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ORIGINAL ARTICLE

Mercury exposure induces proinflammatory enzymes in vascular fibroblasts☆

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KEYWORDS
$HgCl_2$;
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Abstract
Introduction: Previous studies show that mercury exposure increases cardiovascular risk, although the underlying cellular mechanisms have still not been fully studied. The aim of this project is to study, in vascular fibroblasts (VF), the effect of $HgCl_2$ exposure on the expression of enzymes involved in the synthesis of prostanoids and reactive oxygen species (ROS). These molecules have been shown to participate in the inflammatory response associated with cardiovascular diseases.
Material and methods: Adventitial VF cultures of Sprague-Dawley rat aortas, shown to be α-actin negative by immunofluorescence, were exposed to $HgCl_2$ (0.05–5 μg/mL) for 48 h. mRNA and protein levels of cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase 1 (mPGES-1), thromboxane A_2 synthase (TXAS), NADPH oxidase 1 (NOX-1), and 4 (NOX-4) where analyzed using qRT-PCR and western blot, respectively. NOX activity was determined by chemiluminescence.
Results: $HgCl_2$ exposure increased COX-2, mPGES-1, TXAS, and NOX-1 expression and NOX activity, and decreased NOX-4 expression. The increase in NOX-1 and COX-2 expression was abolished by the treatment with inhibitors of COX-2 (10 μM celecoxib) and NOX (300 μM apocynin, 0.5 μM ML-171).
Conclusions: 1) $HgCl_2$ increases the expression of pro-inflammatory enzymes involved in ROS and prostanoid synthesis in VF. 2) There is a reciprocal regulation between COX-2 and NOX-1 pathways. 3) These effects can contribute to explain the increase in cardiovascular risk associated to mercury.

☆ All authors have contributed to the idea and design of this study; to the collection, analysis and interpretation of the data; to the writing of the draft or to the critical review of its contents and to the approval of the final version to be published.

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PALABRAS CLAVE
$HgCl_2$;
Fibroblastos vasculares;
Estrés oxidativo;
NOX;
COX-2

La exposición a mercurio induce la expresión de enzimas pro-inflamatorias en fibroblastos vasculares

Resumen
Introducción: Estudios previos muestran que la exposición a mercurio aumenta el riesgo cardiovascular, sin embargo, los mecanismos celulares subyacentes no han sido esclarecidos completamente. Nuestro objetivo es estudiar el efecto de la exposición a $HgCl_2$ sobre la expresión de enzimas involucradas en la síntesis de prostanoides y especies reactivas de oxígeno (ROS) en fibroblastos vasculares (FV). Se ha demostrado la participación de estas moléculas en la respuesta inflamatoria asociada a enfermedades cardiovasculares.
Métodos: FV de la adventicia de aorta de ratas Sprague-Dawley, caracterizados por inmunofluorescencia como negativos para α-actina, fueron estimulados con $HgCl_2$ (0,05-5 μg/ml) durante 48 horas. Se analizaron los niveles de ciclooxigenasa-2 (COX-2), prostaglandina E sintasa 1 microsomal (mPGES-1), tromboxano A2 sintasa (TXAS), NADPH oxidasa 1 (NOX-1) y 4 (NOX-4) mediante qRT-PCR y *western blot*, respectivamente. La actividad de NOX se determinó mediante quimioluminiscencia.
Resultados: La exposición a $HgCl_2$ aumentó la expresión de COX-2, mPGES-1, TXAS y NOX-1, disminuyendo la expresión de NOX-4. El tratamiento con inhibidores de COX-2 (10 μM celecoxib) y NOX (300 μM apocynin, 0.5 μM ML-171) abolió el aumento de la expresión de NOX-1 y COX-2, respectivamente.
Conclusiones: 1) $HgCl_2$ aumenta la expresión de enzimas proinflamatorias implicadas en la síntesis de ROS y prostanoides en FV. 2) Hay una regulación recíproca entre las vías de COX-2 y NOX-1. 3) Estos efectos pueden contribuir a explicar el aumento del riesgo cardiovascular asociado a la exposición al mercurio.

Introduction

The exposure to different heavy metals such as mercury is a risk factor in the development of different diseases. Mercury is a very toxic environmental pollutant and can be produced by natural or artificial sources. In the environment, there are three main types of mercury: elemental mercury, inorganic mercury and organic mercury, which is the most toxic.[1] It has been observed that mercury exposure is not an uncommon event.[2] In fact, people who consume fish regularly in their diets can reach mercury levels around the established limit.[3] The main and better known toxic effect of mercury exposure is neurotoxicity.[4] However, this metal also induces cardiovascular damage by increasing the risk of hypertension, myocardial infarction, coronary heart disease, generalized atherosclerosis and renal dysfunction.[5–8]

Cyclooxygenases are enzymes that transform arachidonic acid into prostaglandin H_2 (PGH_2). There are two cyclooxygenase (COX) isoforms, while COX-1 is the constitutive form, COX-2 is the inducible one and is usually overexpressed in pathological conditions.[9] A wide variety of stimuli, including mercury,[10,11] can induce the vascular expression of COX-2.[12,13] PGH_2 is transformed by different synthases into specific prostanoids that are responsible for the final response of the vessel. For example, thromboxane A_2 (TXA_2) and prostaglandin E_2 (PGE_2) are important in vascular tone[14,15] and remodeling.[16,17] TXA_2 is produced by TXA_2 synthase (TXAS), while PGE_2 is synthesized by PGE_2 synthases (PGES). There are three PGES isoforms being microsomal PGES-1 (mPGES-1) inducible and the main source of PGE_2 in pathological conditions.[18] In fact, proinflammatory stimuli that induce COX-2 are also able to induce mPGES-1.[19,20]

NADPH oxidase (NOX) is a family of enzymatic complexes that produce mainly superoxide anion ($O_2^{\bullet-}$) as reactive oxygen species (ROS). There are other sources of $O_2^{\bullet-}$ such as cyclooxygenases or xanthine oxidase, but only NOX synthesizes it as its main product using O_2 and NADPH. Seven isoforms of NADPH oxidase have been described in mammals, composed by a catalytic core formed by NOX 1–5 and/or dual oxidase 1–2 (DUOX 1–2) and several regulatory subunits. NOX-1 and NOX-4 are expressed in the adventitia layer of arteries, but their cellular location is different[21] NOX-1 is in the plasma membrane[22] generating $O_2^{\bullet-}$ in pathological conditions.[23] On the other hand, NOX-4 is found in focal adhesions and in endoplasmic reticulum generating $O_2^{\bullet-}$ and H_2O_2 in basal conditions.[21]

Oxidative stress and prostanoids derived from the inducible cyclooxygenase isoform, COX-2 and mPGES-1, are responsible for hypercontractility, endothelial dysfunction and/or vascular remodeling in cardiovascular pathologies, such as in hypertension[20,24,25] by decreasing NO availability and affecting cell migration and extracellular matrix deposition, among other effects.

Previous studies have demonstrated that chronic administration of low doses of mercury to rats induced endothelial dysfunction as a result of the decreased nitric oxide bioavailability induced by increases in oxidative stress.[10,26,27]

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