Effects of Concentrated Ambient Particles on Heart Rate and Blood Pressure in Pulmonary Hypertensive Rats

Tsun-Jen Cheng,[1] Jing-Shiang Hwang,[2] Peng-Yau Wang,[3] Chia-Fang Tsai,[1] Chun-Yen Chen,[1] Sheng-Hsiang Lin,[2] and Chang-Chuan Chan[1]

[1]Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University, Taipei, Taiwan; [2]Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; [3]Institute of Environmental Engineering, National Central University, Chung Li, Taiwan

Epidemiologic studies have shown that increased concentrations of ambient particles are associated with cardiovascular morbidity and mortality. However, the exact mechanisms remain unclear. Recent studies have revealed that particulate air pollution exposure is associated with indicators of autonomic function including heart rate, blood pressure, and heart rate variability. However, this association has not been clearly demonstrated in animal studies. To overcome the problems of wide variations in diseased animals and circadian cycles, we adopted a novel approach using a mixed-effects model to investigate whether ambient particle exposure was associated with changes in heart rate and blood pressure in pulmonary hypertensive rats. Male Sprague-Dawley rats were implanted with radiotelemetry devices and exposed to concentrated ambient particles generated by an air particle concentrator. The rats were held in nose-only exposure chambers for 6 hr per day for 3 consecutive days and then rested for 4 days in each week during the experimental period of 5 weeks. These animals were exposed to concentrated particles during weeks 2, 3, and 4 and exposed to filtered air during weeks 1 and 5. The particle concentrations for tested animals ranged between 108 and 338 μg/m^3. Statistical analysis using mixed-effects models revealed that entry and exit of exposure chamber and particle exposure were associated with changes in heart rate and mean blood pressure. Immediately after particle exposure, the hourly averaged heart rate decreased and reached the lowest at the first and second hour of exposure for a decrease of 14.9 ($p < 0.01$) and 11.7 ($p = 0.01$) beats per minute, respectively. The hourly mean blood pressure also decreased after the particle exposure, with a maximal decrease of 3.3 ($p < 0.01$) and 4.1 ($p < 0.01$) mm Hg at the first and second hour of exposure. Our results indicate that ambient particles might influence blood pressure and heart rate. *Key words:* **ambient particles, blood pressure, heart rate, pulmonary hypertension, radiotelemetry.** *Environ Health Perspect* **111:147–150 (2003). [Online 31 October 2002]**
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Epidemiologic studies have shown that increased concentrations of ambient particles are associated with cardiovascular morbidity and mortality (*1,2*). However, the mechanisms of such associations have not been clearly defined. Recent epidemiologic studies have found associations of increased air particles with increased heart rate and blood pressure and decreased heart rate variability (*3–6*). It has been speculated that particles cause the activation of autonomic nervous system, leading to changes in heart rates (*7*). To clarify the relationship of particles with mortality and morbidity, animal models have been used to investigate the effects of particles (*8,9*). Preliminary studies revealed increased heart rates and arrhythmia in pulmonary hypertensive rats after exposure to concentrated ambient particles (*8,10,11*). However, a recent study revealed a decreased heart rate when residual oil fly ash was instilled into the rats (*12*). To investigate further the mechanisms of particle-induced cardiotoxicity, we used heart rate and blood pressure as outcome indicators to assess their association with concentrated ambient particles in pulmonary hypertensive rats. Studies have also indicated that particles with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) exert greater adverse health effects than coarse particles (*1,13*). To investigate the effects of $PM_{2.5}$ on cardiovascular diseases, we used an ambient particle concentrator, which generated $PM_{2.5}$, to test our hypothesis (*14*).

It is a common practice to divide experimental animals into exposure and control groups to study the effects of air particles. Because of the wide variation among diseased animals, this method requires large numbers of animals to delineate the true effect of air particles (*9*). Furthermore, variation in circadian cycle for individual rats also makes comparisons of heart rate or blood pressure difficult. To overcome this problem, we used each animal as its own control by exposing the individual animals repeatedly to concentrated air and filtered air and successfully detected the effects of particles on heart rate and mean blood pressure in three rats.

