Detection of Cerebral Degeneration in Amyotrophic Lateral Sclerosis Using High-Field Magnetic Resonance Spectroscopy

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Background: Clinical assessment is insensitive to the degree of cerebral involvement in amyotrophic lateral sclerosis (ALS). Regional brain concentrations of N-acetylaspartylglutamate (NAA) plus myo-inositol (Ins), as measured by magnetic resonance spectroscopy, are respectively decreased and increased, suggesting that these compounds may provide a biomarker of the degree of cerebral involvement in ALS.

Objective: To test the hypothesis that the NAA/Ins ratio may provide an index of cerebral involvement in patients with ALS.

Design: High-field (3.0-T) magnetic resonance spectroscopy was performed to determine the NAA/creatine plus phosphocreatine (NAA/Cr), NAA/choline (NAA/Cho), Ins/Cr, and NAA/Ins ratios in the motor cortex.

Participants: Seventeen patients with ALS and 15 healthy control subjects were studied.

Results: In patients with ALS, the greatest abnormality was a 22% decrease in NAA/Ins (71% sensitivity and 93% specificity, \(P = .001\)); Ins/Cr was increased 18% (88% sensitivity and 53% specificity, \(P = .04\)), NAA/Cr was decreased 10% (88% sensitivity and 47% specificity, \(P = .04\)), and NAA/Cho was decreased 14% (53% sensitivity and 87% specificity, \(P = .047\)). Correlation of the ALS Functional Rating Scale with NAA/Ins approached statistical significance (\(R = 0.43, P = .07\)).

Conclusion: The NAA/Ins ratio may provide a meaningful biomarker in ALS given its optimal sensitivity and specificity profile.

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Clinical assessment of upper motor neuron (UMN) signs in amyotrophic lateral sclerosis (ALS) is insensitive, resulting in delays in diagnosis and treatment. Indeed, pathologic evidence of corticospinal tract degeneration has been demonstrated in the absence of UMN signs in patients in whom lower motor neuron signs predominated.

Proton magnetic resonance spectroscopy (MRS) can provide insight into the integrity of UMN pathways in ALS in vivo. The resonance arising from N-acetylaspartate plus N-acetylaspartylglutamate (NAA) is a marker of neuronal integrity. N-acetylaspartate and N-acetylaspartylglutamate levels are decreased in the brain in ALS in a spatially dependent manner that reflects its pathologic distribution. Myo-inositol (Ins) is a cyclitol involved in intracellular signaling. It is a putative spectroscopic glial marker and is increased in the motor cortex in ALS.

Our objective was to determine how well these spectroscopic markers could detect cerebral pathologic features in patients with ALS. Because simultaneous study of cerebral tissue is impossible and because correlation with necropsy material temporally distant to the MRS examination would be invalid because of disease progression, we studied patients with established ALS in whom UMN signs were evident and clearly indicative of the presence of cerebral disease. A favorable accuracy profile in this clinically homogeneous group would merit further trials to evaluate the ability of these spectroscopic markers to detect disease progression and to assist in early diagnosis.

To date, the findings of decreased NAA and increased Ins measured in isolation have suboptimal discriminatory power to distinguish patients with ALS from healthy subjects. Measuring NAA and Ins in the same patient could potentially improve on this. We hypothesized that the NAA/Ins ratio would be a more accurate marker of
cerebral disease because the 2 measures are affected in opposite directions and may reflect distinct pathophysiological processes that are co-occurring in ALS. At a conventional magnetic field strength (1.5 T), the resonances of Ins are difficult to resolve because they originate from a strongly coupled and complex spin system and overlap significantly with resonances arising from glutamate, glutamine, glycine, and taurine. Therefore, subjects were studied using a high-field magnetic resonance system using a sequence tailored to optimize detection of Ins.

METHODS

SUBJECTS

Subjects were recruited from the ALS Clinic at the University of Alberta, Edmonton. Patients met El Escorial criteria for probable or definite ALS; therefore, all had examination findings indicating UMN dysfunction. Patients were administered the ALS Functional Rating Scale (ALSFRS) questionnaire (score range, 0-40). Upper motor neuron functioning was evaluated by measuring finger and foot tapping speed (taps per 10 seconds) and spasticity using the Modified Ashworth Scale. Healthy age-matched control subjects were free of neurological or psychiatric disease. Subjects were required to be able to lie flat for 7.5 minutes for the magnetic resonance examination. All subjects gave informed consent, and the study was approved by the Human Research Ethics Board of the University of Alberta.

