Effect of Creatine Supplementation on the Metabolic Markers of ALS and Motor Neuron Diseases: A ^{1}H Magnetic Resonance Spectroscopic Imaging Investigation

D. C. Shungu[1], X. Mao[1], S. Chan[2], A. M. Ulug[3], M. X. Tang[4], M. L. Del Bene[4], V. Battista[4], M. F. Beal[5], L. P. Rowland[4], H. Mitsumoto[4]

[1]Radiology, Mount Sinai School of Medicine, New York, NY, United States, [2]Radiology, Columbia University, New York, NY, United States, [3]Radiology, Weill Medical College of Cornell University, New York, NY, United States, [4]Neurology, Columbia University, New York, NY, United States, [5]Neurology, Weill Medical College of Cornell University, New York, NY, United States

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disorder of the upper (UMN) and lower (LMN) motor neuron, whose etiology and pathophysiology are yet to be fully elucidated. For the past two and half years, our research group has been conducting a large scale study of ALS and other motor neuron diseases (MND) to establish their natural history, as well as to search for objective and quantitative markers of UMN and LMN involvement, which can be used both for diagnosis and as objective measures of the response to promising therapeutic interventions. Due to its ability to allow direct and noninvasive measurements of a number of metabolites in the motor cortex, magnetic resonance spectroscopy (MRS) has emerged as a potentially powerful technique for measuring UMN involvement in ALS and other MND. Using multi-voxel MRS (MRSI), Pioro *et al.*[1] found a significant focal decrease in NAA/CR in the motor and sensory cortex of ALS patients compared to normal control subjects. In a single-voxel MRS study of patients with ALS and primary lateral sclerosis (PLS, a strictly UMN disease), our group found a significant decrease of motor cortex NAA/CR, not only in ALS subjects but also in PLS subjects, compared to normal controls[2]. Though metabolite ratios (e.g., NAA/CR) are commonly used to express relative changes in MRS data, there is an inherent pitfall in this approach: the underlying assumption that the denominator in the ratios (usually CR) is constant and reliable may not always be true. This is especially important in the study of ALS since, often, subjects enrolled in studies take CR as a supplement, and are usually left on the CR regimen for ethical and moral reasons because of the terminal nature of the illness. Since in our ongoing large scale study of ALS and MND, CR supplementation was not one of the exclusion criteria, we have accumulated data on a number of subjects who were either taking CR or not taking it. This has allowed us in this study to directly investigate the effects of exogenous CR on MRS-derived metabolic markers of ALS and MND.

Methods

The adjoining Table summarizes the sample population of MND and control subjects that we have enrolled into the study thus far. It also indicates the number of subjects in each group who were or were not taking CR during the study. In addition to ALS (a UMN and LMN disease) and PLS (a UMN only disease) subjects, we have also enrolled subjects with progressive muscular atrophy (PMA), which is a LMN only disorder. The rest of the subjects were normal individuals with no history of neurological deficits, who served as controls.

Group	No. on CR	No. not on CR	Total
ALS	7	17	24
PLS	1	3	4
PMA	4	4	8
Control	0	17	17
Total	12	41	53

All the enrolled subjects underwent ^{1}H MRSI using the multislice technique of Duyn *et al.*[3] Two 15-mm brain sections, oriented along the AC-PC line, were selected for analysis, with the most superior slice encompassing the motor and sensory cortex. The MRSI data were acquire with TE/TR 280/2300 ms, 32x32 phase-encoding steps, with circular k-space sampling and 256 time domain points. The resulting data were sorted by slice, zerofilled to 1024 time domain points and 3D Fourier transformed to yield the spectroscopic imaging data. The motor cortex was identified on a high resolution localizer image by a trained neuroradiologist, who also selected the motor cortex voxels for spectral analysis with a frequency-domain fitting software developed in our laboratory. Spectra in voxels from the occipital lobe, which is not involved in MND pathology, were also analyzed to serve as an internal control region. Differences in relative metabolite levels (NAA/CR, CHO/CR, NAA/CHO) in these ROIs between groups were assessed by independent sample t-test.

Results and Discussion

Between group comparisons of the metabolite ratios did not yield any significant differences either for the sensotimotor or for the occipital cortex, although we found a decrease of NAA/CR ratios in the left motor strip for the combined ALS subjects (on and not on CR) to approach significance ($p < 0.06$) compared to the control group. Performing separate comparisons between control subjects and ALS subjects taking CR and those not taking any CR, yielded a significant difference ($p < 0.01$) between controls and ALS subjects taking CR, but not for ALS subjects who were not taking CR. This strongly suggested that CR supplementation, whose effect would be to decrease NAA/CR ratios, strongly influenced the almost significant difference in this ratio between controls and the combined ALS group, as well as the observed difference in the ratio between the control group and ALS subjects taking CR. To more fully test this apparent CR effect, further ratios were computed. For all groups, NAA/CR ratios for the motor cortex were divided by NAA/CR for the occipital lobe – a region not involved in MND pathology – with the assumption that this would decrease or eliminate the effect of supplemental CR from the computed relative metabolite levels. Performing comparisons after taking into account the CR effect with these ratios relative to the occipital cortex, yielded differences which were consistent with what is known about the pathophysiology of ALS and MND. Corrected left motor cortex NAA/CR ratios for the ALS group were found to be significantly lower ($p<0.001$) than those for the control and PMA (a LMN disease) groups. For PLS (a UMN disease), corrected left ($p < 0.016$) and right ($p < 0.002$) motor cortex NAA/CR ratios were significantly lower than those for the control and PMA groups. On the other hand, we found no significant differences ($p > 0.5$) in corrected NAA/CR ratios between the control group and the PMA, indicating no UMN involvement. This is consistent with the strictly lower motor neuron nature of PMA.

Conclusion

The results of this study have demonstrated in a dramatic fashion the potential pitfall of relying on metabolite ratios to assess metabolic changes or differences by MRS among groups, when one of the groups may be taking a compound that could artificially increase or decrease the measured ratios. This is particularly true for ALS, a terminal neurological disease with no cure, for which affected subjects are often supplemented with exogenous compounds, which may offer promise. We are currently re-analyzing these data using absolute concentrations, which should to put the presented results on a stronger footing.

Acknowledgment

This study was supported in part by NIH grant R01 NS41672-01.

References

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