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The cessation of the long-term exposure to low doses of mercury ameliorates the increase in systolic blood pressure and vascular damage in rats

Danize Aparecida Rizzetti[a], João Guilherme Dini Torres[a], Alyne Goulart Escobar[a], Taiz Martins da Silva[a,b,c,d,e], Paola Zambelli Moraes[a], Raquel Hernanz[b], Franck Maciel Peçanha[a], Marta Miguel Castro[c], Dalton Valentim Vassallo[d], Mercedes Salaices[e], Maria Jesús Alonso[b], Giulia Alessandra Wiggers[a,*,1]

[a] Postgraduate Program in Biochemistry, Universidade Federal do Pampa, Uruguaiana, RS, Brazil
[b] Department of Basic Health Sciences, Universidad Rey Juan Carlos, Alcorcón, Spain
[c] Bioactivity and Food Analysis Laboratory, Instituto de Investigación en Ciencias de la Alimentación, Madrid, Spain
[d] Department of Physiological Sciences, Universidade Federal do Espírito Santo, Vitória, ES, Brazil
[e] Department of Pharmacology, Universidad Autónoma de Madrid, Madrid, Spain

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ABSTRACT

This study aimed to verify whether a prolonged exposure to low-level mercury promotes haemodynamic disorders and studied the reversibility of this vascular damage. Rats were divided into seven groups: three control groups received saline solution (*im*) for 30, 60 or 90 days; two groups received $HgCl_2$ (*im*, first dose, 4.6 μg/kg, subsequent doses 0.07 μg/kg/day) for 30 or 60 days; two groups received $HgCl_2$ for 30 or 60 days (*im*, same doses) followed by a 30-day washout period. Systolic blood pressure (SBP) was measured, along with analysis of vascular response to acetylcholine (ACh) and phenylephrine (Phe) in the absence and presence of endothelium, a nitric oxide (NO) synthase inhibitor, an NADPH oxidase inhibitor, superoxide dismutase, a non-selective cyclooxygenase (COX) inhibitor and an AT1 receptor blocker. Reactive oxygen species (ROS) levels and antioxidant power were measured in plasma. $HgCl_2$ exposure for 30 and 60 days: a) reduced the endothelium-dependent relaxation; b) increased the Phe-induced contraction and the contribution of ROS, COX-derived vasoconstrictor prostanoids and angiotensin II acting on AT1 receptors to this response while the NO participation was reduced; c) increased the oxidative stress in plasma; d) increased the SBP only after 60 days of exposure. After the cessation of $HgCl_2$ exposure, SBP, endothelium-dependent relaxation, Phe-induced contraction and the oxidative stress were normalised, despite the persistence of the increased COX-derived prostanoids. These results demonstrated that long-term $HgCl_2$ exposure increases SBP as a consequence of vascular dysfunction; however, after $HgCl_2$ removal from the environment the vascular function ameliorates.

1. Introduction

Mercury (Hg) has become a serious health concern due to its high capacity for bioaccumulation and the variety of its effects on biological systems (Goyer, 1997; Laamech et al., 2014). This metal is ranked a top three priority pollutant by the U.S. Environmental Protection Agency and the Centers for Disease Control (Tchounwou et al., 2012). Recent findings show that the total amount of anthropogenic Hg present in the global oceans has been increased 150% compared to pre-anthropogenic conditions and has become one of the most monitored metals, not only in environment but also in human tissues (Lamborg et al., 2014; Val et al., 2016).

Human exposure to Hg generally occurs in a chronic manner during occupational exposure, through diet (mainly fish intake) and the use or handling of dental amalgam. In these situations, Hg exposure can promote human toxicity by organic (from food), inorganic (from industrial activity) and elemental forms (from dental amalgam restorations) (Kim et al., 2016). Therefore, the different forms of Hg

* Corresponding author.
E-mail address: giuliawp@gmail.com (G.A. Wiggers).
[1] G.A. Wiggers: Giulia Wiggers: – Programa de Pós Graduação em Bioquímica - PPGBioq - UNIPAMPA, BR 472 – Km 592 – postal code 118. Zip Code: 97500-970, Uruguaiana – Rio Grande do Sul, Brazil.

