Proton Magnetic Resonance Spectroscopy (H-MRS) in Chronic Schizophrenia. A Single-Voxel Study in Three Regions Involved in a Pathogenetic Theory

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SUMMARY – *The cognitive dysmetria theory suggests a disconnectivity between the dorsolateral prefrontal cortex, thalami and vermis to explain the pathophysiology of schizophrenia. This study investigated the metabolic integrity of this neurologic circuit in patients with schizophrenia using proton magnetic resonance spectroscopy (H-MRS). Twenty-two patients with schizophrenia and twelve control subjects were studied. Metabolites concentrations were evaluated by a single-voxel technique in the prefrontal cortex, thalami and vermis. To our knowledge, this is the first H-MRS experience with concomitant evaluation of these regions in schizophrenic patients. We found no significant statistical difference in N-AA, Cho and Cr absolute concentrations and N-AA/Cho, N-AA/Cr and Cho/Cr ratios between the schizophrenic patients and control group. At the vermis, we found a constant spectrum with low levels of N-AA and higher levels of Cho and Cr. Our experience does not clearly support or refute the cognitive dysmetria theory. The consistency of metabolic findings in the cerebellar vermis could represent an important datum, highlighting the specificity of metabolic and functional activity in this region.*

Introduction

In schizophrenia, an alteration of the “cortico-cerebellar-thalamic-cortical circuit” (CCTCC) could be responsible for the failure of those neurological processes which allow reception, use and expression of information and experience. The lack of coordination of these mental processes has been called “cognitive dysmetria”¹. Andreasen et al. developed this pathogenetic model suggesting a connection between the left dorsolateral prefrontal cortex, thalami and cerebellar vermis¹⁻³. In particular, she evaluated the correlation of regional cerebral blood flow (r-CBF) by positron emission tomography (PET) with anatomical magnetic resonance (MRI) in patients with schizophrenia and control groups¹⁻³. The aim of our study was to investigate the

regional concentrations of the main cerebral metabolites in schizophrenic patients across the prefrontal cortex, thalami and cerebellar vermis using proton magnetic resonance spectroscopy (1H-MRS). 1H-MRS provides a non-invasive measure of the biochemistry of the brain and detects chemicals containing hydrogen: N-acetylaspartate (NAA), choline-containing compounds (Cho) and Creatine (Cr).

Materials and Methods

Subjects

Twenty-two patients of both sexes (mean age: 37.4 years; median age: 35.5 years) with a diagnosis of schizophrenia after a structured clinical

Figure 1 Axial, coronal and sagittal HASTE images showing the MR spectroscopy voxel placement.

Figure 2 Proton MR spectrum from a cerebellar vermis voxel:
1) N-AA peak at 2.01-2.03 ppm frequency. 2) Cr peak at 3.01-3.03 ppm frequency. 3) Cho peak at 3.21-3.23 ppm frequency.

interview for DSM-IV Axis I Disorders (SCID) and a 12 subject volunteer control group (sex and age-matched) were prospectively studied. All subjects were right-handed. All the schizophrenic patients received stabilized doses of atypical antipsychotic medication. The mean number of years of illness was seven. None of the patients with schizophrenia had a history of head trauma, neurological, major mood or anxiety disorders, alcohol or substance abuse. None of the control subjects had a history of substantial medical illness, head trauma, psychiatric disorders, alcohol or substance abuse. All the control subjects received a structural clinical interview for DSM-III-R-Non-Patient Edition by experienced interviewers to exclude any psychiatric disorders. Written informed consent was obtained from all patients and control subjects.

Clinical Assessment

All patients met DSM-IV TR criteria for schizophrenia diagnosis and the psychopathology was assessed by PANSS (positive and negative symptoms scale). This scale was used to summarize psychopathology in three dimensions: negative, positive and disorganized. Clinical assessment, MR imaging and H-MR spectroscopy examinations were performed within the same week.

Cranial MR Imaging

MRI and H-MRS were performed on a Siemens 1.5-T Magnetom Vision imaging system using a standard quadrature head coil. Brain MR images were acquired to identify any cerebral pathology defined in the exclusion criteria and to position the MR spectroscopy volume. Conventional MRI included: 1) axial Fast spin-echo (FSE) T2-weighted images (TR= 3800 ms, TE=98 ms, NEX=2, echo-train length=11, matrix=190×256, section thickness= 5 mm); 2) sagittal 3D gradient-echo (mpr-GE) T1-weighted images (TR=9.7 ms, TE=4 ms, FA=12, matrix=200×256, slab thickness= 160 mm, eff.thickness=1.25 mm, number partitions=128,); 3) Half-Fourier single shot fast SE (HASTE) images on three-spatial planes (TR=10.9 ms, TE= 87 ms, FA 154, matrix=240×256, thickness= 5 mm).

