Magnetic Resonance Spectroscopic Imaging in Temporal Lobe Epilepsy

Neuronal Dysfunction or Cell Loss?

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Background: Magnetic resonance spectroscopy (MRS) has demonstrated consistent metabolic abnormalities in temporal lobe epilepsy. The reason for decreases in N-acetylated compounds are thought to be related to neuronal hippocampal cell loss as observed in hippocampal sclerosis. However, mounting evidence suggest that the N-acetylated compound decreases may be functional and reversible.

Objective: To establish whether the metabolic changes measured by MRS correlate to hippocampal cell loss in temporal lobe epilepsy.

Subjects and Methods: We prospectively performed quantitative hippocampal MR imaging volumetry and MRS imaging in 33 patients with intractable mesial temporal lobe epilepsy who were undergoing surgery. A neuronal-glial ratio of cornu ammonis and fascia dentata was obtained and correlated while validating the pathologic analysis by comparisons with specimens of age-matched autopsy control-case hippocampus (n=14).

Results: The neuronal-glia ratio of the patient group was statistically significantly lower than in the control group for the cornu ammonis region (P<.001). Correlations of hippocampal volumes with cornu ammonis and neuronal-glia ratio revealed a significant interdependence (P<.01). However, correlations of the resected hippocampal creatine–N-acetylated compound ratio with the cornu ammonis or fascia dentata neuronal-glia ratio showed no significant interdependence (P>.8).

Conclusions: Our findings support the concept that the metabolic dysfunction measured by MRS imaging and the hippocampal volume loss detected by MR imaging volumetry do not have the same neuropathologic basis. These findings suggest that the MRS imaging metabolic measures reflect neuronal and glial dysfunction rather than neuronal cell loss as previously assumed.

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Hippocampal sclerosis (HS) is characterized by neuronal cell loss and astrogliosis in the cornu ammonis (CA) with relative sparing of CA2 area and fascia dentata. The presence of this pathologic entity often correlates with a typical electroclinical syndrome of medial temporal lobe epilepsy and an excellent surgical outcome.

Previous studies have indicated that HS can be diagnosed preoperatively using magnetic resonance imaging (MRI). Hippocampal sclerosis is characterized by the presence of atrophy and abnormal signal intensity from the hippocampus. The MRI-pathologic correlations have shown a direct relationship between hippocampal neuronal cell loss in the CA regions and atrophy measured using MRI volumetry. In contrast, correlative studies with functional imaging techniques such as positron emission tomography have shown a less definitive relationship. A more recent MR technique, proton MR spectroscopy (MRS) has been applied to patients with temporal lobe epilepsy. Studies have demonstrated metabolic abnormalities localized to the epileptogenic temporal lobe. The main metabolite peaks measured with proton MRS are those of N-acetylated compounds (NAs), creatine (Cr), and choline. Studies have demonstrated lower NA concentration or abnormal NA/(choline+Cr) ratios in the epileptogenic areas. Because NAs are primarily localized in neurons, it has been hypothesized that the decrease in NA is a direct reflection of hippocampal neuronal cell loss as present in HS.

In this study we sought to establish the relationship between MRS measurements of the hippocampus obtained preoperatively and the histopathologic findings analyzed quantitatively in patients...
SUBJECTS AND METHODS

SUBJECTS

We prospectively investigated 40 consecutive patients (24 women and 16 men; mean age, 35 years; age range, 18-45 years) who underwent temporal lobectomy for intractable mesial temporal lobe epilepsy at the University of Alabama at Birmingham Epilepsy Center. All patients had pathologic confirmation of mesial temporal epilepsy by qualitative analysis according to established criteria. Patients with other types of lesions such as vascular malformations or dual pathology were excluded from this study. The epileptogenic temporal lobe was identified by prolonged interictal scalp electroencephalogram, ictal electroencephalogram video monitoring with sphenoidal electrodes, and neuropsychological studies in all patients. Nine patients underwent bilateral temporal intracranial electroencephalographic studies prior to resection to confirm the location of the epileptogenic focus in view of conflicting preoperative data.

MRI STUDIES

Magnetic resonance imaging studies were performed using a standard protocol in a 1.5-T unit (Phillips ACS, Best, the Netherlands). All patients and controls were studied using the same imaging protocol. The control population for the MRI volumetric studies consisted of 17 normal volunteers (9 women and 8 men; mean age, 35 years; age range, 23-50 years). For volumetric studies, a volume acquisition (3-dimensional) of the entire brain was acquired using a T1-weighted sequence with an echo time of 6.1 milliseconds, a repetition time of 20 milliseconds, a field of view of 23 cm, and a flip angle of 28° in a plane perpendicular to the long axis of the hippocampus (1.5-mm-thick slices, 0 slice gap).

