Drug-induced parkinsonism in relation to choline-containing compounds measured by $^1$H-MR spectroscopy in putamen of chronically medicated patients with schizophrenia

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Abstract

Extrapyramidal side-effects (EPS), the most frequent and severe side-effects of antipsychotics, sometimes become irreversible and cause severe psychosocial disturbance in patients with schizophrenia. However, the neurobiological basis of EPS has not yet been elucidated. In this study, neurochemical correlates of EPS were examined by $^1$H-MR spectroscopy ($^1$H-MRS). Sixteen medicated patients with schizophrenia and 15 age-, gender- and parental-socioeconomic-status-matched normal controls were examined using single-voxel $^1$H-MRS. Absolute concentrations of N-acetyl aspartate (NAA), choline-containing compounds (Cho), creatine/phosphocreatine, myo-inositol, and Glx (glutamate and glutamine) in the left putamen were evaluated. The patient group showed mild EPS and no significant metabolic abnormalities in this region. The more severe drug-induced parkinsonism assessed by the Simpson–Angus Scale, however, significantly correlated with the higher Cho concentration and tended to be correlated with the higher NAA concentration in the patient group. These results suggest a potential of $^1$H-MRS as a non-invasive monitoring method of neurobiological correlates of EPS associated with neuroleptic treatments in patients with schizophrenia.

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Key words: Antipsychotics, extrapyramidal side-effects (EPS), $^1$H-MR spectroscopy ($^1$H-MRS), schizophrenia, striatum.

Introduction

Treatment with typical antipsychotic drugs (APDs) is accompanied by a high incidence of extrapyramidal side-effects (EPS), including tardive dyskinesia (TD), akathisia, and drug-induced parkinsonism. Although the recently developed atypical APDs have lower risks of developing EPS, they are not completely free from EPS (Tarsy et al., 2002). In addition to the discomfort and distress of patients, EPS may cause poor compliance and ultimately poor treatment outcome. Furthermore, EPS can mimic some aspects of negative symptoms, which complicates differential diagnosis (Kane, 2001). Therefore, it is still important to understand the biochemical mechanisms underlying the generation of EPS in the course of the clinical treatment. Previous positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies showed that EPS are associated with high in-vivo occupancy of D2 dopamine receptors in the striatum (Farde et al., 1992). However, it may be difficult to repeatedly measure the rate of occupancy of neurotransmitter receptors using PET or SPECT because of concerns of excessive exposure to radioactive agents.

Magnetic resonance spectroscopy (MRS) is a non-invasive tool for acquiring neurochemical information in the living brain. Previous studies that employed $^1$H-MRS have indicated high choline (Cho) levels in the left striatum of patients with schizophrenia (e.g. Shioiri et al., 1996). The Cho levels, which reflect
primarily the constituents of cell membranes, increase when turnover or degeneration of membrane phospholipids is accelerated (Hoang et al., 1998). Antipsychotic medication is associated with the increased synaptic spine density primarily in the striatum (Kerns et al., 1992). Moreover, it has been suggested that structural synaptic changes are accompanied by increased membrane phospholipids turnover that should be reflected in Cho changes (Bertolino et al., 2001). Therefore, antipsychotic medication may affect Cho levels in the striatum in parallel with the severity of EPS in patients with schizophrenia.

A limited number of studies have examined the possible relationship between measures of $^1$H-MRS and EPS. Bustillo et al. (2001) reported that performance times in the Grooved Pegboard, a measure of motor dexterity and proxy for parkinsonism, negatively correlated with left frontal N-acetyl aspartate (NAA) level in patients with schizophrenia and controls combined, but not in patients with schizophrenia only. In neurodegenerative disorders, some studies reported that the NAA/creatinine and phosphocreatine resonance (Cr) ratio in the putamen negatively correlated with the severity of parkinsonism (Abe et al., 2000). Possible relationship between measures of $^1$H-MRS in the striatum and EPS in patients with schizophrenia has not yet been clarified by previous studies.

The purpose of this study was to assess whether Cho and/or NAA concentrations in the putamen measured by $^1$H-MRS are correlated with the severity of EPS in chronic, medicated patients with schizophrenia. In addition, we also examined normal controls using $^1$H-MRS to clarify whether or not metabolic abnormalities, particularly increased Cho concentration reported in previous studies, are present in this group of patients with schizophrenia compared to controls.

