Free Radical Biology and Medicine 67 (2014) 366–376

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Low-dose chronic lead exposure increases systolic arterial pressure and vascular reactivity of rat aortas

Edna Aparecida Silveira [a,*], Fabiana Dayse Magalhães Siman [a], Thaís de Oliveira Faria [a], Marcos Vinícius Altoé Vescovi [b], Lorena Barros Furieri [a], Juliana Hott Fúcio Lizardo [c], Ivanita Stefanon [a], Alessandra Simão Padilha [a], Dalton Valentim Vassallo [a]

[a] Department of Physiological Sciences, Federal University of Espírito Santo, Vitória, ES CEP 29040-091, Brazil
[b] Center for Exact Sciences, Chemistry Department, Federal University of Espírito Santo, Vitória, ES CEP 29040-091, Brazil
[c] Department of Morphology, Federal University of Espírito Santo, Vitória, ES CEP 29040-091, Brazil

ARTICLE INFO

Article history:
Received 18 September 2013
Received in revised form
9 November 2013
Accepted 22 November 2013
Available online 2 December 2013

Keywords:
Lead acetate
Hypertension
Rat aorta
NO
ROS
Vasoconstrictor prostanoids
Local renin–angiotensin system
Free radicals

ABSTRACT

Chronic lead exposure induces hypertension affecting endothelial function. We investigated whether low-concentration lead exposure alters blood pressure and vascular reactivity, focusing on the roles of NO, oxidative stress, cyclooxygenase-derived vasoconstrictor prostanoids, and the local angiotensin–renin system. Aortic rings from 3-month-old Wistar rats were treated daily with lead acetate (first dose 4 mg/100 g, subsequent doses 0.05 mg/100 g, im) or vehicle for 30 days. Treatment increased lead blood levels (12 μg/dl), blood pressure, and aortic ring contractile response to phenylephrine (1 nM–100 mM). Contractile response after L-NAME administration increased in both groups but was higher after lead treatment. Lead effects on R_{max} decreased more after apocynin and superoxide dismutase administration compared to control. Indomethacin reduced phenylephrine response more after lead treatment than in controls. The selective COX-2 inhibitor NS398, thromboxane A_2/prostaglandin H2 receptor antagonist SQ 29,548, TXA_2 synthase inhibitor furegrelate, EP_1 receptor antagonist SC 19220, and ACE inhibitor and AT_1 receptor antagonist losartan reduced phenylephrine responses only in vessels from lead-treated rats. Basal and stimulated NO release was reduced and local $O_2^{\bullet -}$ liberation increased in the lead-treated group compared to controls. eNOS, iNOS, and AT_1 receptor protein expression increased with lead exposure, but COX-2 protein expression decreased. This is the first demonstration that blood Pb^{2+} (12 μg/dl) concentrations below the WHO-established values increased systolic blood pressure and vascular phenylephrine reactivity. This effect was associated with reduced NO bioavailability, increased reactive oxygen species production, increased participation of COX-derived contractile prostanoids, and increased renin–angiotensin system activity.

Contents

* Corresponding author. Fax: +55 27 2122 7330.
E-mail address: ednasilveira17@gmail.com (E.A. Silveira).

0891-5849/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.freeradbiomed.2013.11.021

Lead is a common environmental pollutant that affects all organs and systems of an organism and causes several acute and chronic diseases [1]. Previous reports have suggested a close relationship among lead exposure, hypertension, and cardiovascular diseases in humans [2–4] and animals [5–9]. Lead is extensively used in the industrial sector; therefore, all humans usually have lead in their system as a result of exposure to exogenous sources [3]. This exposure occurs during the manufacture of ammunition, batteries, sheet lead, solder, ceramic glaze, caulking, brass and bronze plumbing, circuit boards, military equipment, and surgical equipment and from some medicines (herbal remedies from China and India) and drinking water [3,10]. Several mechanisms have been proposed to cause lead-induced hypertension, such as inhibition of Na^+/K^+-ATPase [11], alterations in calcium exchange [12], increased sympathetic nerve activity, reduced baroreflex sensitivity and parasympathetic tone [13], increased plasma catecholamines [14], reduced vascular β-adrenoceptor density and cAMP levels [15], alterations in the soluble guanylyl cyclase levels in the vascular wall [11], direct activation of smooth muscle protein kinase C [16], increased renin–angiotensin system activity [17], and endothelial dysfunction [18].

