Reduction in temporal N-acetylaspartate and creatine (or choline) ratio in temporal lobe epilepsy: does this ¹H-magnetic resonance spectroscopy finding mean poor seizure control?

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Abstract

Background—Proton magnetic resonance spectroscopy (¹H-MRS) is a potentially useful tool in the in vivo investigation of brain metabolites in intractable temporal lobe epilepsy (TLE). Focal N-acetylaspartate (NAA) reductions have been correlated with mesial temporal sclerosis (MTS) in surgically resected epileptogenic foci.

Objective—To evaluate the abnormalities in the metabolites NAA, creatine+phosphocreatine (Cr), and choline containing compounds (Cho) in the temporal lobe of medically refractory patients with temporal lobe epilepsy, seizure free patients with temporal lobe epilepsy, and normal controls.

Patients and methods—Ten refractory patients, 12 seizure free patients with temporal lobe epilepsy, and 10 age matched normal controls were studied by ¹H-magnetic resonance spectroscopy. All patients had consistently unilateral temporal EEG abnormalities and a normal brain MRI. Proton MR spectra were obtained from an 8 ml volume in the medial temporal lobes in patients with temporal lobe epilepsy (ipsilateral to EEG foci) and the normal controls. The signals measured were expressed in terms of NAA/Cr, NAA/Cho, and Cho/Cr.

Results—When compared with seizure free patients with temporal lobe epilepsy and normal controls, the 10 refractory patients with temporal lobe epilepsy had a lower mean (SEM) NAA/Cr ratio (1.65 (0.53) *v* 2.62 (0.60), and 2.66 (0.73); *p*<0.002 and *p*<0.006) and a lower mean NAA/Cho ratio (1.59 (0.79) *v* 2.83 (1.33) and 2.58 (0.67); *p*<0.02 and *p*<0.007). Furthermore, the two patients showing the

lowest NAA/Cr ratios (1.47 and 1.73) in the seizure free group had had a past period of poor seizure control.

Conclusions—There were reduced temporal NAA/Cr and NAA/Cho ratios, suggesting neuronal loss or damage, associated with past or present poor seizure control in the patients with temporal lobe epilepsy, but it does not exclude the possibility of a future complete seizure control (seizure free patients with temporal lobe epilepsy at the time of ¹H-MRS). This study warrants further ¹H-MRS investigation with a larger series of patients with temporal lobe epilepsy.

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Epilepsy affects 2% to 5% of the population at some time in their lives, intractable seizures occurring in 0.2% to 0.5%. The partial epilepsies are known to be the most intractable, and temporal lobe epilepsy (TLE) is the most common partial epilepsy.¹

Hippocampal sclerosis is the most often encountered cause of temporal lobe epilepsy, accounting for 50% to 70% of cases. Hippocampal sclerosis can be diagnosed using MRI. Quantitative MRI has increased the sensitivity of the MRI diagnosis of hippocampal sclerosis.²

Furthermore, recently proton magnetic resonance spectroscopy (¹H-MRS) has been shown to be a useful tool in investigating in vivo and non-invasively key molecules of brain metabolism relevant to the pathophysiology and neuropathology of mesial temporal structures involved in temporal lobe epilepsy.³⁻⁷ It is possible to analyse abnormalities of metabolites such as N-acetylaspartate (NAA),

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Table 1 Clinical characteristics of refractory patients with temporal lobe epilepsy

Patient No/age/sex	Epilepsy onset (y)	Epilepsy duration (y)	Monthly seizure frequency (mean)	Last seizure	Seizure type	History of poorest seizure control age period
1/15/M	9	6	50	24 h	CPS+S	13-15 y: 2/day
2/53/M	44	9	10	4 days	CPS+sgCPS	49-53 y: 10/month
3/26/F	13	13	4	6 days	CPS+S	21-24 y: 2-3/day
4/16/F	6	10	6	10 days	CPS+S	14-15 y: 10/day
5/26/F	9	17	3	9 days	CPS	
6/26/M	6	20	40	24 h	CPS+S	6-8 y: 6-7/day
7/13/F	2	11	30	48 h	CPS+sgCPS	2-4 y: 5/day
8/14/F	8	6	20	4 days	CPS+sgCPS	8-10 y: 5/day
9/19/F	1	18	2	14 days	CPS+sgCPS	14-15 y: 2/day
10/28/M	7	21	2	15 days	CPS+S+sgCPS	