Materials and Methods

Animals and development of pulmonary hypertension. Male Sprague-Dawley rats 60 days old were obtained from the National Laboratory Animal Breeding and Research Center, housed in plastic cages on Aspen chip bedding, and provided with Lab Diet 5001(PMI Lab, Montville, NJ, USA) and water *ad libitum* except during exposure. Animals were maintained on a 12-hr light/dark cycle at 22 ± 1°C and 55 ± 10 % relative humidity. We injected rats with monocrotaline intraperitoneally at 60 mg/kg body weight to cause them to develop pulmonary hypertension. Fourteen days after monocrotaline injection, animals were ready for experimentation (*15*).

Experimental design. Ambient particles from the Chung-Li area, a suburb of Taipei, were concentrated through a modified ultrafine particle concentrator developed by Sioutas et al. (*14*). Briefly, the particle concentrator used virtual impactor technology in which 110 L/min flow went through a saturator, cooler, impactor, and diffusion dryer to generate concentrated particles with aerodynamic diameters between 2.5 and 0.01 μm. The concentrator output was about 10 L/min at a negative pressure of about 2 mm Hg. Air containing concentrated particles was directed into the nose-only exposure chambers.

We implanted three rats with telemetry transmitters and exposed them to concentrated particles 6 hr each day for 3 consecutive days and then rested them for 4 days. The cycles were repeated three times. The weeks before and after the exposure were used as controls when animals were exposed only to filtered air passing through the concentrator. These three rats were exposed to concentrated particles when they were 10 and 40 weeks old, respectively. We did not include the data obtained when they were 10 weeks old in the analysis because the testing environment was not adequately controlled.

The mean concentration of particles during the 9 days of exposure period was ranged from 108 to 338 μg/m^3 (mean ± SD, 240 ± 77 μg/m^3). The elemental composition of particles collected on polycarbonate filter paper was also characterized using X-ray fluorescence (XRF). Mean concentration of elements

Address correspondence to T.-J. Cheng, Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, 1 Jen-Ai Rd. Sec. 1, Taipei, Taiwan 100. Telephone and Fax: +886-2-2395-7845. E-mail: tcheng@ha.mc.ntu.edu.tw

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during exposure was as follows: aluminum 26.5 μg/m^3; magnesium, 6.8 μg/m^3; sulfur, 2.8 μg/m^3; silica, 2.7 μg/m^3; iron, 1.4 μg/m^3; gallium, 0.7 μg/m^3; phosphorus, 0.5 μg/m^3; zinc, 0.2 μg/m^3; nickel, 0.07 μg/m^3; manganese, 0.03 μg/m^3; copper, 0.02 μg/m^3; cobalt, 0.01 μg/m^3; vanadium, 0.01 μg/m^3.

Heart rate and blood pressure analysis. We used the radiotelemetry system (Data Sciences International, Inc., St. Paul, MN, USA) to monitor and acquire physiologic data including heart rate, mean blood pressure, and core temperature in unanesthetized, unrestrained rats. We obtained the recordings for 10 sec every 5 min. The data were acquired during exposure and subsequently in the rats' plastic cages in a specifically designed, climate-controlled environment within the ranges specified above, with food and water provided *ad libitum*. We calculated the hourly average of heart rate and mean blood pressure for the whole experimental period of 5 weeks.

Statistical analysis. In this experimental design, the rats were exposed to filtered air and concentrated particles between 1000 and 1600 hr, for the first 3 days of weeks 1 and 5, and weeks 2–4. For the rest of time, the rats were exposed to room air. The average response of heart rate and blood pressure at each hour for each rat was calculated for room air, filtered air, and concentrated particles. The hourly mean response to room air exposure was used as a baseline. To adjust the effect of circadian cycle in each rat, we subtracted the hourly baseline responses from the hourly averaged response to filtered air and concentrated particles to obtain the crude effect at each hour since exposure for each rat. Let y_{ijk} be the crude effect at hour k = 1,K,24 on day j = 1,K,35 and for the ith rat. The averages of y_{ijk} across three rats plotted against hours since exposure are given by room air, filtered air, and concentrated particles exposures, respectively, in Figure 1. Note that the rats were moved into the exposure chamber at 0900 hr, which corresponded to hour 0 since exposure. The plots show that the confinement in the chamber had clear effects on both heart rate and blood pressure, and the effects were decreasing through the hours. Moving the animals also had strong effects that lasted for 2 hr. Based on these clear patterns, we modeled the effects for the three rats by the mixed-effects model

