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Mercury-induced vascular dysfunction is mediated by angiotensin II AT-1 receptor upregulation

Danize A. Rizzetti[a], Taiz M. da Silva[a], Alyne G. Escobar[a], Janaina Piagette[a], Franck M. Peçanha[a], Dalton V. Vassallo[b], Maria J. Alonso[c], Mercedes Salaices[d], Giulia A. Wiggers[a,*]

[a] Cardiovascular Physiology Laboratory, Universidade Federal do Pampa, BR 472, Km 592, Uruguaiana, Rio Grande do Sul, Brazil
[b] Cardiac Electromechanical and Vascular Reactivity Laboratory, Universidade Federal do Espírito Santo, Marechal Campos, 1468 Vitória, Espírito Santo, Brazil
[c] Department of Basic Health Sciences, Universidad Rey Juan Carlos, C/ Atenas s/n, Alcorcón, Spain
[d] Department of Pharmacology, Universidad Autónoma de Madrid, C/ Arzobispo Morcillo, 4, Madrid, Spain

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ABSTRACT

Low doses of mercury (Hg) promote deleterious effects on cardiovascular system, but the mechanisms implicated remain unclear. This study analyzed whether angiotensin II AT-1 receptors are involved in the vascular dysfunction caused by chronic exposure to low $HgCl_2$ doses. For this, rats were divided into four groups and untreated (saline by *im* injections and tap water by gavage) or treated for 30 days as follows: Mercury ($HgCl_2$ *im*, first dose of 4.6 μg kg^{-1} and subsequent doses of 0.07 μg kg^{-1} day^{-1}, and tap water by gavage); Losartan (saline *im* and losartan, 15 mg kg^{-1} day^{-1}, by gavage); Losartan-Mercury ($HgCl_2$ *im* and Losartan by gavage). Systolic blood pressure was measured by tail plethysmography, vascular reactivity in aorta by isolated organ bath, oxidative stress by measuring the levels of reactive oxygen species (ROS), malondialdehyde (MDA) and antioxidant capacity (FRAP) and protein expression of AT-1 receptors by Western Blot. As results, co-treatment with losartan prevented the increased aortic vasoconstrictor responses to phenylephrine (Phe), the involvement of ROS and prostanoids on the response to Phe and the reduced negative endothelial modulation by nitric oxide on these responses. Moreover, this co-treatment avoided the increase in plasmatic and vascular oxidative stress and AT-1 protein expression in aorta. In conclusion, these results suggest that AT-1 receptors upregulation might play a key role in the vascular damage induced by Hg exposure by increasing oxidative stress and probably by reducing NO bioavailability.

1. Introduction

Mercury (Hg) can cause deleterious effects on human health, which depend on the chemical form, amount, route of exposure and differences in vulnerability among exposed subjects (Kim et al., 2016; Nedellec and Rabl, 2016). The cardiovascular system can be a sensitive target affected by acute and chronic exposure to Hg, which can induce inflammation, oxidative stress, atherosclerosis and impairment of vascular function (Rossoni et al., 1999; Wiggers et al., 2008a; Lemos et al., 2012; Solenkova et al., 2014), as well as cardiac disorders derived from diffuse fibrosis, myocardial infarction and contractile dysfunction in rodents (Oliveira and Vassallo, 1992; Vassallo et al., 1996; Moreira et al., 2003; Wang et al., 2015; Kamynsky et al., 2016). In humans, it has been proposed that exposure to Hg is associated with increased blood pressure and heart rate variation (Salonen et al., 2000; Choi et al., 2009; Cabana-Munoz et al., 2015).

