Quantitative Proton MR Spectroscopy Findings in the Corpus Callosum of Patients with Schizophrenia Suggest Callosal Disconnection

BACKGROUND AND PURPOSE: The callosal disconnection theory was previously proposed to explain the pathophysiology of schizophrenia. The goal of this study was to investigate the metabolic integrity of the corpus callosum in patients with schizophrenia by proton MR spectroscopy.

MATERIALS AND METHODS: Twelve first-episode and 16 chronic patients meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia and 28 age- and sex-matched control subjects were enrolled in the study. We measured the absolute concentrations of neurochemicals and T2 relaxation times of tissue water (T2b) in the genu of the corpus callosum by using the internal water-reference method. The severity of symptoms in patients was rated by means of psychopathology scales. Differences in neurochemical concentrations and T2b values between the patients and control subjects were assessed. We also investigated the correlation of metabolite concentrations with the severity of symptoms.

RESULTS: N-acetylaspartate (NAA) concentrations were significantly lower in the first-episode as well as in chronic patients, compared with respective control subjects (P < .001). NAA concentrations in the first-episode and chronic patient groups were negatively correlated with both the Brief Psychiatry Rating Scale and the Scale for Assessment of Negative Symptoms scores (P < .001). There was a significant negative correlation between the NAA concentrations and the Scale for Assessment of Positive Symptoms scores in all patients (P = .028). T2b values were significantly higher in the patients, compared with the control subjects (P < .001).

CONCLUSION: Decreased NAA concentration in the corpus callosum correlates with psychopathology in schizophrenia. This finding, together with prolonged T2b values of the corpus callosum, supports the previously proposed callosal disconnection theory concerning the pathophysiology of schizophrenia.

The idea that schizophrenia might be a disorder of functional disconnection between the various regions of brain was first proposed by Wernicke in 1906. More recently, the disconnectivity theory has re-emerged in schizophrenia research. Today, there is increasing evidence pointing to the possibility of an abnormal cortical connectivity in the pathophysiology of schizophrenia. Diffusion tensor imaging (DTI) and magnetization transfer (MT) imaging studies have demonstrated the compromised cerebral white matter tracts in patients with schizophrenia. Altered magnetization transfer (MT) ratios in frontotemporal white matter tracts and the genu of the corpus callosum, which might indicate either an axonal or a myelin-related pathology in schizophrenia, were found in patients with schizophrenia in MT imaging studies. Supporting the results of these structural imaging studies, the genetic studies revealed the altered expression of myelin-related genes, suggesting a disruption in oligodendrocyte function in schizophrenia.

Crow further specified the disconnectivity theory and proposed that schizophrenia might be a disorder of transcallosal disconnection. The corpus callosum is the major interhemispheric white matter tract, which plays very important roles in cognitive processes such as language, memory, and sensory-motor integration. Interhemispheric transmission of information, which allows the functional lateralization of cerebral hemispheres, decreases in schizophrenia. Functional and structural asymmetry in cerebral hemispheres in patients with schizophrenia suggests the possibility of altered interhemispheric connectivity. There are several morphometric studies revealing controversial results on the size and shape differences of the corpus callosum in schizophrenia. In a meta-analysis, Woodruff et al reported a small but significant decrease in the midsagittal area of the corpus callosum. Studies investigating shape differences in the corpus callosum showed that patients with schizophrenia had a more curved corpus callosum. In addition, the corpus callosum was one of the common locations showing a reduced diffusion anisotropy and MT ratio in previous DTI and MT imaging studies investigating white matter integrity in patients with schizophrenia. Moreover, the structural MRI imaging studies conducted in patients with schizophrenia revealed altered signal-intensity changes and decreased attenuation in the corpus callosum.

Proton MR spectroscopy (1H-MR spectroscopy) is a noninvasive method to investigate neurochemical changes in various pathologic and physiologic conditions. 1H-MR spectroscopy can demonstrate subtle neuroaxonal dysfunction, even in normal-appearing cerebral tissue on conventional MR imaging and DTI. The goal of this study was to investigate the metabolic integrity of the corpus callosum in patients with schizophrenia and to test the "callosal disconnection theory" by using 1H-MR spectroscopy. We hypothesized that microstructural abnormalities in the corpus callosum of patients with schizophrenia might cause axonal dysfunction and lead
to changes in neurometabolite concentrations, which could be detected by quantitative $^1$H-MR spectroscopy. We sought to measure the absolute concentrations of major neurometabolites and T2 relaxation times of tissue water (T2$_w$) in the corpus callosum of patients with schizophrenia. Additionally, we investigated the relationship of neurometabolite concentrations with T2$_w$ values and the severity of symptoms. We studied the first-episode and relapsing chronic patients in acute phases of illness.