H-MR Spectroscopy

Single-voxel ¹H-MR spectroscopy examinations were conducted in the same session with conventional cranial MR imaging. For

¹H-MRS, a single-voxel SE sequence (TR=1365 ms, TE=135 ms) was used. It is a double-echo sequence with excitation of three orthogonal slices (one 90° pulse, two 180° pulses). Water suppression was performed via Gaussian pulse by a frequency-selective 90° pulse which flips the longitudinal magnetization of water in the xy plane before the excitation pulse. The ¹H-MRS voxel size was 4 ml³. Voxel placement was performed by the same neuroradiologist.

Left dorsolateral prefrontal cortex, both thalami and cerebellar vermis were studied (Figure 1). In all subjects, post-processing of the raw ¹H-MRSI data was performed as described previously⁴. Resonance intensity values of metabolites were assessed using a combination of Xunspec1 software (Philips Medical Systems, Andover, MA, USA) and free software developed at the Montreal Neurological Institute. Concentrations of Cho (3.21-3.23 ppm frequency), Cr (3.01-3.03 ppm) and N-AA (2.01-2.03 ppm) were evaluated.

N-AA/Cho, N-AA/Cr and Cho/Cr ratios were calculated. Study-specific phantom tests testing the reproducibility of ¹H-MR spectroscopy measurements were performed every week. We used a spherical phantom containing 0.1 molar solution consisting of lithium lactate and sodium acetate.

Statistical Analysis

A one-way multivariate analysis of covariance was performed to investigate the different concentrations for each metabolite (N-AA, Cho, Cr) in patients with schizophrenia and in the control group. Three dependent variables were used: N-AA/Cho, N-AA/Cr and Cho/Cr; group was the independent variable, age the covariate. Preliminary assumption testing was conducted to check for normality, linearity, univariate and multivariate outliers, homogeneity of variance-covariance matrices and multicollinearity, with no serious violations noted. For univariate analysis, a Bonferroni adjusted alpha level of .017 was applied.

Results

Conventional MRI examination showed no brain abnormalities in either the schizophrenic or control group.

¹H-MRS showed a reduced NAA concentration in 14/22 schizophrenic patients (63.63%) in the left dorsolateral prefrontal cortex, in

Table 1 Metabolite concentration mean ratios in four cerebral regions of the patients and control subjects.

Region	Metabolite concentration mean ratios ($\pm$ SD)		
	Cho/Cr	N-AA/Cr	N-AA/Cho
<i>Right Thalami</i>			
Patients (n = 22)	1.14 $\pm$ 0.21	1.01 $\pm$ 0.21	0.91 $\pm$ 0.19
Controls (n = 12)	1.32 $\pm$ 0.27	0.93 $\pm$ 0.20	0.72 $\pm$ 0.20
Significance (uv)	p = 0.049	p = 0.353	p = 0.022
Significance (mv)		p = 0.050	
<i>Left Thalami</i>			
Patients (n = 22)	1.16 $\pm$ 0.27	1.10 $\pm$ 0.26	0.97 $\pm$ 0.24
Controls (n = 12)	1.12 $\pm$ 0.21	0.91 $\pm$ 0.27	0.85 $\pm$ 0.35
Significance (uv)	p = 0.237	p = 0.040	p = 0.382
Significance (mv)		p = 0.159	
<i>Prefrontal Cortex</i>			
Patients (n = 22)	1.11 $\pm$ 0.20	1.00 $\pm$ 0.34	0.91 $\pm$ 0.32
Controls (n = 12)	1.12 $\pm$ 0.11	0.82 $\pm$ 0.32	0.73 $\pm$ 0.27
Significance (uv)	p = 0.540	p = 0.284	p = 0.187
Significance (mv)		p = 0.610	
<i>Cerebellar Vermis</i>			
Patients (n = 22)	1.04 $\pm$ 0.23	0.55 $\pm$ 0.12	0.54 $\pm$ 0.12
Controls (n = 12)	1.07 $\pm$ 0.20	0.50 $\pm$ 0.07	0.49 $\pm$ 0.10
Significance (uv)	p = 0.955	p = 0.311	p = 0.369
Significance (mv)		p = 0.701	

uv: univariate – mv: multivariate

17/22 (77.2%) in the right thalamus and in 11/22 (50%) in the left thalamus. In the control group, NAA peak reduction was demonstrated in 9/12 subjects (75%) in the left dorsolateral prefrontal cortex and right thalamus. In the left thalamus, NAA concentration was reduced in 10/12 subjects (83.3%). In the cerebellar vermis, we found a consistent spectrum with low levels of NAA and higher concentrations of both Cho and Cr, with no significant difference between schizophrenic and normal groups (Figure 2). There was no significant statistical difference in NAA/Cho, NAA/Cr and Cho/Cr ratios between the pathologic and control groups (Table 1- Figure 3: charts I-II-III).

With regards to the clinical assessment, there was a significant correlation between metabolic ratios and different psychopathological dimensions of schizophrenia assessed by PANSS. Specifically, the NAA/Cho ratio was significantly lower in the right thalamus ($p=0.006$) and left thalamus ($p=0.007$) in the positive dimension of schizophrenia. In the

dorsolateral frontal cortex, both NAA/Cho ($p=0.001$) and NAA/Cr ($p=0.003$) ratios were lower in the positive dimension group.

