Anterior cingulate cortex and cerebellar hemisphere neurometabolite changes in depression treatment: A $^1$H magnetic resonance spectroscopy study

Li-Ping Chen, MD,1,3 Hai-Yang Dai, MD,4 Zhuo-Zhi Dai, MD,2 Chong-Tao Xu, PhD1* and Ren-Hua Wu, PhD2

1Department of Mental Health, Shantou University Medical College, 2Department of Medical Imaging, The Second Affiliated Hospital, Shantou University Medical College, Shantou, 3Department of Neurology, Guangzhou First People’s Hospital, Guangzhou, and 4Department of Medical Imaging, Huizhou Municipal Central Hospital, Huizhou, China

Aim: We utilized single-voxel $^1$H magnetic resonance spectroscopy to determine biochemical abnormalities related to major depressive disorder (MDD) in the bilateral dorsolateral prefrontal cortex, anterior cingulate cortex (ACC), and cerebellar hemisphere before and after antidepressant treatment.

Methods: Fifteen adult MDD patients and 15 age- and sex-matched healthy controls were involved. Magnetic resonance spectroscopy of the brain was conducted in all subjects at the beginning of the study and the depressed subjects were reassessed after 8 weeks of antidepressant treatment.

Results: At baseline, N-acetyl aspartate (NAA), total glutamine plus glutamate (Glx) and myo-inositol (MI) levels in the bilateral ACC were significantly lower in MDD patients than in controls ($P < 0.05/3$). MI in the bilateral cerebellar hemisphere were also decreased in patients compared with controls. After the treatment, the lower NAA, Glx and MI in ACC were normalized in MDD patients and the NAA and Glx increased compared to baseline values. The MI levels in the bilateral cerebellar hemisphere were also normalized in patients. MI and choline levels in the right cerebellar hemisphere were elevated compared to those at baseline.

Conclusion: Our study suggests that metabolic abnormalities in the ACC and cerebellar hemisphere are implicated in MDD. Antidepressants may alter the local metabolic abnormalities in these areas.

Key words: choline, glutamate/glutamine, myo-inositol, N-acetyl aspartate, selective serotonin reuptake inhibitor.

Recent developments of neuroimaging technologies have enabled identification of alterations in certain brain regions in patients with major depressive disorder (MDD). The prefrontal areas, particularly anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC), have been suggested to play a key role in the emotional and cognitive processing of MDD. Functional neuroimaging studies provided evidence of dysfunction in the ACC as well as in prefrontal, orbitofrontal and subcortical regions. Proton magnetic resonance spectroscopy ($^1$H-MRS) is a non-invasive procedure that allows for direct, in vivo and non-radioactive measurement of neuronal substrates, including neuronal metabolites (neurometabolites) in the human brain. Previous $^1$H-MRS studies have yielded inconsistent results concerning the metabolic profile of the DLPFC and ACC in adults with MDD. In contrast to
the large number of imaging studies exploring the cerebral cortex in MDD, there is increasing recognition that the cerebellum contributes to cognitive processing and emotional control in addition to its role in motor coordination. A recent meta-analytic study in MDD noted that the cerebellum was one of the most consistently identified regions involved in the pathophysiology of the illness. Moreover, quantitative meta-analysis of a large number of functional neuroimaging studies in depressed subjects revealed decreased activity of the cerebellum, which was ameliorated in patients treated with antidepressants. Neuroanatomical studies have also shown that the cerebellar hemispheres project to the contralateral DLPFC through dentate to thalamic fiber tracts. Cortical regions that receive cerebellar output project back to the cerebellum, thus forming a closed prefronto-cerebellar circuit. Despite these observations, none have focused on the biochemical abnormalities in the cerebellar hemisphere with MDD patients. The purpose of this study was to explore metabolic changes in the bilateral DLPFC, anterior cingulum or cerebellar hemisphere following antidepressant medication in young adult MDD patients.

**METHODS**

**Participants**

The study was approved by the local ethics committee. Written informed consent was obtained from all subjects after the study was fully explained. Fifteen patients with major depression, ranging in age from 18 to 50 years (9 female, 6 male; mean ± SD, 26.2 ± 10.1 years), were recruited from outpatient clinical sites of the Mental Health Center, Shantou University Medical College, China between April 2011 and October 2011. Fifteen age- and sex-matched healthy controls (mean ± SD, 27.6 ± 12.3 years) were enrolled via local advertisements. All depressed patients met DSM-IV criteria for major depression. They were either in an active major unipolar depressive episode or at least had some depressed symptoms. The 17-item Hamilton Depression Rating Scale (HAMD) was used to determine the severity of the illness. All patients had scores of ≥17 on the 17-item HAMD. They had never taken any psychotropic drugs or accepted electroconvulsive therapy (ECT) previously, or were off antidepressants for at least 2 weeks. All subjects were right-handed.

