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Impaired cardiac mitochondrial function and contractile reserve following an acute exposure to environmental particulate matter

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ABSTRACT

Background: It has been suggested that mitochondrial function plays a central role in cardiovascular diseases associated with particulate matter inhalation. The aim of this study was to evaluate this hypothesis, with focus on cardiac O_2 and energetic metabolism, and its impact over cardiac contractility.
Methods: Swiss mice were intranasally instilled with either residual oil fly ash (ROFA) (1.0 mg/kg body weight) or saline solution. After 1, 3 or 5 h of exposure, O_2 consumption was evaluated in heart tissue samples. Mitochondrial respiration, respiratory chain complexes activity, membrane potential and ATP content and production rate were assessed in isolated mitochondria. Cardiac contractile reserve was evaluated according to the Langendorff technique.
Results: Three hours after ROFA exposure, tissue O_2 consumption was significantly decreased by 35% (from 1180 ± 70 to 760 ± 60 ng-at O/min g tissue), as well as mitochondrial rest (state 4) and active (state 3) respiration, by 30 and 24%, respectively (control state 4: 88 ± 5 ng-at O/min mg protein; state 3: 240 ± 20 ng-at O/min mg protein). These findings were associated with decreased complex II activity, mitochondrial depolarization and deficient ATP production. Even though basal contractility was not modified (control: 75 ± 5 mm Hg), isolated perfused hearts failed to properly respond to isoproterenol in ROFA-exposed mice. Tissue O_2 consumption rates positively correlated with cardiac contractile state in controls ($r^2 = 0.8271$), but not in treated mice ($r^2 = 0.1396$).
General Significance: The present results show an impaired mitochondrial function associated with deficient cardiac contractility, which could represent an early cardiovascular alteration after the exposure to environmental particulate matter.

1. Introduction

Epidemiological studies have shown a positive correlation between decreased air quality levels and adverse health effects [1]. Increased cardiovascular morbidity and mortality rates have been found to be associated not only with chronic air pollution exposures [2], but also with short-term daily exposures as well [3]. Interestingly, although the complex nature of air pollution and the coexistence of many compounds which may together contribute to the observed negative health impact, substantial epidemiological data point out that particulate matter (PM) is the main responsible for the health outcomes [4].

PM is comprised of a heterogeneous mixture of solid and liquid particles suspended in air, varying in size, chemical composition and sources of origin [5]. Anthropogenic emissions are the main contributors to environmental PM burden and consist mainly of motor vehicle emissions and fossil fuel combustion during power generation and industrial processes [6]. The inorganic residue that remains after the incomplete oxidation of such carbonaceous materials contributes to PM in urban air and is termed residual oil fly ash (ROFA) [7]. Diverse PM surrogates have been assayed in different animal models in order to study the biological effects of PM exposure. Among them, ROFA has been particularly useful given that it is especially rich in soluble transition metals (namely iron, nickel and vanadium), and because of its low concentration of organic compounds [8]. Therefore, ROFA is the most frequently used combustion-derived particle in order to evaluate the contribution of transitions metals in the biological effects of PM inhalation [9]. Moreover, ROFA particles often present an aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$), a size that have been

Abbreviations: m-CCCP, carbonyl cyanide m-chlorophenylhydrazone; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; ISO, isoproterenol; LVDP, left ventricular developed pressure; NAO, 10-*N*-nonyl acridine orange; PM, particulate matter; ROFA, residual oil fly ash; RCR, respiratory control ratio

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shown to be more closely associated with PM adverse health effects than coarser particles ($PM_{10-2.5}$) [5].

Among adverse cardiovascular effects triggered by PM exposure, daily changes in PM concentration have been associated with increased hospitalizations due to several cardiovascular events such as heart failure, arrhythmias, ischemic heart disease, cerebrovascular disease and peripheral vascular disease [10]. Increased production of reactive O_2 species leading to oxidative damage have been suggested to significantly contribute to the cardiopulmonary toxicity of PM inhalation [11]. Numerous studies in humans and animal models have shown a pulmonary and systemic inflammatory response and oxidative stress associated with PM exposure which can, in turn, alter heart O_2 metabolism and cardiovascular function [12]. Although most of these studies are focused on PM mass, in the last years increased awareness turned to PM chemical composition owing to the relevance of certain PM constituents (e.g. transition metals, organic redox-active compounds and endotoxins) in promoting cardiovascular diseases [4]. It is worth noting that, even though the link between air pollution PM inhalation and cardiovascular adverse effects is quite established, the underlying molecular mechanisms are poorly understood.

Given that mitochondria play an essential role in cellular O_2 and energetic metabolism, several authors suggested that mitochondrial dysfunction is a key feature in the development of cardiac alterations during the exposure to air pollution PM [13,14]. Cardiac contraction and relaxation have a continuous energy requirement, consuming more energy than any other organ. Because of a mismatch in ATP supply and demand, decreased levels of high-energy phosphates have been reported in the failing human heart [15] hampering with the transference of chemical energy to contractile work [16]. Most of this energy is produced in the mitochondria by oxidative phosphorylation, a process that involves electron-transfer reactions through the mitochondrial respiratory chain complexes at the inner mitochondrial membrane. In this context, any alteration triggered by PM inhalation in these multienzymatic complexes, in the electrochemical H^+ gradient that they generate across the inner membrane or in F_0–F_1 ATP synthase activity, could lead to a deficient ATP production resulting in a bioenergetic dysfunction and organ failure [17].

