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In vivo study on the neurotransmitters and their metabolites change in depressive disorder rat plasma by ultra high performance liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

A sensitive and versatile, ultra-high performance, liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method coupled to pre-column derivatization for the simultaneous determination of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), norepinephrine (NE), homovanillic acid (HVA), γ-aminobutyric acid (GABA) and glutamic acid (Glu) was developed and validated in rat plasma. The analytes were dansylated under strong alkaline conditions after protein precipitation extraction, which were analyzed on a BEH C_{18} column using a gradient elution. The lower limit of quantification (LLOQ) values for 5-HT, 5-HIAA, DA, NE, HVA, GABA and Glu were 1.00, 1.00, 0.991, 0.992, 1.02, 1000, and 5030 pmol/mL, respectively. Good linearity was obtained ($r > 0.99$) and the intra- and inter-day precisions of the method (relative standard deviation, RSD%) were lower than 12%. The method was novel, sensitive and specific which can provide an alternative method for the quantification of neurotransmitters and their metabolites in plasma samples.

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1. Introduction

Depression is characterized by low mood status and a decrease in multiple physical activities which results from a variety of factors related to biological and genetic backgrounds, environmental influences, childhood or developmental experiences [1–3]. There are various theories about the etiology of depression and some of them involve neurotransmitters. Neurotransmitters play an important role in transmitting signals from a neuron to a target cell across a synapse, the abnormal alteration of neurotransmitters can affect the incidence of depression [4–7]. Neurotransmitters can be classified into amino acids, monoamines, peptides and others, among them, monoamine neurotransmitters including 5-hydroxy tryptamine (or serotonin, 5-HT), dopamine (DA), noradrenaline (NE) and 5-hydroxyindoleacetic acid (5-HIAA), are found to play an important role in the pathogenesis of depression [8,6,9]. The varied concentrations of brain neurotransmitters and their metabolites can directly reflect the function in the peripheral and central nervous systems; however, it is difficult to obtain the cerebrospinal fluid or brain tissues without certain trauma. It is worth mentioning that the varied concentrations of neurotransmitters and their metabolites in the blood, which can indirectly reflect the status of neurotransmitters in the brain. With the advantages of non-traumatic merits, easy operation, economical application and so on, the monitoring of neurotransmitters in blood have been widely used in the application of biomarker discovery and disease diagnosis [10–12].

Various methods including high performance liquid chromatograph (HPLC) or capillary electrophoresis (CE) coupled with various kinds of detectors, such as UV detector (UV), fluorescence detector (FLD), electrochemical detector (ECD), laser induced fluorescence detector (LIFD) and mass spectrometry detector (MS), have been employed for the determination of neurotransmitters in biological fluids [13–16]. However, these methods have their own limited shortcomings, for example, low sensitivity and selectivity for UV, bad repeatability for ECD due to electrode degradation, interferences after derivatization for FLD, and the difficulty of simultaneously separating monoamine neurotransmitters that have similar electrophoretic behavior for ECD. The methods of

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; DA, dopamine; NE, norepinephrine; HVA, homovanillic acid; GABA, γ-aminobutyric acid; Glu, glutamic acid; ESI, electrospray ionization; UHPLC, ultra-high performance liquid chromatography; LLOQ, lower limit of quantification.

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HPLC-MS was applied mainly to determine the concentration of neurotransmitters in the cerebrospinal fluid or brain tissues, but was deficient in the sensitivity of corresponding neurotransmitters in plasma.

The chronic unpredictable mild stress (CUMS) model has been widely used as an experimental model resembling human depression, which can elicit the symptoms of most human depressive states, such as behavioral despair, reduced food and water intake, anhedonia and cognitive disturbances [17–19]. In the present investigation, a CUMS model was used to identify the potential biomarkers in depression. After that, a positive drug named fluoxetine was further utilized to identify the biomarkers. A UHPLC-MS/MS method was developed and validated to determine the concentrations of monoamine neurotransmitters and amino acid neurotransmitters in plasma from the depression model rats before and after administration of fluoxetine, to identify the possible biomarkers of depression.

2. Experiment

2.1. Chemicals and reagents

5-Hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), norepinephrine (NE), homovanillic acid (HVA), γ-aminobutyric acid (GABA), dansyl chloride and the internal standards of 3,4-dihydroxybenzylamine (DHBA), norleucine (Nle) and 5-hydroxy indole-2-carboxylate acid (5-HICA) were analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA). Glutamic acid (Glu) was supplied by Liaoning Institute for Food and Drug Control (Shenyang, China). The chemical structures can be found in Fig. 1. Acetonitrile (HPLC grade), formic acid (LC/MS grade) were purchased from Tedia (Fairfield, OH). All other chemicals and reagents were of reagent grade or higher and purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Apparatus and operation conditions

Chromatographic analyses were performed in an ACQUITY UPLC system (Waters Corp., Milford, MA, USA), using an ACQUITY UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters) at 30 °C. The samples were separated using a gradient mobile phase consisting of 0.1% formic acid in water (Solvent A) and acetonitrile (Solvent B). Separation was achieved using the following gradient program at a flow rate of 0.2 mL/min for 19.5 min: 35% B at start and increased to 81% within 12.5 min, increased to 95% B for 14.5 min and held for 1.5 min, then the column was equilibrated back to the start conditions for 3.5 min. The injection volume was 20 μL.

