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In vivo gamma-aminobutyric acid and glutamate levels in people with first-episode schizophrenia: A proton magnetic resonance spectroscopy study

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ABSTRACT

Background: Gamma-aminobutyric acid (GABA) dysfunction and its consequent imbalance are implicated in the pathophysiology of schizophrenia. Reduced GABA production would lead to a disinhibition of glutamatergic neurons and subsequently cause a disruption of the modulation between GABAergic interneurons and glutamatergic neurons. In this study, levels of GABA, Glx (summation of glutamate and glutamine), and other metabolites in the anterior cingulate cortex were measured and compared between first-episode schizophrenia subjects and healthy controls (HC). Diagnostic potential of GABA and Glx as upstream biomarkers for schizophrenia was explored.

Methods: Nineteen first-episode schizophrenia subjects and fourteen HC participated in this study. Severity of clinical symptoms of patients was measured with Positive and Negative Syndrome Scale (PANSS). Metabolites were measured using proton magnetic resonance spectroscopy, and quantified using internal water as reference.

Results: First-episode schizophrenia subjects revealed reduced GABA and myo-inositol (mI), and increased Glx and choline (Cho), compared to HC. No significant correlation was found between metabolite levels and PANSS scores. Receiver operator characteristics analyses showed Glx had higher sensitivity and specificity (84.2%, 92.9%) compared to GABA (73.7%, 64.3%) for differentiating schizophrenia patients from HC. Combined model of both GABA and Glx revealed the best sensitivity and specificity (89.5%, 100%).

Conclusion: This study simultaneously showed reduction in GABA and elevation in Glx in first-episode schizophrenia subjects, and this might provide insights on explaining the disruption of modulation between GABAergic interneurons and glutamatergic neurons. Elevated Cho might indicate increased membrane turnover; whereas reduced mI might reflect dysfunction of the signal transduction pathway. In vivo Glx and GABA revealed their diagnostic potential for schizophrenia.

Abbreviations: ^{1}H–MRS, proton magnetic resonance spectroscopy; ACC, anterior cingulate cortex; AUC, area under curve; Cho, choline; CI, confidence interval; Cr, creatine; CRLBs, Cramér Rao Lower Bounds; CSF, cerebrospinal fluid; FFE, fast field echo; FWHM, full width at half maximum; GABA, gamma-aminobutyric acid; GAD67, 67-kDa isoform of glutamic acid decarboxylase; Glu, glutamate; Gln, glutamine; Glx, summation of glutamate and glutamine; GM, grey matter; HC, healthy control; MEGA-PRESS, Meshcher-Garwood point resolved spectroscopy; mI, myo-inositol; NAA, *N*-acetyl aspartate; NMDAR, *N*-methyl-D-aspartic acid-type glutamate receptors; PANSS, Positive and Negative Syndrome Scale; PRESS, point resolved spectroscopy; QUEST, quantification based on quantum estimation; r, Pearson correlation coefficient; ROC, receiver operator characteristics; SVS, single voxel spectroscopy; T1 W, T1-weighted; TE, echo time; TR, repetition time; WAIS-R, Wechsler Adult Intelligence Scale-Revised; WM, white matter.

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1. Introduction

Gamma-aminobutyric acid (GABA) dysfunction and its subsequent imbalance between excitation and inhibition in the cerebral cortex have been implicated in the pathophysiology of schizophrenia (Benes and Berretta 2001; Guidotti et al. 2005; Lewis et al. 2005). In fact, the hypothesis of *N*-methyl-D-aspartic acid-type glutamate receptors (NMDAR) hypofunction has been proposed to be the chief mechanism behind schizophrenia pathophysiology (Nakazawa et al. 2012), and cortical GABAergic interneurons are suggested to be a prime target for NMDAR hypofunction (Olney and Farber 1995).

Post-mortem studies combined with genetic findings have generated compelling evidence suggesting the disruption of the modulation of NMDAR subtypes would contribute to the psychopathology of schizophrenia (Coyle and Tsai 2004). Moreover, reduction in the 67-kDa isoform of the synthetic enzyme for GABA in the cortex, glutamic acid decarboxylase (GAD67), is also a well-replicated molecular finding in schizophrenia (Akbarian et al. 1995; Curley et al. 2011; Gonzalez-Burgos et al. 2010; Guidotti et al. 2000; Hashimoto et al. 2003; Olney et al. 1999), further emphasizing the critical role of GABA in schizophrenia. Indeed, NMDAR hypofunction of inhibitory GABAergic interneurons would consequently cause a reduction of its excitation, and in conjunction with decreased GABA production due to GAD67 reduction, this would thus lead to a disinhibition of glutamatergic neurons, resulting in excessive release of glutamate (Glu) (Lisman et al. 2008), causing a disruption of the modulation between GABAergic interneurons and glutamatergic neurons (Marsman et al. 2014).