Volumetric measurements were performed on the 3-dimensional images using an interactive measuring tool (Gyroview; Phillips ACS). Anatomical guidelines for outlining the hippocampal formation followed previously published methods. Individual variance of the volume of the structure of interest was corrected in every subject according to previous techniques using total intracranial volume. Using this protocol, the raters have an intraobserver test-retest coefficient variation of less than 3%. The interrater variability in our laboratory is 3.1%. Abnormal volumes were defined as 2 SDs below the mean for the control subjects for normalized data.

MRS IMAGING STUDIES AT 4.1 T

All studies were performed prior to surgical implantation or resection and with the approval of the institutional review board. Magnetic resonance spectroscopic imaging (MRSI) data were acquired using a 4.1-T whole body imaging–spectroscopy system and quadrature-driven tunable matchable head coil. Sagittal scout images were acquired using a segmented (8 encodes per inversion pulse) inversion recovery gradient echo sequence (repetition time/inversion recovery delay/echo time, 2500/1000/15 milliseconds). We used the nonselective water signal for whole head shimming to higher than 20 Hz (0.11 ppm at 175 MHz). Further shimming on the MRSI slice achieved 10 to 15 Hz (0.06-0.09 ppm) for water. Contributions from extracerebral lipids were suppressed using an adiabatic inversion pulse followed by dephasing gradients and an inversion recovery delay optimized to suppress lipids (265 milliseconds).

Data were acquired using a repetition time/inversion recovery delay/echo time of 2000/265/50 to 58 milliseconds. The spectroscopic image was acquired using a field of view of 240×240 mm using 32×32-phase encodes with a 1-cm slice thickness. Signal intensity data were 0 filled to 64×64 and filtered using a one-quarter cosine filter in the spatial domain. Nominal voxel size is 1 cc. The spectral domain was processed using a convolution difference of 50 Hz to eliminate broad water components followed by 3 Hz of exponential broadening. Two spatial and 1 spectral Fourier transforms were then performed to generate the 2-dimensional MRSI. Details of the MRSI technique have been previously published.

METABOLITE ANALYSIS

Using the scout anatomical image, a rectangular region of interest was selected that included the midbrain, the hippocampus, and portions of the temporal lobe (both anterior and posterior to the hippocampus). The region of interest (ROI) encompassed approximately 400 to 600 of the 0-filled voxels. The spectra within each voxel were then averaged. Continued on next page

with mesial temporal lobe epilepsy. We also correlated the neuropathologic findings with MRI volumetric measurements of the resected hippocampus while validating the pathologic analysis by comparisons with specimens of age-matched autopsy control cases.

RESULTS

QUANTITATIVE NEUROPATHOLOGIC STUDY

Thirty-two of 33 specimens had evidence of HS (Table). One patient showed evidence of end-folium sclerosis. The N/G ratio was compared between patients and controls using the general linear model procedure (Figure 1). The patient group had a significantly lower N/G ratio than the control group for the CA region in both the first and second hippocampal samples examined (P<.001). In addition, a statistically significant difference between controls and patients was found for the N/G ratio of the FD (P<.001).

CORRELATIONS OF QUANTITATIVE NEUROPATHOLOGIC RESULTS AND MRI VOLUMETRY

Correlations of hippocampal volumes with the CA N/G ratio revealed a statistically significant interdependence (r=0.465, P<.01) (Figure 2A). The correlation between hippocampal volumes and FD N/G ratio, however, was not statistically significant (r=0.012, P<.9).
corrected for B0 shifts by setting the maximum resonance intensity in the vicinity (30 Hz) of the NA resonance (as predicted by the whole slice water resonance position) to 2.02 ppm. All voxels within the ROI were then analyzed using NMR1 software (Tripos, St Louis, Mo) and the line width, resonance area, and chemical shift were determined. From these data the Cr/NA ratios were determined for all pixels.

Typical metabolite ratios for 20 healthy control subjects observed for the structures seen within the ROI were determined from data acquired from the healthy volunteers. The appropriate volumes were chosen using the scout image for anatomical reference. Regional metabolite Cr/NA ratios were determined from the resonance areas of NA and Cr peaks.

