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Alterations of eicosanoids and related mediators in patients with schizophrenia

Dongfang Wang[a], Xiaoyu Sun[a], Jingjing Yan[a], Biao Ren[b], Bing Cao[a], Qingbin Lu[a,c,d], Yaqiong Liu[a,c,d], Jing Zeng[a,c,d], Ninghua Huang[a,c,d], Qing Xie[a,c,d], Haiwei Gu[e,*], Jingyu Wang[a,c,d,**]

[a] Department of Laboratorial Science and Technology, School of Public Health, Peking University, Beijing 100191, PR China
[b] Shimadzu (China) Co., LTD Beijing Branch, Beijing 100020, PR China
[c] Vaccine Research Center, School of Public Health, Peking University, Beijing 100191, PR China
[d] Peking University Medical and Health Analysis Center, Peking University, Beijing 100191, PR China
[e] Center for Metabolic and Vascular Biology, School of Nutrition and Health Promotion, College of Health Solutions, Arizona State University, Phoenix, AZ 85004, USA

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ABSTRACT

Schizophrenia (SCZ) is a multifactorial psychiatric disorder. Currently, its molecular pathogenesis remains largely unknown, and no reliable test for diagnosis and therapy monitoring is available. Polyunsaturated fatty acids (PUFAs) and their derived eicosanoid signaling abnormalities are relevant to the pathophysiology of schizophrenia. However, comprehensive analysis of eicosanoids and related mediators for schizophrenia is very rare. In this study, we applied a targeted liquid chromatography-mass spectrometry based method to monitor 158 PUFAs, eicosanoids and related mediators from enzyme-dependent or independent pathways, in the serum samples of 109 healthy controls, and 115 schizophrenia patients at baseline and after an 8-week period of antipsychotic therapy. Twenty-three metabolites were identified to be significantly altered in SCZ patients at baseline compared to healthy controls, especially arachidonic acid (AA) derived eicosanoids. These disturbances may be related to altered immunological reactions and neurotransmitter signaling. After 8-week antipsychotic treatment, there were 22 metabolites, especially AA and linoleic acid derived eicosanoids, significantly altered in posttreatment patients. Some metabolites, such as several AA derived prostaglandins, thromboxanes, and dihydroxy-eicosatrienoic acids were reversed toward normal levels after treatment. Based on univariate analysis and orthogonal partial least-squares discriminant analysis, anandamide, oleoylethanolamine, and AA were selected as a panel of potential biomarkers for differentiating baseline SCZ patients from controls, which showed a high sensitivity (0.907), good specificity (0.843) and excellent area under the receiver operating characteristic curve (0.940). This study provided a new perspective to understand the pathophysiological mechanism and identify potential biomarkers of SCZ.

1. Introduction

Schizophrenia (SCZ) is a multifactorial psychiatric disorder with a worldwide prevalence of approximately 1% and exhibits a tremendous global disease burden (Saha et al., 2005). It is characterized by psychopathology, cognitive and neurobiological abnormalities, and deficiency in perception, emotion, and social behavior (Addington and Addington, 1999; Gonzalez-Liencres et al., 2014). While most of the previous molecular biology studies of SCZ have focused on neurotransmitters and their receptors (van Os and Kapur, 2009; Vyas et al., 2013), the underlying pathogeneses of this complex disorder have yet to be elucidated. Clinically, there is no reliable diagnostic test available, and current diagnosis of SCZ primarily relies on the subjective identification of clinical symptoms of patients (van Os and Kapur, 2009). During the treatment, heterogeneous responses to antipsychotics are observed attributable to its multifactorial pathoetiology, presentation, and progression (Burghardt et al., 2015). Thus, it is of significant clinical value to identify molecular biomarkers sensitive to the pathological processes of schizophrenia and antipsychotic treatment, which can facilitate the development of objective diagnostic tools and

* Corresponding author. Center for Metabolic and Vascular Biology, School of Nutrition and Health Promotion, College of Health Solutions, Arizona State University, 13208 E. Shea Blvd, CRB 2-221, Scottsdale, AZ 85259, USA.
** Corresponding author. School of Public Health, Peking University, No.38 Xueyuan Road, Haidian District, Beijing 100191, PR China.
E-mail addresses: haiweigu@asu.edu (H. Gu), wjy@bjmu.edu.cn (J. Wang).