**Methods**

**Subjects**

We studied 28 inpatients (19 men and 9 women) with a diagnosis of schizophrenia after **Structured Clinical Interviews for DSM-IV Axis I Disorders (SCID)** data.
section thickness = 5 mm, intersection distance = 1.2 mm); 2) coro-
nal SE T1-weighted images (TR = 530 ms, TE = 30 ms, NEX = 2,
matrix = 196 × 256, section thickness = 3 mm, intersection dis-
tance = 1 mm); and 3) sagittal fast SE T2-weighted images (TR = 5790 ms, TE = 103 ms, NEX = 4, echo-train length = 16, matrix =
196 × 256, section thickness = 3 mm, intersection distance = 1 mm).

**1H-MR Spectroscopy: Data Acquisition and Signal-
Intensity Processing**

Single-voxel 1H-MR spectroscopy examinations of the patients and
control subjects were conducted in the same session with conven-
tional cranial MR imaging. On sagittal images, the corpus callosum
was divided into subregions as described by Witelson33 and Highley
et al14 (Fig 1A). Voxels were placed into the superior and posterior
genu of the corpus callosum by excluding CSF around the corpus
callosum (Fig 1B). The localization of both water-suppressed and
unsuppressed proton spectra were acquired by applying a stimulated
echo acquisition mode (STEAM, TR = 3.5 seconds) sequence. Water
suppression was performed with 3 chemical shift selective saturation
pulses at the water resonance. The number of acquisitions for water-
suppressed and unsuppressed spectra was 246 and 16, respectively. T2
values of metabolites and tissue water had to be calculated to correct
signal-intensity decays caused by T2 (transverse) relaxations. There-
fore, we obtained proton spectra with TE values of 30, 38, 48, 65, 84,
135, and 300 ms with water suppression. TE values for water-unsup-
pressed spectra were 30, 48, 84, 135, 300, 470, and 800 ms. Because T1
(longitudinal) relaxation does not affect the metabolite concentra-
tions measured with TR values above 3 seconds at 1.5T, a TR value of
3.5 seconds, without T1 correction, was used. Spectral postprocessing
included zero filling to 2048 points, an exponential filter correspond-
ing to 1 Hz of line broadening, Fourier transformation, zero order
phase, and automatic baseline corrections by polynomial interpola-
tion. Spectra with a full width at half maximum smaller than 0.1 ppm
were included in the statistical analysis.

Quantification of MR spectroscopy measurements and calcula-
tion of metabolite concentrations were performed by using the inter-
water reference method.33,34 Major metabolite peaks were assigned
to N-acetylaspartate (NAA) between 2.01 and 2.03 ppm, creatine (Cr)
between 3.01 and 3.03 ppm, and choline (Cho) between 3.21 ppm and
3.23 ppm. Calculation of T2 values and T2-corrected signal-intensity
peak areas for water-suppressed and unsuppressed spectra is an opti-
mization problem that involves minimizing the squared error be-
tween the actual values of the measurement and the estimated func-
tion values evaluated at measurement points (MatLab; MathWorks,
Natick, Mass). A monoexponential curve was fit to the metabolite
peak area values. A double-exponential curve-fitting was performed
for unsuppressed water spectra. CSF contamination was measured by
using the difference in T2 decay between the brain tissue and CSF. The
attenuation of brain tissue is accepted as $\rho = 1.04$ kg/L, and metabo-
lite concentrations were calculated in millimoles per kilogram
brain.36

Study-specific phantom tests for calibration measurements and
for testing the reproducibility of 1H-MR spectroscopy measurements
were performed every week. In the phantom studies, we used a spheric
phantom containing 0.1 mol/L/L sodium acetate and 0.1 mol/L/L
lactate. We measured acetate concentration with the internal water-
reference method by using the same TR and TE values. The variation
in the measured acetate concentration in phantom studies was below
7%.