Previously we demonstrated in a rat model that chronic exposure to low doses of $HgCl_2$ for 30 days, mimicking the human exposure, produced endothelial dysfunction and increased vascular reactivity in aorta, mesenteric, coronary and basilar arteries, while blood pressure remained unchanged (Wiggers et al., 2008a; Peçanha et al. 2010; Furieri et al., 2011; Rizzetti et al., 2013; Wiggers et al., 2016). Other findings included the increase in the oxidative stress, lipid peroxidation and the reduction in the local and systemic antioxidant defenses, the rise in the vascular protein expression of pro-oxidant enzymes such as NADPH oxidase, the decrease in the nitric oxide (NO) vascular bioavailability, the increment in the vasoconstrictor prostanoids production from COX-2 as well as in the participation of the renin-angiotensin system (RAS) as a consequence of the rise in the vascular angiotensin converting enzyme (ACE) activity (Wiggers et al., 2008a, 2008b; Peçanha et al., 2010; Furieri et al., 2011; Wiggers et al., 2016). We have recently shown that all these changes seem to be previous to hemodynamic effects since a longer Hg exposure increased SBP; in addition, this cardiovascular damage may be ameliorated after the cessation of the

* Corresponding author.
E-mail addresses: giuliawp@gmail.com, giuliapecanha@unipampa.edu.br (G.A. Wiggers).

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ongoing exposure related to the reduction of the vascular RAS and oxidative stress, despite the persistence of inflammatory factors (Rizzetti et al., 2017).

It has been proposed that angiotensin II (Ang II) may upregulate COX-2 expression and the subsequent vasoconstrictor prostanoids production through the activation of AT-1 receptors in vascular smooth muscle cells (Hu et al., 2002). Additionally, this peptide is also able to stimulate the generation of superoxide anion by increasing the NADPH oxidase activity in vascular smooth muscle cells from normotensive rats (Griendling et al., 1994; Garcia-Redondo et al., 2016) and in hypertension models (Rajagopalan et al., 1996; Touyz et al., 2005; Martinez-Revelles et al., 2013). However, the role of Ang II on the activation of ROS and COX-2 pathways and the associated vascular alterations in the cardiovascular system during exposure to Hg is still unclear. The purpose of the study was to investigate the role of Ang II AT-1 receptors in the vascular damage caused by chronic exposure to $HgCl_2$ at low doses and to clarify the possible interactions between ROS and COX-2 pathways in this damage.

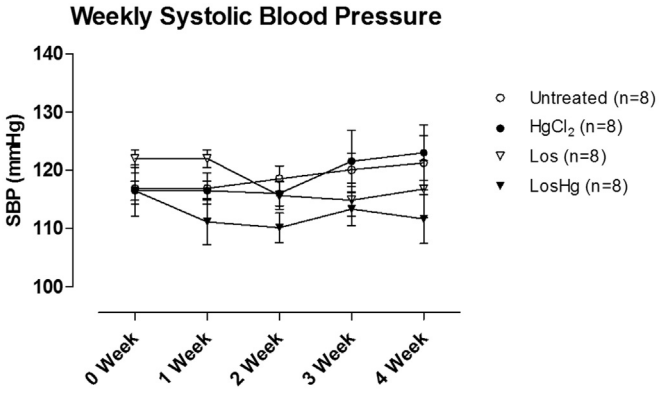

Fig. 1. Effect of losartan on non-invasive systolic blood pressure of rats chronically exposed to low concentrations of $HgCl_2$. Values of weekly systolic blood pressure of rats Untreated and treated with $HgCl_2$, Losartan (Los) or Losartan plus $HgCl_2$ (LosHg). Results are expressed as the mean ± SEM. Two-Way ANOVA $P > .05$.

2. Material and methods

2.1. Animals and ethics statement

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 chow ad libitum. All experiments were conducted in compliance with the guidelines for biomedical research stated by the Brazilian Societies of Experimental Biology and the European legislation on care and use of experimental animals (EU Directive 2010/63/EU for animal experiments; R.D. 53/2013) and approved by the Ethic Committee on Animal Use at Universidade Federal do Pampa, Uruguaiana, Rio Grande do Sul, Brazil (institutional review board 0322013). The experiments were also designed to minimize the number of animals used and their suffering during the execution of the protocols.

