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Authors

PHALEN, RF
MANNIX, RC
KLEINMAN, MT

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EFFECTS OF OXIDANTS, ACIDS AND OTHER AGENTS ON PARTICLE CLEARANCE IN THE RAT*

R. F. PHALEN, R. C. MANNIX and M. T. KLEINMAN

Community and Environmental Medicine, University of California, Irvine, CA 92717, U.S.A.

Abstract—A series of inhalation experiments examining the effects of pollutants on the clearance of monodisperse tracer particles from the rat respiratory tract was performed. Exposures were as brief as 3 h and as long as 4 h per day for 21 days. Pollutant concentrations ranged from community air levels to several times the workplace standards. Short-term clearance was measured by analysing serially-collected faeces. Long-term clearance was measured by repeated, external gamma ray counting of the upper body. Ozone consistently delayed short-term clearance and accelerated long-term clearance. Particulate material usually had effects opposite to those of ozone. Some mixtures had greater effects than expected from the components; others had lesser effects. These studies identified several air pollutants that alter respiratory tract clearance rates. The studies also demonstrated the usefulness of the rat model.

INTRODUCTION

THE MEASUREMENT of inhaled particle clearance efficiency is an important assessment in toxicological evaluations. Failure of this self-cleaning process leads to a build-up of foreign matter in the respiratory tract, perhaps increasing the likelihood of infections and other diseases. This paper deals with the effects we have observed in particle clearance studies using rats during the past 15 years. Acute and repeated pollutant exposures, which represented air pollution episodes were performed. The pollutant concentrations ranged from ambient air levels, up to several times concentrations found in the workplace.

MATERIALS AND METHODS

Animals

Male barrier-reared Sprague-Dawley rats (Hilltop Laboratory Animals, Scottdale, Pennsylvania) weighing about 200 g were delivered in filter-equipped cartons and housed in particle and gas filtering laminar-flow caging systems for about 1 week prior to study. Quality control examinations were performed to establish that the rats were free of lung disease.

Exposures

Among the pollutants studied were ozone, sulphur dioxide, formaldehyde vapour, nitrogen dioxide, nitric acid vapour, acidic aerosols (sulphuric acid and ammonium bisulphate), combustion-generated aerosols (diesel-exhaust soot and propane-flame soot), ferric oxide and sulphate aerosols containing catalytic metals. Pollutants were

*This paper was included in Poster Session 5 and the discussion included in the summary presented in Section 12.

studied both alone and in combination, up to mixture atmospheres with seven components. None of our studies had aerosol exposures likely to produce 'dust overloading' (MORROW, 1988). Prior to 1984, rats were exposed in compartmentalized wire cages inside stainless steel chambers. Since that time rats have been exposed nose-only to prevent neutralization of acids by ammonia and contamination of the atmospheres by dander. During some exposures rats performed treadmill exercise at about twice the resting minute ventilation. Exposures were as brief as 3 h and as long as 4 h per day for 21 days. The temperature was $22 \pm 2^\circ\text{C}$ (SD) and the relative humidity was either high (85%) or low (40%).

Clearance measurement

The procedures used have been described previously (PHALEN *et al.*, 1980; KENOYER *et al.*, 1981; MANNIX *et al.*, 1982; PRASAD *et al.*, 1990). Monodisperse polystyrene latex microspheres (about $1.7 \mu\text{m}$ in diameter) labelled with ^{51}Cr (HINRICHS *et al.*, 1978) were inhaled for 20 min just prior to pollutant exposures, or just prior to a final pollutant exposure, for multi-day studies. Labelled aerosols, generated using a Lovelace-type nebulizer, were dried, diluted and electrically discharged before entering the nose-only exposure chamber (RAABE *et al.*, 1973). After particle deposition the noses were washed and an initial chest count was performed.

Short-term (0–50 h post-deposition) clearance was characterized from 10 serial faecal collections. Faecal excretion curves were fit to a log-normal function and the time at which 50% of the total faecal activity was excreted was determined for each rat. Between 50 and 400 h post-deposition, five chest counts were performed. From these counts a biological half-time for long-term clearance was found for each rat. At 30 days post-exposure the excised lungs were counted for radioactivity. The short-term 50% clearance times, the long-term clearance half-times and the lung count data were grouped with regard to exposure atmosphere and compared using two-tailed *t*-tests.

