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Inorganic mercury and cadmium induce rigidity in eukaryotic lipid extracts while mercury also ruptures red blood cells

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ABSTRACT

Hg and Cd are non-essential toxic heavy metals that bioaccumulate in the tissues of living systems but less is known about their interactions with Eukaryotic lipid bilayers. Microscopy experiments showed that Hg and Cd changed the cell morphology of rabbit erythrocytes while Hg also induced cell rupture. As membranes are one of the first available targets, our study aimed to better understand metal-lipid interactions that could lead to toxic effects. Fluorescence spectroscopy (Laurdan Generalized Polarization) and dynamic light scattering were used to analyze metal-induced changes in membrane fluidity and the size of liposomes composed of Brain (Porcine), Liver (Bovine), Heart (Bovine) and Yeast (*S. cerevisiae*) lipid extracts. Under physiological chloride and pH levels, Hg irreversibly cleaves plasmalogens resulting in an increase in membrane rigidity. These lipids are enriched in Brain, Heart and Erythrocyte membranes and are important in signalling and the protection against oxidative stress. Interestingly, Hg had a heavily reduced effect on the plasmalogen-free Yeast extract membrane. In contrast, Cd induced rigidity by targeting negatively charged phosphatidic acid, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol and cardiolipin in these extracts. Metal-induced liposome aggregation depended on the proportion of negatively charged lipids/plasmalogen and even the order of metal addition. Our results show that data from model systems correlate with trends observed in complex biological extracts and red blood cells and serve as a predictive tool for analyzing metal-lipid interactions. The determination of the specific lipid targets for Hg and Cd provides new insights how these metals exert toxic effects on cell membranes.

1. Introduction

Although the serious adverse effects of Hg and Cd have been known for hundreds of years, the mechanisms of their toxicity are still poorly understood. The industrial revolution resulted in more toxic heavy metals in the environment, which increased the mobilization of Hg and Cd into living systems [1]. Within an organism, one potential target is the lipid bilayer, which is an essential barrier separating the exterior and interior environments of cells. Lipids account for approximately 50% of the dry weight of most cell membranes [2] and represent a significant potential metal target. However, the variation in lipid headgroups and acyl chains results in over 1000 different lipids in a Eukaryotic cell [3]. This prevents a detailed understanding of metal interactions in complex biological membranes without reference data from model systems with well-defined compositions.

Fluidity is one of the essential properties for a functional cell membrane. It reflects the motion of lipid molecules in the bilayer and greatly affects the mobility, conformation and catalytic activity of some proteins as well as membrane permeability [4]. The solvatochromic fluorophore Laurdan was used to monitor membrane fluidity in the absence or presence of Hg and Cd. Due to its hydrophobicity, Laurdan readily incorporates into lipid bilayers and is exposed to more water molecules with increasing temperature. The energy needed for reorientation of the polar water solvent dipoles results in a red shift in the emission intensity which is correlated with membrane fluidity [5]. Laurdan was widely used for liposomes [5], red blood cells [6] and to study lipid rafts in model and plasma membranes [7].

Laurdan results using well-defined model systems clearly showed that these metals decreased membrane fluidity; Hg acted on plasmalogen containing membranes [8] whereas Cd targeted anionic systems [9]. Plasmalogens are enol/ether phospholipids that are enriched in brain, heart and erythrocyte membranes [10]. Their interaction with Hg results in the irreversible transformation into two lipid fragments. While this reaction has been known since 1959 [11], the potential

Abbreviations: DLS, dynamic light scattering; Cd, inorganic Cd; CL, cardiolipin; GP, generalized polarization; Hg, inorganic mercury; LUV, large unilamellar vesicle; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; RBC, red blood cell; TLC, thin layer chromatography

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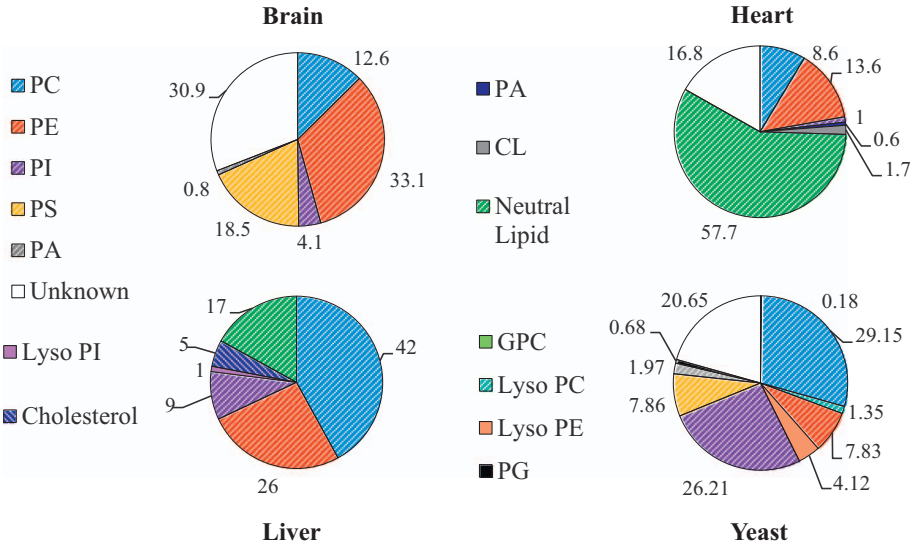