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effective therapies.

Polyunsaturated fatty acids (PUFAs) can be oxygenated in an enzyme-dependent or independent manner into numerous bioactive lipid mediators (Bao et al., 2018). One of the best-studied classes of these mediators are eicosanoids, produced from 20-carbon PUFA oxidation, such as prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs) which are synthesized from dihomo-γ-linolenic acid (DGLA, 20:3, n-6), arachidonic acid (AA, 20:4, n-6), and eicosapentaenoic acid (EPA, 20:5, n-3) (Astarita et al., 2015). The 18-carbon PUFAs including linoleic acid (LA, 18:2, n-6) and α-linolenic acid (ALA, 18:3, n-3) and 22-carbon PUFAs such as docosahexaenoic acid (DHA, 22:6, n-3) are metabolized into corresponding octadecanoids and docosanoids, respectively (Astarita et al., 2015). For the purpose of this study, the use of the terminology of "eicosanoids" has been extended to include octadecanoids and docosanoids. Three major enzymatic pathways involved in the metabolism of PUFA derived eicosanoids are cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) families (Buczynski et al., 2009).

PUFAs and their derived eicosanoid signaling abnormalities are relevant to the pathophysiology of schizophrenia (Horrobin, 1977; Skosnik and Yao, 2003). Eicosanoids perform numerous regulatory functions through interactions with eicosanoid receptors that have been found throughout the brain (Coleman et al., 1994). Eicosanoid receptors have been shown to modulate dopaminergic and serotonergic signaling (Mitsumori et al., 2011; Wacker et al., 2003) which are important neurotransmitter signaling pathways in the pathophysiology of schizophrenia. In addition, eicosanoids also influence immunological processes in schizophrenia (Hallahan and Garland, 2005; Schmidt et al., 2013). Most studies about the association of eicosanoids with schizophrenia focused on AA-derived eicosanoids from COX and LOX pathways, such as PGs and TXs, which are involved in the pathophysiological processes of neuropsychiatric disorders (Huber et al., 2011; Ong et al., 2010; Puppolo et al., 2014), including schizophrenia. For instance, previous studies indicated increased plasma levels of the pro-inflammatory mediator prostaglandin E2 (PGE_2), an eicosanoid produced from AA, due to increased COX-2 expression and activity (Das and Khan, 1998; Kaiya et al., 1989), and decreased anti-inflammatory 15d-prostaglandin J2 (15-d-PGJ2) (Martinez-Gras et al., 2011). Altered levels of PUFAs and eicosanoids were also observed during the antipsychotic treatment (Kim et al., 2012). There are evidences indicating that some second generation antipsychotics may influence PUFAs metabolism via regulating the desaturase gene or mRNA expression (Burghardt et al., 2013; McNamara et al., 2011). Clozapine, a second generation antipsychotic, was reported to decrease COX activity and PGE2 concentration (Kim et al., 2012). However, the evidence on the associations of eicosanoids with schizophrenia remains limited. Hundreds of eicosanoids and related mediators derived from PUFAs represent a complex metabolic network. Therefore, the ability to simultaneously measure a wide range of eicosanoids could further our understanding of their roles in schizophrenia, as well as provide potential biomarkers for schizophrenia.

