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Clinical Study

The ratio of N-acetyl aspartate to glutamate correlates with disease duration of amyotrophic lateral sclerosis

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ABSTRACT

Glutamate (Glu)-induced excitotoxicity has been implicated in the neuronal loss of amyotrophic lateral sclerosis. To test the hypothesis that Glu in the primary motor cortex contributes to disease severity and/or duration, the Glu level was investigated using MR spectroscopy. Seventeen patients with amyotrophic lateral sclerosis were diagnosed according to the El Escorial criteria for suspected, possible, probable or definite amyotrophic lateral sclerosis, and enrolled in this cross-sectional study. We measured metabolite concentrations, including *N*-acetyl aspartate (NAA), creatine, choline, inositol, Glu and glutamine, and performed partial correlation between each metabolite concentration or NAA/Glu ratio and disease severity or duration using age as a covariate. Considering our hypothesis that Glu is associated with neuronal cell death in amyotrophic lateral sclerosis, we investigated the ratio of NAA to Glu, and found a significant correlation between NAA/Glu and disease duration ($r = -0.574$, $p = 0.02$). The "suspected" amyotrophic lateral sclerosis patients showed the same tendency as possible, probable and definite amyotrophic lateral sclerosis patients in regard to correlation of NAA/Glu ratio with disease duration. The other metabolites showed no significant correlation. Our findings suggested that glutamatergic neurons are less vulnerable compared to other neurons and this may be because inhibitory receptors are mainly located presynaptically, which supports the notion of Glu-induced excitotoxicity.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is fatal neurodegenerative disorder characterized by Tar-DNA binding protein-43 inclusion bodies in neurons, which affects upper and lower motor neurons [1]. Glutamate (Glu)-induced excitotoxicity has been implicated in ALS pathogenesis [1]. In this context, a metabotropic glutamate receptor (mGlu) 1 knock-down mouse ALS model showed improved survival [2]. Glu concentration is elevated in the cerebrospinal fluid of sporadic ALS patients [3]. In terms of disease-modifying therapy, riluzole is an inhibitor of Glu release, and is one option to treat patients with ALS; however, the effect is small [1]. A biomarker for early diagnosis and monitoring of disease progression is needed to undertake efficient clinical trials [4]. Glu is considered a good candidate for a new biomarker.

Magnetic resonance spectroscopy (MRS) has recently improved and allows for assessment of metabolites, including Glu and gamma aminobutyric acid (GABA) in the human brain [5]. Indeed, MRS reveals elevated levels of a combined measure of glutamate and glutamine (Glx) in the medulla [6], and elevated levels of Glx and Glu and decreased GABA in the primary motor cortex of patients with ALS relative to healthy volunteers [7,8]. The above-mentioned results support Glu-induced excitotoxicity in ALS. To test the hypothesis that Glu in the primary motor cortex contributes to disease severity and/or duration, the Glu level was investigated using MRS.

2. Materials and methods

2.1. Subjects

Seventeen patients with ALS (13 men and four women, mean age 63.7 ± standard error 2.4) were enrolled in this cross-sectional study. All participants were recruited through the Department of Neurology, Tokushima University Hospital, Tokushima, Japan, between April 2008 and February 2014. Demographic and clinical data from these subjects are provided in Table 1. We evaluated onset features, disease duration and Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) scores. Disease duration was defined as the time from the onset of motor symptoms to the time of MRI. All patients were

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Table 1
Clinical features of amyotrophic lateral sclerosis patients investigated with magnetic resonance spectroscopy

Diagnosis	Onset features	Sex (M/F)	Age (years)	Disease duration at the time of MRI (months)	ALSFRS-R	Deaths
Definite (n = 2)	Limb (n = 2)	1/1	61 ± 4.5	20 ± 1.5	40.5 ± 1.5	1
Probable (n = 2)	Bul (n = 1), limb (n = 1)	1/1	70 ± 2.0	32 ± 18.5	40.5 ± 1.5	0
Possible (n = 11)	Bul (n = 4), limb (n = 5), PLS (n = 1), trunk (n = 1)	8/3	62 ± 3.3	14 ± 3.9	41.6 ± 1.2	1
Suspected (n = 2)	Limb (n = 2)	2/0	71 ± 2.5	15 ± 7.0	44 ± 1.5	0

Data are presented as mean ± standard error unless otherwise indicated.
ALSFRS-R = amyotrophic lateral sclerosis functional rating scale revised, Bul = bulbar palsy, F = female, M = male, PLS = primary lateral sclerosis.

diagnosed according to the El Escorial criteria for suspected, possible, probable or definite ALS. We included two suspected ALS patients who progressively showed only lower motor neuron symptoms because primary muscular atrophy is reported to be similar to ALS clinically and pathologically [9,10]. Informed consent was obtained from all participants under protocols approved by the local Ethics Committee.

