended to be higher for the group with PM_{2.5} exposure above 100 µg/m³ than for the control exposure.

There are very limited human data that can offer any answers on whether the number, the surface area, or the mass of the particles is the most appropriate for measuring dose. Healthy subjects who inhaled cholinergic aerosols with a mass median diameter of 3 or 7 µm required a three to four times higher mass of the large particles than of the small particles to induce the same effect on R_{aw} and FEV_{1.0} in the tracheobronchial tract. This result may be relevant also for at least some particles in ambient air, as another study has shown a strong correlation between the effects of cholinergic aerosols and the effects of sulfuric acid aerosols on asthmatic subjects.

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