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Biological interactions between mercury and selenium in distribution and detoxification processes in mice under controlled exposure. Effects on selenoprotein

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ABSTRACT

Antagonistic interactions between mercury (Hg) and selenium (Se), were evaluated in mouse (*Mus musculus*), as a mammalian model, in a series of controlled exposure experiments. The beneficial effect of Se against Hg toxicity involves a variety of biochemical and toxicological processes that have not been clarified yet. For this purpose, a metallomic workflow based on the use of size-exclusion chromatography (SEC) with inductively coupled plasma mass spectrometry (ICP-MS) detection was complemented with the speciation of selenoproteins and low molecular mass selenium species in serum and liver cytosolic extracts using a multidimensional approach based on SEC-AF-HPLC-ICPMS, using species-unspecific isotope dilution (SUID)-ICP-MS for selenium quantification. The results showed potential interactions between Hg/Se in organs and serum related to accumulation and detoxification processes, in addition to the effects of mercury on selenoproteins in hepatic cytosolic extracts and bloodstream when both elements are administrated at the same time. These results provide information about elements distribution, interactions and homeostasis and reveal the potential of metallomic approaches in exposure experiments.

1. Introduction

Mercury (Hg) is a widespread environmental and industrial contaminant that induces rigorous toxic effects in body tissues of both human and animals [1], depending on its different chemical forms [2]. Mercury exists in three main chemical forms: elemental, as an inorganic salt, or bound to an organic ligand; although all the chemical species are potentially toxic. Divalent inorganic mercury (Hg^{2+}) is one of the strongest thiol-binding elements, which explains its toxicity by the ability to form stable complexes with the sulfhydryl-cysteine groups of proteins (–SH), such as numerous thiol-related enzymes [3]. Hg^{2+} can also give rise to free radicals that induce lipid, protein and DNA oxidation related to oxidative stress [4–6]. On the other hand, organic mercury compounds are mostly metabolized in the liver where they may suffer demethylation [7] or undergo conjugation reactions with glutathione (GSH) [2,8]. It is well known that Se presents a protective effect against Hg toxicity and inhibits oxidative damage caused by Hg in mammals [9–11].

Abbreviations: AF, affinity chromatography; BSA, bovine serum albumin; CRMs, certified reference materials; Cys, cysteine; DNA, deoxyribonucleic acid; eGPx, extracellular glutathione peroxidase; GPx, glutathione peroxidase; GSH, reduced glutathione; HMM, high molecular mass; HPLC, high performance liquid chromatography; ICP-MS, inductively coupling plasma-mass spectrometry; IDA, isotopic dilution analysis; LC, liquid chromatography; LMM, low molecular mass; MS, mass spectrometry; MT, metallothionein; ORS, octopole reaction systems; PMSF, phenylmethanesulfonyl fluoride; RBCs, red blood cells; SeAlb, selenoalbumin; SEC, size exclusion chromatography; SelP, selenoprotein P; SeCys, selenocysteine; SUID, species-unspecific isotopic dilution; SOD, superoxide dismutase; ThxR, thioredoxin reductase; TCEP, tris(2-carboxyethyl)phosphine hydrochloride; 2D, two dimensional.

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The antagonistic interaction between Hg and Se was first reported in 1967 in rats treated with mercury chloride and selenite [11]. All the same, Se is an essential element in mammalian health, with both deficiency and toxicity effects apparent, depending on soil content, due to a fairly narrow range of optimal Se intakes [12–14]. However, it has been reported that simultaneous exposure to both Se and Hg compounds increased whole-body retention of Hg, possibly due to the formation of inert Se-Hg complexes, which has been considered as a preventive mechanism against Hg toxicity [11,15]. Nevertheless, although the Se and Hg co-accumulation in humans and other mammals is well known, the mechanism of interaction between these elements is still unsolved.

Hg^{2+} can also react with selenol groups (–SeH) that constitute a part of selenocysteine (SeCys), and as a consequence they can be incorporated to selenoproteins, prosthetic groups of selenoenzymes and peptides [16–17], since the –SeH in SeCys shows even stronger affinity for Hg that –SH groups, possibly due to the lower pKa of SeCys (~5.4) that provides it a higher reactivity than Cys (pKa~8.0). Moreover, selenoproteins play an important role in the maintenance of cellular homeostasis [18]. In the same way, Hg^{2+} can also react with selenides (Se^{2-}) and hydrogen selenide or selenols to form Hg–Se–S complexes together with glutathione that can finally bond to selenoprotein P (SelP) forming a ternary complex in the bloodstream [19–21]. Se is an essential component of several selenoenzymes, such as glutathione peroxidase (GPx) and thioredoxin reductase (ThxR), which also contains Se as SeCys. In that respect, the perturbation of selenoproteins functions has been related to the development of several diseases such as carcinogenesis [22,23].