IMAGING

Magnetic resonance spectroscopy was performed at 3.0 T using a quadrature birdcage resonator for transmission and reception. Orthogonal gradient-echo images were used to position the volume of interest in the motor cortex contralateral to the most severe UMN findings (Figure 1). In the case of symmetric signs on physical examination, the motor cortex of the dominant hemisphere, as inferred by handedness, was studied. The volume of interest measured $2 \times 3 \times 2$ cm and was centered on and placed parallel to the precentral gyrus to maximize affected tissue content. A stimulated echo acquisition mode sequence was used for single-voxel spectroscopy (repetition time, 3000 milliseconds; 256 averages in 8 separate bins). Using a computational spectrum simulation method, we determined that the optimal sequence timings for measuring Ins distinct from the contaminating background were a mixing time of 40 milliseconds and an echo time of 160 milliseconds. These parameters minimized contributions from glutamate, glutamine, taurine, and macromolecules.

Segmentation of the volume of interest into gray matter, white matter, and cerebrospinal fluid was performed using an inversion recovery 1-dimensional projection method with a point-resolved spectroscopy localization scheme. This method uses simultaneous nulling of the water signal from 2 compartments, leaving only that from the third compartment.

Magnetic resonance spectroscopy data bins were summed off-line in MATLAB (The MathWorks, Inc, Natick, Mass) following automatic phase correction and frequency registration. Automated baseline fitting and metabolite peak quantification were performed using LCModel (available at: http://www.s-provencher.com/pages/lcmodel.shtml), in which simulated spectra were used as the basis spectra. Metabolite resonance peak areas were normalized to creatine plus phosphocreatine (Cr). The error estimate of the fit of a peak (percent standard deviation) was used as a measure of the precision of the quantification.

STATISTICAL ANALYSIS

Group differences in metabolite ratios were analyzed using the Mann-Whitney test. Spearman rank correlation coefficients (R) were computed to evaluate relationships between metabolite ratios and clinical variables. Statistical significance was set at 2-tailed $P < .05$. Receiver operating characteristic analysis was performed for metabolite ratios using MedCalc version 7.4 (MedCalc Software, Mariakerke, Belgium). The cutoff value with the highest accuracy (minimal false negatives and false positives) was determined using MedCalc, and the sensitivity and specificity for a ratio were determined at this cutoff.

RESULTS

Seventeen patients with ALS (3 definite and 14 probable) and 15 healthy control subjects were studied. There was no difference in age or sex distribution (Table 1). All spectra were of good quality (the LCModel fit percent SD for the metabolite peaks was $< 20\%$); none were rejected.
In the ALS group, NAA/Cr, NAA/choline (NAA/Cho), and NAA/Ins were decreased, and Ins/Cr was increased (Figure 2 and Table 2). The greatest difference was a 22% decrease in NAA/Ins. Receiver operating characteristic curves (available in an online eFigure [http://www.archneurol.com]) were similar for NAA/Cr, NAA/Cho, and Ins/Cr. The area under the receiver operating characteristic curve was greatest for NAA/Cr, reflecting its superior sensitivity and specificity profile. The NAA/Cr and Ins/Cr ratios offered the best sensitivity (88%), and the NAA/Ins ratio offered the best specificity (93%).

In 13 patients and 15 control subjects who underwent segmentation, there was no difference in total parenchymal volume (gray matter plus white matter content, mean ± SD, 10.7 ± 0.5 mL vs 10.3 ± 0.8 mL) or in individual fractions (percentages) of gray matter (37.1% ± 16.0% vs 31.6% ± 18.0%), white matter (52.0% ± 16.6% vs 54.6% ± 19.4%), or cerebrospinal fluid (10.8% ± 4.2% vs 13.8% ± 6.3%). Tissue segmentation was incomplete in 4 patients because of fatigue that curtailed the examination session or because of technical difficulties at the time of imaging.

Correlations of the ALSFRS approached statistical significance for NAA/Ins (R = 0.43, P = .07) and were absent for NAA/Cr, NAA/Cho, and Ins/Cr. Magnetic resonance spectroscopic indices did not correlate with symptom duration, contralateral finger or foot tapping rates, or the Modified Ashworth Scale score.