Method

Subjects

Sixteen in- and outpatients with schizophrenia were recruited from the Department of Neuropsychiatry, Tokyo University Hospital, Japan. Ten were male, and six were female. The diagnosis of schizophrenia was determined for each patient according to DSM-IV (APA, 1994) criteria through the Structured Clinical Interview for DSM-IV Axis I Disorder (SCID-I) Clinical Version (First et al., 1997) by a trained psychiatrist (A.I.). The subtypes were catatonic ($n = 5$), paranoid ($n = 10$). All patients had received typical neuroleptics and 15 patients had received anticholinergics for at least 2 yr. Four patients had also received risperidone. Fifteen age- and gender-matched healthy subjects were also examined as controls (Table 1). The socioeconomic status (SES) and parental SES were assessed using the Hollingshead scale (Hollingshead, 1965). All subjects were right-handed (determined using the Edinburgh Inventory; Oldfield, 1971). The laterality index $\geq 0.8$ was used as the cut-off for right-handedness.

The exclusion criteria for both groups were a history of neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, and substance abuse or addiction. Moreover, patients with current moderate or severe TD or akathisia were also excluded to minimize movement artifact in MR examination. Additional exclusion criteria for the control group were a past history of a psychiatric disease and a family history of Axis I disorder in first-degree relatives. After a complete explanation of the study to the subjects, written informed consent was obtained.

Clinical assessments

All schizophrenic subjects were evaluated for EPS by one psychiatrist (H.Y.) using the Simpson–Angus Scale (SAS; Simpson and Angus, 1970) for parkinsonism, the Barnes Akathisia Scale (BAS; Barnes, 1989) for akathisia, and the Abnormal Involuntary Movement Scale (AIMS; Guy, 1976) for TD on the day of MR scanning. Psychiatric symptoms were also evaluated by one psychiatrist (A.I.) using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) within 3 d prior to the MR scanning.

MR examination

MRI and MRS were obtained from all subjects using a 1.5-T MR system (Signa Horizon LX version 8.2, GE Medical Systems, Milwaukee, WI, USA) and a quadrature proton head coil. Each subject was taped under the chin and across the forehead to reduce motion. Sagittal localizer images were obtained first, followed by double-echo spin-echo axial slices of whole brain (TE = 15 and 101 ms, TR = 4000 ms, field of view 22 cm, matrix $256 \times 256$) and T1-weighted axial slices of whole brain (TE = 12 ms, TR = 500 ms, field of view = 22 cm, matrix $256 \times 256$). A trained neuroradiologist (T.F.) evaluated the MRI scans to exclude the subjects with gross morphological abnormalities.

In the axial slice, which showed the largest view of the left putamen, spectroscopic voxels...
were centred on the left putamen, maximizing the view of the grey matter and minimizing that of cerebrospinal fluid (CSF) (Figure 1a). For $^1$H-MRS, a point-resolved spectroscopy (PRESS) sequence (probe-p, GE Medical Systems, software version 5.7; TR = 2000 ms, TE = 35 ms, 128 averages) was applied with fully automated shimming and water suppression adjustments. In all subjects, full width at half-maximum (FWHM) was less than 6 Hz.

**Spectral processing**

Spectral reconstruction and quantification of myoinositol (MI), Cho, Cr and NAA were done automatically using SAGE 7.0 software on a work station. The concentrations of metabolites, namely MI, Cho, Cr and NAA, were estimated using a method similar to that of Michaelis et al. (1993) and Hamakawa et al. (1999). This method is based on the comparison of in-vitro spectra with metabolite solutions in institutional units, being corrected by the saturation factor (SF), gravity of brain tissue, and contributions from capillary ($f_{cap}$) and CSF ($f_{CSF}$) volumes. Briefly, a tissue concentration ($C_{obs}$) of a metabolite was calculated in millimoles per kilogram wet weight brain tissue by calibrating its measured spectral resonance area ($A_{obs}$) with the known concentration ($C_{ref}$) of a reference solution and the respective resonance area ($A_{ref}$) measured in a separate study. The in-vitro