The chromatography was performed on an ACQUITY^{TM} UPLC system (Waters Corp., Milford, MA, USA). Triple–quadrupole tandem mass spectrometric detection was carried out on a Micromass Quattro Micro API mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) interface in positive ion mode. The capillary voltage was 3.00 kV. The flow rate of desolvation gas was 500 L/h. The source temperature and desolvation temperature were 120 and 350 °C, respectively. MRM acquisition settings of the dansylated analytes and its transitions, as well as the cone voltages and collision energies, are shown in Table 1. Data acquisition was acquired and processed using MassLynx 4.1 software (Waters Corp., Milford, MA, USA).

2.3. Preparation of calibration standards, quality controls and internal standards

A mixed stock solution of monoamine neurotransmitters containing 5-HT (4.98 μmol/mL), DA (4.96 μmol/mL), NE (2.98 μmol/mL), 5-HIAA (4.86 μmol/mL) and HVA (5.10 μmol/mL) was prepared by weighing the standards and then dissolved by methanol. Calibration standard working solutions of 5-HT (5.98, 12.0, 29.9, 120, 299, 598, 1196 pmol/mL), DA (5.95, 11.9, 29.7, 119, 297, 595, 1190 pmol/mL), NE (5.95, 11.9, 29.8, 119, 298, 595, 1190 pmol/mL), 5-HIAA (5.84, 11.7, 29.2, 117, 292, 584, 1167 pmol/mL), NE (2.95, 11.9, 29.8, 119, 298, 595, 1190 pmol/mL) were prepared by diluting this stock solution with methanol.

A mixed stock solution of amino-acid neurotransmitters containing GABA (1.20 μmol/mL) and Glu (6.04 μmol/mL) was prepared by weighing the standards and then dissolved by 5% methanol water solution. Calibration standard working solutions of GABA (6.01, 12.0, 30.1, 120, 301, 601, 1202 nmol/mL) and Glu (30.2, 60.4, 150.9, 604, 1509, 3018, 6035 nmol/mL) were prepared by diluting this stock solution with 5% methanol water solution.

A mixed stock solution of internal standards containing DHBA (4.12 μmol/mL), Nle (4.95 μmol/mL) and 5-HICA (7.47 μmol/mL) was prepared by weighing the standards and then dissolved by methanol. Then the working solution of DHBA (0.412 μmol/mL), Nle (0.495 μmol/mL) and 5-HICA (0.747 μmol/mL) was prepared by diluting this stock solution with methanol.

Stock solution of amine derivatization dansyl chloride at the concentration of 4.0 mg/mL was prepared by acetonitrile and stored at −20 °C protected from light.

2.4. Animals

Twenty-four male Sprague-Dawley (SD) rats (180–220 g) were obtained from the Laboratory Animal Center at Shenyang Pharmaceutical University (Shenyang, China) and the protocol was approved by the Animal Ethics Committee of the Institution. The rats were housed in three groups randomly with free access to food and water. Chronic unpredictable mild stress (CUMS) model rats were treated according to the literature [20]. Group I was served as normal control, Group II was served as CUMS model, Group III was served as CUMS model rats treated with 10 mg/kg/d fluoxetine lasting for 2 weeks. The plasma samples were collected after 2 weeks administration and stored at −70 °C until analysis.

2.5. Sample preparation

Sample preparation involved protein precipitation and derivatization. An aliquot of 50 μL IS mixed working solution was piped into a 2 mL centrifuge tube and evaporated under a steady stream of nitrogen to dryness in a water bath at 40 °C. Then 300 μL plasma sample was added and vortexed for 30 s. After the addition of 900 μL of acetonitrile, the mixture was vortex-mixed for 1 min and centrifuged at 13,000 rpm for 15 min. The supernatant of 1100 μL was transferred to another clean tube and evaporated under a steady stream of nitrogen to dryness in a water bath at 40 °C. The residue was redissolved in 100 μL $NaHCO_3$–Na_2CO_3 buffer (0.2 mol/L, pH 11) and 100 μL dansyl chloride (4 mg/mL) and vortexed for 30 s, reacted in a water bath at 65 °C for 20 min protected from light. Then the derivatized sample was removed from the water bath and the action was terminated with 10 μL 15% formic acid solution, the mixture was vortex-mixed for 30 s and centrifuged at 13,000 rpm for 15 min, and 20 μL aliquot of supernatant was injected into the UHPLC–ESI-MS/MS system.

2.6. Method validation

The method validation assays were carried out according to the bioanalytical method validation guidance of United States Food and Drug Administration (FDA) [21]. Calibration linearity was acquired using internal standard spiked calibration solutions at seven concentrations. 50 μL of calibration standard working solutions for

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