and HD mice was observed using the 7 day paradigm. This alteration in Mn accumulation may be related to disease processes, or to changes due to normal aging, or it may be that the Mn transport defect diminishes with disease progression. To examine this in more detail we will measure and image regional brain Mn accumulation using Inductively Coupled Plasma Mass Spectroscopy (ICPMS), laser ablation ICPMS and other novel methods in HD mouse models. In addition, we will examine primary cultured glia and neurons for cell-type differences in the HD-Mn phenotype. Our ultimate goal is to determine whether disease progression influences brain Mn deposition in HD, and if so, what role glia and neurons play in this process. Supported: NIH ES016931, T32 ES007028

PS 1861 Verification of Manganese-Related Choroid Plexus Differentially Expressed Proteins *In Vitro.*

G. J. Li, Y. Dong, J. Liu, Y. Liu, H. Jing, C. Zhao and L. Ma. *Institute for Toxicology, Beijing Centers for Disease Control and Prevention, Beijing Research Center for Preventive Medicine, Beijing, China.*

The regulation of brain manganese (Mn) depends largely on the blood-brain barrier and blood-cerebrospinal fluid barrier (BCB). The latter is constituted by choroid plexus (CP) epithelial cells, which is specialized for cerebrospinal fluid (CSF) production, has been considered as a primary target in Mn-induced neurotoxicity. In our previous study, a total of 32 Mn-related differentially expressed proteins were identified by 2D-PAGE combined with Nano-LC-MS/MS in an in vivo Mn-toxicity rat model, of which 27 were up-regulated, 5 were down-regulated. This study aims to further verify the 7 selected proteins (PHB1, VDAC, β-actin, HSP70, STIP1, TTR, Vimentin) at transcriptional and translational level respectively in immortalized choroidal epithelial Z310 cells in vitro under manganese chloride (MnCl2) exposure. The expressed level of 7 proteins and their mRNA were detected by Western Blot and Real Time RT-PCR, following MnCl2 (0, 50, 100, 200μmol/l)) exposure for 24h or 12h in Z310 cells. The results demonstrated that PHB1, β-actin and STI1 were up-regulated and TTR was down-regulated at both transcriptional and translational levels as compared to controls, which are in accordant with results in in vivo study. Whereas VDAC, HSP70 and Vimentin were down-regulated at both transcriptional and translational levels as compared to controls, which are opposite to the results in in vivo study. Taking together, this study validated that Mn toxic effects on PHB1, β-actin, STIP1 and TTR in CP are accurate and reliable, which provide the valuable clue for elucidating the molecular mechanism of Mn toxicity on choroid plexus epithelial cells (partly supported by NSFC Grant in China #81273108, Capital Development Project 2011-1013-03, and Beijing Health Bureau Project-2011. Corressponding author: Guojun J. Li, guojunli88@yahoo.com).

PS 1862 Expression and Aggregation of a-Synuclein in the Blood-CSF Barrier: New Evidence for the Effects of Toxic Intracellular Manganese and Copper Levels.

C. A. Bates[1], X. Fu[1], D. Ysselstein[2], J. Rochet[2], H. Gu[3], Y. Du[3] and W. Zheng[1]. *[1]Health Sciences, Purdue University, West Lafayette, IN; [2]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN; [3]Neurology, Indiana University School of Medicine, Indianapolis, IN.*

The blood-cerebrospinal fluid barrier (BCB) is responsible for maintaining the homeostasis of a variety of molecules in the brain and cerebrospinal fluid (CSF) including alpha-synuclein (a-Syn). a-Syn plays an integral role in the pathoetiology of Parkinson's disease. Little is known about the role of the BCB in the transport and regulation of a-Syn in the brain and CSF. Previous findings in this lab provided evidence that a-Syn was endogenously expressed in our immortalized Z310 choroidal epithelial cell model, and 100 μM MnCl2 exposure for 24 and 48 hours induced a-Syn aggregation in these cells. The current studies test the hypothesis that the increased a-Syn aggregation in Z310 cells may result from Mn interacting with a-Syn expression and/or its aggregation. qPCR was used to quantify a-Syn expression in Z310 cells after 100 μM MnCl2 incubation for 24 hr. Our data revealed that Mn treatment did not affect a-Syn mRNA expression in Z310 cells. Using the Thioflavin T fibril aggregation assay, 70 μM recombinant a-Syn was incubated with 2 mM MnCl2 and CuCl2 for 7 days in vitro. a-Syn aggregation increased significantly (530% and 634%, respectively) as compared to controls (n=3, $p<0.05$). Finally, we found that 24 hr incubation with 50 and 100 μM CuCl2 induced a-Syn aggregation in Z310 cells. These findings support the hypothesis that cellular aggregation of a-Syn in the BCB is facilitated by exposure to heavy metals. More specifically, Mn exposure induces this effect by two pathways: 1) direct interaction with cellular a-Syn within the cell and/or 2) increasing intracellular Cu levels, shown by our data in literature, leading to Cu-accelerated a-Syn aggregation. (Support by NIH/NIEHS ES08146-S2 Minority Supplemental Award)

PS 1863 Reduced Copper (Cu) Efflux across the Blood-CSF Barrier (BCB) following Manganese Exposure: Effect on Cu Transporters ATP7A and ATP7B.