**Statistical Analysis**

Statistical analyses of the results were performed by using SPSS 11.0
for Microsoft Windows (SPSS, Chicago, Ill). The differences in met-
abolite concentrations and T2B values between each patient and con-
trol group were investigated by analysis of variance. Correlation of
metabolite concentrations with psychopathology scale scores was ex-
amined by using regression analysis. The relationship between metab-
lite concentrations and the duration of illness in chronic patients was
assessed. Correlation between neurometabolite concentrations and
DUP in first-episode patients was also tested. We also investigated the
difference in metabolite concentrations between male and female pa-
tients by using the t test. The Bonferroni correction was applied for
multiple comparisons. In the analyses, a $P$ value of less than .05 was
considered to indicate a statistically significant difference.

**Results**

All the spectra obtained from the patients and control subjects
were deemed to be of good quality (Fig 2A–D). Moreover, the metabo-
lite concentrations measured in the control subjects were consistent with the metabolite concentrations reported in previous studies.35,36 Clinical findings and demographic characteristics of the patients and their control subjects are
presented in Table 1. There was no significant difference in the
SANS and SAPS scores between the first-episode and chronic
patients ($P > .05$). An uncorrected comparison test revealed
significantly higher BPRS scores in chronic patients than in
first-episode patients. However, the significance was lost after
correction for multiple comparisons ($P = .08$).

Mean NAA concentration in all patients (first-episode and
chronic patients together) was 8.91 ± 0.84 mmol/kg brain,
compared with a value of 10.40 ± 0.44 mmol/kg brain in con-
trol subjects (Table 2). The difference in NAA concentrations
between the patients and control subjects was statistically significant \((P < .001)\) (Fig 3). NAA concentration of all patients was negatively correlated with the BPRS \((r = -0.59, P = .001)\) and SANS scores \((r = -0.68, P < .001)\) (Fig 4A, -B). The association of NAA concentrations with SAPS scores was also statistically significant \((r = -0.41, P = .028; \text{Fig 4C})\).

In the first-episode patient group, the mean NAA concentration of patients was 8.97 ± 0.82 mmol/kg brain, compared with a mean value of 10.41 ± 0.45 mmol/kg brain in the respective control group (Table 2). The difference in NAA concentrations between the first-episode patient group and the respective control group was statistically significant \((P < .001)\). In the chronic patient group, the mean NAA concentration of the patients was 8.86 ± 0.89 mmol/kg brain, compared with a mean value of 10.40 ± 0.44 mmol/kg brain in the respective control group \((P < .001)\). The difference between the NAA concentrations of the first-episode and chronic patient groups was not statistically significant \((P = .66)\). In the first-episode patient group, the NAA concentrations of the patients were negatively correlated with the SANS \((r = -0.81, P = .001)\) and BPRS \((r = -0.67, P = .025)\) scores. However, there was no correlation between the NAA concentrations and SAPS scores in the first-episode patient group \((r = -0.27, P = .39)\). On the other hand, the NAA concentrations of patients in the chronic patient group were significantly associated with BPRS \((r = -0.60, P = .014)\) and SANS \((r = -0.76, P = .001)\) scores.

Conversely, there was no relationship between the NAA concentrations and SAPS scores \((r = -0.34, P = .18)\) in this group. In addition, there was no significant association between the NAA concentrations and DUP values in the first-episode patients \((r = -0.23, P = .47)\). The NAA concentrations of the chronic patients did not correlate with the duration of illness \((r = -0.03, P = .89)\).

Cr and Cho concentrations between first-episode and chronic patient groups and their respective control groups showed no significant difference \((P > .05)\) (Table 2). There was no relationship between both Cr and Cho concentrations and psychopathology scale scores \((P > .05)\). The Cr and Cho \((Cr: r = 0.26, P = .31; Cho: r = 0.26, P = .40)\) concentrations did not correlate with the duration of illness in the chronic patients. There was no association between the Cr and Cho \((Cr: r = 0.30, P = .33; Cho: r = 0.25, P = .42)\) concentrations and DUP in the first-episode patients. No sex predilection was noted for the metabolite concentrations \((P > .05)\).