Taking into account that inhaled PM could alter heart oxidative metabolism, the need of an adequate energy supply to sustain proper contractile work, and the crucial role of mitochondria in both O_2 and energetic metabolism, the aim of this work was to evaluate cardiac O_2 metabolism and contractile function, focused on mitochondrial function, in a mice model of acute exposure to PM. The obtained findings could give new insights to the understanding of the biochemical basis of the observed PM-associated cardiovascular effects.

2. Materials and methods

2.1. Drugs and chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, US), except HCl, H_2SO_4 and organic solvents which were purchased from Merck KGaA (Darmstadt, Germany). Mitochondrial fluorescent probes were provided by Molecular Probes (Eugene, OR, US).

2.2. Experimental model

2.2.1. ROFA suspension

ROFA particles were collected from Boston Edison Co., Mystic Power Plant, Mystic, CT, US and were kindly provided by Dr. J. Godleski (Harvard School of Public Health, MA, US). ROFA samples from this source have been previously characterized in terms of elemental composition and particle size [18]. Vanadium, nickel and iron are the predominant metals present as water-soluble sulfates, and particle mean aerodynamic diameter is 2.06 ± 1.57 μm. PM samples were freshly prepared by suspending ROFA particles in sterile saline solution (0.5 mg/mL), followed by a 10 min incubation in an ultrasonic water bath before use.

2.2.2. Animal exposure

Female Swiss mice weighing 20–25 g were anesthetized by an intraperitoneal (i.p.) injection of ketamine (10 mg/kg body weight) and xylazine (0.1 mg/kg body weight), and exposed to ROFA particles (1.0 mg/kg body weight) or saline solution (control group) by intranasal instillation in a single dose. Mice were immobilized in a 60° inclined supine position while 50 μL of the ROFA suspension was delivered dropwise to the nares by the use of an automatic pipette. After 1, 3 or 5 h of exposure, animals were sacrificed and heart samples were collected. Control mice were handled in parallel, instilled with 50 μL of sterile saline solution, and sacrificed at the same time points. Due to the presence of fluid in the mouse nasal cavity, a respiratory reflex is triggered which ensures that the maximum delivered volume reaches the lung [19]. The selected dose falls within the range of concentrations consistently used in several animal studies [11,20,21]. Animal treatment was carried following the 6344/96 regulation of the Argentinean National Drug, Food and Medical Technology Administration (ANMAT) guidelines.

2.3. Tissue samples

2.3.1. Heart tissue cubes

Heart samples were kept in Krebs buffer solution [118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 24.8 mM $NaHCO_3$ and 10 mM glucose (pH 7.4)] at 4 °C. After being washed and weighted, 1 mm^3 tissue cubes were cut by the use of a scalpel [22].

2.3.2. Mitochondrial isolation and preparation of mitochondrial membranes

Heart mitochondrial purified fractions were obtained from tissue homogenates by differential centrifugation in a Sorvall RC5C centrifuge (Sorvall, Buckinghamshire, England). Two mouse hearts were pooled, washed and minced in ice-cold STE buffer [250 mM sucrose, 5 mM Tris–HCl and 2 mM EGTA (pH 7.4)]. A brief digestion was performed in STE medium supplemented with 0.5% (w/v) fatty acid-free BSA, 5 mM $MgCl_2$, 1 mM ATP and 2.5 UI/mL type XXIV bacterial proteinase. After 4 min at 4 °C, samples were homogenized in 1:10 STE buffer with a Potter Elvejhem glass homogenizer and centrifuged at 8000 *g* for 10 min. The obtained pellet was resuspended in ice-cold STE buffer and centrifuged at 700 *g* for 10 min. The sediment was discarded and mitochondria were pelleted from the supernatant by two centrifugation steps at 8000 *g* for 10 min each. Finally, the pellet was washed, rinsed and resuspended in 500 μL of STE buffer. The whole procedure was carried out at 0–4 °C [23]. Purity of isolated mitochondria was assessed by determining lactate dehydrogenase activity; only mitochondria with less than 5% impurity were used. Mitochondrial membranes were obtained by three freeze–thaw cycles of the mitochondrial preparation, followed by a homogenization step by passage through a 29 G hypodermic needle [24]. Protein concentration was measured by the Lowry assay [25] using BSA as standard.

2.4. Oxygen consumption by tissue cubes

A Clark-type O_2 electrode (Hansatech Oxygraph, Hansatech Instruments Ltd, Norfolk, England) for high resolution respirometry was used. Reaction buffer consisted of 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 24.8 mM $NaHCO_3$ and 5.5 mM glucose (pH 7.4). After an initial stabilization period, tissue cubes were added to the reaction chamber and O_2 consumption rates