Although at the moment there are very powerful analytical techniques and sample preparation procedures for element speciation, biological systems require multi-elemental analytical approaches that make possible to characterize processes involving metals interactions, trafficking and homeostasis [24]. For this purpose, metallomics is a relatively new field to decipher changes in metal–biomolecules expression and identification in biological matrices. In this sense, it is necessary to consider that approximately one third of proteins need the presence of metals as cofactors to develop their function [24–27]. On the other hand, ICP-MS is a valuable technique in this field since it allows multi-isotopic analysis, detection capability, high sensitivity, tolerance to matrix and large linearity range of quantification [24], generally coupled to liquid chromatographic arrangements, and parallel molecular mass spectrometry for biomolecules identification in an integrated workflow [25,26]. As a consequence, metallomics provides a good option to deep insight into the fate of elements in exposed organisms to elements, and provides information about element distributions, retentions and interactions [28].

The aim of the present study was to characterize the biochemical response of mice *Mus musculus* in metal distribution and detoxification processes caused by simultaneous mercury exposure and selenium exposure based on suitable metallomic workflows. The metallomic approach included the use of size exclusion chromatography (SEC) coupled to ICP-MS as detector. In addition, the study was complemented by the speciation of selenoproteins and low molecular mass selenium species in serum and liver cytosolic extracts of mice based on species-unspecific isotope dilution (SUID)-ICP-ORS-MS on-line coupled to a SEC in tandem with an AF that integrate the analytical speciation platform.

2. Materials and methods

2.1. Instrumentation

The mineralization of samples to determine the total metal content in biological matrices was carried out by using a microwave accelerated reaction system model MARS (CEM Corporation, Matthews, Carolina del Norte, USA) and MARSXpress vessels.

A cryogenic homogenizer SPEX SamplePrep (Freezer/Mills 6770) was used to prepare tissues homogenates. Metal-containing biomolecules were separated and detected with an inductively coupled plasma mass spectrometer Agilent 7500ce (Agilent Technologies, Tokyo, Japan) which was equipped with an octopole reaction system (ORS). Liquid chromatography separations were performed using a Model 1100 HPLC pump with detector UV (Agilent, Wilmington, DE, USA). ICP-MS conditions (Table 1) to measure in He mode were optimized using a HNO_3 5% (v/v) aqueous solution of ^{59}Co, ^{89}Y, ^{205}Tl (1 μg L^{-1}). A microflow nebulizer (Teflon; model ESI, Ohama, USA) was used to establish the hyphenation of the LC-ICP-ORS-MS system.

2.2. Standard solutions and reagents

All reagents that were used for sample preparation in the metallomic approach were of the highest available purity. Phenylmethanesulfonyl fluoride (PMSF) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (BioUltra grade,>98%) were obtained from Sigma Aldrich (Steinheim, Germany). Helium and hydrogen, which were used as collision and reaction gas (in the ICP-ORS-MS system), were of high-purity grade (>99.999%).

The standards which were used for mass calibration of analytical SEC columns (mass range 600–10 kDa) included horse ferritin (440 kDa) (purity 95%), bovine serum albumin (67 kDa) (purity

Table 1
Operating conditions of chromatographic separations and ICP-ORS-MS detection.

ICP-MS conditions	
Forward power	1500 W
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	1 L min^{-1}
Carrier gas flow rate	0.15 L min^{-1}
Sampling and skimmer cones	Ni
Nebuliser	Microflow (ESI)
Torch	Shield (with long life platinum shield plate)
Q_{oct}	−18 V
Q_p	−16 V
Points per peak	1
Integration time	0.3 per isotope
Replicates	1
Isotopes monitored for total metals determination and SEC	^{63}Cu, ^{65}Cu, ^{64}Zn, ^{66}Zn, ^{103}Rh, ^{80}Se, ^{78}Se, ^{201}Hg and ^{202}Hg.
$H_{e\ flow}$ for total metals determination and SEC	4.0 mL min^{-1}
Isotopes monitored for SUID	^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se, ^{79}Br, ^{81}Br and ^{83}Kr
$H_{2\ flow}$ for SUID	3.7 mL min^{-1}
Dead time detector	47 ns
SEC conditions	
Column	Superdex™-200 (10 × 300 × 13 μm)
Resolution range	600–10 kDa
Mobile phase	Ammonium acetate 50 mM (pH 7.4)
Flow rate	0.7 mL min^{-1}
Injection volume	20 μL
UV detection	254 nm
2D/SEC-AF conditions	
Sample loop	100 μL
Flow rate	1.3 mL min^{-1}
Mobile phase A	0.05 M ammonium acetate pH 7.4
Mobile phase B	1.5 M ammonium acetate pH 7.4
Gradient	0–7 min 100% A, 6–18 min 100% B, 18–20 min 100% A
6-Port valveposition	1–10 min inject 10–17 min load 17–20 min inject