The exclusion criteria for all subjects included: (i) another major psychiatric illness, including bipolar disorder, schizophrenia, or dementia; (ii) alcohol or drug abuse or dependence; (iii) presence of any neurological disease, such as seizures, vascular disease or brain injury; (iv) presence of any physical illness (including hypertension, ischemic heart disease, diabetes, Cushing or obesity) as assessed on personal history, abnormal signs in clinical examination or laboratory data (complete blood count, biochemical tests, lipid profile, thyroidal function tests or electrocardiography screening); (v) the total illness course of depression > 24 months; and (vi) contraindications to magnetic resonance scans.

**Procedure**

Fifteen MDD patients and 15 healthy subjects were involved in the study. An independent doctor who was unaware of this study decided on the patients’ medication, and his instructions were followed. Psychopathology and MRS were performed at the baseline for all subjects. Subsequently, all MDD patients were treated with a selective serotonin reuptake inhibitor (SSRI). Then, depressed subjects were reassessed at 8 weeks of antidepressant treatment.

**Magnetic resonance imaging and spectroscopy**

MRS data were collected using a 1.5-T GE Signa HDx scanner (General Electric Medical Systems, Milwaukee, WI, USA), using an eight-channel head and neck coil. Sagittal T1-weighted spin-echo (relaxation time/echo time [TR/TE], 650/20 ms), axial and coronal T2-weighted turbo spin-echo (TR/TE, 4000/100 ms) was performed to exclude the presence of cranial abnormalities and to locate the voxels of interest for MRS. Upon evaluation by an experienced neuroradiologist, proton magnetic resonance spectra were performed at five single voxels from different brain areas, including the anterior cingulate (2 × 2 × 1.5 cm³), the left DLPFC (2 × 2 × 2 cm³), right DLPFC (2 × 2 × 2 cm³), left cerebellar hemisphere (2 × 2 × 2 cm³) and right cerebellar hemisphere (2 × 2 × 2 cm³) (Fig. 1f1–f5).

The ACC single voxel was placed just in front of the genu of the corpus callosum, which include the regions of the anterior cingulate cortex and medial
Figure 1. Absolute metabolite concentrations (in millimolar, or mM) by region: (a) anterior cingulate cortex (ACC); (b) left dorsolateral prefrontal cortex (DLPFC); (c) right DLPFC; (d) left cerebellar hemisphere; and (e) right cerebellar hemisphere. Dark gray bars are baseline major depressive disorder (MDD) subjects. Mid-gray bars are post-treatment MDD subjects. Light-gray bars are control subjects. *$P < 0.05/3$, baseline MDD vs normal controls (NC) or post-treatment MDD vs NC. (f) Proton magnetic resonance spectra were performed at five single voxels from different brain areas, including the (f1) anterior cingulate, (f2) left DLPFC, (f3) right DLPFC, (f4) left cerebellar hemisphere and (f5) right cerebellar hemisphere. (g) Examples of attained single voxel spectra in the ACC by LCModel evaluation of metabolite values: (g1) Baseline MDD (g2) Post-treatment MDD. Cho, choline; Cr, creatine; Glx, total glutamine plus glutamate; MI, myo-inositol; NAA, N-acetyl aspartate.
prefrontal cortex. The DLPFC voxel was placed just in front of either the left or the right anterior limb of the internal capsule, which includes the superior frontal gyrus and part of the anterior cingulate and prefrontal cortex; and the cerebellar hemisphere voxel was placed in the largest slice of either the left or right cerebellar hemisphere, just in the middle of the level of the middle cerebellar peduncle. Single voxel MRS was performed using a point-resolved spectroscopy acquisition mode (PRESS) sequence (TE = 30 ms, TR = 3000 ms, with 128 averages, duration 6 min 24 s). Water suppression (≥298%) and shimming (linewidth, <10 Hz) were automatically achieved using a variable pulse power and optimized relaxation delay scheme. The concentrations of N-acetyl aspartate (NAA), total glutamine plus glutamate (Glx), choline (Cho) and myo-inositol (MI) were measured and compared between groups.