2.2. MRI acquisition and analysis

The present study was performed using a 3.0 Tesla MRI scanner (GE, Milwaukee, WI, USA) with a standard head coil for both MRI and MRS measurements. Proton MR spectra were measured with a stimulated echo acquisition mode sequence with parameters of repetition time (TR) = 5 s, echo time (TE) = 15 ms and number of signals averaged = 48. We used relatively short TE and long TR because metabolite T2 values are altered in the brains of ALS patients [11]. Metabolites were quantified using LCModel software (version 6.3), and included *N*-acetyl aspartate (NAA), creatine (Cr), choline (Cho), inositol (Ins), Glu and glutamine (Gln) concentrations. NAA is contained almost exclusively within neurons and is considered an *in vivo* marker of neuronal loss (that is, reduced NAA concentration indicates neuronal loss) [12]. T2-weighted images were acquired before the ^{1}H-MRS examination, and placed a single 6.0 ml (20 × 20 × 15 mm) volume of interest in each primary motor area separately (Fig. 1). The metabolites were quantified with Cramer-Rao lower bounds, which the estimated error of the metabolite quantification. The measured values with standard deviation below 20% were included in the analysis. For each metabolite, averages of measured values in both sides were used for statistical analysis.

2.3. Statistics

We performed partial correlation between each metabolite concentration or NAA/Glu ratio and disease severity or duration using age as a covariate. *P* value less than 0.05 was considered as statistically significant. Furthermore, if there was significant correlation, we planned to perform bootstrapping to calculate 95% confidence intervals (CI). The number of bootstrap smaples was 10,000, and the result of bootstrapping was expressed as theta. Partial correlation and bootstrapping were carried out using the Statistical Package for the Social Sciences version 21 (IBM, Armonk, NY, USA) and R software (http://www.r-project.org/), respectively.

3. Results

Considering our hypothesis that Glu is associated with neuronal cell death in ALS, we investigated the ratio of NAA to Glu, and found a significant correlation between NAA/Glu and disease duration ($r = -0.574$, $p = 0.02$; Fig. 2A). Intriguingly, the ALS patients diagnosed as "suspected" showed the same tendency as others including possible, probable and definite ALS (Fig. 2A). There was a significant correlation even if the suspected patients were removed from the analysis ($r = -0.549$, $p = 0.04$; Fig. 2B). The bootstrap analysis showed that the most frequent theta was around −0.6 and 95% CI were from −0.76 to −0.33 (Fig. 2C). The other metabolites showed no significant correlation with disease duration at the time of MRI (NAA: $r = -0.331$, $p = 0.210$;

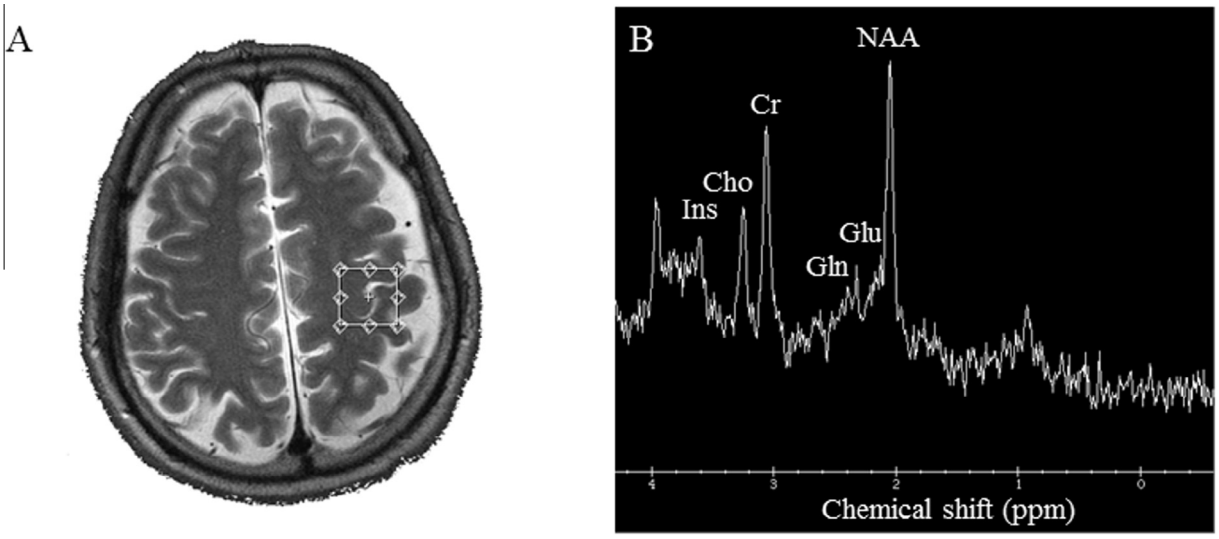

Fig. 1. MR spectroscopy acquisition. (A) Axial view showing that the volume of interest (VOI; square) was in the primary motor cortex. A similar VOI was used in the primary motor cortex on the other side. (B) ^{1}H-MR spectroscopy spectra. Cho = choline, Cr = creatine, Gln = glutamine, Glu = glutamate, Ins = inositol, NAA = *N*-acetyl aspartate, ppm = parts per million.