Influence of Paramagnetic Changes in Cytochrome c on T2-weighted MRI of G93A-SOD1 Transgenic ALS Mice


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Introduction. Amyotrophic lateral sclerosis (ALS) is a progressive and fatal motor neuron disease characterized by selective degeneration of motor neurons in the brain stem, spinal cord, and motor cortex. Approximately 5–10% of ALS cases are familial, and 15–20% of these are linked to a mutation in Cu/Zn superoxide dismutase (SOD1) gene. Mice expressing mutant G93A-SOD1 (glycine to alanine substitution in SOD1) develop symptoms and pathology similar to those in human ALS. In this study, low temperature X-band EPR spectroscopy was used to monitor the alterations in paramagnetic heme protein (e.g., cytochrome c) in brain stem, spinal cord and muscle tissues in G93A-SOD1 mice. The effect of EPR paramagnetic changes on alterations in T2-weighted MRI of brain stem in G93A ALS mice will be reported.

The onset and progression of neurodegenerative diseases (e.g., ALS, Parkinson’s, Alzheimer’s) is characterized by mitochondrial dysfunction. Mitochondria are abundant in paramagnetic metal-rich Fe-S clusters, heme and binuclear Cu(II) and Fe(III)-Cu(II) present at the active sites of the electron-transport chain. We used low temperature EPR spectroscopy to investigate specific paramagnetic changes to heme proteins of G93A-SOD1 transgenic ALS mice. We hypothesized that alterations in paramagnetism of endogenous heme proteins in normal and pathological tissue will influence T1 and T2 values, leading to tissue hyper- or hypointensity in MRI. We monitored T2-weighted MRI (T2-W MRI) of G93A-SOD1 transgenic mice at 60, 80 and 115 days. The objectives here were to investigate the following: 1) the paramagnetic alterations in heme protein (e.g., cytochrome c) during ALS progression in G93A mice using low temperature EPR, and 2) the effect of the paramagnetic changes on T2-W MRI.

Methods. Transgenic mice: B6SJL (G93A-SOD1) mice (Jackson Laboratories, Bar Harbor, USA) were crossed with non-transgenic B6SJL mice. They were identified by polymerase chain reaction (PCR). Age-matched non-transgenic litter mates were used as controls. The X-band EPR of tissues of normal and G93A-SOD1 mice were recorded at liquid helium temperatures on a Bruker E500 ELEXYS spectrometer with a 100 kHz field modulator, and equipped with an Oxford Instruments ESR-9 helium flow cryostat. Temperature and microwave power dependence EPR spectra were obtained over the range of 4-50 K and 0.1-159 mW. EPR spectra were analyzed using the equation:

\[ H = D \hat{S}_z^2 - \frac{S(S+1)}{3} + E(\hat{S}_z^2 - \frac{1}{2}) + g_B \vec{B} \cdot \vec{S} + \beta_B \hat{S}_z \hat{B} \]

where the terms D, E and A correspond to zero-field splitting, electronic and nuclear Zeeman splitting, and hyperfine coupling, respectively.

MRI imaging: MRI experiments were performed on a Bruker Biospec scanner at 9.4 T/31 cm free bore with a BGA 120 mm diameter solenoid or BGA 60 mm gradient system. Axial and sagittal T2-W spin echo images were obtained sequentially using a rapid acquisition relaxation enhanced (RARE) sequence with the following parameters: TR 5000 ms, TE 13 ms, slice thickness 500 mm, FOV 20x20x20, matrix 256x256, RARE factor 8, NEX 4. The total scanning time was 17 min. Data were processed and analyzed using the AFNI software program.

Results and Discussion. The ex vivo EPR spectra of spinal cord, cortex, cerebellum, brain stem, and muscle tissues of wild-type (A) and G93A mice (B) are shown in Figure 1. There is a marked increase in g=6 high spin heme signal in brain stem, cortex, spinal cord, cerebellum, and muscle of G93A mice showing ALS symptoms (Fig. 1B). At higher resolution, the g=3 signal due to low spin heme (III) was replaced by a low spin heme EPR of cyt-c in aqueous buffer (not shown). Previously, increased formation of vacuoles containing cyt-c in membrane-bound vesicles was reported. Taken together, these results suggest that cyt-c release from mitochondria during neurodegeneration caused a T2 increase in brain stem ofALS mouse. The mechanism of T2 hyper/hypointensity in MRI and its potential correlation to spin conversion (from S=1/2 to S=5/2) of paramagnetic cyt-c heme Fe(III) centers in mitochondria during ALS progression will be reported.

Conclusions. The EPR results show that cyt-c release from mitochondria correlated with a T2 increase observed in brain stem in ALS mice and phantom of cyt-c in lipids as determined by T2-W MRI. This T2 hyperintensity in MRI is attributed to changes in hyperparamagnetic cyt-c from a low spin iron (S=1/2) to a high spin iron (S=5/2).