The effects of lead on human health depend on blood levels and on exposure duration. The Agency for Toxic Substances and Disease Registry [19] recommends that blood lead concentrations should be under 60 μg/dl in occupationally exposed adults [3,15,20]. Nevertheless, increased arterial pressure has been reported for individuals with blood lead concentrations between 31.4 and 53.5 μg/dl [21,22]. Recent experimental studies of lead toxicity demonstrated blood lead concentrations between 31.8 and 58.7 μg/dl [6,23], which are similar to those found in lead-exposed workers. However, little attention has been given to the effects of lead in the blood at concentrations below those found in humans with occupational lead exposure. Because of a lack of knowledge in these areas, we developed an experimental model of controlled lead exposure in rats that produces blood concentrations below that found in humans with occupational lead exposure. Thus, the aim of this study was to investigate the effects of 30-day, low-concentration lead acetate exposure on systolic blood pressure and to investigate aortic ring vascular reactivity and the possible roles of nitric oxide (NO), oxidative stress, cyclooxygenase-derived prostanoid vasoconstrictors, and the renin–angiotensin system.

Materials and methods

Animals and treatment

The study was performed on male rats (250–300 g). All of the experiments were conducted in accordance with the guidelines for biomedical research as stated by the Brazilian Societies for Experimental Biology and were approved by the Health Science Center of Vitória Institutional Ethics Committee (CEUA-EMESCAM 004/2007). All of the rats had free access to water and were fed rat chow ad libitum. The rats were randomly divided into control (vehicle–saline, im) or lead acetate-treated groups for 30 days (first dose 4 mg/100 g, subsequent doses 0.05 mg/100 g, im).

After treatment, the rats were anesthetized with pentobarbital (35 mg/kg, ip) and were killed by exsanguination. The thoracic aortas were carefully dissected out, and connective tissue was removed. For vascular reactivity experiments, the aortas were divided into 4-mm cylindrical segments in length. For protein expression analysis, some arteries were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

Blood pressure measurements

Indirect systolic blood pressure was measured weekly using tail-cuff plethysmography (IITC Life Science, Inc.). Conscious rats were restrained for 5–10 min in a warm, quiet room and conditioned to numerous cuff inflation–deflation cycles by a trained operator. Systolic blood pressure was measured, and the mean of three measurements was recorded [23].

Blood lead level measurements

Blood lead levels were determined according to the protocol developed by Koresková-Sysalová [24]. In both groups of animals the lead concentrations in whole blood samples after 30 days of treatment were measured in duplicate by atomic absorption spectrometry (Model AAS5 EA with graphite furnace; Carl Zeiss, Oberkochen, Germany) at the Center for Exact Sciences, Chemistry Department (Federal University of Espírito Santo).

Vascular reactivity study

Aortic segments were mounted between two parallel wires in an organ bath containing Krebs–Henseleit solution (in mM: 124 NaCl, 4.6 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 0.01 EDTA, and 23 $NaHCO_3$, pH 7.4) at 37 °C under 95% O_2–5% CO_2. Arterial segments were stretched to an optimal resting tension of 1 g. Isometric tension was recorded using a force displacement transducer (TSD125C; Biopac Systems, Santa Barbara, CA, USA) that was connected to an acquisition system (MP100A; Biopac Systems).

After 45 min of equilibration, all of the aortic rings were initially exposed twice to 75 mM KCl. The first exposure assessed their functional integrity, and the second exposure assessed the maximal tension developed. Afterward, endothelial integrity was assessed with acetylcholine (10 μM) in segments that had been previously contracted with phenylephrine (1 μM). A relaxation equal to or greater than 90% was considered demonstrative of the functional integrity of the endothelium. After a 45-min washout, phenylephrine concentration–response curves were determined. One single concentration–response curve was performed for each segment.

The role of the endothelium in phenylephrine-induced contraction was evaluated in aortic rings that had been subjected to