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Lead (Pb) and selenium (Se) deposition in brain of Pb exposed mice using synchrotron x-ray fluorescence

Alexis Webb[1], Kathryn Spiers[2], Gerald Falkenberg[2], Yansheng Du[3], Wei Zheng[1], and Linda Nie[1]

[1]School of Health Sciences, Purdue University, West Lafayette, IN, USA; [2]DESY Research Center, Hamburg, Germany; [3]Indiana University School of Medicine, Indianapolis, IN

INTRODUCTION

Heavy metals are found in high concentrations in the environment due to their wide use in industry. Some of these metals serve no biological purpose, and many have been connected to a number of neurological diseases [1]. The presence of lead (Pb) in the environment through the use of leaded gasoline and paint over the years in combination with water contamination from lead pipes, poses a major health concern.

Alzheimer's disease is one of the leading causes of dementia, affecting 4.5 million people in the United States [2], and 13% of people over 65 in developed countries around the world. Late-onset Alzheimer's disease, which represents 98-99% of AD cases [3], has recently been linked to lead exposure. Our previous studies have shown an increased b-amyloid in brain tissues and CSF [4]. X-ray fluorescence studies also show high levels of lead present in beta amyloid plaques—one of the key indicators of the disease [2]. Lead is transported by the blood and deposits in bones, teeth and soft tissue, including the brain for its ability to penetrate the blood-brain barrier [1].

Synchrotron X-ray Fluorescence (SXRF) offers high spatial resolution, on the order of microns or nanometers, with the ability to detect and quantify multiple elements in one scan. SXRF is becoming a valuable imaging tool in various fields to reveal elemental distribution and mapping in tissues. Another benefit of SXRF is its non-destructive characteristics. The nature of this imaging technique allows for the preservation of the sample so that it can be used for more than one experiment, if needed.

This study uses SXRF technology to measure the metal distribution and concentration in the brains of mice that had been exposed to lead. SXRF scans were run on the P06 beamline at the Deutsches Elektronen-Synchrotron (DESY) facility. Coarse scans of the entire brain were run in order to locate the cortex and hippocampus regions, after which scans with smaller step sizes and better resolution were run in these areas. The results, for the first time, showed that lead deposited in localized spots in the cortex and hippocampus of both the exposed and unexposed samples. There were significantly more spots in exposed mouse brain than in unexposed mouse brain. In addition, there was an evident positive correlation between lead and selenium in exposed mouse brain samples. This co-deposition may be due to the antioxidant effects of selenium, which act through different pathways to reduce lead toxicity [1].

METHOD

Transgenic PDAPP mice were divided into two groups: one fed a lead-doped diet and one with saline as the control. Their brains were extracted and frozen before being sliced along the sagittal plane. Frozen brains were sliced using a microtome to a thickness of 10 micrometers, then fixed on to a slide. Slides were composed of a plastic holder and Ultralene thin film for XRF.

Scans were run on the P06 Beamline at the DESY facility. The beamline had a maximum energy of approximately 40 keV; a beam energy of 18 keV was chosen for this experiment. A beam of approximated 10^{10} photons/s was focused down to a 350 nm spot size. The beam spot size for our experiments was about 700 nm.

For each sample, a coarse overview scan of the entire sample was performed initially. From this scan, a smaller region in the cortex was located for a finer scan. In the exposed samples, an even smaller region in the hippocampus was scanned as well. For the overview scans, 25 microns and 10 ms were used for the step size and dwell time, respectively. For the finer scans, the step sized ranged from 1-4 microns and the dwell time ranged between 100-150 ms. The length of scanning time and pertinent required resolution were taken into consideration when choosing the step size and dwell time.

The P06 beamline is equipped with the Maia detector. The Maia detector has 384 detector elements which allows for high spatial resolution. GeoPIXE imaging analysis software was used for the data analysis. Spectrum analysis was completed to determine the elements present. Other analysis tools were utilized to determine the locations of and relationships between the various elements.

RESULTS

Pb Spots in Cortex

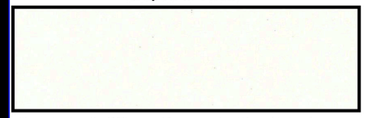

Figure 1a. Results of fine scan of cortex region (Figure 3) showing locations of lead deposits. Black dots indicate lead.

Se Spots in Cortex

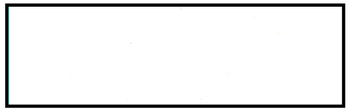

Figure 1b. Results of fine scan of cortex region (Figure 3) showing locations of selenium deposits. Black dots indicate selenium.

Pb and Se Co-deposition in Cortex

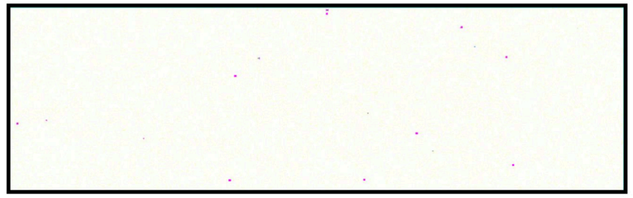

Figure 1c. Shows the areas in the cortex region (Figure 3) in which both lead and selenium are present. Purple dots represent the existence of both lead and selenium. Black dots represent lead alone.