CPS=complex partial seizure; SPS=simple partial seizure; sgCPS=secondarily generalised complex partial seizure; CPS+S=complex and simple partial seizures.

Table 2 Clinical characteristics of seizure free patients with temporal lobe epilepsy

Patient No/age/sex	Epilepsy onset (y)	Epilepsy duration (y)	Monthly seizure frequency (mean)	Last seizure	Seizure type	History of poorest seizure control age period
11/35/F	5	30	0	5 y	SPS+sgCPS	
12/14/M	10	4	0	3 y	CPS+C+sgCPS	
13/20/F	7	13	0	3 y	SPS+sgCPS	
14/40/M	38	2	0	1 y	CPS	
15/45/M	6	39	0	6 months	CPS+sgCPS	
16/12/F	8	4	0	2 y	CPS+sgCPS	
17/18/M	8	10	0	3 y	CPS+S+sgCPS	12-14y: 4/month
18/24/F	2	22	0	3 y	CPS+sgCPS	19-21y: 2/month
19/26/F	2	24	0	1 y	sgCPS	
20/50/M	43	7	0	3 y	CPS	
21/23/F	14	9	0	1 y	CPS+sgCPS	
22/29/F	25	4	0	1 y	sgCPS	

CPS=complex partial seizure; SPS=simple partial seizure; sgCPS=secondarily generalised complex partial seizure; CPS+S=complex and simple partial seizures.

creatine+phosphocreatine (Cr), and compounds containing choline (Cho) by acquiring signals in proton spectra from regions located in temporal lobes that are generated by those metabolites.⁶ Reductions in NAA, only found in neurons, can be interpreted in terms of neuronal loss or dysfunction, and increases in Cho and Cr may be a reflection of astrocytosis.⁶

Proton MR spectroscopy has been shown to detect abnormalities in medial temporal lobes of medically intractable patients with temporal lobe epilepsy (reduced NAA/Cr ipsilateral to

the ictal EEG focus) with a normal MRI volumetric study of the hippocampus and a histopathology (after surgery) showing mild mesial temporal sclerosis (MTS).⁷

The purpose of this study was to evaluate abnormalities in metabolites in terms of NAA/Cr, NAA/Cho, and Cho/Cr in the medial temporal lobe ipsilateral to electrically defined focus (interictal scalp EEG) in 10 medically refractory patients, 12 seizure free patients with temporal lobe epilepsy, and 10 normal control subjects matched for age and sex.

This approach considers two questions: (1) whether the reduced NAA/Cr (correlated with mesial temporal sclerosis) in the medial temporal lobes is an exclusive or specific marker of medically refractory patients with temporal lobe epilepsy; (2) If reduced NAA/Cr is found in the medial temporal lobes of seizure free patients, whether these patients form a special subgroup in terms of their previous seizure control history.

Table 3 Summary of demographic data statistics from control subjects and from patients as a whole and as groups 1 and 2

	Age (y, mean (SEM))	Sex (M/F)	Duration of epilepsy (y, mean (SEM))
Control (n=10)	32.7 (16.8)	6/4	
Group 1 + 2	26.0 (11.9)	8/14	13.6 (20.0)
Group 1 (n=10)	23.6 (11.8)	4/6	13.1 (5.6)
Group 2 (n=12)	28.0 (12.2)	4/8	14.0 (12.0)

Group 1=patients with refractory temporal lobe epilepsy (patients 1–10); group 2=seizure-free patients with temporal lobe epilepsy (patients 11–22).