Recently, metabolomics has emerged as a powerful approach to measure a wide breadth of small molecules in the context of physiological stimuli or disease states (Buas et al., 2016; Fiehn, 2002; Gu et al., 2015; Lindon and Nicholson, 2014). Targeted metabolomics, one of metabolomics strategies, is the measurement of defined groups of chemically characterized and biochemically annotated metabolites (Roberts et al., 2012). This approach takes advantage of the comprehensive understanding of a vast array of metabolites, and the known biochemical pathways to which they contribute (Roberts et al., 2012). Over the past decade, targeted metabolomics has been widely used for the integrated analysis of multiple biochemical pathways disturbed by psychiatric disorders, such as bipolar disorder, depression, and SCZ (Liu et al., 2015; Sethi and Brietzke, 2015; Sethi et al., 2017). However, there is still a lack of comprehensive metabolic profiling of eicosanoids and related mediators for schizophrenia.

In this study, we reported the first metabolomics analysis of eicosanoids and related mediators for schizophrenia. We applied a targeted liquid chromatography-mass spectrometry (LC-MS) based method to detect 158 eicosanoids and related mediators which are metabolized from PUFAs and related precursors via enzyme-dependent or non-enzymatic pathways in the serum samples of healthy controls, schizophrenia patients at baseline and the patients after an 8-week period of antipsychotic therapy. Our study aims to provide more evidence of eicosanoid disturbances to understand the pathophysiology of SCZ, and to find potential biomarkers associated with the diagnosis and therapeutic monitoring of SCZ.

2. Methods

2.1. Subjects

A total of 115 patients meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia were recruited from the inpatient clinic of the Weifang Mental Health Center (Shandong Province, China). All subjects met the following criteria: (1) no diabetes, hyperlipidemia, cardiovascular disease, or other severe physical diseases; (2) no additional mental disorders, alcoholism, or other substance abuse disorders. Of these, 27 patients were first-episode and drug-naïve, while the remaining schizophrenia subjects ($n = 88$) were recurrent and had not taken any antipsychotic drugs for at least 1 month before hospitalization. During the same time period, we recruited 108 healthy controls who matched the patients in age (± 5 years), gender, and ethnicity in frequency. All patients were hospitalized and received antipsychotic treatment for eight weeks. The Positive and Negative Syndrome Scale (PANSS) was used to evaluate psychopathology during weeks 0 and 8. During the trial, all patients received a standard diet from a menu of limited food choices provided by the hospital and abstained from alcohol or smoking.

The ethical committee of Peking University Health Science Center reviewed and approved the protocol of this study (IRB00001052-14071) and the procedures employed for sample collection. All participants signed a written informed consent before any procedure was carried out. If patients cannot make rational decisions, the consents were signed by their family members on behalf of the patients. This study was conducted in accordance with the Helsinki Declaration as revised 1981.

2.2. Sample preparation

All blood samples were collected from subjects after overnight fast at baseline and after an 8-week therapy. Serum samples were separated and stored at −80 °C until use. For LC-MS analysis, 200 μL of each serum sample was aliquoted into a labeled 2 mL Eppendorf vial. Protein precipitation and metabolite extraction were performed by adding 1 mL of methanol solvents containing 14 internal standards (ISs) (the detailed ISs and their concentrations see Supplementary Table S1). The mixture was then vortexed for 10 s and gently mixed for 10 min at 4 °C, and then centrifuged at 12,000 × g for 10 min. The supernatant was diluted with 4 mL of 0.1% formic acid in water and loaded onto preconditioned solid-phase extraction cartridge (33 u Polymeric Reversed Phase, 10 mg/1 mL, Strata-X). The cartridge was washed with 1 mL each of 0.1% formic acid and then 15% ethanol. Then, the metabolites were eluted with 200 μL of methanol. The eluent was evaporated by vacuum evaporator and reconstituted in 50 μL of methanol. After centrifuged at 12,000 × g for 5 min, 40 μL of supernatant was collected into a 150 μL vial insert for analysis. All samples were randomized during the sample preparation. A pooled sample from all the samples was extracted using the same procedure as described above. This sample was used as the quality control (QC) sample and was analyzed once for every 10–15 study samples.