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Aluminum exposure for one hour decreases vascular reactivity in conductance and resistance arteries in rats

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ABSTRACT

Aims: Aluminum (Al) is an important environmental contaminant; however, there are not enough evidences of Al-induced cardiovascular dysfunction. We investigated the effects of acute exposure to aluminum chloride ($AlCl_3$) on blood pressure, vascular reactivity and oxidative stress.
Methods and results: Male *Wistar* rats were divided into two groups: *Untreated*: vehicle (ultrapure water, *ip*) and *$AlCl_3$*: single dose of $AlCl_3$ (100 mg/kg,*ip*). Concentration-response curves to phenylephrine in the absence and presence of endothelium, the nitric oxide synthase inhibitor L-NAME, the potassium channel blocker tetraethylammonium, and the NADPH oxidase inhibitor apocynin were performed in segments from aortic and mesenteric resistance arteries. NO released was assessed in aorta and reactive oxygen species (ROS), malondialdehyde, non-protein thiol levels, antioxidant capacity and enzymatic antioxidant activities were investigated in plasma, aorta and/or mesenteric arteries. After one hour of $AlCl_3$ exposure serum Al levels attained 147.7 ± 25.0 μg/L. Al treatment: 1) did not affect blood pressure, heart rate and vasodilator responses induced by acetylcholine or sodium nitroprusside; 2) decreased phenylephrine-induced vasoconstrictor responses; 3) increased endothelial modulation of contractile responses, NO release and vascular ROS production from NADPH oxidase; 4) increased plasmatic, aortic and mesenteric malondialdehyde and ROS production, and 5) decreased antioxidant capacity and affected the antioxidant biomarkers non-protein thiol levels, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase and catalase enzymatic activities.
Conclusion: $AlCl_3$-acute exposure reduces vascular reactivity. This effect is associated with increased NO production, probably acting on K^+ channels, which seems to occur as a compensatory mechanism against Al-induced oxidative stress. Our results suggest that Al exerts toxic effects to the vascular system.

1. Introduction

Aluminum (Al) is the third most abundant element in the Earth's crust and the most common metal, representing approximately 8% of the Earth's surface (Sparling and Lowe, 1996). Al is not an essential metal for life (Kawahara and Kato-Negishi, 2011); however its high availability points to this metal as an important environmental contaminant and increases the potential toxicity for human exposure (Prabhakar et al., 2012; Bohrer et al., 2014; Veiga et al., 2013). The major sources of Al exposure include food additives, antacids, vaccines, hemodialysis solution (HD) and parenteral nutrition (PN) (Yokel and McNamara, 2001; Shaw et al., 2013). Specifically, the presence of Al in intravenous solutions, such as PN and HD fluids, is responsible for the high exposure to this metal. The Al concentration in PN can reach 135 μg/L (Yokel and McNamara, 2001), while the maximum considered safe by the Food and Drug Administration (FDA) is 25 μg/L(Food and Drug Administration (FDA), 2014). Moreover, acutely Al-intoxicated HD occurs when serum Al concentration exceed 60 μg/L in HD patients (Anon., 2003), and it can reach 237 μg/L (Anon., 2008).

Al compounds can be accumulated in several organs (Veiga et al., 2013), and it has been associated with osteopenia (Li et al., 2011), microcytic anemia (Barata et al., 1996) and neurological disorders (Shaw et al., 2013; Walton, 2006). Concerning the cardiovascular system, there are not enough evidences for Al-induced cardiovascular dysfunction; however, it is known that Al can be strongly accumulated in cerebral arteries (Minami et al., 1996; Bhattacharjee et al., 2013).

The underlying mechanisms of Al-induced toxicity are not entirely clear; however, they are apparently related to their pro-oxidant effects.

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Al can induce oxidative stress by promoting an imbalance between enzymatic/non-enzymatic antioxidant defenses and reactive oxygen species (ROS) generation, thus facilitating peroxidative reactions in membranes (Kumar et al., 2009). In vessels, the most important sources of ROS are NADPH oxidase, xanthine oxidase, mitochondria and the uncoupled endothelial nitric oxide synthase (NOS) (Montezano and Touyz, 2012).

Although there are few studies devoted to the cardiovascular effects of Al poisoning (Minami et al., 1996; Lind et al., 2012; Ezomo et al., 2009; Gomes et al., 1994; Granadillo et al., 1995), to the best of our knowledge, the vascular responses to Al exposure remain unknown. Therefore, the aim of this study was to investigate whether the acute exposure to aluminum chloride ($AlCl_3$) affects blood pressure, vascular reactivity and oxidative stress in rats.

2. Materials and methods

2.1. Animals and reagents

Three-month-old male *Wistar* rats (354–482 g) were obtained from Central Animal Laboratory of the Federal University of Pelotas, Rio Grande do Sul, Brazil. During treatment, rats were maintained at a constant room temperature, humidity, and light/dark cycle, and they had access to water and feed *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 0,282,013). The experiments were designed to minimize the number of animals used and their suffering during the execution of the protocols.

All reagents were high purity and purchased from Sigma-Aldrich (St. Louis, MO, USA), and all of the solutions were prepared with purified water by a Milli-Q high purity water device (Millipore, Bedford, MA, USA). To avoid Al contamination from glass, only plastic materials were used. For this, the laboratory equipment was immersed for at least 24 h in a 10% (*v*/v) HNO_3/ethanol solution and washed with ultrapure water shortly before use. To reduce contamination from the air, all of the steps in the preparation of the samples and reagents were conducted on a Class 100 clean bench.