Data from the patients were collected prior to surgical resection and analyzed without knowledge of the clinical, electroencephalographic, and MRI data. To visualize those regions that displayed statistically significant differences from normal data, all voxels within the selected ROI were analyzed as described previously in detail.17 Regions of interest showing significant increases in Cr/NA ratios, more than 2 SDs exceeding the normal 95% confidence interval, higher than any of the voxels measured from the healthy volunteers were highlighted and overlayed on the anatomical image. Details of the methods have been described previously.17,30

SURGERY AND PATHOLOGIC STUDY

All temporal lobe resections were performed by the same neurosurgeon (R.M.) using a standard technique. A modified temporal resection is done at our institution. The resection includes removal of the uncus, amygdala, and anterior 2.5 to 3.0 cm of the anterior hippocampus. Representative tissue samples of the hippocampus were routinely obtained for analysis. Specimens were fixed in formalin and embedded in paraffin. Histologic sections of the hippocampus and temporal lobe were stained with hematoxylin-eosin and glial fibrillary acidic protein. The neuropathologist (C.P.) selected and quantified cells in an ROI. The appropriate volumes were chosen using the scout image for anatomical reference. Regional metabolite Cr/NA ratios were determined from the resonance areas of NA and Cr peaks.

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QUANTITATIVE HIPPOCAMPAL CELL COUNTS

Cell counts were accomplished by 6-µm-thick paraffin sections stained with hematoxylin-eosin and glial fibrillary acidic protein. Slides were viewed with a microscope equipped with a videocamera interfaced to a computer containing proprietary image analysis software designed at the University of Alabama at Birmingham. The cell counter is a custom-made, object-oriented technology enabling the identification and quantification of selected cells. The neuropathologist (C.P.) selected and quantified cells in an ROI. The software does not permit counting of the same cells more than once. All cells in an identified ×40 high-power-field window were counted using the computerized method described herein earlier. Individual cells were selected by the neuropathologist enabling elimination of microglial and endothelial cells. In the CA regions, neurons were identified as large, pyramidal-shaped cells with a nucleus and nucleolus. Glial cells were defined by nuclear cytoplasmic characteristics and glial fibrillary acidic protein–positive staining. Granule cells in the fascia dentata (FD) were identified by anatomical location and by being round with small nuclei and stippled chromatin. Neuronal and glial cell counts were obtained separately and a neuronal-glial (N/G) ratio was calculated.

An N/G ratio was determined for CA though it is likely that CA1 was the most sampled section of the hippocampus owing to the recognition of the adjacent subiculum in most cases and the presence of typical pyramidal-shaped cells of CA1. To increase reliability, a second CA sample from each specimen was analyzed using identical methods. In addition, samples from the FD were identified and examined for neurons and glial cells. Cells in the FD were counted in a similar fashion to CA using 2 separate samples from each patient to increase the reliability of the data. Hippocampal control data were obtained from 14 individuals who died from nonneurological causes. The autopsy control group was age- and sex-matched according to the patient distribution. Tissue processing, staining, and cell counts followed the same protocol as described previously herein.

The N/G ratios in the control group were obtained from 30 patients who died from nonneurological causes. The autopsy control group was age- and sex-matched according to the patient distribution. Tissue processing, staining, and cell counts followed the same protocol as described previously herein.

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These findings indicate that hippocampal atrophy computed by volumetry correlates with hippocampal CA N/G ratios in the resected hippocampus of patients with temporal lobe epilepsy. However, the metabolic measurements obtained with MRSI do not correlate with the same hippocampal N/G ratios. These findings suggest that the MRS metabolic measures reflect neuronal and glial dysfunction rather than absolute neuronal cell loss as previously assumed.

Previous MRI-based studies have reported a good correlation between neuronal density cell loss and hippocampal volumes. Cascino et al16 showed a correlation between CA1 and CA3 cell loss with MRI-based volume...
loss. Another study using quantitative neuronal and glial cell counts found a correlation between CA1 and CA4 cell loss and hippocampal atrophy by volumetry.\textsuperscript{13} However, hippocampal volumes did not correlate with granule cell density loss in the FD\textsuperscript{14}. Our results agree with the above studies\textsuperscript{8,13} demonstrating a correlation with CA but not with FD counts and indicate that hippocampal volumetry predominantly correlates with cell loss in the CA region of the hippocampus in patients with mesial temporal lobe sclerosis. Our results serve to validate the quantitative method used in our study for MRSI correlations.