The mean \(T_{2b}\) value of all patients was 77.23 ± 11.97 ms, compared with a mean value of 59.25 ± 7.35 ms in the control subjects \((P < .001)\). The \(T_{2b}\) values in both first-episode \((P < .001)\) and chronic patient \((P < .001)\) groups also differed significantly from those of the respective control subjects. There was no significant difference in \(T_{2b}\) values of first-episode and chronic patients \((P = .5)\). Likewise, no association between the \(T_{2b}\) values and duration of illness was present in the chronic patients \((r = 0.16, P = .55)\). The \(T_{2b}\) values did not correlate with the DUP in the first-episode patients \((r = 0.28, P = .37)\). The \(T_{2b}\) values did not correlate with the psychopathology scale scores in any of the patient groups \((P > .05)\).

**Discussion**

Reciprocal and proper interaction of different cortical regions is required for information processing in the brain. Functional integration of spatially remote cognitive events is achieved by corticocortical connections. A disruption of corticocortical connections may lead to cognitive impairments. The disconnectivity theory involving the pathophysiology of schizophrenia is based on the hypothesis that an abnormality in cerebral corticocortical interactions could cause or contribute to cognitive disturbances and symptoms of schizophrenia.4-7 Hoffman and McGlashan37 defined a neural network computer simulation model of reduced corticocortical connectivity in schizophrenia. Their simulation model could explain some of the positive psychotic symptoms of schizophrenia such as auditory hallucinations. The results of previous DTI and MR spectroscopy studies pointed to the presence of an axonal and/or a myelin-related pathology in schizophrenia.5-10,38 DTI measures diffusibility of water molecules in brain parenchyma. Dense packing of axonal fibers and myelination of axons decrease the diffusion rate of water molecules perpendicular to white matter tracts and leads to anisotropy. Thus, anisotropy reflects the structural integrity of axonal fibers and myelination. Pathologies causing demyelination or axonal loss may result in decreased anisotropy. The corpus callosum was found to be one of the common locations with reduced anisotropy in patients with schizophrenia.5-8 Because some of the structural studies selectively indicated abnormalities in the genu of the corpus callosum, we investigated the superior and posterior parts of the genu.5,17,25,26 Axonal fibers crossing the
neuronal activity in both physiologic and pathologic conditions may cause an increase in NAA concentration. Thus, neuroaxonal activity–dependent changes in NAA concentration demonstrate a close relationship between neuroaxonal functional status and NAA. Decreased NAA concentration in the corpus callosum in our patients may be due to decreased axonal attenuation, axonal dysfunction, or both. Histologic studies conducted to investigate the corpus callosum of patients with schizophrenia revealed controversial results.

Although most of them showed no difference between patients and control subjects, Highley et al reported a significant sex-specific decrease in both the cross-sectional area and axonal fiber attenuation of the corpus callosum favoring female schizophrenics. No sex predilection was noted for the metabolite concentrations in our study. The correlation between decreased NAA concentration and severity of psychotic symptoms suggests that the pathology causing a decrease in callosal NAA concentration plays a role in the pathophysiology of schizophrenia.

Schizophrenia is accepted as a genetically mediated neurodevelopmental disease. The neurodevelopmental theory of schizophrenia states that genetically mediated pathogenetic factors, long before the onset of formal psychotic symptoms, lead to abnormal changes in the normal course of neurodevelopment, resulting in subtle abnormalities in neurons and neural circuits. The early presentation of cognitive dysfunctions before the onset of psychotic symptoms supports the neurodevelopmental theory. The difference in NAA concentrations between first-episode and chronic patients was not significant in our study. This finding shows that the pathology leading to decreased NAA concentration in the corpus callosum is present at the beginning of illness. Furthermore, the lack of a relationship between NAA concentrations and the duration of illness suggests that neuroaxonal pathology leading to the decreased NAA concentration is not a progressive process during the clinical course of schizophrenia.