RESULTS

A summary of our results is presented in Table 1. Ozone alone delayed short-term clearance (above about 0.6 ppm) and accelerated long-term clearance (no apparent lower threshold) in resting rats. When other pollutants were added to the ozone atmosphere, the effects were in the same direction as those produced by ozone alone, but the intensity of the effects was often not as pronounced, depending upon the relative humidity and the co-pollutants present. Ozone at 0.6 ppm in combination with 2.5 ppm of nitrogen dioxide produced effects (delay in short-term clearance, acceleration in long-term clearance) far in excess of those expected for ozone alone. In addition, ozone at 0.4 ppm, in combination with 2.5 ppm of nitrogen dioxide and 0.5 mg m^{-3} of sulphuric acid and with exposure during exercise, produced effects like 0.8 ppm ozone at rest. Sulphate aerosols alone (ammonium sulphate, ferric sulphate, sulphuric acid) usually had no significant effect on short-term clearance, but tended to delay long-term clearance—more so at 40% than at 85% relative humidity. Formaldehyde and sulphur dioxide exposures at rest delayed short-term clearance at 20 ppm. Exposure to 10 ppm formaldehyde did not produce significant effects; however, this same concentration during exercise caused a delay in short-term clearance. A delay in long-term clearance was produced by a 5 day (5 h per day) exposure to 0.5 mg m^{-3} propane-flame

TABLE 1. SUMMARY OF CLEARANCE STUDY FINDINGS. A POSITIVE CHANGE IN HALF-TIME INDICATES A DELAYED AND A NEGATIVE CHANGE INDICATES AN ACCELERATED RATE OF CLEARANCE. THE NUMBER IN PARENTHESES IS THE RANK WITH RESPECT TO MAGNITUDE OF THE EFFECT OF THE EXPOSURE ON CLEARANCE

(Rank) atmosphere	% change in clearance half-time		
	Exposure duration	Short term	Long term
1 ppm O ₃	4 h	+43* (1)	-49* (1)
0.8 ppm O ₃	4 h	+5* (12)	-21* (8)
0.6 ppm O ₃	4 h	+2 (19)	-11 (15)
0.4 ppm O ₃	4 h	+2 (18)	-12 (14)
0.8 ppm O ₃ + 20 ppm SO ₂ + 6 mg m ⁻³ A	4 h	+16* (4)	-43* (2)
0.8 ppm O ₃ + 5 ppm SO ₂ + 1 mg m ⁻³ sulphate aerosol†	4 h	+11* (6)	-21* (7)
0.8 ppm O ₃ + 3.5 mg m ⁻³ sulphate aerosol†	4 h	+7* (9)	-23* (5)
0.6 ppm O ₃ + 2.5 ppm NO ₂ ± co-pollutants	4 h	+20* (3)	-19* (9)
0.6 ppm O ₃ + 3.5 mg m ⁻³ NH ₄ HSO ₄	4 h	-8* (8)	+22* (6)
0.6 ppm O ₃ + 5 ppm SO ₂ + 2.5 ppm NO ₂ + 1 mg m ⁻³ sulphate aerosol† + 0.3 mg m ⁻³ Fe ₂ O ₃	4 h day ⁻¹ × 21 days	-15* (5)	+10 (16)
0.6 ppm O ₃ + 10 ppm HCHO	4 h	+5 (13)	-18* (12)
0.4 ppm O ₃ + 2.5 ppm SO ₂ + 0.5 mg m ⁻³ H ₂ SO ₄ ‡	3 h Ex	+5 (11)	-25* (4)
0.35 ppm O ₃ + 2.5 ppm SO ₂ + 1.3 ppm NO ₂ + 0.5 mg m ⁻³ propane soot + 1 mg m ⁻³ sulphate aerosol†	4 h	+2 (20)	-10 (17)
0.5 mg m ⁻³ propane soot + 0.35 mg m ⁻³ mg m ⁻³ HNO ₃ + 0.15 mg m ⁻³ H ₂ SO ₄	5 h day ⁻¹ × 5 days	+1 (21)	+27* (3)
0.5 mg m ⁻³ diesel soot + 0.35 mg m ⁻³ HNO ₃ + 0.15 mg m ⁻³ H ₂ SO ₄	5 h day ⁻¹ × 5 days	-5 (14)	+6 (18)
20 ppm HCHO and 20 ppm SO ₂	4 h	+10* (7)	-3 (20)
10 ppm HCHO	3 h Ex	+26* (2)	+0.4 (23)
10 ppm HCHO	4 h	+3 (16)	+19 (10)
10 ppm HCHO + 1 mg m ⁻³ (NH ₄) ₂ SO ₄	4 h	-1 (22)	+19 (11)
5 ppm SO ₂ + 1 mg m ⁻³ (NH ₄) ₂ SO ₄ ‡ + 0.3 mg m ⁻³ Fe ₂ O ₃	4 h	+3 (17)	-2 (22)
5 ppm SO ₂ + 1 mg m ⁻³ sulphate aerosol†	4 h	-7 (10)	+2 (21)
1 mg m ⁻³ H ₂ SO ₄	2 h day × 20 days	-4 (15)	+6 (19)
3.5 mg m ⁻³ sulphate aerosol†	4 h	+0.3 (23)	+16* (13)

*Statistically significant, $P < 0.1$, two-tailed t -test.

