Determining the Effects of Tetrathiombolydate on the Copper Levels in Amyloid Aggregates of Cerebral Amyloid Angiopathy Using X-ray Fluorescence Microscopy

Ashwin Ambi¹,², Tiffany W. Victor², Aleksandra Stanisavljevic³, William Van Nostrand⁴, and Lisa Miller¹,²

¹Department of Chemistry, Stony Brook University; ²National Synchrotron Light Source, Brookhaven National Laboratory; ³College of Pharmacy, University of Rhode Island

ashwin.ambi@stonybrook.edu; lmiller@bnl.gov

Cerebral amyloid angiopathy (CAA) is a neurodegenerative condition that is characteristic of amyloid β-protein (Aβ) accumulation on the walls of cerebral blood vessels. Recent reports have shown elevated Cu levels bound to the Aβ aggregates and this interaction may produce neurotoxic reactive oxygen species through Fenton reaction. In this study, we administered a Cu-specific chelator, tetrathiombolydate (TTM), through IP injection to a transgenic rat model of CAA (rTgDI) to lower the Cu content in the Aβ deposits. We hypothesize that the copper levels bound to amyloid will be potentially sequestered and attenuated using TTM. The Cu and molybdenum concentrations were determined using synchrotron-based X-ray fluorescence microscopy (XFM). The results surprisingly showed (1) the Cu levels in the vascular Aβ aggregates in the TTM-treated rTgDI rats were elevated by 1.5x relative to the untreated rTgDI rats (2) similarly, the relative Cu concentration in the healthy unaffected blood vessels was higher by 2x in the TTM-treated rTgDI rats compared to the untreated rTgDI rats and (3) there were molybdenum hotspots (< 5 ppm) colocalized with sequestered Cu that appeared to form a Mo-Cu complex bound to the blood vessels in the TTM treated rTgDI and WT rats. The results signify that TTM was not only ineffective with reducing the levels of Cu present in the Aβ deposits, but also increased the Cu levels bound to the amyloid and the healthy blood vessels in rTgDI. Furthermore, the TTM administration led to formation of Mo-Cu complexes by potentially sequestering labile copper or loosely bound copper in proteins such as albumin from the blood stream. Overall, the study highlights the ineffectiveness of TTM stems from the difference in the relative affinity of amyloid aggregates and TTM for Cu. Additionally, TTM exacerbates the situation by carrying Cu in the vicinity of the Aβ deposits.