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$PM_{2.5}$ induces acute electrocardiographic alterations in healthy rats ☆

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Abstract

There is evidence that changes of the autonomic control of the heart are among the potential mechanisms responsible for pollution-related cardiac mortality. The objective of this work is to assess the acute effects of urban particulate matter of $\leqslant 2.5\,\mu m$ ($PM_{2.5}$) particles on heart rate (HR) and HR variability. Forty-seven healthy Wistar rats were anesthetized, submitted to tracheal intubation, and instilled with 1 mL of four different solutions: saline, blank filter, and 50 or 100 μg of $PM_{2.5}$. $PM_{2.5}$ was collected in glass fiber filters using a high-volume sampler. Electrodes for obtaining electrocardiograms were implanted subcutaneously in a Lead II configuration. HR and the standard deviation of the intervals between normal beats (SDNN) were assessed immediately before and 30 and 60 min after instillation. HR decreased significantly ($P<0.001$) with time, but no significant effect of treatment or interaction between time and treatment was observed. In contrast, there was a significant SDNN interaction between time and treatment ($P = 0.025$). The SDNN decreased 60 min after instillation with a $PM_{2.5}$ of 50 and 100 μg. In conclusion, the injection of an aqueous suspension of $PM_{2.5}$ induced a reduction of SDNN in healthy rats. The effect was observed 1 h after instillation and in a concentration of $<100\,\mu g$.

Keywords: $PM_{2.5}$; Electrocardiogram; Heart rate variability; Rats; Air pollution

1. Introduction

A series of recent studies have reported epidemiological associations between daily concentrations of air pollution and mortality (Laden et al., 2000; Pope et al., 1995; Schwartz et al., 2002) due to cardiovascular events (Dockery, 2001; Liao et al., 1999; Goldberg et al., 2001). Epidemiologic studies have shown a stronger association between cardiovascular deaths and air pollution during the first 24 h after a pollution increase. Braga et al. (2001) recently showed that a 10-μg/m^3 increase in particulate matter of $\leqslant 10\,\mu m$ (PM_{10}) is associated with cardiovascular death on the same day of the pollution event. Furthermore, cardiac patients with implanted cardioverter defibrillators experience an increased risk of arrhythmias shortly after increases in air pollution (Peters et al., 2000).

Several cardiovascular effects have been documented in response to exposure to PM, including the disruption of autonomic nervous system activity by increased (Tarkiainen et al., 2003; Magari et al., 2002) and decreased heart rate variability (HRV) (Gold et al., 2000; Pope et al., 2004; Magari et al., 2001). Arterial vasoconstriction (Batalha et al., 2002; Brook et al., 2002), cardiac arrhythmias (Peters et al., 2000; Watkinson et al., 1998), and cardiac events including myocardial infarction (Peters et al., 2001), hospitalization

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(Lin et al., 2003; Schwartz, 1999), depression of the ST segment in coronary heart disease patients during exercise (Juha et al., 2002), and systemic and inflammatory changes have also been documented (Kodavanti et al., 1998; Schins et al., 2004; Saldiva et al., 2002).

There is evidence that changes of the autonomic control of the heart are among the potential mechanisms responsible for pollution-related cardiac mortality (Pope et al., 1999). Analysis of beat-to-beat HRV is one of the noninvasive methods to quantitatively assess cardiac autonomic activity and has been used in patients with a variety of cardiac and noncardiac diseases (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Low HRV is associated with a higher risk of all-cause mortality in survivors of an acute myocardial infarction (Sosnowski et al., 2002; Carney et al., 2001) and sudden death (La Rovere et al., 2003).

It has been suggested that $PM_{2.5}$, the fine component of particulate matter ($\leqslant 2.5\,\mu m$), may mediate some of the adverse cardiac effects reported by epidemiologic studies (Seaton et al., 1995; Frampton, 2001; Utell and Frampton, 2000). However, the mechanisms of particle-induced cardiovascular damage are not yet defined. Information about the time course and specific constituents of $PM_{2.5}$ responsible for cardiac effects, including dose–response relationships, is still needed, either from human or animal studies. In this context, recent studies in rats have provided evidence that combustion-derived particles induce alterations of heart rhythm in animals with previous cardiovascular disease (Wellenius et al., 2002; Wichers et al., 2004).

We have continued this line of study, assessing whether the administration of urban $PM_{2.5}$ particles promotes alterations of detectable acute electrocardiogram (ECG) parameters such as heart rate (HR), standard deviation of the intervals between normal beats (SDNN), or disturbances of heart rhythm. To address these questions we used a single instillation of two concentrations of $PM_{2.5}$ in healthy adults Wistar rats.

2. Methods

2.1. Animals

Adult male Wistar rats (3 months of age) weighing ~250 g were obtained from the vivarium of the School of Medicine at the Universidade of São Paulo. They were maintained at 22–23 °C, controlled humidity, and a 12 h dark to 12 h light cycle. Food and water were available ad libitum.

2.2. Particle sampling and analysis

We employed a high-volume sampler (HIVOL, Energética, Brazil) coupled with an inlet (Tisch Environmental Inc., USA) that allows the separation of $PM_{2.5}$ at a flow rate of 1.1 m^3/min. The particle sampler was located on the roof of our Medical School (about 15 m above ground level), which is situated at an intersection with heavy traffic in downtown São Paulo. Particles were collected in glass fiber filters, which were dried for 24 h at 50 °C and weighed before and after particle collection. Particles were sampled in September 2003.

Neutron-activation analysis was used to determine the trace-element content of the filtrate. Filter samples and elemental standards were irradiated under thermal neutron flux of the IEA-R1 nuclear research reactor for 30 s and 16 h. After adequate decay times, the irradiated samples and standards were measured using a hyperpure Ge detector coupled to a multichannel analyzer. Concentrations of elements were calculated by comparative methods. Blank filters were analyzed under the same experimental conditions as for the analysis of filter samples. The contribution from the blank filter was subtracted from the results, and final results were expressed as a function of the volume of air collected. The sulfur content was determined by X-ray fluorescence analysis, using the nonexposed area of filters as a blank filter.

2.3. Filter extracts

Aqueous suspensions of filters were prepared within 24 h of collection. Because of the inherent difficulty of extracting PM from high-volume filters, subcomponents of the filter were submerged in distilled water and the filtrate was removed via agitation by an ultrasound water bath of 8 h. The concentration of PM was determined by the weighing of the filters before and after extraction. The weight of the extracted filter was determined after drying for 24 h at 50 °C.

2.4. Particulate matter instillation

Forty-seven rats were submitted to tracheal instillation of 1 mL of the following solutions:

SAL ($n = 12$), sterile saline solution;
BF ($n = 12$), blank filter solution obtained by ultrasonication of a blank filter in distilled water;
PM_{50} ($n = 12$) solution obtained by ultrasonication of a filter containing PM submersed in distilled water and containing 50 μg of $PM_{2.5}$; and
PM_{100} ($n = 11$) solution obtained by ultrasonication of a filter containing PM submersed in distilled water (the same procedure as for PM_{50}) containing 100 μg of $PM_{2.5}$.

The instillation procedure was conducted under anesthesia with 3% sodium pentobarbital (30 mg/kg body wt., ip). The rats were submitted to tracheal intubation using an adapted pediatric laryngoscope, and