Systolic (SBP) and diastolic blood pressure (DBP) were assessed in anesthetized rats (urethane 1.2 g/kg, *ip*) by carotid artery cannulation using a polyethylene catheter (PE 50, Clay-Adams, NY, USA), filled with saline and heparin (50 U/mL). Blood pressure was obtained by a pressure transducer (TSD104A) connected to a preamplifier and to an acquisition system (MP150 Biopac Systems Inc., Goleta, *CA*, USA). After 30 min of stabilization, ultrapure water (*ip*) or $AlCl_3$ (100 mg/kg, *ip*) were injected and SBP, DBP and heart rate (HR) were measured immediately before administration and 60 min after injection. Next, rats were euthanized by decapitation, and blood and part of the aorta and mesenteric arteries were collected for biochemical analysis. Serum Al concentration was measured after dilution with purified water 1:1 by graphite furnace atomic absorption spectrometry (GFAAS) according to Noremberg et al. (2015) using an ANALYTIK Jena AG (Jena, Germany) model ZEEnit 600.

2.2. Reactivity experiments

The thoracic aorta and the third-order mesenteric resistance arteries (MRA) were dissected and placed in Krebs-Henseleit solution (pH 7.4) (in mM: 124 NaCl, 23 $NaHCO_3$, 4.6 KCl, 1.2 $MgSO_4$ $7H_2O$, 2.5 $CaCl_2 \cdot 2H_2O$, 1.2 KH_2PO_4, 11.1 glucose and 0.01 Na_2EDTA) at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2. The surrounding connective tissue was carefully removed and the vessels were cut into 2 mm in length. Aortic segments were horizontally mounted under isometric conditions by inserting two stainless-steel wires (75 μm in diameter) into the lumen and stretched to an optimal resting tension of 1.5 g. Tension was recorded by an isometric force transducer (TSD125BX8 Biopac Systems Inc.) connected to a recorder (MP150WSW-SYS Biopac Systems Inc.).

MRA segments were mounted in a small-vessel dual chamber myograph (Multi Wire Myograph System, DMT620, ADInstruments do Brasil, São Paulo, SP, Brazil) for measurement of isometric tension, according to Wiggers et al. (2008). Segments were stretched to their optimal lumen diameter for active tension development. This was determined based on the internal circumference-to-wall tension ratio of the segments by setting their internal circumference, L_o, to 90% of what the vessels would have if they were exposed to a passive tension equivalent to that produced by a transmural pressure of 100 mmHg.

After a 45-min equilibration period, aorta and MRA were respectively exposed to 75 and 120 mM KCl to check their functional integrity. Concentration-response curves to phenylephrine (0.1 nM–3.5 mM) were performed after 60 min of a new stabilization period. The effects of N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 100 μM), a nitric oxide synthase (NOS) inhibitor, apocynin (0.3 mM), a presumed NADPH oxidase inhibitor, and tetraethylammonium (TEA, 2 mM), a non-selective potassium channels blocker, were investigated by their addition to the bath 30 min before the concentration-response curves to phenylephrine. The role of the endothelium on the contractile responses was studied by its removal. Effectiveness of endothelium removal was confirmed by the inability of 10 μM acetylcholine (ACh) to induce relaxation. To investigate the endothelium-dependent and -independent relaxation, concentration-response curves to ACh (0.1 nM–3.5 mM) and sodium nitroprusside (SNP, 0.1 nM–3.5 mM), were respectively performed in segments pre-contracted with phenylephrine at a concentration that produce approximately 50% of the contraction induced by K^+-KHS in each case.

2.3. Nitric oxide release

NO release was measured as previously described (Avendano et al., 2014). After an equilibration period of 60 min in Krebs-HEPES buffer at 37 °C, aortic segments were incubated with 4,5-diaminofluorescein (DAF-2, 2 μM) for 45 min. Thereafter, the medium was collected to measure basal NO release. Subsequently, the induced NO release was measured after segments were incubated with phenylephrine1 μM and relaxed with ACh 10 μM. The fluorescence was measured using a spectrofluorometer (SpectraMax M5 Molecular Devices, Sunnyvale, *CA*, USA) with excitation at 492 nm and emission at 515 nm. The induced NO release was obtained by subtracting basal NO release from that evoked by ACh. Blank samples were measured in the medium without segments to subtract background emission. The amount of NO released was expressed as arbitrary units/g tissue. Data were expressed as percentage of results obtained for Untreated rats.

2.4. Pro-oxidants assays

For biochemical assays, segments of aorta and MRA were homogenized in 50 mM Tris-HCl, pH 7.4, (1/10, *w*/*v*), centrifuged at 2400*g* for 10 min at 4 °C and the resulting supernatant fraction was frozen at −80 °C for further assay. ROS levels in plasma, aorta and MRA were determined by spectrofluorimetric method, as previously described by Loetchutinat et al. (2005). For this, samples were diluted (1:1000 for plasma and 1:5 for aorta and MRA) 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 nonfluorescent DCHF, which is then rapidly oxidized to form highly fluorescent 2′, 7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS formed. The DCF fluorescence intensity emission was recorded at 520 nm (with 488 nm excitation) for 60 min at 15 min intervals. The