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PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation ☆

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Abstract

Epidemiological studies show that increases in particulate air pollution (PM) are associated with increases in cardiopulmonary morbidity and mortality. However, the mechanism(s) underlying the cardiac effects of PM remain unknown. We used pharmacological strategies to determine whether oxidants are implicated in PM-dependent cardiac dysfunction and whether PM-induced increase in autonomic stimulation on the heart mediates cardiac oxidative stress and toxicity. Adult Sprague–Dawley rats were exposed to either intratracheal instillation of urban air particles (UAP 750 μg) or to inhalation of concentrated ambient particles (CAPs mass concentration 700 ± 180 μg/m^3) for 5 h. Oxidative stress and cardiac function were evaluated 30 min after UAP instillation or immediately after exposure to CAPs. Instillation of UAP led to significant increases in heart oxidants measured as organ chemiluminescence (UAP: 38 ± 5 cps/cm^2, sham: 10 ± 1 cps/cm^2) or thiobarbituric acid reactive substances (TBARS, UAP: 76 ± 10, Sham 30 ± 6 pmol/mg protein). Heart rate increased immediately after exposure (UAP: 390 ± 20 bpm, sham: 350 ± 10 bpm) and returned to basal levels over the next 30 min. Heart rate variability (SDNN) was unchanged immediately after exposure, but significantly increased during the recovery phase (UAP: 3.4 ± 0.2, Sham: 2.4 ± 0.3). To determine the role of ROS in the development of cardiac malfunction, rats were treated with 50 mg/kg N-acetylcysteine (NAC) 1 h prior to UAP instillation or CAPs inhalation. NAC prevented changes in heart rate and SDNN in UAP-exposed rats (340 ± 8 and 2.9 ± 0.3, respectively). To investigate the role of the autonomic nervous system in PM-induced oxidative stress, rats were given 5 mg/kg atenolol (β-1 receptor antagonist), 0.30 mg/kg glycopyrrolate (muscarinic receptor antagonist) or saline immediately before exposure to CAPs aerosols. Both atenolol and glycopyrrolate effectively prevented CAPs-induced cardiac oxidative stress (CL_{ATEN}: 11 ± 1 cps/cm^2, CL_{GLYCO}: 10 ± 1 cps/cm^2, $TBARS_{ATEN}$: 40 ± 6 pmol/mg protein, $TBARS_{GLYCO}$: 38 ± 6 pmol/mg protein). These data indicate that PM exposure increases cardiac oxidants via autonomic signals and the resulting oxidative stress is associated with significant functional alterations in the heart.

Keywords: Reactive oxygen species; Oxidative stress; Particulate air pollution; CAPs

1. Introduction

Acute and chronic exposure to respirable ambient particulate matter (PM) is positively associated with increased cardiovascular morbidity and mortality [1,2]. Epidemiological studies indicate that acute exposures to PM increase the number of hospital admissions for arrhythmia [3], myocardial infarction [4,5], and congestive heart failure [6,7]. However, the mechanism(s) leading to PM cardiac effects are not yet known.

Ambient particles may elicit cardiovascular effects, in part, through the autonomic nervous system. Support for this hypothesis has come from a number of studies showing that short-term exposure to particles is associated with changes in autonomic function as assessed by heart rate variability [8–14]. Measures of heart rate variability derived from electrocardiographic recordings provide a quantitative,

Abbreviations: PM, particulate matter; CAPs, concentrated ambient particles; TBARS, thiobarbituric acid substances; ROS, reactive oxygen species; TCA, thricloroacetic acid; NAC, N-acetylcysteine; CL, chemiluminescence; HAPC, Harvard Ambient Particle Concentrator; UAP, urban air particles; ROFA, residual oil fly ash

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non-invasive marker of cardiac autonomic nervous system control (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). These studies have generally found that increased levels of PM are associated with changes in heart rate variability that are consistent with perturbations of both sympathetic and parasympathetic activity leading to relative sympathoexcitation.

