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Mercury and cadmium rigidify eukaryotic lipid extracts but mercury also ruptures red blood cells

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 leading to increased bioaccumulation of Hg and Cd. Within an organism, one potential target is the lipid bilayer, which is an essential barrier separating the outside and inside environments of cells. One of many important properties for a functional cell membrane is its fluidity, which reflects the movement of lipids and greatly affects the mobility, conformation and activity of some proteins. We utilized the fluorescent probe Laurdan to monitor the membrane fluidity of complex lipid extracts including Brain (Porcine), Heart (Bovine), Liver (Bovine) and Yeast (*S. cerevisiae*) in the absence or presence of Hg and Cd.

Laurdan readily incorporates into lipid bilayers and acts as a polarity sensor. Laurdan fluoresces more at 440 nm in tightly packed bilayers that are less accessible to water. On the other hand, Laurdan fluoresces more at 490 nm in a less tightly packed membrane as seen upon rising temperatures due to increased water exposure. These fluorescence changes can be related to membrane fluidity and quantified by using the generalized polarization (GP) equation (Eq. 1).

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

Previous laurdan results using well-defined 1-2 lipid model systems clearly showed that these metals decreased membrane fluidity; Hg acted on plasmalogen containing membranes whereas Cd targeted negatively charged lipids. Plasmalogens are enol/ether lipids that are enriched in brain, heart and red blood cell membranes while Hg reacts by irreversibly cleaving them into two lipid fragments. This destructive interaction is important as plasmalogens fulfill many biological roles including antioxidation, signal transduction and the mediation of membrane fusion. To further study the Hg-plasmalogen interaction, red blood cells were investigated since they contain appreciable amounts of plasmalogens and are readily available and can be easily manipulated. This was done using light microscopy.

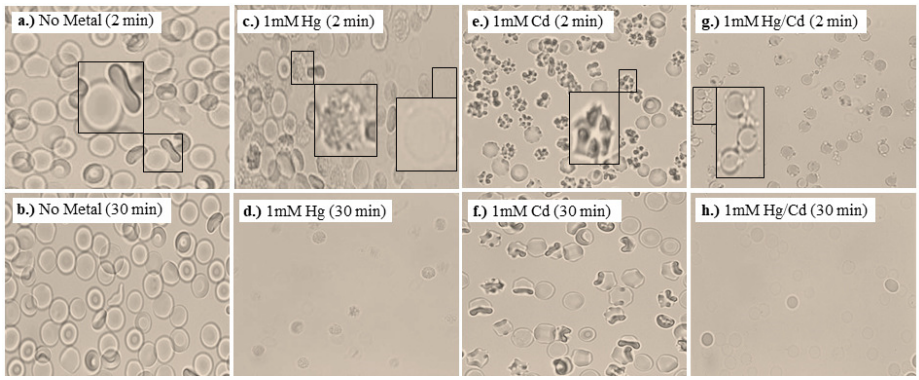

Fig. 1. Images of rabbit red blood cells in the absence of Hg and Cd at indicated incubation times. Images were collected using a light microscopy at 1000x magnification at room temperature.

Red blood cells (RBCs) in the absence of any metal had a normal biconcave shape (Fig. 1A, B) while after 22 mins incubation with Hg, over 95% of RBCs were not visible within the microscope field of view strongly indicating Hg-induced hemolysis (Fig. 1D). A 2 min incubation with 1 mM Cd led to the formation of echinocytes with characteristic spicules or bulges (Fig. 1E, F). However, no lysis of cells was observed at any time with Cd. Lysis of RBCs was observed after 15 mins with a 0.5 mM Hg + 0.5 mM Cd mixture (Fig. 1H) showing faster lysis from the metal mixture and highlighting the toxic effects of these metals on RBCs.

While Laurdan results showed that Cd decreased membrane fluidity in all extracts, the magnitude of the effect correlated with the fraction of negatively charged lipids in the order (highest to lowest) Heart > Brain > Yeast > Liver (Fig. 2). While Hg also decreased membrane fluidity, it primarily targeted plasmalogen rich extracts for which the largest changes occurred in Heart and Brain extracts (Fig. 2). Liver and Yeast extracts with less plasmalogen were largely unaffected by Hg (Fig. 2).

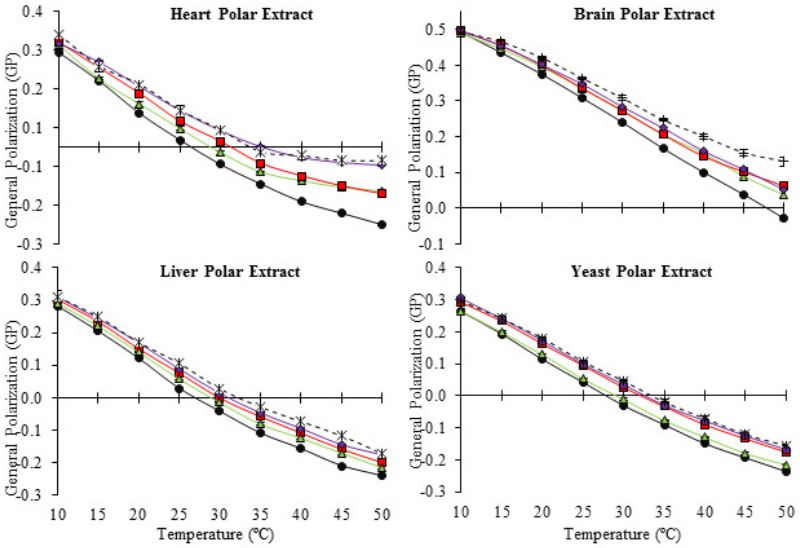

Fig. 2. Generalized Polarization (GP) values for Laurdan incorporated into Heart, Brain, Liver and Yeast polar lipid extracts commercially available from Avanti Polar Lipids. Data was collected with no metal (black circles), 2 mM Hg (Green Triangles), 2 mM Cd (Red Squares), 2 mM Hg + 2 mM Cd (Purple Diamonds) and 2 mM Hg + 2 mM Cd Calc (Dotted black line). Results are the average of 3 replicates with 4 measurements/replicate. In most cases error bars are within the symbol size.

In summary, these data show a two-fold impact of the toxic metals Cd and Hg on the fluidity of complex membranes. A concentration dependent increase in membrane rigidity was observed for both Hg and Cd, which will also change other membrane properties like permeability and cell morphology. Potentially more importantly, the Hg induced cleavage of the plasmalogens, in part, led to the destabilization and rupture of RBCs.

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Publication

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