**Post-processing and metabolite quantification**

Post-processing, including phase and frequency correction was performed using SAGE software. Analysis was conducted with LCModel (LCMODEL, Oakville, Ontario, Canada). LCModel normalizes the metabolite spectra obtained using an unsuppressed water peak as a reference. Only metabolites with a Cramer-Rao SD of less than 20% were included for analysis. Absolute concentrations of NAA, Glx, Cho and MI were derived and compared between groups. An external standard phantom, containing detectable compounds of known concentrations, was used to calibrate the metabolite concentrations.

**Statistical analysis**

Baseline demographic differences between healthy control subjects and depression patients were calculated by χ² and t-tests. Paired-samples t-test was chosen to test HAM-D scores within the depressed group. The χ² and t-tests were used to compare the MDD and HC groups with respect to demographic and clinical variables. Data are presented as means and SD. All the metabolite levels exhibited a normal distribution as documented by the Kolmogorov–Smirnov test. The two-tailed significance level was set at P < 0.05. The Student’s t-test was used to compare metabolite levels between healthy subjects and patients. Metabolite levels and clinical scores of patients at baseline and after antidepressant treatment were compared with a paired sample t-test. Pearson’s correlation coefficient was used to correlate age and clinical variables within the measured metabolite levels. SPSS 13.0 (SPSS, Chicago, IL, USA) was used. To consider type 1 errors in the multiplicity of statistical analyses, P < 0.05/3 is significant, and the others are treated as trend levels.

**RESULTS**

There were no significant differences between patient and control groups in age and sex status. Seven of the patients had their first episode and were drug-naïve before inclusion in the study. The remaining patients were free of psychotropic drugs (including anxiolytics) for a mean of 35.6 days (Table 1).

All MDD patients were treated with a SSRI and the number of patients taking each medication was as follows: 50 mg/day sertraline (n = 2), 10 mg/day escitalopram (n = 6), 20 mg/day paroxetine (n = 5), and 20 mg/day fluoxetine (n = 2). Three patients on escitalopram dropped out of the study, so that 12 MDD subjects were reassessed after 8 weeks of antidepressant treatment. The predetermined criterion of remission was achieved after the duration of acute treatment with SSRI for 8 weeks. Depression symptoms improved in MDD patients, and the 17-item HAMD scores of MDD patients decreased more than 50% before the second MRS scanning (mean ± SD, 10.17 ± 4.69). Of the 12 MDD patients who took antidepressants and participated in the second MRS scan, only one patient, who had been on 20 mg/day fluoxetine for 8 weeks, showed no improvement. This may be due to his higher severity of depression (HAMD = 29) and the longer duration of depressive episodes (duration = 18 months total). The metabolite concentrations in each voxel in our study are displayed in Figure 1a–e. In the cingulum, baseline NAA (t = −3.202, P = 0.004), Glx (t = −4.550, P = 0.000) and MI (t = −3.228, P = 0.006) levels were significantly lower in depressed patients compared to those of healthy controls (Fig. 1a). However, there were no significant differences between control and pre-treatment MDD subjects for any metabolites in the bilateral DLPFC (P > 0.05). After treatment, the lower NAA (t = 0.086, P = 0.932), Glx (t = 1.174, P = 0.259) and MI (t = −0.888, P = 0.384) levels in the ACC were normalized in MDD patients and no statistically significant differences existed between post-treatment and controls. The ACC Glx (t = −3.111, P = 0.017) level increased significantly compared to
those prior to baseline level and the NAA \( (t = -2.629, P = 0.025) \) also showed a prominent ascendant trend (Fig. 1a). The MI levels (left: \( t = -1.615, P = 0.121 \); right: \( t = 0.853, P = 0.404 \)) in the bilateral cerebellar hemisphere were normalized in patients after treatment. Noteworthy is the increase of MI \( (t = -2.820, P = 0.026) \) and Cho \( (t = -2.389, P = 0.036) \) in the right cerebellar hemisphere compared to baseline values (Fig. 1e), while no significant metabolite changes were found between baseline and post-treatment MDD subjects in the bilateral DLPFC. No correlations between metabolite levels and HAMD scores before and after antidepressant treatment were observed. An example spectrum in the ACC is presented in Fig. 1 (g1, Baseline MDD; g2, Post-treatment MDD).