Rats were divided into four groups and untreated – saline by *intramuscular (im)* injections and tap water by gavage; or treated for 30 days with: Mercury ($HgCl_2$) – $HgCl_2$ *im*, first dose of 4.6 μg kg^{-1} and subsequent doses of 0.07 μg kg^{-1} day^{-1} to cover daily loss, using a model previously described (Wiggers et al., 2008a) and tap water by gavage; Losartan (Los) – saline *im* and losartan, 15 mg kg^{-1} day^{-1}, by gavage, whose dose was previously reported (Alvarez et al., 2007) and Losartan-Mercury (LosHg) – $HgCl_2$ *im* and Losartan by gavage.

2.2. Systolic blood pressure

Indirect systolic blood pressure (SBP) was weekly measured in conscious rats using non-invasive tail-cuff plethysmography (AD Instruments Pty Ltd, Bella Vista, NSW, Australia). Before measurement, rats were kept at 37 °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 of them was obtained. To minimize stress-induced variations in blood pressure all measurements were taken by the same person in a warm and quiet room.

2.3. Blood and aorta collection and reactivity experiments

Rats were anesthetized with a combination of ketamine and xylazine (87 mg kg^{-1} and 13 mg kg^{-1}, respectively, *ip*), and after loss of the righting reflex rats were submitted to a surgical procedure to expose the abdominal aorta; then blood was subsequently collected to obtain plasma for the biochemical determinations. Thereafter, rats were euthanized by decapitation; a segment of the abdominal aorta was collected and kept in −80 °C until the biochemical and molecular analysis. The thoracic aorta was carefully dissected out, cleaned of fat and connective tissue and divided into cylindrical segments of 2 mm in length for the vascular reactivity experiments.

Aortic segments were mounted under isometric conditions in an isolated tissue chamber containing 5 mL of Krebs-Henseleit solution (pH 7.4, 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) at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2, maintaining an optimal resting tension of 1.5 g. Tension was recorded by an isometric force transducer (TSD125BX8 – Biopac Systems, Inc, Santa Barbara, USA) connected to a recorder (MP150WSW-SYS – Biopac Systems, Inc, Santa Barbara, USA).

After a 45-min equilibration period, aortic rings were exposed to 75 mM KCl to check their functional integrity and to assess the maximal-induced tension (30 min). Endothelial integrity was subsequently tested with acetylcholine (ACh, 10 μM) using segments previously contracted with phenylephrine (Phe, 1 μM). Relaxation equal to or greater than 80% represented a positive demonstration of the functional integrity of the endothelium. After a washout period (30 min), a concentration-response curve to Phe (0.01 nM – 3.5 mM) was generated.

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. To evaluate the participation of NO, ROS, or prostanoids 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) and indomethacin (1 μM) were investigated in vessels with intact endothelium by their addition 30 min before Phe.

To evaluate the relaxation dependent and independent of the endothelium, concentration-response curves to ACh (0.01 nM – 300 μM) and sodium nitroprusside (SNP, .01 nM – 300 μM) were respectively performed, in segments precontracted with Phe.

2.4. Biochemical analysis

Segments of abdominal aorta were homogenized in 50 mM Tris-HCl, pH 7.4, (1/10, w/v); thereafter the homogenate and blood were centrifuged for 10 min at 2500 rpm and 4 °C. The supernatant and plasma were collected for oxidant and antioxidant analysis.

ROS levels in plasma and aorta were determined by spectrofluorometric method as previously described (Loetchutinat et al., 2005). For this, samples were diluted (1:400 for plasma and 1:5 for aorta) in 50 mM Tris-HCl (pH 7.4) and 2′,7′-dichlorofluorescein diacetate (DCHF-DA; 1 mM) was added to the medium. DCHF-DA is enzymatically hydrolyzed by intracellular esterases to form