†Sulphate aerosol = ammonium sulphate, ferric sulphate, or sulphuric acid.

‡Iron and manganese catalysts.

Ex, exercise at 8 m min⁻¹.

A, ammonium sulphate, sodium vanadate and cupric chloride.

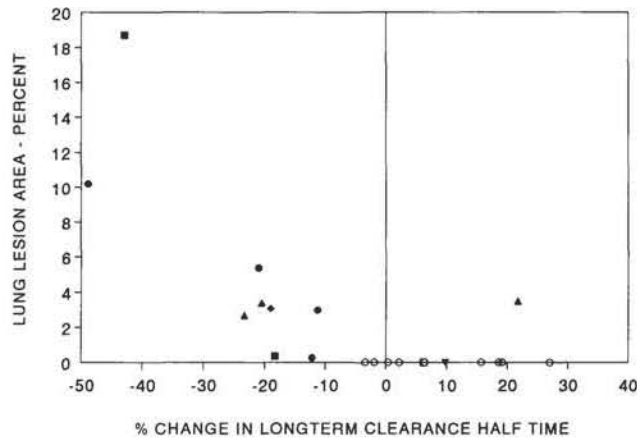


FIG. 1. The relationship between histopathologic focal alveolar lesions (wall thickening \pm free macrophages and other cells in alveolar lumens) and the change seen in long-term clearance half-time (a negative change indicates an acceleration of clearance). Dark symbols indicate ozone's presence and open symbols indicate ozone's absence. Symbols are as follows: \bullet , ozone; Δ , ozone + sulphate and/or sulphur dioxide; \blacksquare , ozone + a vanadium containing mixture or formaldehyde; ∇ , ozone in a seven-component mixture; \circ or \square , sulphate, soot, sulphur dioxide and/or formaldehyde alone or combined; \blacklozenge , ozone + nitrogen dioxide + other components.

soot + 0.35 mg m⁻³ nitric acid + 0.15 mg m⁻³ sulphuric acid and exposure to the same atmosphere, but with diesel-exhaust soot substituted for the propane-flame soot, did not cause the effect. An extended exposure (4 per day for 21 days) to 0.6 ppm ozone + 5 ppm sulphur dioxide + 2.5 ppm nitrogen dioxide + 1 mg m⁻³ ammonium sulphate (with iron and manganese catalysts) + 0.3 mg m⁻³ ferric oxide significantly accelerated short-term clearance.

Because histopathological examinations were performed in nearly all studies, we were able to correlate lung lesions with changes in particle clearance. Focal lesions were correlated with changes in long-term clearance ($r^2=0.8$) and clearance changes were frequently seen when no lung lesions were present, but not vice versa (Fig. 1). Data for single 3 or 4 h exposures to ozone or sulphate aerosols (alone or in combinations) were analysed using multiple linear regression. Ozone demonstrated slowing of short-term clearance (slope \pm standard error, $b=22.6\pm 10\%$ per ppm) and a significant acceleration of long-term clearance ($b=-32.0\pm 6.6\%$ per ppm). Sulphate did not significantly alter short-term clearance, but significantly slowed long-term clearance [$b=6.6\pm 2.8\%$ per (mg m⁻³)] in a dose-dependent manner.

DISCUSSION

The studies identified several ambient air pollutants that affect respiratory tract clearance. Ozone, which produced a delay in short-term clearance, followed by an acceleration in long-term clearance, tended to dominate mixtures. Particulate material (sulphate aerosols, etc.) often had an effect on long-term clearance opposite to that of ozone, perhaps due to impairment of the alveolar macrophages. Formaldehyde and sulphur dioxide, upper respiratory tract irritants, delayed short-term clearance, but had no effect on long-term clearance. This may be because these gases are soluble in the

mucus lining the upper airways and they do not reach the lung region in significant quantities.

A 21-day study of a mixture which included ozone showed effects in the opposite direction to the expected 'signature' effects of ozone. The rats exposed to this atmosphere demonstrated a significant acceleration in short-term clearance. This surprising effect may have resulted from the subchronic nature of the study, which allowed time for the rat respiratory system to adapt to ozone, allowing the remaining components to dominate.

The rats were useful subjects. They adapted well to three modes of exposure (whole body, nose-only and on a treadmill), they remained healthy and were sensitive enough to the pollutants that significant effects could generally be observed with groups of 30 or fewer rats.

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