Lack of dystrophin results in abnormal cerebral water exchange and perfusion in vivo

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PURPOSE: The blood-brain barrier (BBB) ensures the proper environment for brain function by preventing the free diffusion of large molecules. Dystrophin, the main component of the dystrophin-glycoprotein complex, plays a role in the formation of the BBB in vitro. Importantly, dystrophin is necessary for the anchoring of the water channel, aquaporin 4 (aqp4), in astrocytes and the tight junctions in endothelial cells[1]. The absence of dystrophin has also been associated with enhanced arteriogenesis in skin and muscle[2]. Overall, the absence of dystrophin has been shown to lead to a leaky BBB and fluid retention in vitro[3]. To elucidate the function of dystrophin in maintaining water balance and perfusion at the BBB in vivo, we characterized the effect of dystrophin disruption on cerebral perfusion and water exchange across BBB using arterial spin labeling (ASL) and diffusion-weighted MRI (DWI) in dystrophin-deficient mice (mdx) versus wild-type (WT).

METHODS: Imaging studies were performed on 2 and 10 month mdx (dystrophin$^{-/-}$, Jackson Labs, n=5) and C57/BL6 WT (Jackson Labs, n=5) mice on a 7T Bruker Biospec MRI scanner (Billerica, MA). ASL images were acquired with a novel ASL-FISP (ASL - Fast Imaging in Steady Precession) acquisition which combines a FAIR (Flow-Sensitive Alternating Inversion Recovery) preparation with a FISP imaging readout (TI = 1600ms, TR/TE= 2.4ms/1.2ms, NSA = 20). Diffusion-weighted EPI images were acquired with the following parameters: TR, 5000 ms; B values, 0, 500 s/mm^2; NSA, 5). After the ASL/DWI imaging data were acquired, the mouse brains were excised, fixed in paraformaldehyde, and frozen. Indirect immunofluorescence against CD31 (Platelet endothelial cell adhesion molecule 1) was performed on the fixed frozen mdx and C57 mouse brains to count cerebral vasculature.

RESULTS & DISCUSSION: Baseline DWI studies established the mean diffusivity in young WT mice as 0.884x10^{-3} mm^2/s. Disruption of dystrophin in 2 month mdx mice resulted in a cerebral mean diffusivity of 0.751x10^{-3} mm^2/s (Fig. 1a, $p<0.05$). In addition, there was a 17% decrease in cerebral perfusion in 10 month mdx as compared to WT (Fig. 1b, $p<0.05$). No significant difference in cerebral perfusion was observed in the 2-month mice. There was no difference in the amount of cerebral vasculature (number of PECAM1 positive cells) between the young mdx (144±14) and WT (147±8). Interestingly, there was enhanced arteriogenesis in the aged (10 month) mdx mice (190±7) as compared to the controls (168±10) (Fig. 1c, $p<0.05$).

CONCLUSIONS: Our study demonstrates the alterations in cerebral water diffusion and perfusion associated with the absence of dystrophin. The reduction in water diffusivity in mdx mice is likely due to an increase in cerebral edema or the existence of large molecules in the extracellular space from a leaky BBB, which was supported by in vitro studies. This is possibly due to the absence of properly functioning aqp4 channels, which would prevent the removal of water. Interestingly, the 10 month mdx mice demonstrated a decrease in cerebral perfusion despite an increase in arteriogenesis as measured by immunohistochemistry. One potential explanation for this result is the necessity to maintain a constant mean transit time (MTT), thus decreasing perfusion in the setting of increased vasculature.

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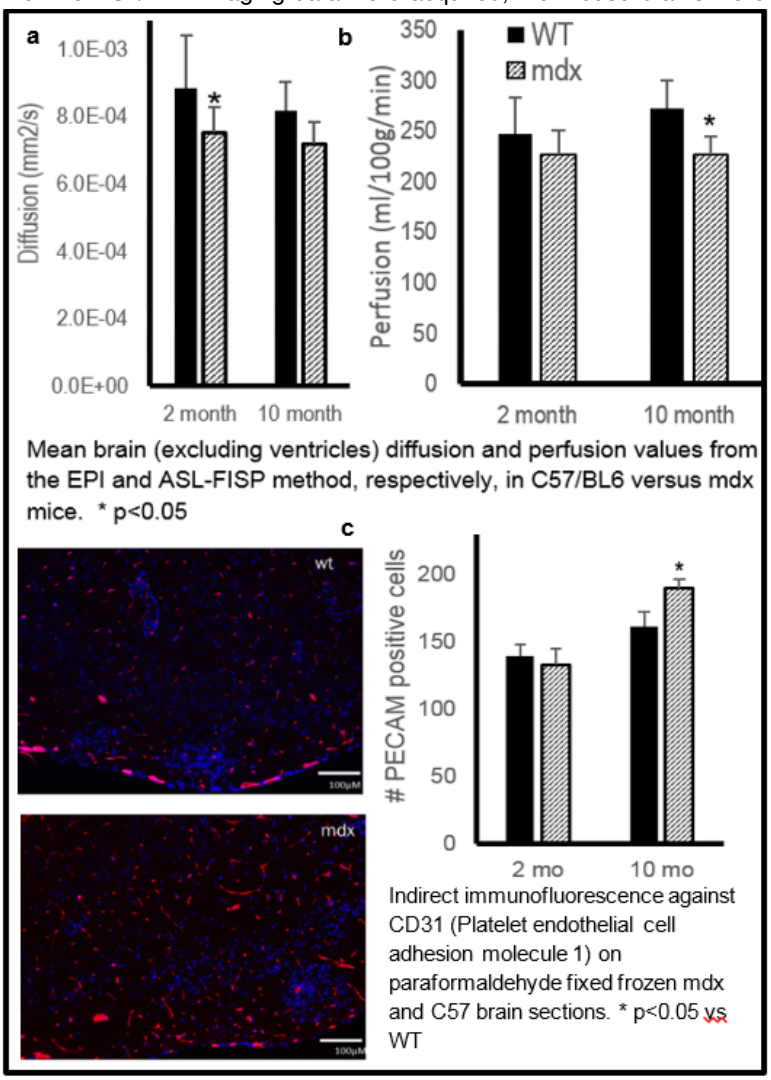

Figure 1

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