| Table 1. Subject characteristics, symptom scores and metabolite concentrations |
|---------------------------------|-----------------|-----------------|----------------|
| Variable                        | Schizophrenic patients ($n = 16$) | Control subjects ($n = 15$) | $t$ tests |
| Age (range)                     | Mean (S.D.)     | Mean (S.D.)     | d.f. | t value | p   |
| Male/female                     | 30.7 (22–51)    | 28.4 (22–36)    | 29   | 1.14    | 0.25 |
| Education (yr)                  | 12.4 (1.4)      | 16.4 (1.2)      |     | 8.56    | <0.001 |
| SESa                            | 4.4 (0.8)       | 1.8 (0.7)       |     | 9.16    | <0.001 |
| Parental SESa                   | 2.6 (0.70)      | 2.3 (0.72)      |     | 1.38    | 0.17 |
| Height (cm)                     | 165 (9.4)       | 165 (6.1)       |     | 0.05    | 0.95 |
| Body weight (kg)                | 65.9 (13)       | 59.2 (9.6)      |     | 1.56    | 0.13 |
| Neuroleptic doseb (mg/d)        | 543 (538)       | – –             |     | – –     | – – |
| Anticholinergicsc (mg/d)        | 4.04 (2.53)     | – –             |     | – –     | – – |
| Onset of illness, years         | 22.6 (7.6)      | – –             |     | – –     | – – |
| Duration of illness, years      | 8 (2–20)        | – –             |     | – –     | – – |
| PANSS                           |                |                 |     |         |     |
| Positive subscale               | 14.5 (5.7)      | – –             |     | – –     | – – |
| Negative subscale               | 19.5 (4.1)      | – –             |     | – –     | – – |
| General psychopathology subscale| 34.5 (6.5)      | – –             |     | – –     | – – |
| Simpson–Angus Scale (Parkinsonism) | 3.4 (4.1)   | – –             |     | – –     | – – |
| AIMS (Tardive dyskinesia)       | 0.6 (1.3)       | – –             |     | – –     | – – |
| Barnes (Akathisia)              | 0.2 (0.5)       | – –             |     | – –     | – – |

Metabolite concentration (mmol/kg)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Schizophrenic patients ($n = 16$)</th>
<th>Control subjects ($n = 15$)</th>
<th>$t$ tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>9.30 (1.46)</td>
<td>9.54 (1.43)</td>
<td>28 0.004 3.84</td>
</tr>
<tr>
<td>Cho</td>
<td>3.21 (0.62)</td>
<td>3.26 (0.57)</td>
<td>28 0.019 0.354</td>
</tr>
<tr>
<td>Cr</td>
<td>9.81 (1.15)</td>
<td>9.81 (0.92)</td>
<td>28 0.881 19.7^d</td>
</tr>
<tr>
<td>MI</td>
<td>6.18 (1.50)</td>
<td>6.05 (1.15)</td>
<td>28 0.081 0.057</td>
</tr>
<tr>
<td>Glx</td>
<td>13.9 (3.05)</td>
<td>12.6 (2)</td>
<td>28 3.28 3.7</td>
</tr>
</tbody>
</table>

a Socioeconomic status, assessed using the Hollingshead scale. Higher scores indicate lower status.
b Based on chlorpromazine equivalents.
c Based on biperiden equivalents.
d $p < 0.001$. 

(15 × 15 × 15 mm³) were centred on the left putamen, maximizing the view of the grey matter and minimizing that of cerebrospinal fluid (CSF) (Figure 1a). For $^1$H-MRS, a point-resolved spectroscopy (PRESS) sequence (probe-p, GE Medical Systems, software version 5.7; TR = 2000 ms, TE = 35 ms, 128 averages) was applied with fully automated shimming and water suppression adjustments. In all subjects, full width at half-maximum (FWHM) was less than 6 Hz.
examination was performed at a similar magnetic-field homogeneity and the receiver gain was set at the same value as that in the in-vivo examination. Additional corrections were performed as follows:

\[
C = \frac{C_{\text{ref}} \times A_{\text{obs}} / \text{SF}_{\text{obs}} / \text{SF}_{\text{ref}}}{(1 - f_{\text{CSF}}) \times (1 - f_{\text{cap}}) \times \rho_{\text{brain}}}
\]

Finally, the concentration was converted to millimoles per kilogram wet weight by dividing with \(\rho_{\text{brain}} = 1.04 \text{ kg/l}\) for the specific gravity of brain tissue.

To quantify the concentration of Glx (a mixed peak mainly containing glutamate, glutamine, and GABA), a single NAA peak at 2.02 ppm generated by the fitting data noted above was subtracted from the measured \(^1\text{H-MR}\) spectrum. This spectrum was fitted by the following peaks: a broad macromolecule peak located between 1.0 and 2.0 ppm, multiple Glx peaks between 2.0 and 2.5 ppm, the other NAA peak at 2.6 ppm, Cr (3.0 ppm), Cho (3.25 ppm), MI (3.6 ppm), and Cr (3.9 ppm). It was confirmed that the residual spectra, which was generated by the subtraction of synthesized spectrum from the raw data, showed only noise component. The methodology of the Glx quantification was reported elsewhere (Fukui, 2001).

These post-processings were performed by a rater (T.F.) who was blind to the clinical data. Inter-assay reliability was examined in six healthy subjects who were examined again on another occasion. The duration of the two experiments was 16–30 d.