Discussion

Previous ^1H -MRS studies showed conflicting results regarding changes in brain metabolites, such as N-acetylaspartate (NAA), in structures involved in schizophrenia⁵⁻¹⁵. Several authors reported a decrease in NAA in some cerebral regions, like the thalamus, frontal lobes, hippocampus or corpus callosum in schizophrenic patients⁵⁻¹⁴. A reduction in NAA concentration is thought to represent a loss of neurons and/or axons, as well as neuronal or axonal dysfunction or damage. Other studies to date have failed to detect any difference in metabolic concentrations between schizophrenic and control subjects^{7,12,15}. Furthermore, only a few articles focused on the role of the cerebellar vermis^{7,16,17}.

Figure 3 Charts I-II-III. The box-plot graphs demonstrate the distribution of N-AA/Cho, N-AA/Cr and Cho/Cr ratios around the median values of control subjects (nor) and patients (pat).

The specificity of our experience is the concomitant *in vivo* evaluation by single voxel ^1H -MRS of the following four regions: left dorsolateral prefrontal cortex, both thalami and cerebellar vermis. The present study explored the neural circuitry according the pathogenetic hypothesis of cognitive dysmetria, investigated by Andreasen et al.². Their investigation was based on PET functional studies integrated with morphological MRI showing, in schizophrenic patients, r-CBF abnormalities in cortical, cerebellar and thalamic regions. According to these results, the cognitive dysmetria theory was pro-

posed to explain the pathophysiology of schizophrenia. A cortico-cerebellar-thalamic-cortical circuit (CCTCC) alteration was suggested¹⁻³.

The main finding in our study is that there was no overall difference in NAA/Cho, NAA/Cr and Cho/Cr ratios between the patients and control group in the explored regions mentioned above. In particular, we noted a statistically significant interindividual variability of the main brain metabolite peaks in the supratentorial regions (frontal cortex and thalami), in contrast to a consistent spectrum in the cerebellar vermis. In all subjects, we obtained a cer-

ebellar spectrum featuring higher Cho and Cr levels compared to lower NAA concentration.

This evidence of a relative cerebellar NAA decrease is well known¹⁸⁻²⁰. However, the consistency of the finding could be an important datum in our series, highlighting the specificity of metabolic and functional activity of the cerebellum. In particular, the consistent spectrum obtained at the cerebellar vermis could reflect the absolute metabolic stability of this region.

The lack of global significance in our experience between the schizophrenic patients and the control group is consistent with literature data reporting normal ¹H-MRS spectral analysis in schizophrenia. However, we noted a statistically significant correlation between metabolic ratios and different psychopathological dimensions of schizophrenia. In the positive dimension group, the NAA/Cho ratio was significantly lower in both thalamic regions. Moreover, in this group of schizophrenic patients, both NAA/Cho and NAA/Cr ratios were lower in the dorsolateral frontal cortex. These findings could suggest a more serious involvement of both thalami and prefrontal cortex in the pathogenesis of positive symptoms of schizophrenia.

A possible limitation of our findings is the technical acquisition of spectral data. Spectroscopic voxels typically contain varying mixtures of gray and white matter (partial volume effect), which often complicates interpretation of the metabolic changes. Furthermore, with regard to technical precision, we used the absolute consistency of cerebellar spectral data as a further quality control of our acquisition system.

Another possible limitation of our investigation is the medical therapy. All patients in our sample received stabilized doses of atypi-

cal neuroleptics and this might have resulted in changes in locoregional metabolism. Nevertheless, some authors reported there was no relationship between the metabolites, duration of illness and antipsychotic treatment¹⁵. More recently, however, other reports demonstrated a relationship between antipsychotic treatment and H-MRS metabolite value. Szulk et al.²¹ observed a significantly decreased Glx/Cr ratio in the temporal lobe and a trend for an increase in the NAA/Cr ratio in the thalamus in a schizophrenic population after antipsychotic treatment, studied with H-MRS with short echo-time.

H-MRS with short echo-time, especially using high field (3-4T) MR equipment has the advantage of measuring other metabolites like glutathione (GSH), glutamine (Glx), GABA and glutamate (Glu), likely to be involved in the pathogenesis of schizophrenia. Many experiences reported a decrease of Glu concentration in the medial frontal region and an increase in Glx and GABA concentrations in patients with schizophrenia compared with healthy individuals²²⁻²⁴. Interestingly, both Glu and Glx levels in the frontal region decrease progressively with age in patients with schizophrenia, which could suggest a progressive loss of synaptic activity²².

In conclusion, the findings of our study neither support nor refute the cognitive dysmetria theory. The connectivity between frontal cortex, thalamus and cerebellum could be better investigated using other techniques. Recently, for example, diffusion tensor tractography with MRI was used to evaluate the white matter connectivity between cerebellum and thalamus in a group of schizophrenic patients and in a control group^{25,26}.

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