We have previously shown that short-term exposure to concentrated ambient particles (CAPs) significantly increases oxidant levels in rat heart and lung [15]. Moreover, these changes were associated with oxidant-dependent lung and heart edema, and with significant increases in the serum levels of LDH and of CK. These results indicate a harmful effect of CAPs at environmentally relevant concentrations.

We also found that lung oxidants increased immediately upon exposure to CAPs, while significant oxidative stress in the heart developed only after a 1-h lag [15]. We hypothesize that this observation reflects pulmonary to cardiac signaling via either nervous or systemic mediators.

In the current study, we sought to: (1) characterize the functional changes associated with PM-dependent increases in the production of ROS in the heart; (2) to determine the role of ROS in the development of cardiac malfunction after PM exposure; and (3) to identify the pathway(s) leading to cardiac oxidative stress and toxicity by PM.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (300 g body weight) were maintained and studied in accordance with the National Institutes of Health guidelines for the care and use of animals in research. All protocols were approved by the Harvard Medical Area Standing Committee on Animals.

2.2. Urban ambient particles (UAP) instillation

Standard Reference Material 1649 (Urban Dust, NIST, Washington, USA) was used. UAP composition is listed in Table 1. Rats were anesthetized with 5% halothane (Vaporizator, Colonial Medical Supply Co. Inc., USA) and intratracheally instilled with 750 μg UAP suspended in 300 μl sterile saline (Abbott Laboratories, USA). Control animals were instilled with 300 μl saline. Sham and exposed animals were assayed for the steady-state concentration of oxidants in the heart (organ chemiluminescence: CL) and cardiac function (ECG). After measuring, CL and cardiac function heart samples were collected and stored for biochemical analyses (lipid peroxidation). Tissue samples were flash frozen in a dry-ice bath.

Table 1
Average UAP composition*

	Mass fraction (μg/g)
Na	ND
Mg	9.2±0.3
Al	0.06±0.01
Si	ND
S	32.7±9
Cl	2.8±0.1
K	ND
Ca	ND
Ti	ND
V	0.34±0.01
Cr	0.21±0.01
Mn	0.24±0.08
Fe	29.8±0.7
Ni	0.17±0.01
Cu	0.22±0.01
Zn	1.68±0.04
Br	11.9±0.01
Cd	0.02
Ba	0.57±0.02
Pb	12.4±0.4

* National Institute of Standards and Technology. Standard Reference Material 1649a, ND=not determined.

2.3. Concentrated ambient particles (CAPs)

The Harvard Ambient Particle Concentrator (HAPC) concentrates ambient air particles for subsequent aerosol exposure of animals [16]. Particles remain in suspension for inhalation exposures or for collection onto filters for mass concentration and composition analysis. Mass concentrations were determined gravimetrically and the size of the particles was measured using a microorifice impactor. Trace metal concentrations were determined using X-ray fluorescence (Chester LabNet, Tigrad, Oregon). The CAPs total mass and elemental composition of the CAPs used in this study is presented in Table 2. Rats were exposed to CAPs aerosols (CAPs) or filtered air (sham) in the chamber of the HAPC at 25 °C as described [15]. The animals were awake and unrestrained during the exposures. The CAPs and sham groups were exposed and tested simultaneously using 2 animals per group. After 5 h, the animals were removed from the chamber and assayed for the steady-state concentration of oxidants in the heart (organ chemiluminescence: CL) and cardiac function (ECG). After measuring, CL and cardiac function heart samples were collected and stored for biochemical analyses (lipid peroxidation). Tissue samples were flash frozen in liquid nitrogen.

2.4. Treatments

2.4.1. Autonomic nervous systems stimulation

Rats were injected with 10 μg/kg (i.v.) isoproterenol, 3 μg/kg (i.v.) acetylcholine, or muscarine (0.03 μg/kg i.v.). Drugs were injected into the tail vein using a small