**Effect of TrkB agonist therapy on astrogliosis following hypoxia ischemia in neonatal mice**

Nagendren L1,2, Hagen M1,2, Bischel C1,2, Kintner D1,2, Karahan N1,2, Ozaydin B3, Cengiz P1,2

1Waisman Center, University of Wisconsin, Madison, WI, USA; 2Department of Pediatrics, Division of Critical Care Medicine, University of Wisconsin, Madison, WI, USA; 3Department of Neurosurgery, Division of Critical Care Medicine, University of Wisconsin, Madison, WI, USA

**BACKGROUND**

Neonatal hypoxia ischemia (HI) related encephalopathy is a major contributor to perinatal morbidity and mortality. Recently, we showed that the neuroprotectin tyrosine kinase B receptor (TrkB) agonist, 7,8-dihydroxyflavone (7,8-DHF) is neuroprotective in females but not in males following HI1. Any brain insult can result in astrogliosis leading to inflammation and fibrosis in developing brains. Here we investigated the role of TrkB agonist 7,8-DHF in astrogliosis as a neuroprotective mechanism post-HI in neonatal mice brains.

**METHODS**

HI was induced in P9 male and female mice using Vannucci’s HI model2. Mice were treated daily with 7,8-DHF or vehicle control, perfuse-fixed 3 days post-HI. Three coronal sections through hippocampi were stained for glial fibrillary acidic protein (GFAP-astrocyte marker) and microtubular associated protein 2 (MAP2-neuronal dendritic marker). Whole brain stained epifluorescent images were obtained. Using Image J, we defined the isocortex, hippocampus and thalamus in the MAP2 image (green) (Fig. 1). These defined regions were then transferred to the GFAP image (red) and the mean intensity with pixels above 2.5x background was calculated using Image J. The percent change in the mean GFAP intensity between ipsilateral to contralateral sides was determined with the formula below:

\[ \text{Ipsilateral hippocampus} = \frac{\text{Contralateral hippocampus}}{\times 100} \]

An one-way ANOVA was used to analyze and compare the changes in percentage of GFAP mean intensity. *p<0.05 vs corresponding sham; # p<0.05 vs corresponding male.

**RESULTS**

In this preliminary study, we found a significant increase in ipsilateral GFAP expression in all regions of the brain at day 3 post-HI of both male and female mice. The increase was most dramatic in the hippocampus and thalamus compared to the isocortex. However, in isocortex, astrogliosis was significantly increased in female compared to male. 7,8-DHF therapy did not significantly decrease the GFAP expression in any of the groups post HI, although there was a trend for reduction in female brains in the isocortex and thalamus. We will increase our sample size and investigate the GFAP expression in mice at 90 days post-HI to assess the effect of TrkB agonist therapy in long-term neuroprotection.

**CONCLUSIONS**

There is a differential early inflammatory response to HI in neonatal brain with increased astrogliosis in hippocampal and thalamic tissue relative to isocortex. While astrogliosis was increased in female isocortex compared to male, 7,8-DHF failed to significantly reduce GFAP-staining in either sex. Further studies are needed to understand the importance of the role of TrkB agonist and inflammation following HI. This is particularly important given our observation that TrkB agonist therapy results in improved memory and learning that persists to adulthood in female mice only.

**ADDITIONAL KEY INFORMATION**

**Figure Legend**

Figure 1: Effect of TrkB agonist in regional GFAP expressions 3 days post-HI in neonatal mice. Summary graphs of the % increase in ipsilateral GFAP staining intensity in the hippocampus (A), isocortex (C) and thalamus (E). Data is mean ± SEM. N= 4–7. Regions of interest were outlined in MAP2 images (B, D, F) (dotted line) and then transposed to the corresponding GFAP image (Bb, Dd, Ff). The mean GFAP intensity in the pixels above 2.5 X background in each region was calculated. Scale bar = 1 mm.

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**References**