Now It Can Be Said: N-Acetylaspartate IS a Neuronal Marker --- A HRMAS proton MR spectroscopy and Stereology Study

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Introduction

With the ever increasing applications of in vivo MRS in clinical radiology, n-acetylaspartate (NAA), as a widely used neuronal marker, has been playing an important role in diagnoses of human brain disorders. Although changes in NAA have been shown to accompany a large number of neurological conditions, this observation alone does not prove that NAA is located in neurons, and hence is a valid neuronal marker. Such a proof is obviously crucial in the efforts to solidify the foundation of brain MRS and its applications in clinical neurology.

The major aims of this report are: 1) to establish a quantitative correlation between NAA and adult neurons; and 2) to evaluate the sensitivity of NAA in differentiating human brain with Alzheimer disease (AD) from the control group. High resolution magic angle spinning proton MR spectroscopy (HRMAS 1HMRS) is used to quantify the concentration of NAA from intact brain specimens. Stereological pathology is employed to provide brain neuronal counting.

Materials and Methods

To date, we have obtained frozen brain specimens of superior temporal sulcus from nine subjects (five ADs, and four age-matched controls) for HRMAS 1HMRS analysis. Among them, three ADs and four controls have been subjected to the statistically unbiased stereological neuronal counting. Stereological optical dissection was performed on 50um-thick sections that had been fixed in 4% paraformaldehyde, placed in a cryoprotection 15% glycerol buffered solution, and Nissl-stained. Data were recorded with a Bioquant Image Analysis System (Nashville, TN). The HRMAS experiments were performed at 3°C on an MSL400 NMR spectrometer (9.4T) by using a BD-MAS probe custom modified for HRMAS analysis (Bruker Instruments, Inc. Billerica, MA). Sample spinning rate was stabilized at between 2.2 and 2.5kHz (+/- 2Hz) for individual samples. A rotor-synchronized Carr-Purcell-Meibom-Gill pulse sequence [90-(t-180-t)n-acquisition] was used as a T2-filter. The value of n for each sample was adjusted according to sample spinning rates in order to create a T2 filter time of 2nt = 20ms. Spectra were collected both with and without presaturation of resonance signals from tissue water. The tissue water signal, measured from spectra without presaturation, was used as an internal standard for the estimation of the concentration of brain cellular metabolites.

Results and Discussion

Figure 1 shows the statistically significant linear correlation observed between the number of neurons and the concentration of NAA for all seven subjects that have been measured (dotted line). An even better correlation of statistical significance, also plotted in the figure, can be reached with four control subjects alone (solid line, open circles). However, due to possible variation of condition under which the tissue was preserved, neither the NAA-neuron correlation, nor NAA concentrations or neuronal counts can differentiate ADs from controls among these cases. Nevertheless, the most important finding demonstrated by this figure is that the extrapolation of the linear correlation obtained with least squares fitting intercepts with zeros for both the NAA and the neuronal counts. The observation provides the first direct experimental evidence that NAA resides in the neurons alone.

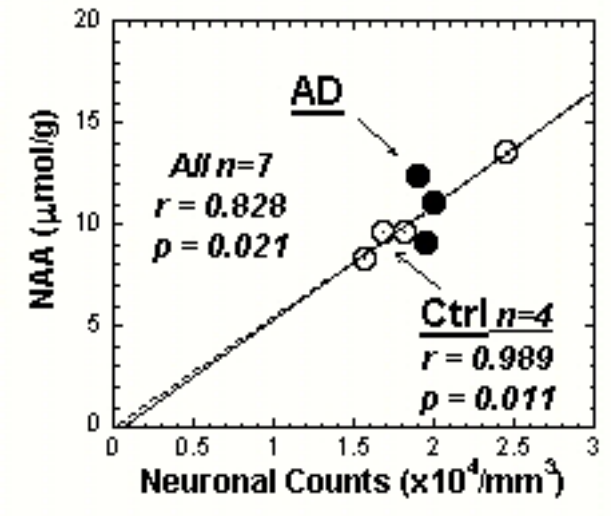

Figure 1. NAA concentration is proportional to the number of neurons.

Figure 2, on the other hand, demonstrates that by using the metabolic ratio of NAA:Cr, the examined AD cases can be clearly differentiated from controls with statistical significance. This is because a ratio of metabolites is generally less susceptible to the influence of tissue conditions. For instance, the loss of tissue water can result in the overestimation of the concentrations for both metabolites, but the ratio of them remains unchanged.

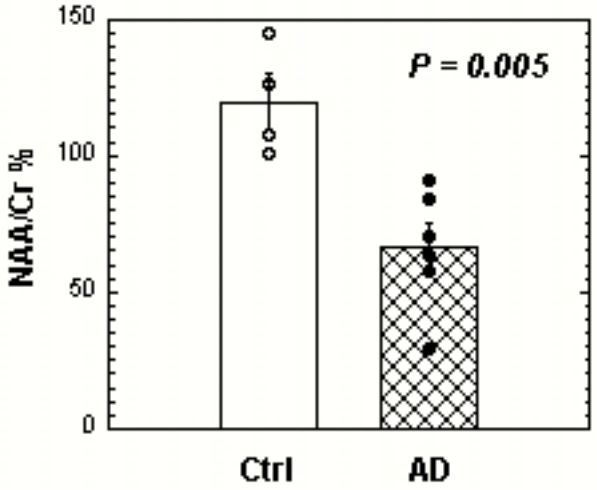

Figure 2. NAA/Cr differentiates ADs from controls.

Conclusion

We have demonstrated the one-to-one relationship between NAA and neurons, and the sensitivity of NAA in AD diagnosis; we will also present our results on other brain metabolites, their relationship to neuronal counts, and their significance in AD diagnosis.

Supported in part by Mass ADRC, PHS grants CA77727, NS34626, RR13213, and MGH NMR Center.