**Statistical analyses**

All statistical tests were performed with SPSS 10.1 software. Student’s \(t\) test was used to test for group differences in clinical variables (age, years of education, SES, parental SES, height, body weight). Group differences in metabolites were tested by multivariate analysis of covariance (MANCOVA) with the concentrations of metabolites (NAA, Cho, Cr, MI and Glx) as the dependent variable and age (which showed a trend of negative association with Cr; see the Results section) as the covariates. This test was followed by univariate \(F\) tests. Although multiple comparisons were performed, we did not use the Bonferroni correction, because we examined the hypothesis that there is an increase in Cho concentrations. The associations between extrapyramidal symptoms and metabolite concentrations in patients with schizophrenia were tested for significance using Spearman’s correlation coefficient. In this analysis, only the SAS scores were used, because the AIMS scores (mean = 0.6, s.d. = 1.3) and BAS scores (mean = 0.2, s.d. = 0.5)

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**Figure 1.** (a) T2-weighted magnetic resonance image in a control subject. The square indicates the volume of interest (VOI), \(1.5 \times 1.5 \times 1.5 \text{ cm}^3\) voxel in the left putamen. (b) Average of proton magnetic resonance spectra from control subjects and (c) from patients with schizophrenia. NAA, N-acetyl aspartate; Glx, glutamate, glutamine and GABA; Cr, creatine; Cho, choline-containing compounds; MI, myo-inositol.
were too low to calculate the correlation. We also did not use the Bonferroni correction, although multiple tests were performed, because we calculated the correlation under the hypothesis that either the Cho or NAA concentration in the putamen measured by $^1$H-MRS is correlated with the severity of EPS in the patients with schizophrenia.

Additionally, Spearman’s rho was calculated for exploring correlations between the clinical measures (age, height, body weight, SES, parental SES, years of education, onset of illness, duration of illness, anticholinergic and neuroleptic dose) and metabolite levels in each group separately. We considered $p<0.0007$ as denoting statistical significance [Bonferroni correction for 80 correlations (50 for schizophrenia group (5 metabolites x 10 clinical measures); 30 for control group (5 metabolites x 6 clinical measures))].

**Results**

All 16 patients with schizophrenia and 15 control subjects completed MR examination. MRI scans showed no gross morphological abnormalities in any subjects. There were no significant differences between the schizophrenic subjects and controls in age, male/female proportion, parental SES, height, body weight, except for the significantly lower SES in the controls than the schizophrenic subjects (lower rating indicates higher status, $T=9.16$, d.f. = 2.29, $p<0.001$). While the total SAS score had a tendency of correlation with the dose of neuroleptics ($p=0.488, p=0.082$), no correlation with the duration of illness was found ($p=0.039, p=0.885$).

The results in schizophrenia and controls showed a significant positive correlation between the total SAS score and Cho concentrations in the schizophrenia group ($\rho=0.515, p=0.041$) (Figure 2). From Figure 2, it is evident that this positive correlation between Cho concentration and SAS score is partly driven by a patient with high SAS and Cho values. However, even if this patient is omitted, there remains a tendency of positive correlation ($\rho=0.422, p=0.117$).

The total SAS score showed no significant correlations with any other metabolite concentrations ($0.302 \geq \rho \geq -0.138, p \geq 0.254$), except for a trend of positive correlation with NAA concentrations ($\rho=0.470, p=0.065$) (Figure 3). There were no significant correlations between metabolite levels and age, height, body weight, SES, parental SES, years of education, onset of illness, duration of illness, anticholinergic or neuroleptic dose ($0.602 \geq \rho \geq -0.602$, $p \leq 0.05$)
Discussion

The present study showed no significant differences in any metabolite concentrations in the putamen between patients with schizophrenia and normal controls. On the other hand, the severity of drug-induced parkinsonism significantly correlated with the Cho concentration in the left putamen of patients with schizophrenia measured by $^1$H-MRS. It is noteworthy, however, that the Cho concentration correlated with neither the anticholinergic nor the neuroleptic dose.

As noted above, there were no significant metabolic changes in the left putamen of patients with schizophrenia. This result suggests that there exists slight or no pathological change in this region of this patient group. This result is consistent with those of four studies that evaluated the putamen, which failed to show metabolite abnormalities in patients with schizophrenia (Bertolino et al., 1996, 1998; Heimberg et al., 1998; Ohara et al., 2000). On the other hand, studies that have evaluated the caudate consistently reported elevated Cho levels in patients with schizophrenia compared to normal controls (e.g. Shioiri et al., 1996). In this study, patients with severe TD or akathisia were excluded from the assessment, which might have caused no apparent difference in metabolite levels.

