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Abstract We studied whether N-acetylaspartate (NAA), a neuronal marker, is reduced in the brain of 14 patients with clinically definite amyotrophic lateral sclerosis (ALS) and whether NAA levels in the motor area and frontal lobe correlate with the clinical features, including frontal lobe function. We also studied 14 normal controls were evaluated. We obtained peak integrals in 1H magnetic resonance spectroscopy (MRS) for NAA, creatine (Cr), and choline-containing compounds (Cho). Severity of the disease was determined using the manual muscle strength test, and the Norris limb and bulbar scales. In the patients, the NAA/Cr ratio was reduced in the motor area and frontal lobe, while the Cho/Cr ratio was normal throughout the brain. There were significant correlations between the NAA/Cr ratio in the motor area and the Norris limb scale (r = 0.50; P < 0.01) and between the NAA/Cr ratio in the frontal lobe and the number of categories achieved in the Wisconsin Card Sorting test (r = 0.71; P < 0.05), implying frontal lobe dysfunction. These correlations suggest that a reduced NAA/Cr ratio is a marker of cortical neuronal loss and dysfunction in ALS.

Keywords Motor neurone disease · N-acetylaspartate · Magnetic resonance spectroscopy

Introduction

Amyotrophic lateral sclerosis (ALS) is a motor neurone disease (MND) characterised by progressive degeneration of upper and lower motor neurones. Cortical involvement in patients with ALS has been well known, but correlations between cortical dysfunction and clinical features are uncertain. Cognitive function in ALS has recently been a focus of attention [1, 2, 3, 4, 5, 6] and some workers have differentiated cases of ALS with dementia from those of classic ALS. However, even patients with classic ALS have been shown to develop cognitive, especially frontal lobe dysfunction [7].

Magnetic resonance spectroscopy (MRS) enables us to investigate neuronal metabolism in vivo and studies on patients with ALS have been reported [8, 9, 10, 11, 12, 13]. However, correlations between biochemical markers and neuronal function have not been clarified [9, 14, 15].

Our primary objective was therefore to see whether levels of N-acetylaspartate (NAA), a neuronal marker [16, 17], are reduced in ALS, and whether the levels in the motor area and frontal lobe correlate with clinical features.

Subjects and methods

We studied 14 patients with clinically definite ALS by El Escorial criteria [18] two with bulbar and twelve with limb onset: eight men, six women, mean age 65.8 ± 9.9 years, range 48–72 years, mean duration of disease 2.58 ± 1.7 years, range 1–6 years, plus 14 age- and sex-matched normal controls without neurological deficits (eight men six women, mean age 59.3 ± 12.8 years, range 44–72 years), using 1H MRS. No of patient had taken riluzole. All subjects gave informed consent in written form, according to our institutional guidelines.

All patients were neurologically examined and the severity of disease was assessed using the manual muscle strength test (full
score 110) [19], and the Norris limb (full score 63), and bulbar (full score 39) scales [20]. Cognitive function was screened by the Mini Mental State Examination (MMSE) [21]. To assess frontal lobe function, we used the Wisconsin Card Sorting Test (WCST) [22]; these tests have been standardised as Japanese versions [6]. In order to exclude the possibility that neuropsychological abnormalities in the patients were caused by associated depression or anxiety, patients and controls were rated with Zung’s self-rating depression scale (SRS) [23].

MRI and ¹H MRS studies were performed with a 1.5 tesla system, using a standard quadrature head coil. A neuroradiologist (Y.W.) who had no knowledge of the subject’s diagnosis selected 2 ml volumes of interest (VOI) in the motor area, in the frontal lobe, including Brodmann’s areas 6, 8 and 46 by, in the parietal lobe including areas 39 and 40 and in the occipital lobe, including areas 18 and 19 with care to avoid partial-volume effects due to parenchymal atrophy and to fit the VOI entirely within the same neuroanatomical structures (Fig. 1). We located two VOI in each of the motor area, frontal, parietal and occipital lobes to minimise the location effect. Only when we found no difference between two VOI in each group, did we use the mean value. After localised shimming of the VOI, ¹H-MRS was performed using a stimulated echo acquisition mode sequence for volume selection with a chemical-shift selective presaturation for water suppression. Acquisition parameters were repetition time (TR) of 1500 ms, echo time (TE) of 140 ms, with 1024 acquisition points, and 256 acquisitions. The procedure including preparation, MRI, and MRS lasted approximately 30 min. After acquisition, the MRS data were transferred to a workstation and processed using a SA/GE software. The process included zero-filling to 2048 data-points, 3 Hz line-broadening and fast Fourier transformation (FFT). After zero-order phase correction and cubic baseline correction, Lorenzian fit for the peak area, determined by the Marquardt-Levenberg method [24] was used to measure the areas of spectra from each VOI. We measured NAA, Creatine (Cr) and choline-containing compounds (Cho) and calculated the metabolite signal-intensity ratios (NAA/Cr or Cho/ Cr) for each VOI. Nonparametric statistics, the Kruskal-Wallis and Mann-Whitney tests were used to assess differences in ratios between patients and controls. Spearman’s rank correlation was used to test a possible linear relationship between the clinical rating scores and the ratios. All statistical analyses were carried out by a statistics software, using a microcomputer. Statistical significance was defined as $P < 0.05$.

**Results**

There was no correlation between NAA/Cr ratios and age in patients or controls. The patients showed lower ratios than the controls in the motor area $(1.69 \pm 0.14$ vs $2.23 \pm 0.26$, $P < 0.01$) and frontal lobe $(1.74 \pm 0.23$ vs $2.27 \pm 0.15$, $P < 0.01$) (Fig. 2), but not in the parietal $(2.05 \pm 0.19$ vs $2.19 \pm 0.24$) or occipital $(1.99 \pm 0.15$ vs $2.02 \pm 0.19$) lobes. There was no significant difference in Cho/Cr ratio between patients and controls in the motor area $(1.03 \pm 0.21$ vs $1.08 \pm 0.26$), or the frontal $(1.12 \pm 0.16$ vs $1.05 \pm 0.15$), parietal $(1.09 \pm 0.15$ vs $1.19 \pm 0.14$) or and occipital $(1.05 \pm 0.20$ vs $1.04 \pm 0.16$) lobes.

The patients had lower scores than the controls on the manual muscle strength test $(75.7 \pm 6.6$ vs $110 \pm 0.0$, $P < 0.001$), and Norris limb $(51.0 \pm 4.5$ vs $63 \pm 0.0$, $P < 0.001$), and bulbar $(28.6 \pm 3.4$ vs $39 \pm 0.0$, $P < 0.001$) scales. There was a significant correlation between the NAA/Cr ratio in the motor area and the Norris limb scale ($r = 0.50; P < 0.01$) (Fig. 3), consistent with the neuronal loss. There was no significant difference between patients and controls concerning in total MMSE score $(28.8 \pm 1.2$ vs $29.4 \pm 0.91$). However, the patients with ALS had lower scores on the WCST $(2.64 \pm 1.7$ vs $5.86 \pm 0.73$, $P < 0.001$), and there was a significant correlation between the NAA/Cr ratio in the frontal lobe and the WCST score ($r = 0.71; P < 0.05$) (Fig. 4).

**Discussion**

Degeneration of upper motor neurones in the cerebral cortex is a fundamental pathological change in ALS [25, 26, 27, 28, 29]. However, there have been controversies concerning reduction of NAA in the motor area and frontal lobe in this disease. In spinal cords obtained at autopsy, the NAA level, measured by high-performance liquid chromatography (HPLC) was 40% lower in pa-