Pure myelin-related pathologies may cause a decrease in NAA concentration at 1H-MR spectroscopy. Decreased callosal NAA concentration observed in our patients may result from a myelin-related pathology or oligodendrocyte dysfunction. Histologic studies suggesting the presence of a myelin-related pathology or oligodendrocyte dysfunction in schizophrenia are present in the English literature. In an electron phrase.
A decrease in attenuation of oligodendrocytes in the frontal cortex of patients with schizophrenia was found in another postmortem study. The gene expression analyses revealed differential expression of myelination-related genes, suggesting a disruption in oligodendrocyte function in schizophrenia. A link between schizophrenia and the reduced expression of 2',3'-cyclic nucleotide 3'-phosphodiesterase, a marker of myelin forming cells, has been demonstrated in a recent study. Oligodendrocytes and myelin increase neuronal conduction velocity by their insulating properties and provide extrinsic support for axons. Myelin-related pathologies impair neuroaxonal conduction in white matter tracts and consequently cause corticocortical disconnections. Metachromatic leukodystrophy, for instance, is a dysmyelinating metabolic white matter disease, which demonstrates the pathophysiologic linkage between corticocortical disconnection and psychotic symptoms. Patients with adult-onset metachromatic leukodystrophy frequently present psychotic symptoms. Similarly, a myelin-related pathology causing reduced corticocortical connectivity may lead to the symptoms of schizophrenia.

Pure water has a T2 relaxation time of around 3 seconds. Owing to an interaction of water molecules with nonaqueous molecules in brain parenchyma, the T2 relaxation time of brain T2 is much shorter. In brain parenchyma, myelin-water, intracellular/extracellular water, and CSF contribute to T2. Intra- and extracellular water constitutes the major tissue-water fraction in brain and has a T2 relaxation time of approximately 80–100 ms. Water molecules trapped between myelin bilayers (myelin water) constitute a smaller fraction of tissue water and have a shorter T2 relaxation time (approximately 20 ms) than intra- and extracellular water and CSF. Because water trapped between myelin bilayers is proportional to the myelin content of the tissue, T2 can provide indirect measurement of the myelin content of this tissue. Demyelinating diseases decrease the myelin water fraction and prolong the T2 value. In our study, the T2 values in patients were significantly prolonged compared with those of control subjects. A myelin-related pathology or decrease in axonal attenuation may be the cause of prolonged T2 in our patients. Theoretically, any factor or pathology that is able to alter the intrinsic magnetic field within tissue may change T2 relaxation times. Prolonged T2 relaxation time in the genu of the corpus callosum was reported in a recent MR imaging study of patients with schizophrenia. T2 relaxation times in the frontal white matter of patients with schizophrenia was also prolonged. In these studies, the authors proposed that decrease in the myelin water fraction might be the cause of prolonged T2 relaxation times. In our study, the accompaniment of a prolonged T2 value with reduced NAA concentration suggests the presence of an axonal or myelin-related pathology in the corpus callosum in patients with schizophrenia. A prolonged T2 value was associated neither with the duration of illness nor with the severity of psychopathology in our study. This finding is consistent with the possibility that the pathology altering the T2 value has a neurodevelopmental basis.

Phosphorylcholine and glycerophosphorylcholine, which are the precursors of cell membrane synthesis and the breakdown products of myelin and cell membrane, contribute to Cho peak at proton MR spectra. The lack of difference in Cho concentration between the patients and control subjects showed that there was no active demyelination ongoing in the corpus callosum of the patients with schizophrenia.

All of our patients were in the acute phase of illness. Therefore, all the patients except 2 were under antipsychotic treatment during the 1H-MR spectroscopy examinations. Due to ethical considerations, we could not increase the number of drug-naive patients to compare metabolite concentrations of antipsychotic-naive patients and the patients under antipsychotic treatment. Hence, we could not exclude the potential effect of antipsychotic drugs on neurometabolite concentrations. This may be accepted as a limitation of our study. However, lack of a difference in NAA concentrations between the first-episode and chronic patients demonstrated that chronic antipsychotic treatment did not lower the NAA concentration in the corpus callosum of the patients. There are controversial results in the previous literature involving the effect of antipsychotic drugs on NAA concentration. In one of the longitudinal 1H-MR spectroscopy studies, Bertolino et al reported an elevation of NAA/Cr ratio in response to antipsychotic...
medication. However, this finding could not be confirmed in the other studies. In addition, recently reported animal studies have shown that antipsychotic medication does not alter neuro-metabolite concentrations measured by ¹H-MR spectroscopy. ⁵⁶

In conclusion, the prolonged T2 relaxation time of tissue water in the corpus callosum and the correlation of decreased NAA concentration with the severity of psychopathology are the outstanding findings of this study. Finally, our results support the previously proposed callosal disconnection theory in schizophrenia.

References

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