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determine the route of exposure, absorption, distribution, and target organ toxicity (Park and Zheng, 2012).

Earlier findings have shown deposition of Hg in many organs, mainly in the kidney, liver and brain, leading to such serious adverse effects as kidney damage (Magos and Clarkson, 2006), gastroenteritis (Vojdani et al., 2003), pulmonary fibrosis (Haddad and Stenberg Jr, 1963), reduction of reproductive function and infertility (Davis et al., 2001; Martinez et al., 2014a), as well as cardiovascular dysfunction (Vassallo et al., 1996; Frustaci et al., 1999; Drescher et al., 2014). At the cardiovascular level, Hg exposure has been associated with the development of atherosclerosis, hypertension, coronary artery disease and myocardial infarction (Virtanen et al., 2005; Houston, 2007; Wennberg et al., 2012; Kamynsky et al., 2016).

Previously, we have demonstrated that, in the cardiovascular system, long-term exposure to low doses of $HgCl_2$ for 30 days induces oxidative stress, decreases the bioavailability of nitric oxide (NO), increases the production of reactive oxygen species (ROS) and cyclooxygenase (COX)-derived vasoconstrictor prostanoids in the aorta and the mesenteric, coronary and basilar arteries, leading to endothelial dysfunction and increased vasoconstriction in rats (Wiggers et al., 2008, 2016; Pecanha et al., 2010; Furieri et al., 2011). However, despite the vascular damage no changes in blood pressure were observed in this experimental model. Recently, we have also demonstrated that co-treatment with the NADPH oxidase inhibitor apocynin prevented the increase of ROS caused by Hg exposure without changes in the COX-contractile prostanoids production, indicating that oxidative stress is due mainly to the superoxide anion from NADPH oxidase and that the activation of COX pathway is independent of oxidative stress (Rizzetti et al., 2013).

Despite the severity of the damage caused by exposure to Hg in almost all human organs, only a few studies have investigated whether this toxic effect can be minimised or even eliminated after the cessation of the metal contact; however, these studies were focused on neurological and behavioural effects caused by Hg, with differences due to the level and time of exposure (Kishi, 1978; Kishi et al., 1993; Yoshida et al., 2006). Thus, the aim of this study is primarily to verify whether a prolonged *in vivo* exposure to $HgCl_2$, similar to human occupational contact to this metal, promotes haemodynamic disorders as a consequence of vascular and biochemical harm previously observed *in vitro* in this model. Moreover, we also propose to analyse the reversibility of this Hg-induced vascular damage after the cessation of the exposure in rats.

2. Material and methods

2.1. Animals

Three-month-old male *Wistar* rats (240–300 g) were obtained from the Central Animal Laboratory of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. During treatment, manipulation of the animals was performed following the appropriate safety procedures. Rats were housed at a constant room temperature, humidity, and light cycle (12:12 h light:dark), with free access to tap water and fed with standard food *ad libitum*. All experiments were conducted in compliance with the guidelines for biomedical research stated by the Brazilian Societies of Experimental Biology and approved by the Ethics Committee on Animal Use of the Federal University of Pampa (CEUA/UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil (Protocol Number: 013/2013). Rats were divided into seven groups (eight animals each): a) untreated for 30, 60 or 90 days (Untreated 30 d, Untreated 60 d and Untreated 90 d), receiving intramuscular (*im*) injections of saline solution 0.9% during the respective treatment time; b) treated with $HgCl_2$ for 30 or 60 days ($HgCl_2$ 30d and $HgCl_2$ 60 d), receiving *im* injections of $HgCl_2$ dissolved in a saline solution vehicle; first dose, 4.6 μg/kg, with a subsequent dose of 0.07 μg/kg/day during the respective treatment time to cover daily loss by metal removal via urine and faeces, using the model previously described (Wiggers et al., 2008); c) treated with $HgCl_2$ for 30 or 60 days (*im*, first dose, 4.6 μg/kg, subsequent doses 0.07 μg/kg/day) followed by a washout period of 30 days (30+30 d Washout and 60+30 d Washout).