Fig. 1. Brain, Heart, Liver and Yeast polar extract lipid profiles available from Avanti Polar Lipids at: https://avantilipids.com/product-category/products/natural-lipids/extracts/. PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol), PS (phosphatidylserine), PA (phosphatidic acid), CL (cardiolipin), GPC (glycerophosphorylcholine), PG (phosphatidylglycerol).

impact on membrane fluidity is unknown and was investigated in this study. This destructive interaction is important as plasmalogens fulfill many biological roles including antioxidation, signal transduction and the mediation of membrane fusion [12]. A depletion of plasmalogens has been previously associated with varying diseases such as Alzheimer's [13], Down syndrome [14] and Zellweger syndrome [15]. It has to be emphasized that Hg accumulates in the brain and a link between Hg and Alzheimer's has been proposed [16].

To address the Hg-plasmalogen interaction in a more complex system, red blood cells (RBCs) were investigated since they contain appreciable amounts of plasmalogens, are readily available and can be easily manipulated. Interestingly, Hg exposure of intact RBCs strongly indicated lysis. To better assess this interaction and the role of lipid classes, complex Eukaryotic extracts were studied. Brain extract was selected as both Hg and Cd have neurotoxic effects inducing a disruption of cognitive thinking and memory [17]. Heart and liver polar extracts were studied as Hg and Cd have also been linked to increased risk of cardiovascular disease [18] and are known to accumulate in the liver [19]. Lastly, yeast Polar Extract represents a plasmalogen-free system to test our hypothesis that plasmalogens are the predominant Hg target in Eukaryotic membranes.

Dynamic light scattering (DLS) was used to analyze changes in LUV (large unilamellar vesicles) size as extensive aggregation of LUVs based on electrostatic metal-lipid interactions has been observed [9]. Both the size and lipid composition of LUVs can be carefully controlled making them popular in the study of different biomolecule-membrane interactions as well as drug delivery vehicles [20]. The presented results suggest that data from model systems can predict metal interactions in different Eukaryotic membranes as Hg and Cd target the same lipids in all matrices.

2. Materials and methods

2.1. Materials

Brain polar extract (Porcine), Heart polar extract (Bovine), Liver polar extract (Bovine) and Yeast polar extract (*S. cerevisiae*) obtained from Avanti Polar Lipids were used to prepare LUVs. $HgCl_2$, $CdCl_2$ and 6-dodecanoyl-2-dimethyl-aminonaphthalene (Laurdan) were purchased from Sigma-Aldrich (Oakville, ON). All reagents and lipid solutions were made using doubly distilled water filtered by a Millipore Synergy 185 water purification system (Billerica, MA). All chemicals were used without further purification.

2.2. Rabbit erythrocyte microscopy

Blood was obtained from a fasted New Zealand White rabbit (Riemens Fur Ranches Ltd., St. Agatha, ON) and kept on ice in vials containing heparin (Mississauga, ON). 25 μL of blood was added to 225 μL Hg, Cd or Hg:Cd (1:1) in 150 mM NaCl pH 7.4 at a final metal concentration of 1 mM. 10 μL of the blood-metal sample were deposited onto a microscope slide and erythrocytes were viewed at 1000 × magnification using a Motic BA200 compound microscope (Richmond, BC).

2.3. LUV preparation

Commercially available brain, heart, liver and yeast extracts with well characterized lipid profiles (see Fig. 1) were stored as dry lipid powders and dissolved in 7:3 (v/v) chloroform: methanol mixtures and dried under argon to form a lipid film. The structures of the most abundant lipids and Laurdan are shown in Fig. 2. Samples were kept under vacuum overnight to ensure a complete removal of organic solvent. Lipid films were then resuspended in 100 mM NaCl adjusted to pH 7.4 followed by intermittent vortexing and sonication. LUVs were made using an extruder (Avanti Polar Lipids, Alabaster, AL) by passing the suspension 21 times through two 100 nm Nucelopore polycarbonate membranes (Whatman, Maidstone, UK). Each solution was kept at 50 °C throughout the extrusion. Finally, the phospholipid concentration was determined by using the Ames inorganic phosphate assay [21].

2.4. Laurdan Generalized Polarization

Laurdan was added to LUVs in DMSO at a dye/lipid molar ratio of 1/550 whereby the dye locates at the glycerol interface region of the bilayer. Changes in hydration can be correlated to membrane fluidity as shown for emission spectra of Laurdan in brain extract LUVs at different temperatures (Fig. S1) and quantitated by generalized polarization (Eq. (1)) [5]

$$GP = \frac{I_{440\,nm} - I_{490\,nm}}{I_{440\,nm} + I_{490\,nm}} \quad (1)$$

Laurdan GP experiments were performed as previously published [8,9]. Fluorescence spectroscopy was done using a Varian Cary Eclipse Spectrofluorometer and circulating water bath (Agilent Technologies, Santa Clara, CA) that controlled the sample temperature to within ± 0.1 °C. Laurdan was added at a dye/lipid molar ratio of 1/550, which provided sufficient signal without disrupting the bilayer structure [22].