$$\begin{aligned} y_{ijk} = {} & \left[(\alpha_0 + a_{0i}) + (\alpha_1 + a_{1i})(k-9)\right] \\ & \times I(\text{in}_{jk}) + (\gamma_1 + c_{1i}) \times I(\text{out}_{1jk}) \\ & + (\gamma_2 + c_{2i}) \times I(\text{out}_{2jk}) \\ & + \textstyle\sum_{h=0}^{15} (\beta_h + b_{hi}) \times I(\text{PM}_{jk}) \\ & + (\delta + d_i) \times T_{ijk} + \varepsilon_{ijk} \end{aligned} ,$$

where $I(\text{in}_{jk})$ = 1 when the rats were in the chamber and 0 otherwise; $I(\text{out}_{1jk})$ = 1 and $I(\text{out}_{2jk})$ = 1 when the rats were moved out of the chamber and the next hour, respectively, and 0 otherwise; $I(\text{PM}_{jk})$ = 1 when the jth day the rats were exposed to concentrated particles. The body temperature was denoted by T_{ijk}. The error term ε_{ijk} was chosen to be autoregressive moving average process. The line with intercept α_0 and slope α_1 described the mean chamber effects over the 7 hr in the chamber. The mean moving effects were measured by γ_1 and γ_2. The parameters β_0,K,β_{15} were of most interest, as they represented the expected effects of concentrated particles for hours since exposure. Because the three rats were randomly selected from a population, the effects obtained from each rat would be different from the population mean effects because of different characteristics of the sampled rats. The variations of the effects among the rats was modeled by random components a_{hi}, b_{hi}, and c_{hi}. These random coefficients were all assumed to be normally distributed with mean 0 and some constant variances. We used statistical software S-Plus (Insightful Corp., Seattle, WA, USA) to estimate the parameters and standard errors.

Results

Hourly averaged crude effects of heart rate and blood pressure during and after exposures to concentrated ambient particle and filtered air, using measurements during exposure to room air as a baseline, are shown in Figure 1. Heart rate in rats with filtered air exposure increased when rats were placed into and moved out of the tube of chamber; then heart rates gradually returned to baseline. During particle exposure, heart rate had an early decrease and then gradually increased to the maximal during the 11th hr from the beginning of the exposure, compared to those with exposure to filtered air.

Hourly averaged crude effect of heart rate was fit into the linear mixed-effects model as a function of entry and exit of animal holders for nose-only exposure, core temperature, and particle exposure. Core temperature was not associated with the changes of heart rate. Entry [40.4 beats per minute (bpm), p < 0.01] and exit (69.4 bpm, p < 0.01) were associated with an increase in heart rate. The hourly averaged heart rate decreased by 14.9 bpm (p < 0.01) at first hour of particle exposure as compared to exposures to filtered air, by 11.7 bpm (p = 0.01) at the second hour (Figure 2). The effect of particle exposure on heart rate then gradually increased and reached its maximum at the 9th hour from the particle exposure (8.6 bpm, p <0.05).

Similar results were also observed with the linear mixed-effects model for mean blood pressure (Figure 1). Entry (15.3 mm Hg, p < 0.01) and exit (11.4 mm Hg, p < 0.01) were also associated with an increase in blood pressure. The hourly averaged blood pressure decreased by 3.3 mm Hg (p < 0.01) at first hour of particle exposure as compared to those of filtered air, by 4.1 mm Hg (p < 0.01) at second hour (Figure 2). The blood pressure was then gradually returned to the levels of those with exposure to filtered air.