X. Fu, Y. Zhang, W. Jiang and W. Zheng. *School of Health Sciences, Purdue University, West Lafayette, IN.*

Increased Cu levels in blood, saliva and brain are found in Mn-exposed animals and humans. However, the underlying mechanism is unknown. ATP7A and ATP7B are Cu-ATPases that function to maintain intracellular Cu homeostasis by exporting excess Cu from the cytosol to extracellular space. This study was designed to test the hypothesis that Mn exposure disrupted the Cu transport across the BCB by interrupting the intracellular trafficking of ATP7A and ATP7B. Rats received ip injections of 6 mg Mn/kg as MnCl2 or saline, 5 d/wk for 4 wks. Increased Cu and Mn levels in serum and CSF were observed following Mn exposure. An in situ ventriculo-cisternal perfusion by infusing [64Cu] and [14C]-sucrose into brain ventricle was conducted to determine Cu clearance by the BCB. Mn exposure significantly increased [64Cu] radioactivity by 2.6 fold in the CSF outflow, as compared to controls, suggesting a reduced Cu removal by the choroid plexus. Confocal images exhibited both Cu-ATPases distributed in perinuclear region in normal plexus tissues and Z310 cells. Incubation of plexus tissues with Mn or Cu caused translocation of ATP7A from the cytosol toward the apical membrane facing the CSF, whereas ATP7B relocated toward the basal membrane facing the blood. Exposing Z310 cells to 100 μM MnCl2 for 24 hr led to a significant decrease in ATP7A and ATP7B fluorescent intensities, which was consistent with their significant mRNA and protein expression reductions in tissue and Z310 cells. The two-chamber Transwell transport studies showed a reduced Cu efflux from the CSF to the blood following Mn exposure or when ATP7A or ATP7B expression were knocked down by siRNA. Collectively, these data suggest that Mn exposure reduces Cu efflux by the BCB which appears to be due to the reduction of ATP7A and ATP7B. A decreased clearance of Cu by Mn exposure may result in the buildup of Cu in the brain. Opposite translocation of ATP7A vs. ATP7B is interesting, yet how this may help interpret a decreased Cu efflux via the BCB remains uncertain. (Supported by NIH/RO1-ES008146)

PS 1864 Bone Manganese (Mn) Concentrations in Sprague-Dawley Rats following Subchronic Manganese Exposure.

L. Hong, S. O. Neal, L. Nie and W. Zheng. *Purdue University, West Lafayette, IN.*

Occupational exposure to Mn causes a Parkinson-type disorder called Manganism, a neurodegenerative disease currently without reliable biomarkers for body burden estimation and for pre-symptomatic toxicity assessment. Data in literature suggest that Mn deposited in bone accounts for 43% of total body Mn. The objective of this study was to determine if bone Mn levels were parallel to Mn concentrations in brain regions known to be the targets of Mn toxicity. Groups of rats (6-7 each) received daily dose of 50 mg Mn/kg as MnCl2 PO for 0, 2, 4, 6, 8 or 10 weeks before tissue dissection. The metal concentrations of Mn, zinc (Zn), iron (Fe), and copper (Cu) in bone tissues, body fluids, brain tissues, as well as other organs were analyzed by atomic absorption spectrophotometry. Following Mn exposure, bone tissues including femur, tibia, humerus and skull bones showed a dose-time-dependent increase in Mn concentrations, with 1.0-1.6 μg/g of bone mass at week 10, which were about 2.3-3.6 fold higher than those in controls (~0.4 μg/g) at day 0 ($p<0.01$). A statistically significant relationship exists between bone Mn concentrations and Mn levels in brain tissues such as striatum ($r=0.755$, $p<0.001$), hippocampus ($r=0.782$, $p<0.001$) and choroid plexus (0.652, $p<0.001$), and in brain fluid such as cerebrospinal fluid ($r=0.720$, $p<0.001$). Interestingly, in vivo exposure to Mn also led to significantly increased Fe (152-372%) and Zn (194-230%) concentrations in bone tissues except the skull bones, with no statistically significant effect on bone Cu (58-95%). These results suggest that bone is a significant storage site for body Mn; the good correlation between bone Mn and brain Mn alludes to bone Mn being an internal source of Mn long-term exposure. Further experimentation for noninvasive quantitation of Mn in bone is well warranted for Mn neurotoxicological research. (Supported in part by R21 OH010044, and RO1 ES008146)
Keywords:
Manganese, Manganism, Biomarker, Bone, Correlation, Pharmacokinetics

PS 1865 Effects of Chronic Manganese Exposure on Cognitive Functioning in Nonhuman Primates.

J. Schneider[1], C. Williams[1], M. Ault[1] and T. R. Guilarte[2]. *[1]Thomas Jefferson University, Philadelphia, PA; [2]Columbia University, New York, NY.*

Exposure to elevated levels of manganese (Mn) can result in neurological dysfunction in humans including effects on cognitive functioning, particularly those that depend on the integrity of frontal cortex. Our group is performing longitudinal

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