2619

Neuroregenerative effect of Mesenchymal Stem Cell following Hypoxia-Ischemia in the pup mouse brain assessed by Diffusion Tensor Imaging

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Introduction:

Premature infants are at risk of white matter injury and altered development resulting in a chronic disturbance of myelination. Cerebral Hypoxia-Ischemia (HI) then reperfusion in the premature infant and bacterial infection in the mother and/or fetus represent the two major causes for perinatal white matter injury [1]. The resulting altered brain damage not only disrupts white matter but also modifies grey matter development [1]. Recently, van Velthoven at al. [2] showed that repeated Mesenchymal Stem Cell (MSC) treatment after neonatal HI had distinct effects on formation and maturation of new neurons and oligodendrocytes leading to restoration of altered brain structures, corticospinal motor tract activity, and sensorimotor function. Diffusion tensor imaging (DTI) derived parameters as directional diffusivities ($D_{//}$ and $D_{\perp}$) and Fractional Anisotropy (FA) are commonly used to probe brain microstructure. The goal of this study was to assess the neuroregenerative effect of MSC treatment in neonatal mouse brain following HI by DTI derived parameters.

Materials and Methods:

Animal preparation has been described previously [2]. Briefly, 9 day-old mice (P9) underwent HI injury under isoflurane anesthesia. Right carotid artery occlusion was performed followed by 45 minutes hypoxia under 10% O_2. At P12 and P19, 100,000 MSCs in 2µl of PBS (MSC group, n=8) or vehicle (HI group, n=8) were infused into the ipsilateral hemisphere at 2 mm caudal to bregma, 2 mm right from midline, and 2 mm below dural surface under isoflurane anesthesia. Sham controls (Sham group, n=6) underwent anesthesia and incision only. At P37, mice were sacrificed and brains were formalin-fixed for subsequent *ex-vivo* MRI.

All experiments were performed on an actively-shielded horizontal 9.4T/31cm magnet (Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs) with a transmit-receive 25-mm birdcage RF coil. After manual adjustment of the first and second order shims (water linewidth ~ 20 to 40 Hz) a Spin-Echo sequence with addition of the Stejskal-Tanner diffusion gradients was used. Diffusion gradients were applied along twelve spatial directions: dual diffusion gradient sampling scheme [3] as well as the six opposite directions to cancel *b*-value cross terms [4]. Intensity, duration and diffusion time were set to 22 G/cm, 3 ms and 19 ms respectively, given a *b*-value of 1185 s.mm^{-2}. A field of view of 15 × 15 mm^2 was sampled on a 128 × 128 cartesian grid. Multi-slice DW images were acquired (12 slices of 0.5 mm thickness) in the axial plane with 10 averages and TE/TR = 35/2000 ms. Using in house Matlab script (Mathworks, Natick, MA), diffusivity values ($D_{//}$ and $D_{\perp}$) as well as FA was derived from the tensor. The program allows manual delineation of region of interest (ROI) on the Direction Encoded Color (DEC) maps. The corpus callosum (CC) was analyzed at six different image-planes: Genu, Body1 to 4 and Splenium (See fig.1 for correspondence). Significant differences of diffusivity and FA values between the groups were assessed by a Mann-Whitney test.

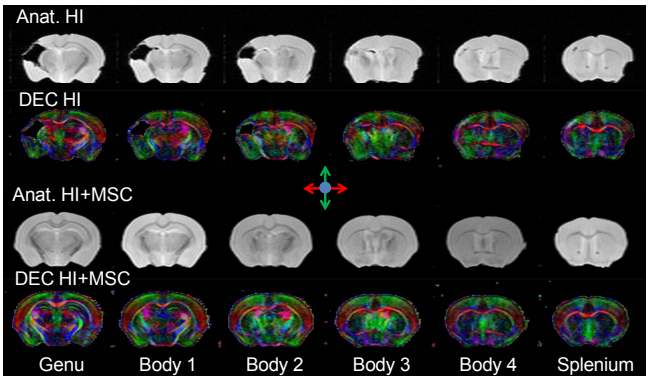

Figure 1: Anatomic images and direction encoded color maps of a typical HI mouse brain as well as a typical HI+MSC mouse brain: anatomical recovery is obvious.

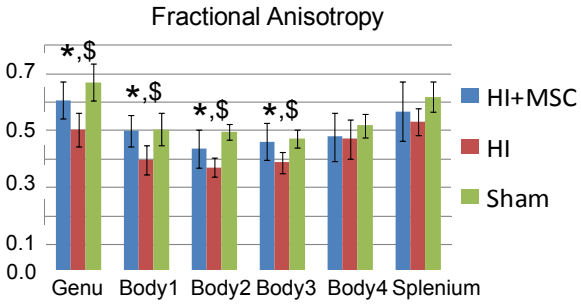

Figure 2: Histogram of FA ± SD at different image-planes of the CC (Genu, Body 1 to 4 and Splenium) for the three groups. (*;$: p<0.05, HI vs. Sham and HI vs. HI+MSC, respectively)

Results and Discussion:

Typical DT images are presented in Fig. 1. In the caudal part of the brain, FA in the corpus callosum of the HI animals showed a significant decrease (Fig. 2) compared with the sham group and with the HI+MSC group (Genu, Body1 to 3). No significant difference was observed in the CC between HI+MSC and Sham groups. FA decreases in the HI group were principally due to a decrease of $D_{//}$ sometimes associated with an increase of $D_{\perp}$. The axial diffusivity decrease is likely to be attributed to axonal stretching, misalignment [5] or decrease of axonal density [6] whereas the radial diffusivity increase is commonly related to myelination defect [7] or decrease of axonal density [6]. To conclude, our study confirmed white matter damages following neonatal HI on mouse brain. Further, it corroborates the neuroregenerative effect of MSC treatment on the HI induced white matter damage. Additional studies will assess the effect of MSC in the cortex as well as the quantification of the anatomical recovery following MSC.

References: [1] Volpe JJ. Lancet Neurol 2009; [2] van Velthoven C. et al. J. Neurosci 2010; [3] Basser PJ. et al. MRM 1998; [4] Neeman M. et al. MRM 1991; [5] Song S.K. et al. Neuroimage 2003; [6] Barzany D. et al. Brain 2010; [7] Song S.K. et al. Neuroimage 2002.

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