White Matter Injury in Neonatal Rats after a Mild Cerebral Hypoxic- Ischemic Insult: MR Confirmation Prior to Assessment of Injury Markers

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Introduction

Regarding the mechanisms of injury and neuroprotection following cerebral hypoxia ischemia, there has been extensive focus on injury in gray matter with less emphasis on the diagnosis and mechanisms of selective injury in white matter despite selective white matter injury being relatively frequent in pre-term and near-term infants. Cerebral hypoxia-ischemia is a cause of cerebral palsy and associated with developmental deficits in motor, perceptual, visual or cognitive functions in later life [1]. Recently, we have developed a perinatal model of selective white matter injury in 7-day-old rats by exposing them to cerebral hypoxia-ischemia of shorter duration at a slightly lower body temperature [2]. There is potential for MRI using T_2 imaging to detect selective white matter injury thereby allowing a non-invasive confirmation of severity of injury and the ablitiy to investigate differences in mechanisms of moderate and selective white matter injury using various markers such as those for oligodendrocyte maturity, free radical injury specific for white matter, inflammation and cell death. We hypothesized that in animals with selective white matter injury detected on MRI, markers or mediators of injury would differ from those with more severe injury.

Material and Methods

Cerebral hypoxia-ischemia or sham procedures were performed in 7-day-old rats (n=58) as described previously [2]. Briefly, the right carotid artery was occluded under isoflurane anaesthesia with subsequent exposure to 8% oxygen either for 45-50 minutes at a body temperature of approx. 36-37^{o}C (chamber temperature of 34.5 ^{o}C) in a mild model or for 65-70 minutes at 37-38^{o}C (chamber temperature of 35.5 ^{o}C). MRI was used to confirm selective white matter injury. T_2 maps were acquired at 48hrs following hypoxia-ischemia using a 9.4T/21cm MR imaging system [3]. T_2 maps were collected from a set of T_2 weighted spin echo images (32 echoes, TR=2500ms, TE=10ms between echoes, FOV=3cm^2, 128×128 matrix). According to changes in T_2 maps, animals with marked gray matter injury after a mild hypoxia ischemia were excluded from the study. After the MR imaging, brains were frozen and cut at 20µm at various times (48 hours,1 week and 4 weeks) following the hypoxia ischemia and assessed histologically for cell death with TUNEL, neuronal viability with microtubule-associated protein (MAP2), reactive microglia with ED1 and oligodendrocyte injury with O4 and myelin basic protein (MBP).

Results

At 48hrs aftert a mild hypoxia ischemia there were striking increases in T_2 within white matter ipsilateral to the occlusion of carotid artery whereas there were relatively modest changes in the gray matter (fig. 1). Consistent with the MR, there were spongiform changes in white matter but only patches of neuronal loss in the cerebral cortex within MAP2 stained slides at 48 hrs following a mild hypoxia ischemia (fig. 1). Evidence for permanent selective white matter injury following mild hypoxia ischemia was observed as an increased TUNEL positive labelling of cells in white matter at 48hrs and a thinner external capsule (white matter tract) when stained with MBP and O4 at 1 and 4 weeks post the insult (fig. 2). In contrast following moderate hypoxia ischemia, there was extensive cell death (TUNEL), hypomyelination (MBP and O4) and extensive loss of MAP2 stain in both gray and white matter at 48hurs post and at 1 week post. Following 4 weeks of a moderate hypoxia ischemia, a majority of animals had a hemispheric cyst. A notable difference between mild and moderate hypoxia-ischemia was that reactive microglia (ED1) were observed selectively in the whiter matter as early as 48hrs post a mild hypoxia ischemia whereas in moderate hypoxia ischemia it occurred at 1-4 weeks post in both gray and white matter.

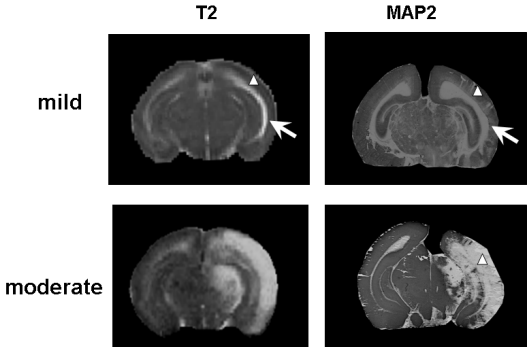

Fig.1. T_2 and MAP2 maps of rat brains at 48hrs post a mild or moderate hypoxia ischemia. There was a selective white matter (arrow) injury with little gray matter injury (triangle) following a mild hypoxia ischemia.

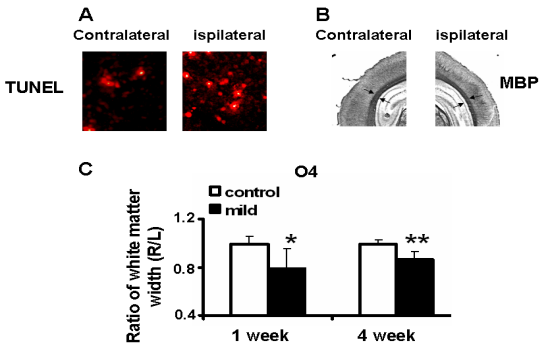

Fig.2. Selective white matter injury shown in TUNEL(A, 48hrs), MBP (B, 4 weeks) and O4 (C, 1&4 weeks) stains in the external capsule (arrows) following a mild hypoxia ischemia. *P<0.05,** P<0.01 vs the sham control using students t-test.

Conclusions and Discussions

Following exposure of neonatal brain to a relatively mild unilateral hypoxia-ischemia, there is a selective white matter injury that can be detected using T_2 MRI. Much of this white matter injury appears irreversible as demonstrated histologically by an increased number of dead cells detected with TUNEL and a reduced myelination subsequently detected with O4 and MBP. Injury of both gray and white matter was detected when animals were exposed to a moderate hypoxia ischemia with a longer hypoxia ischemia and slightly higher body temperature indicating that white matter is more susceptible than gray matter to a hypoxic ischemic insult. Inflammatory responses associated with microglia activation could play an important role in the selective white matter injury considering their predominance in white matter at 48 hr following the insult. Bearing in mind there is some variability in the response to a mild hypoxic-ischemic insult, MR imaging is useful non-invasive imaging tool to confirm severity of injury to allow grouping of animals with selective white matter injury appropriately. *(Supported by the Heart and Stroke Foundation of Alberta).*

References

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