Concentrations of ambient particles, however, were not associated with either heart rate or mean blood pressure in the model.

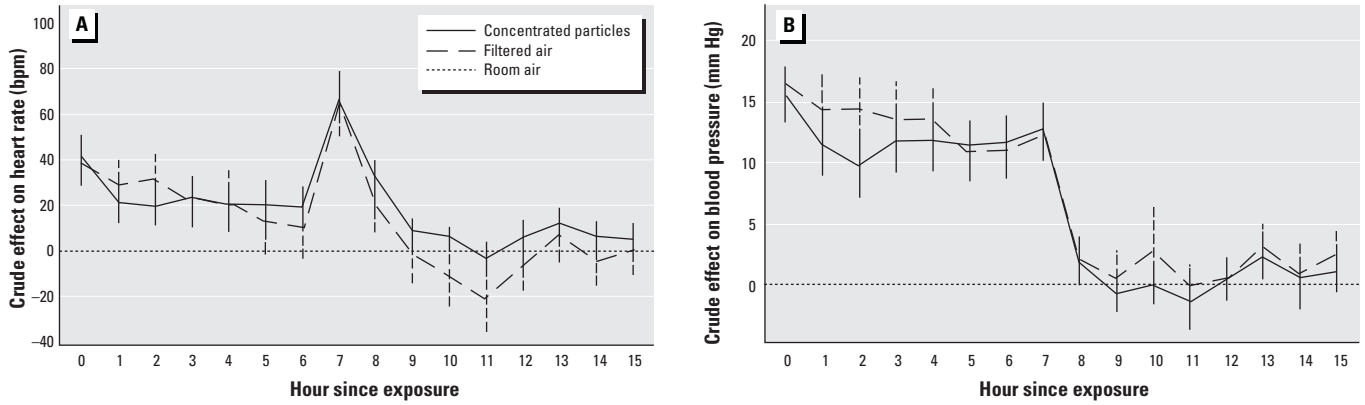

Figure 1. Crude effects of concentrated particles and filtered air on (*A*) heart rate and (*B*) blood pressure by hours since exposure, using measurements during exposure to room air as a baseline. The vertical bars indicate the 95% confidence intervals of the response. 0 represents 1 hr before the exposure.

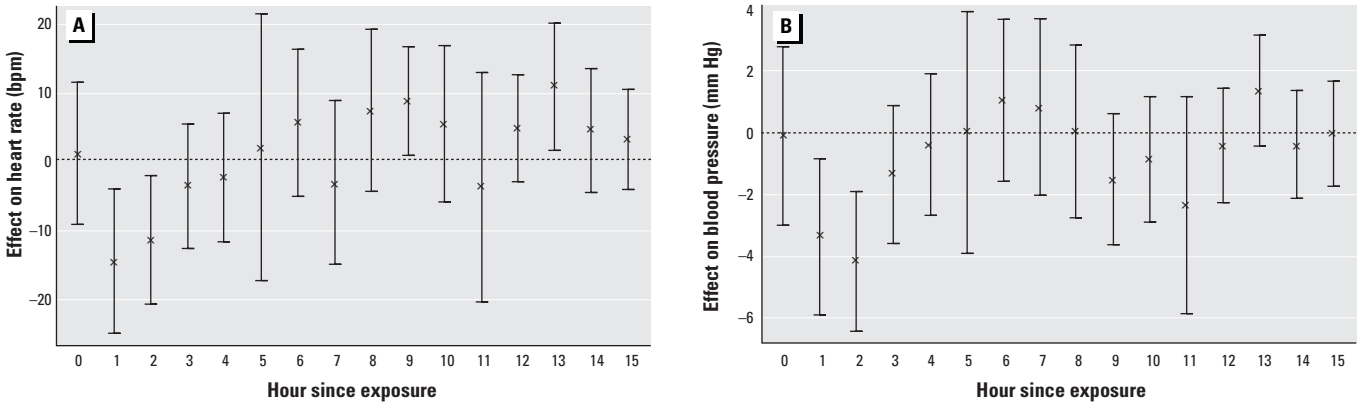

Figure 2. The effects of concentrated particles exposure on (*A*) heart rate and (*B*) blood pressure obtained from mixed-effects models. The vertical bars indicate the 95% confidence intervals of the response; 0 represents 1 hr before the exposure.