**DISCUSSION**

The remarkably diminished anterior cingulated Glx concentrations in our research replicate the results of previous MRS studies in MDD,\(^9\) suggesting dysfunctional glutamatergic neurotransmission. However, some studies indicated that due to the high-energy demands of the neural–astrocyte interaction in glutamate metabolism, alteration of Glx level may not represent abnormal neurotransmission, but may rather indicate impaired gial function.\(^{10,11}\) It is believed that normal glutamate metabolism depends on intact neuronal and glial cell function. A meta-analysis predicted that of all regions studied in functional brain imaging studies, including functional magnetic resonance imaging (fMRI), single photon emission computed tomography (SPECT) and positron emission tomography (PET), the ACC was most consistently associated with functional differences compared to controls.\(^7\) Our findings of the changes in absolute values of Glx observed with the depressive symptoms reduction are consistent with previous structural and functional studies and can be explained by the glutamate/astrocytic dysfunction in the ACC of MDD patients. Furthermore, clinical recovery of MDD is associated with the low Glx concentration returning to normal. It has been reported that chronic administration of antidepressant drugs could induce delayed structural and molecular adaptations at glutamatergic forebrain synapses that may be implicated in the antidepressant actions.\(^12\) Our findings of the significant increase Glx concentrations in the ACC after treatment in MDD patients are consistent with previous studies and suggest that the glutamatergic system plays an important role in the neurobiology and treatment of MDD.\(^13\)

We observed an NAA decrease significantly at baseline across all patients compared with control subjects in the ACC. The NAA reduction was reported in the caudate in unipolar depressed patients\(^{14}\) and in patients with MDD\(^{15}\) in the cingulum. NAA is an amino acid highly localized to neurons and often considered a marker of neuronal density and integrity. Studies of other diseases known to involve neuronal and/or axonal loss (e.g. infarcts, brain tumors, multiple sclerosis plaques) have uniformly shown NAA to be decreased.\(^{16}\) Accumulating evidence suggests that

| Clinical variables | Patient group \( n = 15 \) (mean ± SD) | Control group \( n = 15 \) (mean ± SD) | Comparison |
|-------------------|----------------------------------------|----------------------------------------|------------|
| Age (years)       | 27.87 ± 11.67                          | 27.53 ± 11.89                          | \( t = -0.077, \text{d.f.} = 28, P = 0.939 \) |
| Sex (M/F)         | 6/9                                    | 6/9                                    | \( \chi^2 = 0.00, \text{d.f.} = 1, P = 1.000 \) |
| Education (years) | 11.80 ± 2.57                           | 13.47 ± 3.34                           | \( t = -1.533, \text{d.f.} = 28, P = 0.136 \) |
| Duration of depressive episodes (months, total) | 14.80 ± 9.94 | - | - |
| Hamilton Depression Rating Scale | - | - | - |
| Baseline          | 23.33 ± 4.36*                          | 3.07 ± 1.21                            | \( t = 10.253, \text{d.f.} = 11, P = 0.000 \) |
| After treatment   | 10.17 ± 4.69*                          | 3.07 ± 1.21                            | \( t = 5.101, \text{d.f.} = 11, P = 0.000 \) |

Values are expressed as mean ± SD.

*\( P < 0.01 \), pre-treatment major depressive disorder vs normal controls or post-treatment major depressive disorder vs normal controls.
decreases in regional NAA levels are not static and can also represent reversible neuronal or mitochondrial dysfunction. Thus the decreased NAA levels might not reflect neuronal death per se but the role of NAA as an early functional state marker. Koolschijn also indicated that volume reductions in MDD patients are prominent in the ACC. The lower NAA exhibited reduced density of neurons and neuron dysfunction in the bilateral ACC in our MDD patients at baseline. Furthermore, studies have demonstrated that decreased NAA concentrations are reversible and NAA is sensitive to processes that affect neuronal function. Following antidepressant treatment, the NAA levels increased in the ACC compared to MDD patients at baseline. Our results suggest that antidepressants may ameliorate or normalize NAA metabolite abnormalities in patients with MDD. The increment in NAA levels can be interpreted as an improvement of neuronal viability and integrity in the ACC.

Myo-inositol is traditionally considered to be a marker of the glial cells, which play an important role in signal regulation and neurogenesis. The role of glia in the cause of MDD has not yet become clear. Our study found a significant decrease in ACC MI concentrations in MDD patients at baseline and the lower MI was normalized after treatment. Previous studies reported that MI or MI/creatinine (Cr) ratios were significantly reduced in the ACC/PFC of MDD patients. Postmortem studies have shown a decrease in glial density, a finding that has been replicated in ACC. Thus, our results might indicate that MI responds to antidepressants of MDD.

