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

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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[1]; 2) takes part in the regulation of intracellular calcium and the cascade of calcium- related events[2]; 3) works in force and signal transduction[3]; 4) influences the aggregation of neurotransmitter receptors[4]; 5) prevents excessive generation of reactive oxygen free radical species[5]. 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 ^{1}H MRS to investigate the alterations of the structure and the neurometabolites in adult male *mdx* mice.

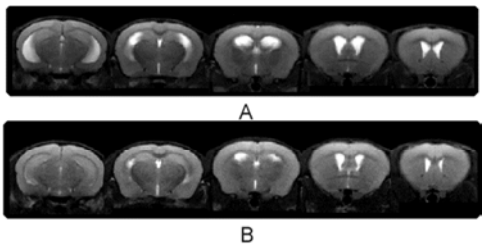

Fig 1. T_2-weighted MRI images of the axial view from the brain of a **A**. *mdx* mouse; **B.** WT mouse.

Materials and Methods

Five dystrophic mice (*mdx*, C57BLScSn-DMD*mdx*) 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 ^{1}H 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 O_2 (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^{o}C using a warm water circulation. Both Proton-density-weighted and T_2-weighted images were obtained using a 2D rapid acquisition with relaxation enhancement (RARE) sequence in the axial plane ($TR/TE_{eff1}/TE_{eff2}$ = 5500/19/58 msec, RARE factor = 4, field of view = 20 x 20 mm^2, slice thickness = 1 mm, in-plane resolution = 114 x 114 μm^2, number of averages (na) = 1). A customer-modified short-TE PRESS pulse sequence[6] (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 mm^3). LCModel package was used for quantification of the MRS data. The reliability of the major metabolites was estimated in the Cramér-Rao lower bounds (CRLB) from the LCModel 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.

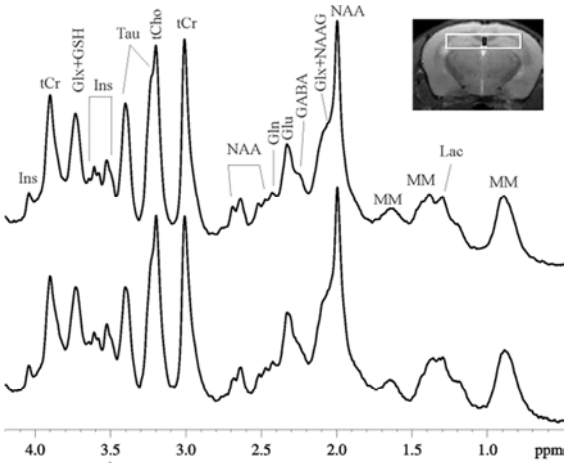

Fig 2. *In vivo* ^{1}H MR spectra and corresponding hippocampal voxel location depicted on the anatomic images from the sum of the five *mdx* mice (top raw) and WT mice (bottom raw). GABA=γ–aminobutyric acid, Glu=glutamate, Gln=glutamine, Glx=glutamate + glutamine, Ins=myo-inositol , NAA=*N*-acetylaspartate , Tau=taurine, tCho=total Choline , NAAG=NAA+*N*-acetylaspartateglutamate, tCr=total Creatine

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 T_2-weighed images (Fig.1). The imbalance between how much CFS 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[7] and animal models[8,9] 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 (Ca^{2+}) 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 Ca^{2+} in the brain tissue. Studies have showed that Ca^{2+} homeostasis is perturbed in *mdx* muscle[10,11], elevated in skeletal muscle at all ages in *mdx* mice. The high level of Tau may be a response to the abnormal level of Ca^{2+}, which may indicate a chronic osmotic stress in hippocampus of *mdx* mice.

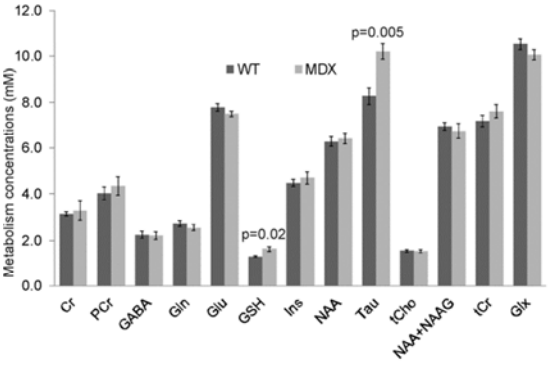

Fig 3. Metabolites concentrations in hippocampus from WT and *mdx* mice; data expressed as mean ± standard error.

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

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