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Low-level lead exposure changes endothelial modulation in rat resistance pulmonary arteries

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ABSTRACT

Lead exposure induces hypertension and endothelial dysfunction. However, the effects on the pulmonary vasculature have not been explored. In this study, rats exposed to lead acetate for seven days (4 μg/100 g on the 1st day and 0.05 μg/100 g/day i.m. subsequently) had lead blood level of 3.9 ± 0.7 μg/dL and increased right ventricular pressures. There was an increased Pb deposition and superoxide anions production in the pulmonary arteries, associated with reduced vasoconstriction but unchanged endothelium-dependent vasodilatation to acetylcholine (ACh). In both groups, inhibition of the nitric oxide (NO) synthase with L-NAME blocked the response to ACh, while indomethacin (cycloxygenase inhibitor) had no effect. Incubation with nonspecific potassium channel blocker (tetraethylammonium) reduced the ACh-induced vasodilatation only in the Pb group. Apamin (SK_{Ca} channel blocker) and 4-aminopyridine (K_v channel blocker), but not iberiotoxin (BK_{Ca} channel blocker), also inhibited this response in the Pb group. The vasodilatation to exogenous NO was reduced by Pb, while relaxation to the cGMP analogue was similar between groups. Concordantly, the protein level of soluble guanylate cyclase (sGC) was reduced. In conclusion, short-term and low-level exposure to Pb changes pulmonary haemodynamic and increases oxidative stress. The pulmonary vasculature exhibited increased hyperpolarization by the K_v and SK_{Ca} channels, probably as a compensatory mechanism to the decreased responsiveness to NO.

1. Introduction

Lead is an abundant metal in the Earth's crust and can be found naturally as a result of volcanic emissions and geochemical weathering. In addition, its intense use in human activities has significantly increased its emission into the atmosphere [1], resulting in important environmental and occupational contamination levels [2]. Thus, lead is currently named as a pollutant, implying high risks to public health (Menke et al., 2006). The Agency for Toxic Substances and Disease Registry (ATSDR) proposes an acceptable level of lead in blood below 60 μg/dL in occupationally exposed adults because its effects on human health depend directly on plasma levels and exposure duration.

Lead affects the central nervous system [1], renal system [3] and the cardiovascular system, where it induces arterial hypertension [4], increases the production of free radicals and decreases the antioxidant reserve [5–7], induces cardiac dysfunction [8] and increases sympathetic activity [9,10].

We recently demonstrated that, although rats exposed to a low lead dose for a short term exhibited blood concentrations below the safety recommendations, there was activation of the renin-angiotensin system, increased vascular oxidative stress and impaired vasomotor response in the aorta [11]. It is worth noting that vascular dysfunction is also involved in the pathophysiology of pulmonary circulation disorders, contributing to the occurrence of pulmonary artery aneurysm, thrombosis, lung infarction, primary pulmonary hypertension, and ventilation-perfusion disturbances [12]. Thus, in the present study, we aimed to investigate the effects of 7-day lead exposure at a low dose on the right ventricular function and on the reactivity of resistance pulmonary arteries to assess the pathways potentially involved.

2. Material and methods

2.1. Animals and experimental model of lead exposure

Male Wistar rats (250–300 g) were used, and all experiments were conducted in accordance with the Brazilian Guidelines for the Care and Use of Animals for Scientific and Educational Purposes and were approved by the Institutional Ethics Committee on Animal Use (CEUA-UFES 018/2011). During treatment, rats were kept in cages with free

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access to water and food under conditions of controlled temperature and humidity and were subjected to a 12-h light-dark cycle.

The animals were divided into two groups: control (Ct), which received daily intramuscular injections of vehicle (NaCl 0.9%); and lead (Pb), injected with lead acetate (1st dose of 4 μg/100 g and subsequent doses of 0.05 μg/100 g/day to cover daily losses) following the experimental model previously described [11].

2.2. Haemodynamic study of the right ventricle

The haemodynamic evaluation was performed on a heated operating table (37 °C) under adjusted anaesthesia (urethane chloride, 1.5 g/kg; Sigma, St. Louis, USA). A polyethylene angled catheter (PE 50, ClayAdams; Becton Dickinson & Co., Parsippany, USA) was inserted from the right jugular vein into the right ventricular cavity to assess the intraventricular pressure using a pressure transducer (TSD104A; Biopac Systems, Santa Barbara, USA). The following data were acquired and analysed (Acknowledge software; Biopac Systems, Santa Barbara, USA): RV systolic (SP) and end-diastolic pressures (EDP) and heart rate (HR).

2.3. Blood and pulmonary lead level measurements

Lead concentrations were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (NexIon 300-D; Perkin Elmer, Germany) [13]. Lung samples were dried at 70 °C for 12 h before digestion. Briefly, 0.1 g of the lung or blood samples were digested with 3 mL of conc. HNO_3, 1 mL of 30% *v*/v H_2O_2 (m/m) and 2 mL of ultrapure water (Elga — Purelab, Marlow, UK) using a microwave oven equipped with PTFE vessels (Multiwave 3000 microwave, Anton Paar, Graz, Austria). The final volume was adjusted with ultrapure water up to 15 mL. For ICP-MS analysis, argon (99.999% Air Liquid; Rio de Janeiro, Brazil) was used for plasma generation, as the auxiliary gas and for sample nebulization. The sample introduction system comprised a cyclonic spray chamber and a Meinhard® nebulizer. The quality control requirements were followed closely to demonstrate accurate quantification of lead in the samples used in this study. The mass-to-charge ratio (*m*/*z*) was monitored with isotope 208Pb, and 193Ir was used as an internal standard. The ICP-MS operating conditions were: a generator frequency of 40 MHz, a plasma gas flow rate of 16 L•min^{-1}, an auxiliary gas flow rate of 1.2 L•min^{-1}, a nebulizer gas flow rate of 1.02 L•min^{-1} and an RF 173 power of 1.55 kW. Accuracy was checked using recovery tests. The limit of quantification (LOQ) for lead in the samples analysed by ICP-MS was 5.6 ng•g^{-1}. The LOQ was determined for each analysis performed.