In contrast to our findings in the ACC, bilateral DLPFC metabolites did not differ in unmedicated MDD patients and healthy controls. In addition, no changes occurred in these regions before and after treatment. This contrasts previous MRS findings reporting alterations of metabolite levels in the DLPFC and neurometabolite changes seen with response to antidepressive treatment in these regions of depressed patients. However, it should be noted that Kumar et al. and Michael et al. showed in the left DLPFC of elderly depressed patients, whereas Farchione et al. investigated 11 depressed pediatric patients, of whom seven had another psychiatric disorder. The discrepancies between these studies and ours may be due to differences in the patient samples involved. On the contrary, our sample was composed of relatively young euthymic or mild-to-moderately depressed patients with illness onset in adulthood. Therefore, these investigations are not comparable in terms of age range (geriatric and pediatric groups), treatment modalities, and psychiatric history. Furthermore, differences in acquisition and post-processing parameters may also account for some discrepancies between our findings and other \(^1\)H spectroscopy controlled studies.

Recent evidence indicates that the cerebellum is involved in emotional control. A PET study noted activation of the bilateral cerebellum during provocation of happiness, sadness and disgust in healthy volunteers. An fMRI study also showed reduced activation in lateral cerebellum during anticipation of the noxious stimulus in women who had recovered from major depression, suggesting that depression may impart a permanent and irreversible change in cerebellar function. Interestingly, the MI levels in the bilateral cerebellar hemisphere in our unmedicated MDD patients were lower than health controls and were normalized after treatment, which provide important evidences that suggest that the cerebellar hemisphere might be involved in the cause of MDD.

Additionally, the increased MI and Cho after treatment in our study might indicate the influence of antidepressant treatment on the probable damage in the glial cells and membrane turnover. We know that glial cells have their own trophic factors and play a role in signal regulation and neurogenesis. Monoamines released to the synapse by antidepressants could also be transported to the glial cells. Choline is an essential precursor of the neurotransmitter acetylcholine, as well as the membrane lipids phosphate dylcholine and sphingomyelin. An elevated Cho signal most likely reflects an increase in membrane turnover. We found metabolism changes before and after antidepressant treatment in the cerebellar hemisphere. Little et al. have also shown that never-hospitalized depressed patients responding to bupropion had pretreated hypermetabolism in cerebellum. Furthermore, high-frequency repetitive transcranial magnetic simulation was applied in a sham and occipital-stimulation-controlled within-subjects design to investigate the role of the cerebellar vermis in subconscious responses to emotional stimuli. That study found that exclusively after medial cerebellar stimulation, participants showed significant increased emotional responses to happy facial expressions. Our results are consistent with the previous neuroimaging evidences and predict that the cerebellar hemisphere might be a key region implicated in antidepressant treatment in MDD.
The present study has some limitations that should be noted. The major issue in our study is the small sample size and type-1 errors might inevitably be included. Second, inhomogeneous antidepressant treatment was another limitation. All drugs, however, belonged to the same group: SSRI. Third, the imaging protocol introduces additional sources of error. As usually was the case in preliminary researches, we defined acquisition parameters as TR = 3000 ms and TE = 30 ms. In short-echo spectra, macromolecule signals affect the quantification of metabolite concentrations at baseline, and the longer TR time can result in a higher SNR. Also noteworthy, TR time mainly affects the scanning time: the longer the TR is selected, the more scanning time consumed and it is difficult for our subjects to remain still in a long-duration MRS scan. Furthermore, the lack of grey matter correction also restricts the analysis of the MRS data in this study. As in the single-voxel MRS technique, the ROI selected covers a field of brain tissue and it is almost inevitable to incorporate both gray matter and white matter. It may be more accurate to acquire the metabolites concentrations in either gray matter or white matter, for example using Matlab and SPM software in further studies. However, in the data post-processing stage, metabolite concentrations were automatically adjusted for cerebrospinal fluid and ratio of gray to white matter using LCModel software.

In conclusion, the present data provide evidence for neurochemical alterations in the anterior cingulate cortex and cerebellar hemisphere in patients with major depression. This is the first longitudinal study investigating effects of antidepressant medication using {sup}1{H}-MRS in the bilateral DLPFC, ACC and cerebellar hemisphere in relatively young adults with MDD. An increase in MI and Cho levels in the right cerebellar hemisphere following treatment provided further evidence to support a role of the cerebellum involved in the pathophysiology of the MDD.

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