Metallothionein 1,2 Deficiency Worsens Outcome Following Neonatal Hypoxia-Ischemia

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Introduction: Metallothioneins (MT) are small sulfur-rich proteins that complex free-zinc and are induced in brain by hypoxic preconditioning (1). Free-zinc is known to perpetuate injury following ischemic brain (2) insults and chelation of free-zinc can mitigate the injury (3). We hypothesized that knock-out of a functional MT 1 and MT 2 protein would lead to a worse outcome following neonatal hypoxia-ischemia (HI) than seen in wild type (WT) animals exposed to the same HI stress.

Methods: A total of 72 (4 groups, 18 per group) WT and homozygous MT1,2 deficient C57Bl/6 X 129T2 F1 hybrids were randomized to either sham HI or 45 minutes of HI on post natal day 10 (P10). HI insult is accomplished by ligating the right common carotid artery and 2 hours later exposing the mice to 10% oxygen in nitrogen while maintaining normo-thermia (4). The surviving mice were subjected to a battery of behavioral tests beginning on P50. These included week 1 locomotor activity and novel object, week 2 rest, week 3 cued water maze, week 4 hidden water maze, week 5 reduced water maze, week 7 delayed probe trial and apomorphine challenge. Binary outcomes were analyzed using chi-squared statistics, water maze data was analyzed using repeated measures general liner models with variance leveling transforms, as indicated by residual analysis. Post hoc comparisons were made using either the Tukey or Games-Howell technique depending on variance homogeneity (Levine's test) .

Results: There were no differences in mortality between WT and MT1,2 deficient mice following P10 HI. Body weights at the time of death (P110) were not different among groups. The brain weight for the MT 1,2 deficient HI mice were significantly less than both the WT and MT 1,2 deficient sham HI mice ($p = 0.0003$ and 0.0373 respectively). No differences among groups were observed on novel object recognition testing. The sham HI MT 1,2 deficient mice exhibited less spontaneous activity than other groups during the first one-third of the activity testing protocol.

All groups had similar learning curves on the cued maze, however, the MT 1,2 deficient HI had longer latencies on the cued maze than both the MT1,2 deficient sham-HI mice and the WT sham-HI mice ($p=0.001$ and 0.012 respectively). The MT 1,2 deficient HI mice had learning curves which were different from WT shams on both the hidden ($p=0.037$) and reduced mazes ($p=0.011$). The same group also had the longer latencies and slower swim speeds than both the MT 1,2 deficient sham-HI and WT sham-HI mice. The latencies for the MT 1,2 deficient HI mice were longer and swim speeds slower than the WT HI mice but the differences were statistically significant ($p = 0.102$-.174). Path length, a better measure of cognitive function than latency, was shortest for the WT sham HI mice on the hidden maze ($p < 0.04$ vs MT 1,2 deficient sham; $p< 0.001$ vs. WT and MT 1,2 deficient HI). The path length comparisons for the reduced maze showed that only the MT 1,2 deficient HI and WT HI mice differed significantly from WT sham HI mice ($p=0.013$ and 0.037 respectively). The MT 1,2 deficient sham-HI did not differ from the WT sham-HI mice in path length on the reduced maze. No differences were noted among groups on the delayed probe trial.

On apomorphine challenge only the HI mice circled. No difference was noted in the circling incidence between WT and MT 1,2 deficient HI mice.

Discussion: Mice lacking a functional MT1,2 protein in brain develop a more severe injury following a mild to moderate HI insult than seen in wild type mice. Both motor and spatial-cognitive functions are adversely affected. The evidence herein is consistent with the concept that increased free-zinc is deleterious following an ischemic insult. Interestingly, there is evidence of behavioral abnormalities in the MT 1,2 deficient sham-HI mice compared to wild-type sham-HI mice. The reasons for this observation are unclear at this time.

References: 1. Bernaudin et al. J.Biol. Chem. 277:39728,2002
2 Choi and Koh. Ann. Rev Neurosci. 21:347,1998
3. Calderone et al. J. Neurosci. 24:9903,2004
4. McAuliffe et al. Brain Research, 2006 in press