Figure 1 Coronal image showing the position of the 2×2 cm region selected for spectroscopy.

Patients and methods

PATIENTS AND CONTROLS

Thirty two subjects were studied using ¹H-MRS, including 10 medically refractory patients (group 1), 12 seizure free patients with complex partial epilepsy (group 2), and 10 age matched subjects as a control group.

All patients (or their close relatives) gave informed consent.

All patients met the following selection criteria: attendance of epilepsy clinic (Hospital S João) for at least the past 3 years; complex partial seizures of presumed clinical temporal lobe origin; consistently unilateral temporal EEG foci (at least three interictal EEG recordings); and a normal previous cranial MRI study. All patients had been seizure free at least 24 hours before the ¹H-MRS study. No ictal event occurred during the ¹H-MRS examination.

The demographic and clinical characteristics of these groups are shown in tables 1–3. The patients were included in group 1 because of a mean monthly seizure frequency higher than 1 and in group 2 when seizure free for the past 6 month period before ¹H-MRS study.

MRI STUDY

Previous independent routine cranial MRI investigations were performed using a conventional 1.5 Tesla imager (Signa) and interpreted by a neuroradiologist. The following images

Figure 2 Typical ^1H MR spectrum from a control subject. NAA=N-acetylaspartate; Cr=creatine+phosphocreatine; Cho=compounds containing choline.

were obtained: sagittal T1 weighted images, 5 mm thick, 1.5 mm gap, TR 600/ TE 10, axial T2 weighted images, 5 mm thick, 2 mm gap, TR 2500–3000/ TE 30–90, coronal T1 weighted images, 2 mm thick, 0 mm gap TR 17.2/ TE 4.2 and coronal T2 weighted images, 4 mm thick, 1 mm gap TR 2300–4000/ TE 126–133. No special protocol was used for optimised imaging of the mesial temporal region.

PROTON MRS STUDY

MRI and MRS were performed in a whole body 1.5 Tesla Magnetom (Siemens, Erlangen, Germany), using an imaging head coil and standard gradient system (10 mt/m maximum strength).

Spin echo T2 weighted images (TR=2100, TE=20, or 90 ms) in different planes, immediately preceded spectroscopy to locate the volume of interest (VOI). The automated calculation of grey and white matter volumes was not performed, because we lack the appropriate software. All images were acquired with a 5 mm thickness, 256×256 matrix, and a FOV of 230.

Localised proton MR spectra were obtained using STEAM sequences^{8,9} (TR=3000 ms;

TE=270), from aVOI measuring 2×2×2 cm centred on the medial portions of the temporal lobes, as shown in figure 1. Spectra were reconstructed with one dimensional Fourier transformation followed by first order phase correction. Baseline was also applied.

Signal intensities at 2.0 ppm (primarily due to N-acetylaspartate, NAA), 3.0 ppm (creatine and phosphocreatine, Cr), and 3.2 ppm (compounds containing choline, Cho) were measured from the peak areas by integration (fig 2). The intensity of the resonance of the total water proton from the 8 ml VOI was constant across all patients, as the integral of the non-suppressed water peak area did not differ among all volumes of interest.

Total examination time for MRI and ^1H -MRS was 60–90 minutes.

Data are presented in terms of NAA/Cr, NAA/Cho, and Cho/Cr.

STATISTICS

^1H -MRS data are expressed as mean (SEM). and a Mann-Whitney test was used for comparison between different groups of data. Continuous variables were correlated applying the Spearman rank test. Statistical significance was considered to be present for $p < 0.05$.

Results

CHARACTERISTICS OF PATIENTS AND NORMAL CONTROL SUBJECTS

Demographic and clinical data are shown in tables 1–3. The patients as a whole and as groups 1 and 2 did not differ significantly from control subjects in terms of age. Sex ratios (M:F) were 4:6, 4:8, and 6:4 respectively for groups 1, 2, and control subjects (table 3). Groups 1 and 2 had a similar mean duration of epilepsy of 13.1 years and 14.0 years respectively, as presented in table 3.