To examine Glu and GABA levels in schizophrenia, in vivo measurements could be achieved using proton magnetic resonance spectroscopy (^{1}H–MRS). However, unambiguous detection of GABA cannot be accomplished by conventional pulse sequences due to the overlapping of resonance peaks of GABA with other detectable metabolites. Yet, with the advent of technology, a Meshcher-Garwood point resolved spectroscopy (MEGA-PRESS) pulse sequence was developed to detect GABA (Edden and Barker 2007). Recently, a number of ^{1}H–MRS studies investigating in vivo GABA in patients with schizophrenia yielded mixed results, with the majority reporting reduced GABA (Goto et al. 2010; Kelemen et al. 2013; Marsman et al. 2014; Rowland et al. 2013; Rowland et al. 2016; Thakkar et al. 2017; Yoon et al. 2010), while the remaining showing either increased (Kegeles et al. 2012; Ongur et al. 2010) or no change (Chen et al. 2014; Stan et al. 2015; Tayoshi et al. 2010) in GABA in schizophrenia patients, compared with healthy controls (HC). Whereas for Glu, due to the overlapping resonances of Glu and glutamine (Gln) at field strength of ≤3.0 T, it was commonly expressed together with Gln as Glx [summation of Glu and Gln], and prior studies also showed controversial results of elevated (Bustillo et al. 2010; Kegeles et al. 2012; van Elst et al. 2005), reduced (Liemburg et al. 2016; Natsubori et al. 2014; Rowland et al. 2013; Tayoshi et al. 2010; Theberge et al. 2003), or no change (Marsman et al. 2014) in Glx in schizophrenia patients, compared with HC. Although it has been hypothesized that the modulation between GABAergic interneurons and glutamatergic neurons is disrupted in schizophrenia, there were only a few studies investigating GABA and Glu simultaneously in the same cohort reporting diverse results, i.e. elevations in both GABA and Glx (Kegeles et al. 2012), reductions in both GABA and Glx (Rowland et al. 2013; Thakkar et al. 2017), reduced GABA but no change in Glx (Marsman et al. 2014), and no change in GABA but decreased Glx (Tayoshi et al. 2010), and these studies failed to demonstrate the disrupted modulation.

Of all the brain regions, the anterior cingulate cortex (ACC), which exhibits extensive connections and plays a critical role in cognitive and emotional processes (Paus 2001; Walton et al. 2007), has been repeatedly reported to exhibit abnormalities in schizophrenia patients in both neuropathological and neuroimaging studies, showing alterations in GAD67 levels (Woo et al. 2004), morphological change (Baiano et al. 2007; Fujiwara et al. 2007; Zetzsche et al. 2007), and reduced activation during cognitive tasks (Brune et al. 2008; Koch et al. 2008; Liddle et al. 2006; Yucel et al. 2007).

In the current study, in vivo GABA and Glx were measured in the ACC of first-episode schizophrenic patients and compared with HC using ^{1}H–MRS at 3.0 T. Based on the involvement of GABA and Glu in schizophrenia pathophysiology, we hypothesized that the disruption of modulation between GABAergic interneurons and glutamatergic neurons might be reflected by the levels of GABA and Glx. Furthermore, the diagnostic potential of various metabolites as upstream biomarkers for first-episode schizophrenia was also assessed.

2. Methods

2.1. Subjects

Nineteen subjects with first-episode schizophrenia (mean duration of illness ± standard deviation = 17.58 ± 14.79 months) and fourteen HC participated in this study. Clinical subjects were recruited from outpatient clinics of an early psychosis intervention programme in Hong Kong, and all were diagnosed with DSM-IV (APA 2000) schizophrenia. The inclusion criteria were (1) 18–40 years of age; and (2) duration of illness of <5 years (since the onset of first-episode schizophrenia). Exclusion criteria were (1) a lifetime history of substance abuse; (2) history of undergoing electro-convulsive therapy in the past 6 months; (3) presence of co-morbid Axis I DSM disorders; (4) history of head injury; (5) presence of neurological disorders; and (6) epilepsy. Diagnosis was ascertained by two qualified psychiatrists using structured clinical interview (First et al. 1996). The severity of clinical symptoms was measured using the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987). All clinical subjects were receiving follow-up at the early psychosis clinic. At the time of scanning, all schizophrenia subjects have not experienced any schizophrenia relapse, as ascertained by the treating psychiatrists, nor they have experienced any exacerbation of psychotic symptoms which necessitate medication adjustments. Clinical profile of each first-episode schizophrenia patient is shown in Table 1. HC were recruited from neighbouring community, and interviewed by a qualified psychiatrist using structured interviews to ascertain that they had no family or lifetime history of any DSM-IV psychiatric disorder. They did not have any history of substance abuse in the past 6 months before scanning.

Demographics and clinical characteristics were gathered from case-notes review, and the dosage of antipsychotics was converted to chlorpromazine equivalence (mg/day) (Gardner et al. 2010). The first-episode schizophrenia subjects and HC did not differ in age ($p = 0.481$) and gender ($p = 0.710$) (see Table 2). All subjects with first-episode schizophrenia were medicated and clinically stable at the time of this study, as evidenced by the low ratings on the PANSS, which was assessed within three weeks of the day of scanning. Prorating method was used to estimate intelligence, based on the Chinese version of the Arithmetic, Similarities and Digit Span subscales of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Gong 1992). All subjects gave their informed consent. The study was approved by the Institutional Review Board of The University of Hong Kong/Hong Kong West Cluster.

2.2. MR scanning

2.2.1. Structural

All MR scans were performed using a 3.0 T scanner (Achieva TX, Philips Healthcare, Best, The Netherlands). A sensitivity encoding-head-8-coil was used. A standardized T1-weighted (T1 W) 3D volumetric fast field echo (FFE) sequence was employed with the following imaging parameters: repetition time (TR)/echo time (TE) = 7.0/3.2 ms, voxel size = $1 \times 1 \times 1$ mm^3, field of view = $240 \times 240 \times 160$ mm^3, reconstruction matrix = 256, and turbo field echo factor = 240. Images acquired from T1 W 3D FFE were employed for the positioning of single

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