To our knowledge, this is the first study correlating quantitative hippocampal histopathologic abnormalities and MRSI in patients with mesial temporal lobe epilepsy. The MRSI ratios did not correlate with the N/G ratios of the resected hippocampi in either CA or FD subregions. Our results disagree with the findings of Duc et al\textsuperscript{31} who reported a linear correlation between NA concentrations and HS. The study by Duc et al, however, included only a few patients (n=8) and it is likely that these data were influenced by restriction of range. More important, however, is the expected inaccuracy of their qualitative determination of HS that may have resulted in an apparent correlation.\textsuperscript{31}

A limitation of our study is the histologic variability present in CA subregions of the hippocampus in mesial temporal sclerosis and our inability to specifically perform the histologic measurements in one or more hippocampal subregions.\textsuperscript{13} It would be optimal to have the entire hippocampus for analysis, but in our center, as in most centers, en bloc resections are not routinely performed and, thus, the specimens are limited. However, we believe that the histologic sampling limitations do not invalidate our results for several reasons. First, although CA1, CA3, and CA4 regions are mostly affected in HS, CA2 also demonstrates statistically significant neuronal cell loss when compared with controls.\textsuperscript{32} Second, our histologic analysis was buttressed by sampling each CA and FD region twice and showing no difference between the 2 samples. Third, hippocampal N/G ratios demonstrated statistically significant differences between patients and controls. Finally, the pathologic findings are validated by the demonstration of statistically significant correlations with MRI-based volumetric atrophy.

Several studies have demonstrated that neuronal density is not a direct reflection of the total number of neuronal cells without reference to the volume in which the neurons are counted.\textsuperscript{33} To overcome this problem, we adopted a previously validated method of N/G ratios as a more reliable technique since it is independent of the total measured volume.\textsuperscript{33} The use of N/G ratios may be important for other reasons particularly when correlating with MRSI data. First, it has been shown that HS is not only characterized by neuronal cell loss but is also associated with reactive astrogliosis.\textsuperscript{1,13,32,34} Second, neuronal cell loss is coupled to decreases in NA concentrations while reactive astrogliaosis induces Cr level changes.\textsuperscript{21,35} Several studies have reported increases in Cr and choline levels ipsilateral to the temporal lobe fo-
noma,14 This could be secondary to the decreases in glucose metabolism do not correlate with the fascia dentata hippocampi N/G ratio (r = 0.485, P < .01). B, Linear regression and 95% confidence interval analysis of creatine-N-acetylated compounds (Cr/NAs) ratio and hippocampal CA N/G ratio. No correlation was present (r = −0.04, P < .8). MRS indicates magnetic resonance spectroscopic imaging.

Figure 2. A, Linear regression and 95% confidence interval analysis of magnetic resonance imaging (MRI) hippocampal volumes and the hippocampal cornu ammonis (CA) neuronal-glial (N/G) ratio for resected hippocampus. The hippocampal volumes positively correlated with the CA N/G ratios but not with the fascia dentata hippocampi N/G ratio (r = 0.485, P < .01). B, Linear regression and 95% confidence interval analysis of creatine-N-acetylated compounds (Cr/NAs) ratio and hippocampal CA N/G ratio. No correlation was present (r = −0.04, P < .8). MRS indicates magnetic resonance spectroscopic imaging.

Thus, we elected to use a histologic ratio that is independent of volume and is intrinsically better suited for MRSI-derived metabolic correlations.

Several animal and human studies support our findings that the metabolic ratios measured by MRSI do not directly reflect neuronal cell loss in epileptic tissue. First, data derived from volumetric-MRSI correlations have concluded that both techniques are lateralizing and concordant but that the degree of metabolic change does not directly correlate with hippocampal volume loss.30 Second, positron emission tomography studies suggest that the decreases in glucose metabolism do not directly correlate with CA1 neuronal cell loss.14 This could be secondary to the fact that hypometabolism in positron emission tomography reflects synaptic density changes leading to hypometabolism rather than cell loss. Third, findings from recent correlative studies suggest that hypometabolism measured by positron emission tomography and MRS changes do not directly correlate.36,37 Even more relevant to the results presented here, however, are MRS data suggesting that the metabolic abnormalities may reflect an N/G ratio dysfunction rather than neuronal cell loss. A study reported metabolic abnormalities in regions that showed no significant neuronal cell density or N/G ratio differences when compared with control brain samples in patients with epileptogenic developmental malformations.38 Furthermore, several studies have indicated that after cessation of seizures, MRSI metabolism normalizes in the peri-ictal region or in contralateral regions such as the hippocampus.39,40 Finally, animal experiments suggest that NA changes are detected prior to neuronal cell loss and reflect metabolic changes that can be reversible.41 Together, the lack of a direct relationship between the histopathologic findings and MRSI values and the results of previous studies strongly support the functional nature of the proton MRS metabolic changes in epilepsy.

Our findings support the concept that the metabolic dysfunction measured by MRS and the hippocampal volume loss detected by MRI volumetry do not have the same neuropathologic basis. These observations support the use of proton MRSI not only as a functional imaging technique that provides lateralization in epilepsy but also as a tool that provides further information about the status of brain function.

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