Regional N-Acetylaspartate Reduction in the Hippocampus Detected With Fast Proton Magnetic Resonance Spectroscopic Imaging in Patients With Alzheimer Disease

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Objective: To detect regional metabolic changes that resemble the expected spatial pattern of neuronal loss in patients with Alzheimer disease (AD).

Methods: Thirty-four patients with AD and 22 healthy control subjects were included in the study. Single-slice fast proton spectroscopic imaging was performed in parallel an- gulation to the temporal lobes. Proton spectra were selected from the hippocampus, the lateral temporal lobe, and the occipital lobe of both hemispheres to determine metabolite concentration of N-acetylaspartate (NAA), total creatine (tCr), including phosphocreatine and creatine, and choline-containing compounds (Cho). The metabolic ratios of NAA/tCr and Cho/tCr were calculated and compared between patients with AD and healthy volunteers.

Results: The NAA/tCr ratios were significantly reduced in the left (F1,1 =4.34, P =.04) and right hippocampus (F1,1 =9.96, P =.003) in patients with AD. The Cho/tCr ratios remained unchanged in both hippocampi. There was no significant change of either NAA/tCr or Cho/tCr in the lateral temporal and occipital lobes of patients with AD.

Conclusion: This study provides evidence that fast proton spectroscopic imaging may detect the regional pattern of disturbed neuronal integrity in patients with AD with high spatial resolution in a short acquisition time.

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PATIENTS, MATERIALS, AND METHODS

PATIENTS

Thirty-four patients (mean age, 70 years; SD, 8 years; 10 men, 24 women) who met the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders criteria for probable AD and 22 healthy subjects (mean age, 69 years; SD, 9 years; 12 men, 10 women) were included in the study. The Mini-Mental State Examination (MMSE)34 and the cognitive part of the AD Assessment Scale (ADAScog)35 were applied for disease staging. The MMSE scores range from 0 to 30 points, with 30 points being the best score, whereas the ADAScog scores range from 0 to 70 points, with 70 points expressing most severe cognitive deficits.

At the time of the MRS examination, mean ± SD scores for patients with AD were 20.1 ± 4.5 (range 10-27) on the MMSE and 23.6 ± 12.8 (range, 8-36) on the ADAScog. The control subjects underwent the same neuropsychological examination to ensure normal cognitive functioning (MMSE score, 28.6 ± 2.1; ADAScog score, 7.2 ± 1.6). The study protocol was approved by the ethics committee of the University of Bonn and is in accordance with the Declaration of Helsinki. All patients and control subjects gave informed consent before participation.

MAGNETIC RESONANCE EXAMINATIONS

Magnetic resonance investigations were performed on 1.5-T whole-body magnetic resonance system Gyroscan (ACSN TPT2000; Philips Medical Systems, Best, the Netherlands) using a mirror head coil suited for magnetic resonance imaging and fast spectroscopic imaging. Coronal T2-weighted turbo spin-echo sequences with repetition time (TR)/TE of 2700/120 milliseconds, axial proton density and T2-weighted spin-echo sequences with TR/TE1, TE2 of 2400/20, 90 milliseconds, and axial, sagittal, and coronal T1-weighted spin-echo sequences with TR/TE of 300/15 milliseconds were obtained for image-guided localization of the single-slice turbo spectroscopic imaging (TSI).

An axial slice 2 cm thick on the hippocampal level was selected for the TSI acquisition, and a field of view of 20 to 22 cm was covered by 32 × 32 phase encoding steps. Lipid signals from the skull base and from the retro-orbital space were eliminated by polygonal outer volume presaturation36 using 10 MREST (multiple regional saturation technique) slabs. This scheme was combined with slice-selective 90° excitation, resulting in a volume of interest with contours matching the frontal, occipital, and lateral borders of the temporal lobe (Figure 1A-B). A TE of 272 milliseconds was chosen for further reduction of lipid artifacts and for in-phase detection of lactate signals. With a TR of 2000 milliseconds and with 3 phase-encoded echoes per excitation (turbo factor, 3), the acquisition took 11 minutes, including a non–water-suppressed data set for susceptibility shift correction.30 With symmetric echo registration within an acquisition interval of 250 milliseconds, a frequency resolution of 4 Hz was obtained. Signal processing of the TSI data set included Lorentz-Gauss spectral and cosine spatial filtering, susceptibility correction, zero filling, and digital shift subtraction for improved water suppression. Metabolic maps displaying the local concentrations of NAA (Figure 1C), Cho, tCr, and lactate were calculated by gray-level encoding of the respective peak integrals in the B0-corrected TSI spectra (B0 indicates the main magnetic field). Metabolite ratios NAA/tCr and Cho/tCr were determined in TSI spectra from voxels selected in the hippocampal, lateral temporal, and occipital region of both hemispheres (Figure 1D). To increase quantification accuracy, a representative spectrum with improved signal-to-noise ratio was generated for each region by averaging the signals from 6 adjacent 1-ml voxels by time-domain superposition (Figure 2). Metabolite ratios for these cerebral areas were compared with the values obtained from healthy controls using the same acquisition and processing protocol.