MAGNETIC RESONANCE SPECTROSCOPY FINDINGS

^1H MRS spectra were obtained from the medial temporal lobes ipsilateral to EEG foci in the 22 patients with temporal lobe epilepsy and in 13 medial temporal lobes in the 10 control subjects (in three subjects, both temporal lobes were chosen for ^1H -MRS).

EEG and ^1H -MRS data from all patients are shown in table 4.

Table 5 presents the statistical analysis of the ^1H -MRS data from control subjects and from patients as a whole and divided into groups 1 and 2. The mean NAA/Cr (SEM) from group 1 was 1.65 (0.53) and differed significantly from that of group 2 ($p < 0.002$) showing a mean NAA/Cr ratio of 2.62 (0.60) and from control subjects ($p < 0.006$) with a mean NAA/Cr ratio of 2.68 (0.73) as shown in fig 3.

The mean (SEM) NAA/Cho ratio was also found to be significantly reduced (1.59 (0.79) in comparison with that of seizure free (2.83 (1.33); $p < 0.02$) and normal controls (2.58 (0.67); $p < 0.007$). The Cho/Cr ratios did not differ significantly among all the groups.

Two patients (17 and 18 from tables 2 and 4) in the seizure free group had the two lowest NAA/Cr ratios: 1.47 and 1.73 respectively. Both figures were close to the NAA/Cr ratio

Table 4 EEG, and ^1H -MRS data

Patient No	EEG	^1H -MRS ipsilateral metabolite ratios		
		NAA/Cr	NAA/Cho	Cho/Cr
1	LT	2.45	3.06	0.80
2	LT	1.06	0.50	2.16
3	LT	1.34	1.47	0.91
4	LT	1.11	0.45	2.45
5	LT	1.18	1.44	0.82
6	RT	1.20	1.50	0.80
7	RT	1.73	1.49	1.17
8	RT	2.00	1.86	1.09
9	RT	2.14	1.67	1.29
10	RT	2.26	2.49	0.91
11	LT	2.50	2.55	0.98
12	LT	2.50	2.50	1.00
13	LT	2.61	4.50	0.58
14	LT	2.72	4.36	0.62
15	LT	2.79	3.28	0.85
16	LT	3.03	3.72	0.81
17	RT	1.47	1.57	0.94
18	RT	1.73	1.61	1.07
19	RT	2.30	1.63	1.38
20	RT	3.00	4.50	0.67
21	RT	3.17	2.71	1.17
22	RT	3.66	3.70	0.99

EEG=electroencephalogram; ^1H -MRS=proton magnetic resonance spectroscopy; NAA=nacetylaspartate; Cr=creatine+phosphocreatine; Cho=compounds containing choline; LT=left temporal lobe; RT=right temporal lobe.

Table 5 Metabolite ratios for normal controls and patients: statistics

	NAA/Cr (mean (SEM))	NAA/Cho (mean (SEM))
Group 1	1.65 (0.53)	1.59 (0.79)
Group 2	2.62 (0.60)	2.83 (1.33)
Control	2.68 (0.73)	2.58 (0.67)
Group 1 v control		
Difference	-1.03	-0.99
p Value	< 0.006	< 0.007
Group 1 v group 2		
Difference	-0.97	-1.24
p Value	< 0.002	< 0.02
Group 2 v control		
Difference	-0.06	+0.25
p Value	> 0.05	> 0.05

Group 1=medically refractory patients; Group 2=seizure free patients.

Figure 3 Temporal NAA/Cr ratios among all patients with temporal lobe epilepsy and normal controls. NAA=N-acetylaspartate; Cr=creatine+phosphocreatine.

mean obtained for refractory patients with temporal lobe epilepsy. In this group, two patients (1 and 8 from tables 1 and 4) had high NAA/Cr ratios and were patients with two of the poorest seizure control conditions.

No correlation between the time elapsed from the last seizure and the NAA/Cr ratio was found.