2.4. Vascular reactivity of the resistance pulmonary artery

With the animals under anaesthesia, the chest was opened for the removal of the cardiopulmonary block, which was placed in a Petri dish containing ice-cold Krebs-Henseleit solution (KHS, in mM: 115 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4.7H_2O$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 11.1 glucose and 0.01 Na_2EDTA) gassed with 95% O_2 and 5% CO_2 (pH 7.4). Then, under a dissecting microscope, the pulmonary artery was dissected from the right ventricular outflow tract, and the third-order arterial branches corresponding to the resistance pulmonary arteries (RPAs) were isolated and cut into segments approximately 2 mm in length. Briefly, two tungsten wires (40 μm in diameter) were introduced through the lumen of the segments and mounted in a small-vessel myograph chamber (Model 410 A; Danish Myo Tech, JP-Trading I/S, Aarhus, Denmark) for isometric tension recordings, acquired by hardware connected to a computer (Powerlab/800 ADInstruments, Castle Hill, Australia) and analysed by software (Labchart 8 ADInstruments, Castle Hill, Australia). After a 30-min equilibration period in oxygenated KHS at 37 °C and pH 7.4, the segments were stretched to their optimal lumen diameter for active tension development according to the internal circumference/wall tension curve. The internal circumference (L_0) was set to 90% of what the vessels exhibit as a passive tension, equivalent to the transmural pressure of 25 mm Hg (L_{100}). The internal diameter (I_1) was determined according to the equation $I_1 = L_1/\pi$, using specific software for normalization of the resistance arteries (DMT Normalization Module; ADInstruments, Castle Hill, Australia). Thereafter, the RPA segments were washed with KHS and left to equilibrate for 30 min, and then the smooth muscle responsiveness was assessed by measuring contraction in response to 80 mM KCl. Next, the vessels were washed with KHS and left to equilibrate for 1 h before initiating protocols with vasoconstrictor and vasodilator agents to test the vascular response. The vasocontractile responses were performed by the cumulative addition of serotonin (5-HT 10^{-8} to 10^{-2} M). To evaluate the vasodilator response, RPAs pre-constricted with 5-HT (10^{-3} M) were exposed to the cumulative addition of endothelium-dependent vasodilator acetylcholine (ACh, 10^{-13} to 10^{-3} M), an endothelium-independent vasodilator, the nitric oxide donor sodium nitroprusside (NPS, 10^{-13} to 10^{-3} M), or a membrane-permeable activator of cyclic guanosine monophosphate (cGMP)-dependent protein kinases and cGMP-gated ion channels, to test the response of RPA to exogenous cGMP (8-pCPT-cGMP, 10^{-8} to 10^{-4}). Additionally, to assess the involvement of the main endothelial vasodilators in the RPA rings from lead-injected rats, some experiments of ACh-induced vasodilatation were carried out in the presence of inhibitors of the three pathways involved in the endothelial-dependent relaxation: L-NAME 10^{-4} M (nitric oxide synthase inhibitor), indomethacin 10^{-5} M (non-selective cycloxygenase inhibitor) or tetraethylammonium (TEA) 10^{-3} M (non-specific potassium channel blocker). To specifically evaluate the potassium channel subtypes responsible for the endothelial modulation of RPA tone in rats exposed to lead, we pre-incubated RPA rings with different specific inhibitors: 4-aminopyridine 10^{-1} M (selective blocker of voltage-gated potassium channel - K_v), apamin 10^{-4} M (selective blocker of calcium-activated small conductance potassium channel - SK_{Ca}) and iberiotoxin 3×10^{-5} M (selective blocker of calcium-activated large conductance potassium channel - BK_{Ca}). All vasodilatations were elicited after a pre-contraction with 5-HT.

2.5. In situ production of nitric oxide and superoxide anion in resistance pulmonary arteries from lead-exposed rats

Local nitric oxide (NO) production was determined using 4,5-diaminofluorescein (DAF-2) as previously described [14,15]. RPAs were dissected and embedded in a freezing medium (Killik-OCT EasyPath; Erviegas Ltda, SP, Brazil). Transverse arterial sections (10 μm) were obtained using a cryostat and were equilibrated under identical conditions for 30 min at 37 °C with phosphate buffer (0.1 M) containing $CaCl_2$ (0.45 mM). After 30 min, the sections were incubated in a light-protected humidified chamber at 37 °C with 8 μM DAF-2 in phosphate buffer (0.1 M) containing $CaCl_2$ (0.45 mM).

The oxidative fluorescent dye dihydroethidium (DHE) was used to evaluate $O_2^{\bullet-}$ production *in situ* as previously described [14]. Digital images were collected on a Leica DM 2500 fluorescence microscope with a Leica DFC 310 FX camera using the same imaging settings for the control and lead-exposed groups. For quantification, five frozen tissue segments per animal were sampled for each experimental condition and averaged. The mean fluorescence densities in the target region were calculated using free software (ImageJ software 1.44p; National Institutes of Health, USA).

2.6. Western blotting analysis of soluble guanylate cyclase

Proteins from homogenized arteries were separated by 10% SDS-PAGE. Proteins were transferred to PVDF membranes and then incubated overnight with a rabbit polyclonal antibody against the Guanylate Cyclase β1 subunit (1:1000; Cayman Chemical, Ann Arbor, MI, USA). After washing, the membranes were incubated with anti-rabbit

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