Spectrum of Pb Spot 1 (Pb peaks)

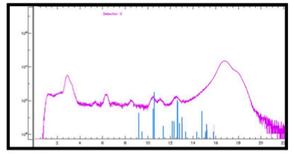

Figure 3a.

Spectrum of Pb Spot 1 (Se peaks)

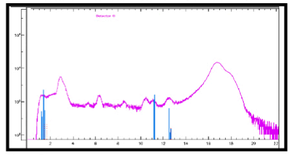

Figure 3b.

Spectrum of Pb Spot 2 (Pb peaks)

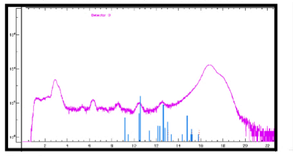

Figure 3c.

Spectrum of Pb Spot 2 (Se peaks)

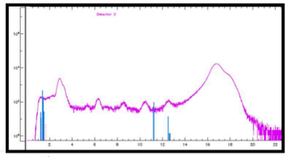

Figure 3d.

Fig. 3a and **3b** show the spectrum for a single lead spot in the cortex region. **Fig. 3a** shows the spectrum with lead lines indicating the presence of lead. **Fig. 3b** shows the spectrum with selenium lines indicating the presence of selenium. **Fig. 3c** and **3d** show the spectrum for a different lead spot in the cortex region. Again, **Fig. 3c** shows the presence of lead, but **Fig. 3d** shows the absence of selenium.

Total Spectrum of Exposed Sample

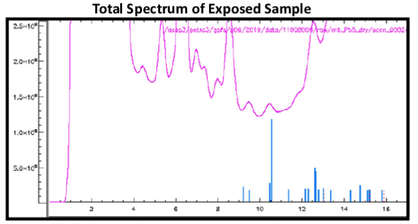

Figure 4a. Total spectrum of exposed sample with Pb lines.

Total Spectrum of Exposed Sample

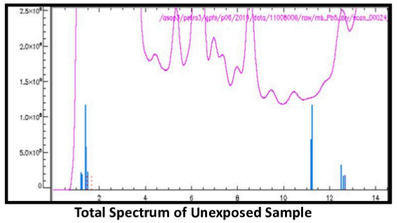

Figure 4b. Total spectrum of exposed sample with Se lines.

Total Spectrum of Unexposed Sample

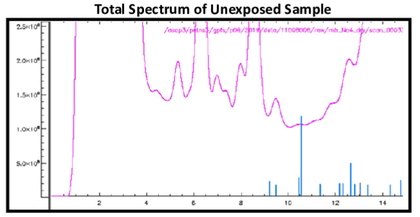

Figure 4c. Total spectrum of control sample with Pb lines.

Total Spectrum of Unexposed Sample

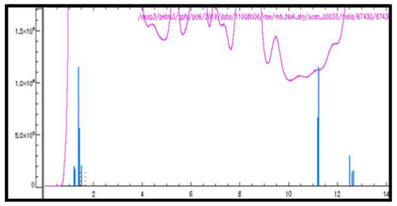

Figure 4d. Total spectrum of control sample with Se lines.

Se vs. Pb Exposed Sample 1

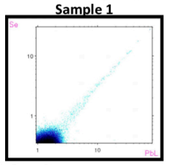

Figure 2a.

Se vs. Pb Exposed Sample 2

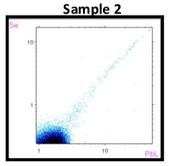

Figure 2b.

Se vs. Pb Unexposed Sample 1

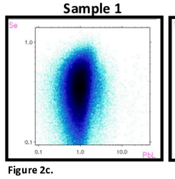

Figure 2c.

Se vs. Pb Unexposed Sample 2

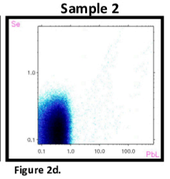

Figure 2d.

Figures 2a-2d show the correlation between selenium and lead in four different samples, two exposed and two control. A positive correlation is seen in the two exposed samples (**Figs. 6a.** and **6b.**), whereas the relationship in the control samples (**Fig. 6c.** and **6d.**) is less clear.

CONCLUSIONS

Our results show that lead deposits in localized spots and that lead co-deposits with selenium in the mouse brain, with much more Pb and Se spots in exposed mouse brain samples than those in controls. The clear association between lead and selenium in the brain is supported by the literature. High levels of selenium are toxic, but it's possible that at lower levels it reduces the cytotoxicity induced by lead, as well as other heavy metals [1]. These protective effects may be due to the formation of selenium-lead complexes. It's also been proposed that selenium prevents cellular damage against the formation of subcellular free radicals [5]. Data in literature also suggest that selenium may attenuate beta-amyloid production and subsequently beta-amyloid induced neurotoxicity, suggesting that it plays a role in AD protection [6].

Future work will be done to confirm these results as well as to further investigate the relationship between lead, selenium, and other elements. We are working to compare the SXRF results with images obtained from other techniques, which will allow us to determine which anatomical structures the elements are depositing in and the mechanisms of Pb toxicity and its relation to neurodegenerative diseases such as AD.

REFERENCES

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CONTACT INFORMATION

Alexis Webb: webb134@purdue.edu

Linda Nie: hnie@purdue.edu