2.2. Systolic blood pressure

Indirect systolic blood pressure (SBP) was measured weekly in conscious rats using non-invasive tail-cuff plethysmography (AD Instruments Pty Ltd, Bella Vista, NSW, Australia). Before the measurement, rats were kept at 30 °C for 15 min to make the pulsations of the tail artery detectable. To establish the value of SBP, 10 measurements were taken, and the average of all was obtained. To minimise stress-induced variations in blood pressure, all measurements were taken by the same person in a warm and quiet room.

2.3. Blood collection and reactivity experiments

Rats were anaesthetised with a combination of ketamine and xylazine (87 mg/kg and 13 mg/kg, respectively, *ip*), and after loss of the righting reflex the rats were submitted to a surgical procedure to expose the abdominal aorta; blood was subsequently collected to obtain plasma for the biochemical experiments. To prepare the plasma samples, blood was centrifuged for 10 min at 2500 rpm and 4 °C and the resulting plasma was kept at −80 °C until used to determine ROS levels and antioxidant capacity. Thereafter, rats were euthanised by decapitation, and the thoracic aorta was carefully dissected out and cleaned of fat and connective tissue. For reactivity experiments, the aorta was divided into cylindrical segments of 2 mm in length.

Segments of thoracic aorta were mounted in an isolated tissue chamber containing 5 ml of Krebs-Henseleit solution (in mM: NaCl 118; KCl 4.7; $NaHCO_3$ 23; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; glucose 11 and EDTA 0.01), gassed with 95% O_2 and 5% CO_2 (pH 7.4) and maintained at a resting tension of 1.5 g at 37 °C. Isometric tension was recorded using an isometric force transducer (TSD125BX8, Biopac Systems, Inc, Santa Barbara, CA, USA) connected to an acquisition system (MP150WSW-SYS, Biopac Systems). After a 45-min equilibration period, aortic rings were exposed twice to 75 mM KCl, first to check their functional integrity and again to assess the maximal tension developed. Afterwards, endothelial integrity was tested with acetylcholine (ACh, 10 μM) in segments that were previously contracted with phenylephrine (Phe) at a concentration that produced close to 50% of the contraction induced by 75 mM KCl. Relaxation equal to or greater than 80% was considered demonstrative of the functional integrity of the endothelium. After 60 min of washout, a single concentration-response curve to Phe (0.01 nM – 300 μM) was performed.

To evaluate the role of the endothelium in the vasoconstrictor responses to Phe, some rings had their endothelium mechanically removed, and its absence was confirmed by the inability of ACh to induce relaxation greater than 10% of the previous contraction due to Phe. To evaluate the participation of NO, ROS, prostanoids or AT1 receptors on Phe responses, the effects of N_ω-nitro-L-arginine methyl ester (L-NAME 100 μM), apocynin (0.3 mM), superoxide dismutase (SOD 150 U/ml), indomethacin (1 μM) and losartan (10 mM) were investigated by their addition 30 min before Phe in vessels with intact endothelium.

To evaluate the relaxation dependent and independent of the endothelium, concentration-response curves were also performed with ACh (0.01 nM–300 μM) and sodium nitroprusside (SNP, 0.01 nM–300 μM), respectively, in segments precontracted with Phe.

2.4. Mercury quantification

Total Hg concentration was determined in blood samples by an Hg analyser (SMS 100, PerkinElmer, Inc., Shelton, CT, USA) in the Atomic Spectrometry Service at the Universidad de Málaga, Spain, using the