Discussion

¹H-MRS is an emergent in vivo technique that allows us to obtain neurochemical/metabolite information in a selected volume of brain (a *metabolic biopsy*) relevant for the investigation of several neurological disorders, including temporal lobe epilepsy.^{10 11}

Our study using single volume ¹H-MRS in the metabolite investigation of patients with complex partial seizures showed a significant reduction in NAA/Cr and NAA/Cho signal ratios from the temporal lobe ipsilateral to the EEG focus of patients with complex partial seizures uncontrolled by medication compared with patients seizure free for a considerable time and normal control subjects. However, no definite conclusion can be drawn from these preliminary findings as there were few patients in the study but we are including new patients in our current ¹H-MRS studies. Otherwise, we have spectra from the two temporal lobes in only three normal subjects, our first three healthy volunteers; this methodological option, considered by us to have no practical implication, is supported by consistent absence of side

differences between the temporal lobes in control subjects for NAA/metabolites values reported by several authors, either using, as we did, single volume ¹H-MRS⁵ or multiple volume ¹H-MRS,^{4 12} and is explained by the difficulties in obtaining the cooperation of the volunteers for acquisition of the two spectra, particularly in view of the long duration of the ¹H MRS experiment.

Keeping in mind those considerations, we suggest, in this preliminary study, that the abnormal NAA/Cr (or Cho) ratio will potentially provide a useful indicator of a poor seizure control condition in patients with temporal lobe epilepsy. Several relevant publications^{3-5 7} suggest that loss of the NAA signal is consistent with selective neuronal loss or damage and increase in Cho and Cr may reflect reactive astrogliosis (mesial temporal sclerosis), even when MRI volumetric studies show normal hippocampi.⁷ Focal NAA reductions have been shown to be consistent with pathology finding of mesial temporal sclerosis in the surgically resected epileptogenic foci.

¹H-MRS will eventually prove useful in the investigation of intractable temporal lobe epilepsy, by providing presurgical information on the lateralisation/localisation of intractable epileptogenic foci.

Another issue that has been considered in the recent literature^{13 14} is whether and to what extent, if any, the level of NAA/metabolite ratios correlates with the frequency of seizures over the past life of the patient. One piece of evidence, although based on a small sample of patients studied, suggested that the degree of reduction of NAA/Cr did not correlate with the frequency of seizures over the years preceding the MRS examination.¹³ This study is in agreement with MRI volumetric studies of the hippocampus, which indicated that repeated seizures throughout life did not seem to cause an increased measurable atrophy of the hippocampus. The atrophy shown by volumetric study showed a good correlation with neuronal loss in the postoperative histopathology.¹⁴ Our own findings showed that the degree of NAA/Cr reduction did not correlate with the time elapsed from the last seizure and therefore to the related seizure frequency; moreover, two of our patients (patients 1 and 8 from tables 1 and 4) with a high average number of seizures a month had high NAA/Cr ratios. All these findings support the hypothesis that the abnormalities in the NAA, Cr, and Cho signals do not result only from the seizures.

Two patients (17 and 18 from tables 2 and 4) in the seizure free group were found to have low NAA/Cr ratios similar to the mean value (1.56) shown by the refractory group and an underlying mesial temporal sclerosis may be speculated for those two patients. This comment is of particular relevance when considering that those patients have, contrasting with all the others in the seizure free group, a 2 year period in the past of poor seizure control. However, these results need to be confirmed in a larger series.

In conclusion, our proton MRS findings show that a significantly reduced NAA/Cr and

NAA/Cho ratios in temporal lobes ipsilateral to EEG foci are associated with a poor seizure control at some time in the life of patients with temporal lobe epilepsy, whether the patient has his seizures uncontrolled or is seizure free at the time of the ¹H-MRS study. Therefore the abnormal NAA ratios of the temporal lobes, that have been shown to correlate with mesial temporal sclerosis on postoperative histopathology,^{3-5,7} do not indicate an absolute inability to achieve a good seizure control.

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