Abnormalities in brain structure and biochemistry associated with mdx mice measured by in vivo MRI and high resolution localized ¹H MRS

Su Xu¹, Da Shi², Stephen JP Pratt¹, Wenhujun Zhu¹, Andrew Marshall¹, and Richard M Lownering²

¹Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, Baltimore, MD, United States, ²Department of Orthopaedics, University of Maryland School of Medicine, Baltimore, MD, United States

Introduction

In addition to the well-known muscle wasting, it is generally accepted that some patients with Duchenne muscle dystrophy (DMD) have cognitive impairments. Dystrophin, normally expressed in hippocampal neuronal and glial cells, is absent in DMD patients as well as its homologue murine model (mdx). This suggests that the mental deficit is a result of the missing protein; however the precise function of dystrophin is still unknown. In the muscle, it has been suggested that dystrophin: 1) supports the sarcolemma against mechanical stress and stabilizes it in the course of contraction–relaxation cycles; 2) takes part in the regulation of intracellular calcium and the cascade of calcium-related events; 3) works in force and signal transduction; 4) influences the aggregation of neurotransmitter receptors; 5) prevents excessive generation of reactive oxygen free radical species. Some of these mechanisms may also be applied in the brain to a significant degree. Brain is a dystrophin-targeted organ; solving the mystery of dystrophin function in the brain is a prerequisite for introduction of any effective therapy for DMD. In this study, we used in vivo MRI and high resolution localized ¹H MRS to investigate the alterations of the structure and the neurometabolites in adult male mdx mice.

Materials and Methods

Five dystrophic mice (mdx, C57BLScSn-DMDmdx) and five aged-matched wild type mice (WT, C57BL/10ScSn) were obtained from Jackson Laboratory, Bar Harbor, ME. The in vivo MRI and MRS acquisitions were performed on mice close to one year of age. The experiments were performed on a Bruker BioSpec 70/30USR Avance III 7T scanner. A Bruker four-element ¹H surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. Each mouse was anesthetized in an animal chamber using a gas mixture of O₂ (1 L/min) and isoflurane (3 %) then later maintained at 1-1.5% isoflurane during scanning. An MR compatible small-animal monitoring and gating system was used to monitor the animal respiration rate and body temperature. The animal body temperature was maintained at 36-37°C using a water warm circulation. Both Proton-density-weighted and T2-weighted images were obtained using a 2D rapid acquisition with relaxation enhancement (RARE) sequence in the axial plane (TR/TE/µTE = 5500/19/58 msec, RARE factor = 4, field of view = 20 x 20 mm², slice thickness = 1 mm, in-plane resolution = 114 x 114 µm², number of averages (na) = 1). A customer-modified short-TE PRESS pulse sequence (TR/TE = 2500/10 ms, NA = 356) was used for MRS data acquisition with the voxel centered on the hippocampus (1.5 x 6.0 x 0.9 mm³). LCMModel package was used for quantification of the MRS data. The reliability of the major metabolites was estimated in the Cramè-Rao lower bounds (CRLB) from the LCMModel analysis. All experimental procedures were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. In vivo MRI and MRS results from mdx and WT mice were compared using t-tests.

Results and Discussions

The mdx mice showed obviously enlarged lateral ventricles (Fig. 1.) and this finding was confirmed by volumetric measurements; (lateral ventricle volume/brain volume: mdx [0.053±0.007] vs. WT [0.027±0.003], p = 0.007). The enlargements indicate a buildup of excess cerebrospinal fluid (CSF), which was displayed by the high signal intensity on the T₁-weighted images (Fig. 1). The imbalance between how much CSF the brain makes and resorbs may be caused by the lack of dystrophin. In vivo MRS spectra in the hippocampus from the two groups of mice are showed in Fig. 2. Compared to WT mice, mdx mice demonstrated significant elevations in glutathione (GSH, p=0.02) and taurine (Tau, p=0.005) (Fig. 3). The high GSH level in mdx mice may be explained by an upregulation due to the high levels of glutathione reductase, glutathione peroxidase, and glutathione S-transferases found in both human and animal models of muscular dystrophy. It might indicate a chronic oxidative stress in the hippocampus in the mdx mice. Tau is a hydrolyte attributed to efficiently transporting potassium, sodium, and calcium (Ca²⁺) in and out of the cell. It is also known to play a significant role in many physiological activities, including maintaining a stable, constant condition of Ca²⁺ in the brain tissue. Studies have showed that Ca²⁺ homeostasis is perturbed in mdx muscle12,13, elevated in skeletal muscle at all ages in mdx mice. The high level of Tau may be a response to the abnormal level of Ca²⁺, which may indicate a chronic osmotic stress in the hippocampus of mdx mice.

Conclusions

We report enlarged brain lateral ventricles and elevations in glutathione and taurine in the hippocampus of the one-year-old mdx mice. Such findings indicate a structural change, an altered cellular antioxidant defense, and a chronic osmotic stress in the brain. The current study provides the evidences to extend our understanding the dystrophin function in brain, which is very important for developing any effective therapy for DMD.

References