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Research paper

Plasma metabonomics study of first-Episode schizophrenia treated with olanzapine in female patients

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HIGHLIGHTS

- Tests of blood glucose and lipids were conducted at baseline and after 4 weeks of treatment with olanzapine.
- Compared with the control group, the case group showed significant changes in plasma metabolites.
- In the therapeutically effective cases, metabolites appeared to change towards the normal trend.
- The metabonomics that we found can be potential biomarkers for the diagnosis and treatment of schizophrenia.

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ABSTRACT

Schizophrenia is a persistent chronic mental illness with an unknown pathogenic mechanism; no empirical laboratory-based tests are available to support the diagnosis of schizophrenia or to identify biomarkers correlated with the therapeutic effect of olanzapine.

For this study, 15 female first-episode, drug-naïve patients with schizophrenia and 15 healthy female volunteers were recruited. Tests for blood glucose and lipids were conducted at baseline and after 4 weeks of treatment with olanzapine. UPLC–MS based metabonomic analysis was performed on both case and control groups to identify biomarkers of schizophrenia at baseline and to explore which biomarkers correlated with the therapeutic effect of olanzapine after a 4-week treatment. Compared with the control group, the case group showed significant changes in plasma metabolites. Thirteen distinct metabolites were identified. Among all the therapeutically effective cases, levels of these metabolites appeared to shift towards the normal trend; 8 of the identified 13 metabolites changed dramatically. The metabolites that we found are potential biomarkers for the diagnosis and treatment of schizophrenia.

1. Introduction

Schizophrenia is a persistent chronic mental illness with common symptoms of psychotic behaviour (delusions and hallucinations), disorganisation, dysfunction in normal affective responses and altered cognitive functioning [1]. Several hypotheses for the pathogenesis of schizophrenia have been proposed, including abnormal alterations in embryonic neurogenesis due to molecular disturbances during brain development [2], and abnormalities in dopaminergic, serotonergic, glutamatergic and muscarinic signaling [3–6]. Oxidative stress-mediated cell damage might also be involved [7]. In-depth studies of schizophrenia have found that the prevalence of insulin resistance in schizophrenia is significantly higher than that of the general population [8]. Schizophrenia as well as the effects of antipsychotic drugs can cause or aggravate metabolic disorders.

Metabonomics is an important component of genomics, transcriptional genomics and proteomics which aims to investigate changes of the body's response to external stimuli. A metabonomic analysis is capable of detecting analytes with relatively low molecular weight (<1000 Da), such as amino acids, oligopeptides,

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sugars, steroids, biliary acids, fatty acids, and intermediates of many biochemical pathways [9,10]. Metabonomics is an emerging analytical technology that has been widely used in disease diagnosis, toxicity screening and drug efficacy assessments [11]. Ultraperformance liquid chromatography-mass spectrometry (UPLC–MS) is a convenient tool for high-throughput metabolomics profiling due to fast scanning capabilities, accuracy of mass measurements and widespread availability [12].

Therapeutic drug monitoring is a useful tool for the clinical management of patients receiving pharmacotherapy, particularly in psychiatry. We analyzed clinical blood samples via UPLC–MS-based metabonomics to search for early diagnosis biomarkers for schizophrenia and to identify metabonomic markers of olanzapine efficacy.

2. Methods

This study was scrutinized and approved by the institutional review board of the Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from all participants and the patients' guardians.

2.1. Participants

A total of 30 female subjects participated in this study. Fifteen cases were diagnosed as first-episode drug-naïve schizophrenia according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) by two certificated psychiatrists from the Shanghai Mental Health Center in China. Patients of childbearing potential were required to have a negative pregnancy test at the time of screening. Exclusion criteria were any chronic untreated medical condition; substance dependency; and any other psychiatric disorder in Axis I or II.

During the same time period, healthy control subjects whose demographic data (height, weight, age, race.) matched the case patients were recruited from the staff of the Shanghai Mental Health Center, who were interviewed to ascertain that there was no psychiatric illness in their first-degree relatives. There were no significant differences in age, height, weight or BMI between patients and healthy controls (28.25 ± 5.34 vs. 27.63 ± 6.34 years, $p = 0.83$; 161.12 ± 5.87 vs. 164.63 ± 3.78 cm, $p = 0.18$; 53.19 ± 5.95 vs. 55.06 ± 6.60 kg, $p = 0.56$; 20.54 ± 1.54 vs. 20.19 ± 2.10 kg/m^2, $p = 0.87$, respectively). Subjects with evidence of organic brain disorders, alcohol or drug abuse, pregnancy, or any severe physical illness, such as brain tumour or epilepsy, were excluded from the study. Physical examinations to establish weight, height and blood pressure as well as face-to-face interviews were conducted before blood was collected from the 15 control subjects.