Discussion

In this study we observed decreased heart rate and mean blood pressure in pulmonary hypertensive rats during exposure to concentrated ambient particles. Heart rate and mean blood pressure reached their lowest values at the first and second hour of PM exposure.

An increase in heart rate and blood pressure was detected during the hour when the animals were held in the nose-only exposure chambers. Heart rate and blood pressure subsequently returned to the baseline after the animals were removed from the animal holders of nose-only exposure chambers. Sharp increases for heart rate were also noted when rats were removed from the chambers. This phenomenon was expected because of the stress on these animals.

Our results revealed that heart rate decreased at the beginning of particle exposure. A recent study conducted by Watkinson et al. (*12*) also revealed similar findings: Bradycardia developed in rats 0–6 hr after intratracheal instillation of residual oil fly ash. Inhalation of wood smoke immediately causes airway irritation through the stimulation of vagal pulmonary C fiber in rats (*16*). We speculate that particles may also exert these effects through the afferent nerves to the vasomotor center in the brainstem, leading to increases of parasympathetic tone of the heart and peripheral vascular system (*17,18*). Reactive oxygen species have been postulated as a factor mediating the inflammatory response in previous studies (*19,20*). Irritation caused by wood smoke leading to bradycardia has also been demonstrated as a result of hydroxyl radicals (*16,21*). In the present study, cumulative effects were not observed after 3 days of exposure. It appears that the heart rate and blood pressure gradually return to the baseline during exposure, when the tolerance to particles develops.

Interestingly, we observed an increase in heart rate 9 hr since the beginning of exposure. An animal study revealed that heart rate increased in pulmonary hypertensive rats after they were exposed to ambient concentrated particles (*11*). Epidemiologic studies also reported that increased heart rate (*3,4*) and decreased heart rate variability (*5,22,23*) were associated with increased particles in air measured 1–2 days earlier. It seems that prolonged exposure to ambient particles may also induce increased heart rate, in contrast to a decreased heart rate at the beginning of particle exposure. Further study with a wider range of particle concentrations and larger number of animals may shed light on the exact mechanisms of particle-induced changes in heart rate and blood pressure.

The fact that the heart rate and mean blood pressure were highly correlated at the beginning of particle exposure indicated that decreases in heart rate and mean blood pressure might be mediated through the same mechanism. The data on the relationship between blood pressure changes and particle exposure are limited. A recent population study revealed that the increased systolic blood pressure was associated with total suspended particles in those with increased heart rate and elevated viscosity (*6*). Again, the discrepancy between animal studies and epidemiologic studies indicates a precaution in extrapolating the results of animal study to humans and warrants further investigation.

The concentrations of ambient particles were not associated with either heart rate or blood pressure in the current study, probably due to the small variation of the concentrated particles in this experiment. Acidity (*24,25*), transition metals (*26–28*), endotoxin (*29*), and particle size (*30,31*) have been associated with the inflammatory responses of airways. However, it is not clear if components and size of particles affect heart rate and blood pressure through similar mechanisms. Further study is needed to answers these questions.

With our novel design, we successfully observed the cardiovascular effects of ambient particles using a relatively small number of animals. Our results suggest that a mixed-effects model is useful for studying hemodynamic changes caused by particles in animals. Although we observed decreased heart rate and blood pressure with increased levels of particles in hypertensive animals, it is not clear whether similar phenomena will be observed in healthy or in young, hypertensive rats. In addition, the role of airway inflammation on cardiovascular diseases remains unclear. Further study is needed to elucidate the exact mechanism in which particles may exert the effects on the cardiovascular system.

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