STATISTICAL ANALYSIS

Group comparisons of metabolic ratios were performed using a 2-factorial analysis of variance (diagnosis and sex). Even though age did not differ significantly between groups (t=7.48, P=.46), it was included as a covariate because of recent reports of age effects on the metabolic ratios in various brain regions.37 Correlations of MMSE and ADAScog scores with metabolite ratios were evaluated using Spearman rank correlation. Calculations were performed with the SPSS 9.0 computer software package (SPSS Inc, Chicago, Ill).
pect a difference between the patients and the control group in the occipital lobe. Finally, we expected a correlation of the degree of NAA reduction with disease severity.

RESULTS

Data sets of 31 patients and 19 volunteers were of sufficient quality to be included in the statistical analysis. In some of these cases, however, the TSI spectra of certain regions, which are particularly sensitive to susceptibility artifacts (eg, the anterior parts of the temporal lobe slice), had to be discarded because of extensive line broadening. In 3 patients with AD and 3 controls, the entire data set had to be discarded because of extensive movement of the subject during the TSI acquisition. However, there was no relationship between the severity of dementia and the technical success rate of the applied technique. The number of cases used for the group comparison is given in the Table for each region.

Analysis of TSI spectra revealed a significant reduction of NAA/tCr in the left ($F_{1,1}=4.34, P=.04$) and right hippocampus ($F_{1,1}=9.96, P=.003$) in the patients with Alzheimer disease. The N-acetylaspartate metabolite image, interpolated to $256 \times 256$ matrix, with overlay of brain contour taken from T2-weighted turbo spin-echo magnetic resonance image (B). D, Anatomic T2-weighted magnetic resonance image with the position of selected TSI spectra indicated by squares. Numbers 1 through 6 indicate hippocampal spectra; 7 through 12, lateral temporal spectra; and 13 through 18, occipital spectra.

Figure 1. Acquisition of a turbo spectroscopic imaging (TSI) data set from a 71-year-old man with Alzheimer disease. A, Image-guided selection for single-slice TSI with polygonal outer volume suppression using 10 presaturation slabs (hatched areas with vertical lines, center of each slab indicated by a circle) displayed on a sagittal T1-weighted spin-echo magnetic resonance image. Selected slice is positioned parallel to the temporal lobe and includes both hippocampi. B, Same slice selection displayed on an axial T2-weighted turbo spin-echo magnetic resonance image. C, N-acetylaspartate metabolite image, interpolated to $256 \times 256$ matrix, with overlay of brain contour taken from T2-weighted turbo spin-echo magnetic resonance image (B). D, Anatomic T2-weighted magnetic resonance image with the position of selected TSI spectra indicated by squares. Numbers 1 through 6 indicate hippocampal spectra; 7 through 12, lateral temporal spectra; and 13 through 18, occipital spectra.
AD compared with healthy control subjects. Mean NAA/tCr of patients with AD was reduced by 11% in the left hippocampus and 16% in the right hippocampus. No significant differences were found for Cho/tCr ratios calculated from spectra selected in both hippocampi. Group comparison of metabolite ratios determined for the occipital and lateral parts of the temporal lobes yielded no significant differences between patients with AD and controls (Figure 3). There were no age or sex effects on any metabolite ratio, and there were no significant cor-

Figure 2. Spectra selected from the processed turbo spectroscopic imaging data set of the same patient as in Figure 1. Left column shows proton spectra (corresponding to the outlined numbers in Figure 1D) selected from the hippocampal, the lateral temporal, and the occipital regions. Right column shows the summation of these spectra for each region. NAA indicates N-acetylaspartate; Cho, choline-containing compounds; and tCr, total creatine, including phosphocreatine and creatine. Metabolic ratios (NAA/tCr and Cho/tCr) calculated from the summation spectra are as follows: hippocampal, 2.08 and 1.21; lateral temporal, 4.37 and 1.32; and occipital, 4.27 and 0.95.
relations between any metabolite ratio and the MMSE or ADAScog scores. Means and SDs of all metabolite ratios are summarized in the Table.

The main finding of this study is a significant decrease of NAA/tCr in the left and right hippocampi of patients with AD compared with healthy control subjects. The expected reduction of NAA/tCr in the lateral temporal lobe did not reach significance, and there was no significant change of NAA/tCr in the occipital lobes. Since the metabolite ratio NAA/tCr reflects neuronal integrity, this pattern mirrors the neuropathologic features of mild-to-moderate AD. The results of this metabolic mapping study of a temporally angulated entire brain section are in agreement with other MRS studies that reported a reduction of NAA in the hippocampus of patients with AD. The present approach, however, extends previous reports by several points. In addition to the study by Schuff et al,19 which only reported NAA alterations in the hippocampus, the present study also included unaffected control regions, which allows the detection of the specific pattern of neuronal damage in AD. The approach of including an unaffected control region was already presented in recent studies by our group,29,38 which investigated the medial temporal lobe and the precentral and postcentral region. That study, however, used single-voxel MRS with limited spatial resolution, thus including the hippocampus plus adjacent nontarget tissue in the voxel of interest. In the present study, the spatial resolution was improved by using MRS imaging, which allows the examination of the hippocampus and other areas in much greater detail and reduces partial volume effects of nontarget regions. Whether the inclusion of unaffected control regions will contribute to the discrimination of AD from other dementing disorders will have to be assessed in future studies.