2.2. Procedures

Patients were treated with olanzapine and received daily doses ranging from 5 to 30 mg, doses commonly used by clinicians. It is reported that olanzapine can rapidly improve the acute symptoms of schizophrenia, within 2–4 weeks [13,14]. Plasma specimens were collected from the patients at baseline and 4 weeks after olanzapine monotherapy. All samples were analyzed by UPLC–MS to collect metabonomics data. The subjects were allowed to take certain drug combinations (such as benzhexol hydrochloride, benzodiazepines, zolpidem), while other drugs, such as other antipsychotics, antidepressants, and mood stabilising agents, were not allowed. Blood glucose and lipids (including levels of triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein) were tested at baseline and at the end of the 4 weeks of treatment.

The Positive and Negative Syndrome Scale (PANSS) [15] was applied to rate the severity of schizophrenia and the efficacy of treatment. Based on the therapeutic effect, we planned to divide the patients into effective and ineffective groups, and then compare their plasma metabolites to the control group.

UPLC–MS-based metabonomics analysis was performed on patients at baseline and after 4 weeks of treatment. Both of these scores were compared to the control group. A low-protein and low-fat food intake was ensured three days before sample collection for both case and control groups, and the diet of hospitalized patients was strictly followed through the hospital cafeteria's nutrition criteria. The patients' physical activity was limited to 4–6 h per week.

2.3. Efficacy assessment

An efficacy assessment was conducted, and for treatment to be deemed effective, patients needed to show a $\geq$50% reduction in the PANSS score from baseline to day 28 (the end of week 4) [16].

Two senior psychiatrists received training on the PANSS and then conducted the efficacy assessment. After passing a test on the use of the PANSS and getting high internal concordance (an internal concordance of 0.70 [17] was the minimum required), both psychiatrists were qualified as scorers.

2.4. Blood sample collection

Fasting blood samples were collected from the antecubital vein on the morning of day 0 as a baseline. At the end of the fourth week, samples were again drawn in the morning, approximately 12 h after the last dose of olanzapine. All blood samples were centrifuged at 3000 g for 5 min at 4 °C and the sera were frozen in 2 ml cryovials at −80 °C until analysis. A 100 μl aliquot of each serum sample was added to 300 μl methanol, and the mixture was vortexed vigorously for 3 min. The samples were then centrifuged at 12,000 rpm for 15 min at 4 °C to separate the supernatant, which was stored at 4 °C until UPLC–MS.

2.5. UPLC–MS

Gradient elution was performed with an Agilent UPLC 1290–6538 Q/TOF MS (Agilent, USA). An Acquity UPLC BEH HILIC 2.1×100 mm, 1.8 μm column (Waters Corp., USA) was used. Column temperature was set to 40 °C. Gradient mobile phase conditions were used, with the two solvents composed of phase A (water with 0.1% formic acid) and phase B (acetonitrile containing 0.1% formic acid). A flow rate of 0.4 ml/min was applied and the injection volume was 4 μl. The gradient elution consisted of maintaining the mobile phase composition at 2% B from 0 to 2 min, then ramping up to 95% B from 2 to 17 min, then maintaining at 95% B from 17 to 19 min. Total chromatographic run time was 19 min.

Mass spectrometry was performed using a 6538 Q/TOF (Agilent, USA) with an electrospray ionization (ESI) source. A mass spectrum was acquired through a full scan analysis over an m/z range of 100–1100 using an extended dynamic range and stored in centroid mode.

The instrument was operated in positive and negative ion modes with a gas temperature of 350 °C, drying gas flow of 11 l/min, nebulizer pressure at 45 psi, capillary voltage of 4000 V (positive) or 3000 V (negative), skimmer 1 voltage of 60 V, octopole RF peak voltage of 750 V, and reference masses at m/z 121.0509 and 922.0098 (positive) or 112.985587 and 1033.988109 (negative).

2.6. Quality control (QC)

An unsupervised principal component analysis (PCA) model was established based on the levels of metabolite markers. PCA clearly