**COMMENT**

High-field MRS at 3.0 T was used to study neurochemical abnormalities in the motor cortex in patients with ALS. Relative representations of NAA and Ins were evaluated as the NAA/Cr, NAA/Cho, Ins/Cr, and NAA/Ins ratios. We hypothesized that NAA/Ins would be the most accurate at detecting disease given that previous reports have revealed decreased NAA and increased Ins in ALS. Indeed, NAA/Ins was the most abnormal, with a 22% decrease. Decreased NAA/Cr and increased Ins/Cr had high sensitivity but low specificity. Conversely, decreased NAA/Cho had low sensitivity but high specificity. The NAA/Ins ratio had moderate sensitivity (72%), the highest specificity (93%), and the best sensitivity and specificity profile among the 4 metabolite ratios. The NAA/Ins ratio has been evaluated in other neurodegenerative disorders; however, to our knowledge, this is the first report of NAA/Ins measurement in ALS.

The best correlation with the ALSFRS, a validated clinical rating scale and end point in clinical trials, was with NAA/Ins. Although this result only approached statistical significance, it would suggest that cerebral degeneration contributes to disability as measured by the ALSFRS. Correlation was not found with tapping rates, contrary to a previous report, or with the Modified Ashworth Scale spasticity score, as has been described elsewhere.

Whereas NAA is an established marker of neuronal integrity, the pathophysiological basis for an increase in Ins in ALS is unknown. The popularity of Ins as a putative spectroscopic glial marker is based on a cerebral distribution that strongly favors glial cells and on the observation of increased Ins in several disorders in which astrogliosis is prominent. This has particular relevance in ALS where motor neuron degeneration is commonly accompanied by astrogliosis. Evidence is emerging that astrocytes may play an active role in the pathogenesis of ALS. Glial glutamate transporters are decreased in the motor cortex of patients with ALS. In SOD1 transgenic mouse models of ALS, proliferating astrocytes interact intimately with neurons. Mutant SOD1 expression in motor neurons or astrocytes alone does not lead to neurodegeneration, implicating an apparent necessity of an interaction between neuronal and glial or other non-neuronal cells for degeneration to occur.

Myo-inositol is a precursor to the phosphatidylinositol second messenger system; altered signal transduction in ALS is supported by the observation of increased phosphatidylinositol 3–kinase activity and an elevated protein kinase C level. Increased Ins may also be a consequence of glutamate-mediated excitotoxicity, for which there is considerable evidence as a pathogenetic process in ALS. Glutamate activates the phosphatidylinositol cycle by stimulation of metabotropic receptors. This, in addition to cell depolarization and increased cellular calcium, up-regulates neuronal and astrocytic cell surface expression of a proton-coupled Ins transporter.

Myo-inositol also functions as an osmolyte, with increased cellular uptake occurring as a protective response to hypertonicity. Because this is absent in ALS, it would be an unlikely mechanism leading to increased Ins.

We sought to specifically evaluate the NAA/Ins ratio because of the greater experimental accuracy and technical ease of measurement associated with ratio determination that permits easier application of our results to further clinical investigation. Although absolute quantitation allows measurement of individual metabolite concentrations, this may reduce precision and limit application to clinical evaluation. Future studies could address the role of individual metabolites in ALS. Assessment of metabolite relaxation times could also be considered to explore their effect on observed changes in and the pathophysiological relevance of neurochem-
cal perturbations in ALS. Measurement of glutamine, an astrocyte marker, concurrent to Ins may help clarify the cellular specificity of Ins. With further inclusion of glutamate, such studies would contribute valuable knowledge to in vivo derangements in glutamate and glutamine metabolism, involvement of astrocytes, and glutamate-mediated excitotoxicity. However, the protocols will need to be tailored to minimize the duration of data acquisition because patients with ALS cannot tolerate protracted studies.

This study amplifies previous investigations of spectroscopic markers of disease in ALS. The metabolites studied could provide insight into aberrant intracellular signaling and astrogliosis, and they were significantly abnormal so as to accurately differentiate patients with an established clinical diagnosis from healthy controls. The utility of the NAA/Ins as a biomarker will require further investigation. Specifically, to determine its disease specificity and predictive ability with early diagnosis of ALS, a study is required that includes patients with progressive lower motor neuron syndromes in whom UMN signs are absent (ie, progressive muscular atrophy) or insufficient (ie, "possible ALS" by El Escorial criteria) to make a firm clinical diagnosis. A longitudinal study with sequential MRS examinations will be essential to establish the sensitivity of the NAA/Ins ratio to disease progression.

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Additional Information: The eFigure is available at http://www.archneurol.com.

REFERENCES

Web-only Figure

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eFigure. Receiver operating characteristic curves for the metabolite ratios. The N-acetylaspartate plus N-acetylaspartylglutamate (NAA)–myo-inositol (Ins) ratio has the greatest area under the curve, reflecting its superior sensitivity and specificity profile. Cho indicates choline; Cr, creatine plus phosphocreatine.