Furthermore, we improved the acquisition technique compared with our earlier study6 by using a non-rectangular slice in temporal angulation, which covers larger regions of the tissue of interest, and by reducing the acquisition time for the entire slice from 30 to 11 minutes, which makes the examination much more feasible for patients with AD.

The present approach, however, also has limitations. Less than optimal B0-field homogeneity and contamination from lipid-containing structures, especially in the anterior parts of the temporal slice, affect the quality of spectra. The enhanced sensitivity of fast spectroscopic imaging to susceptibility artifacts associated with a decreased signal-to-noise ratio resulted in the failure to obtain valid spectra in some patients and control subjects. These limitations may also be the reason for the failure to detect a correlation of the NAA/tCr reduction with the cognitive dysfunction in the group of patients with AD. A further limitation of the present protocol is the inability of gray-white matter differentiation within single spectra. Because the white matter proportion is larger in the lateral temporal lobe than, for example, in

### Table: Regional Distribution of Metabolite Ratios for Patients With AD and Healthy Controls

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of Patients</th>
<th>Left Hemisphere</th>
<th>Right Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAA/tCr</td>
<td>Cho/tCr</td>
<td>NAA/tCr</td>
</tr>
<tr>
<td><strong>Hippocampal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>22</td>
<td>2.33 (0.49)†</td>
<td>1.22 (0.24)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>2.25 (1.43-3.21)</td>
<td>1.22 (0.64-1.71)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>2.61 (0.45)</td>
<td>1.26 (0.29)</td>
<td>13</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>2.54 (1.80-3.47)</td>
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<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>4.34, .04</td>
<td>0.33, .57</td>
<td>9.96, .001</td>
</tr>
<tr>
<td><strong>Lateral temporal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>21</td>
<td>3.20 (0.49)</td>
<td>1.28 (0.26)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.24 (2.26-3.99)</td>
<td>1.23 (0.75-1.90)</td>
<td></td>
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<tr>
<td>Median (range)</td>
<td>3.26 (0.60)</td>
<td>1.42 (0.25)</td>
<td>15</td>
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<tr>
<td>Mean (SD)</td>
<td>3.40 (2.29-3.93)</td>
<td>1.33 (1.06-1.89)</td>
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<tr>
<td>Median (range)</td>
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<td>0.93,.34</td>
<td>0.68,.42</td>
</tr>
<tr>
<td><strong>Occipital</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>31</td>
<td>3.28 (0.48)</td>
<td>0.95 (0.17)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.21 (2.40-4.43)</td>
<td>0.96 (0.60-1.24)</td>
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<tr>
<td>Median (range)</td>
<td>3.34 (0.39)</td>
<td>0.97 (0.18)</td>
<td>19</td>
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<tr>
<td>Mean (SD)</td>
<td>3.39 (2.41-3.88)</td>
<td>0.94 (0.77-1.37)</td>
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<tr>
<td>Median (range)</td>
<td>0.44,.51</td>
<td>0.00,.99</td>
<td>0.07,.79</td>
</tr>
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</table>

*AD indicates Alzheimer disease; NAA, N-acetylaspartate; tCr, total creatine, including phosphocreatine and creatine; and Cho, choline-containing compounds. P values refer to a 2-factorial analysis of variance (diagnosis and sex) with age as a covariate; significant values are in boldface.
†Significantly different from mean of controls.
the hippocampus, this would confound a direct comparison of these 2 regions within a single group of subjects. Therefore, each region was separately analyzed only with respect to group differences.

In summary, we present a metabolic mapping technique that covers the crucial brain regions in patients with AD in a short examination time. With this technique, we were able to detect a reduction of the neuronal marker NAA in both hippocampi but not in control areas in patients with AD. Future developments will have to focus on increasing the accuracy and stability of the method to recognize pathologic conditions not only by a group comparison but also on an individual patient scale in the diagnostic workup of dementia.

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Author contributions: Study concept and design (Drs Block, Jessen, Traber, Flacke, Keller, Heun, and Schild); acquisition of data (Drs Block, Jessen, Traber, Manka, Lamerichs, and Heun); analysis and interpretation of data (Drs Block, Jessen, Traber, Flacke, and Heun); drafting of the manuscript (Drs Block and Jessen); critical revision of the manuscript for important intellectual content (Drs Jessen, Traber, Flacke, Manka, Lamerichs, Keller, Heun, and

Figure 3. Box plots displaying the distribution (median, quartiles, and range, outliers as circles) of the metabolic ratios NAA/tCr and Cho/tCr acquired from the hippocampal, lateral temporal, and occipital regions (right hemisphere) of patients with Alzheimer disease (AD) compared with healthy controls. NAA indicates N-acetylaspartate; Cho, choline-containing compounds; and tCr, total creatine, including phosphocreatine and creatine.
REFERENCES


