



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

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BACKGROUND

Neonatal hypoxia ischemia (HI) related encephalopathy is a major contributor to perinatal morbidity and mortality. Recently, we showed that the neurotrophin 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 stitched 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:

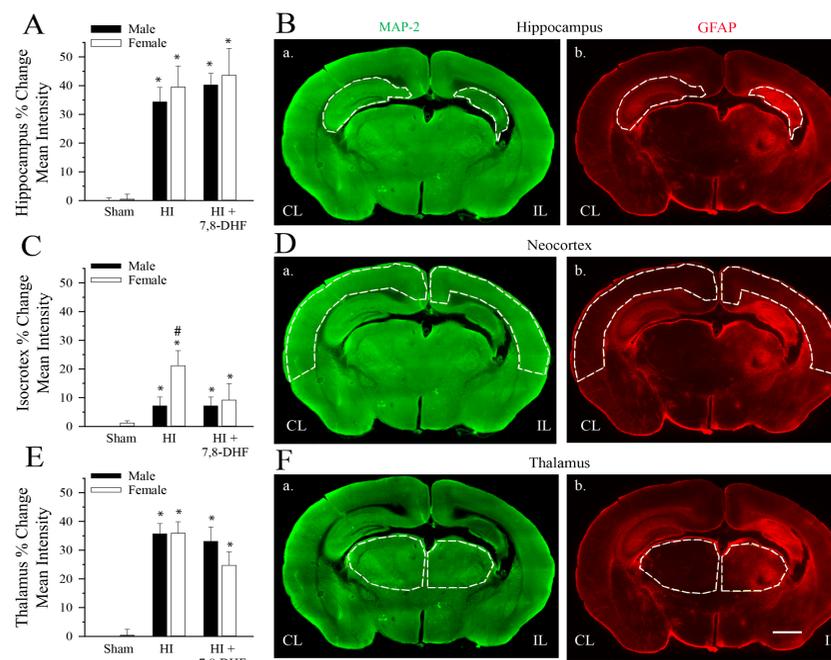
$$\frac{\text{Ipsilateral hippocampus} - \text{Contralateral hippocampus}}{\text{Contralateral hippocampus}} \times 100$$

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.

- HI induces significant increase in astrogliosis.
- This increase is most profound in hippocampus and thalamus compared to isocortex.
- Astrogliosis was significantly increased in female compared to male isocortex.
- 7,8-DHF failed to significantly reduce hippocampal astrogliosis.
- 7,8-DHF maybe decreasing astrogliosis in female isocortex and thalamus.

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 P60+ 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_a, D_a, F_a) (dotted line) and then transposed to the corresponding GFAP image (B_b, D_b, F_b). 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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