To our knowledge, this study provides the first evidence that the Cho concentration in the striatum measured by $^1$H-MRS may be related to the severity of EPS in patients with schizophrenia. This finding is partly in accordance with those of a limited number of previous studies that reported the relationship between EPS and $^1$H-MRS data in other regions of the brain and/or in other diagnostic groups (Abe et al., 2000; Bustillo et al., 2001). These studies indicated that the decreased NAA level may be related to EPS. In contrast, we found that the more severe drug-induced parkinsonism correlated with the higher Cho and NAA concentrations in the left putamen of schizophrenic subjects. Several studies have reported morphological changes in the striatum of subjects receiving long-term administration of typical APDs. In the striatum of rodents, an increase in the number of perforated axospinous synapses associated with vacuous chewing movements (Meshul and Reassen, 1996) and an increase in the total number of synapses (Kerns et al., 1992) have been reported. For in-vivo studies, an increase in the volume of striatum after treatment with typical APDs has been reported (Corson et al., 1999; Keshavan et al., 1994). Since NAA is known to reflect numbers and integrity of neurons, these structural changes might have caused apparent positive correlation between NAA and the severity of EPS. Since it has been suggested that structural synaptic changes are accompanied by increased membrane turnover and cause an increase in Cho levels (Bertolino et al., 2001), these lines of evidence are consistent with the correlation between severity of drug-induced parkinsonism and Cho concentration in the left putamen of chronic medicated patients with schizophrenia. Although the present examination included patients with mild EPS and without significant changes in the Cho concentration compared to healthy subjects, Cho concentrations correlated with drug-induced parkinsonism. Therefore, it is logical to expect that the Cho concentrations measured by $^1$H-MRS sensitively reflect the neurochemical mechanisms underlying the development of EPS. Recently, Ando et al. (2002) examined patients with schizophrenia with or without TD by $^1$H-MRS. They showed increased Cho/Cr peak ratio in patients with TD compared to healthy controls. Furthermore, Cho/Cr peak ratio tended to be correlated with the severity of TD. Although there was no significant difference in NAA/Cr peak ratio, it was somewhat higher in patients with TD than controls. These results are in accordance with our results.

Here, we address the methodological considerations of our study. First, in the present examination, contributions of globus pallidus and white matter within the volume of interest (VOI) to the resonance were not eliminated. The partial volume effect within the VOI should be considered whenever possible, as we did in our previous MRS study of anterior cingulate (Yamasue et al., 2002). However, in the case of basal ganglia, it is difficult to distinguish the volume of globus pallidus from white matter, since the signal intensities of globus pallidus are intermediate. Although this methodological issue made it difficult to interpret the present finding, the partial volume effect by white matter may be small due to the small size of VOI in the present examination. Secondly, some of the mean metabolite concentrations calculated in this study seem to be higher than the values presented in previous studies. The concentrations calculated vary depending on the methodological differences. For example, as shown in Table 1, the mean Cho concentration of the healthy control group was 3.26 mmol/kg in this study, while the values ranged between 1.3 and 2.9 mmol/kg in previous reports even though they examined slightly different brain regions in different
populations (Hamakawa et al., 1998, 1999; Michaelis et al., 1993; Pouwels et al., 1999). The correction for CSF/capillary contribution might have caused this discrepancy (Michaelis et al., 1993). As this study compared calculated concentrations between patients and controls, the conclusion of the present study is not affected.

Here, we address the limitations and future directions of our study. First, this study could not assess whether or not patients with severe or moderate EPS have metabolic abnormalities in the putamen. Secondly, the sample size was not large. Thirdly, the present examination did not employ patients treated with atypical APDs only. Since atypical APDs show different effects on the resonances of $^1$H-MRS (Goff et al., 2002) and the generation of EPS (Kane, 2001), the employment of a pharmacological comparison group should be considered in future studies. Fourthly, since the present study did not examine intra-individual changes of $^1$H-MR spectra with the course of illness, it is not clear whether the $^1$H-MRS finding represents vulnerability to EPS or reflects a metabolic change associated with neuroleptic medication. Last, since only one region was studied, this study cannot totally exclude the possibility that a similar association might also be seen in other brain areas as well.

In conclusion, the severity of drug-induced parkinsonism correlated with the Cho and NAA concentration in the left putamen of patients with schizophrenia, although this patient group showed only mild EPS and no significant changes in any metabolic levels in this region. Even though the neurobiological mechanism responsible for this relationship is unclear, $^1$H-MRS may have a potential for monitoring EPS non-invasively at the neuronal level.

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