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Brain Metabolomics Study on the Protective Effects of Ginsenosides Rg1 and Rb1 in an Alzheimer's Disease Mouse Model

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Alzheimer's disease (AD) is a serious neurodegenerative disease in aging populations with no effective method for the diagnosis or for the treatment. Although some physiological and pathological functional parameters have been studied, little knowledge about the changes of small metabolites in biofluids has been reported, which may result in poor diagnosis and treatment for AD. Ginsenoside Rg1 and Rb1, the pharmacologically active ingredients of ginseng, were known to have anti-AD effects, while, their mechanism remain unclear completely. This study was designed to explore globally metabolomic character of AD induced by Aβ1-42 in brain and the holistic efficacy of ginsenoside Rg1 (GRg1) and ginsenoside Rb1 (GRb1) on AD. Morris water maze was performed to examine the behavioral changes in mice. Global metabolic profiling with UPLC/MS (ultra-high-performance liquid chromatography-mass spectrometry) and principal component analysis (PCA) were performed to discover differentiating metabolites. A total of 9 potential biomarkers were identified that were associated with the metabolism of lecithin, purine, and sphingolipids in AD mice. The peak intensities of lysophosphatidylcholine,dihydrosphingosine,hexadecasphingan ine, phytosphingosine were lower, while that of hypoxanthine and ceremide were higher, in AD than in control mice. GRg1 and GRb1 treatment affected lecithin and sphingolipid pathways, while not purine metabolism. These results provide the first evidence of a link between metabolite imbalance and AD, and reveal a molecular basis for the therapeutic benefits of ginsenosides in AD treatment. Alzheimer's disease (AD) is among the most debilitating neurodegenerative diseases in aging populations, and is characterized by progressive memory loss and the impairment of behavioral, language, and visuospatial skills . AD affects millions of people both in developed and developing countries and has become a major medical and social problem all over the world , while there is no effective method for its treatment to date. Thus, there is a critical need to identify agents that can prevent AD progression. Ginseng, a key agent in traditional Chinese medicine, is widely used to improve memory and delay senescence. Currently, many in vivo and in vitro studies have shown its beneficial effects in aging, central nervous system (CNS) disorders, and neurodegenerative diseases, such as AD . Ginsenosides Rg1 (GRg1) and Rb1 (GRb1) as the main pharmacologically active ingredients of ginseng have been proved in their effectiveness in AD prevention and treatment . In addition, previous studies have demonstrated that they exert its effects on multiple sites of action like Regulation of neurite outgrowth , inhibition of Neuroinflammation , reducing the level of Aβ and so on. To date, previous studies have mainly focused on the biochemical and pathological changes that occur in AD, while few studies have examined changes in metabolite profiles upon treatment with GRg1 and GRb1, or examined how these agents affect metabolism. Metabolomics, based on the comprehensive and simultaneous analyses of multiple metabolites in biological samples, demonstrates a great potential in health survey for the study of disease pathology, discovery of biomarkers and drug development since metabolites represent the end point of biological reactions, reflecting well the interactions between genes, proteins and the environment . Thereby, several metabolomics studies have been performed in the last years for the investigation of AD . Rodents injected with Aβ1-42 have been used as a classical AD animal model for drug screening . The administration of Aβ peptide induces memory loss , and acute injection of Aβ1-42 into the brain leads to dysfunction followed by neurodegeneration and also impairs learning and memory in a process similar to that observed in AD . In this study, a metabolomics platform based on complementary analysis by reversed-phase ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS) was used to investigate metabolic perturbations in the brain of AD mice and to investigate protective effects of GRg1 and GRb1. A principal component analysis (PCA) was carried out to estimate the changes in brain metabolite levels and identify highly sensitive and specific biomarkers for AD. All these studies would provide a theory and practice basis for the early diagnosis and treatment of AD. GRg1 and GRb1 (purity ≥ 98%) were purchased from Shanghai Yuanye Bio-Technology Co. (Shanghai, China). High performance LC grade methanol and acetonitrile were purchased from Fisher Scientific (Bridgewater, NJ, USA). Water was purified by redistillation and filtration through a 0.22 μm membrane. Aβ1-42 peptide (Sigma, St. Louis, MO, USA) and analytical grade formic acid were from the Department of Pharmaceutics, Shenyang Pharmaceutical University (Shenyang, China). Male 12 week old Kun Ming mice weighing 18-22 g were purchased from the Central Animal House of Shenyang Pharmaceutical University (Shenyang, China). Mice were housed five per cage under controlled conditions (temperature 20°C ± 2°C, relative humidity 55% ± 10%, 12:12 h light/dark cycle with lights on from 07:00 to 19:00 h) with free access to food and water. Experiments were conducted in accordance with the regulations for animal experimentation issued by the State Committee of Science and Technology of China. Mice were anesthetized with diethyl ether and brains were collected and weighed. 1.0 ml water was added to 0.1 g of brain tissue, and then the mixtures were homogenized in an ice bath. An aliquot of 600 μL of ice-cold methanol was then added to 150 μL aliquots of cerebral homogenate to precipitate protein, and the tubes was vortexed for 5 min followed by centrifugation at 13,000 rpm for 10 min at 4°C. The supernatant was transferred to another tube and evaporated to dryness at 30°C under a gentle stream of nitrogen. The dried residue was then reconstituted in 100 μL of acetonitrile-water (2:98, v/v) and 5 μL of this solution

was injected into the UPLC-MS/MS system. Mice were anesthetized with chloral hydrate (200 mg/kg) and placed in a Kopf stereotaxis (David Kopf Instruments, Tujunga, CA, USA). Aggregated Aβ1-42 peptide (3 μl) was then unilaterally injected into the hippocampal region (anterior-posterior, -2.00 mm; mediallateral, 1.50 mm; dorsal-ventral, 1.0 mm). Mice in the control group were injected with saline. The Aβ1-42 peptide was dissolved and diluted in saline to a concentration of 10 mg/ml and incubated at 37°C for 5 days to obtain the fibrillized form of the peptide before injection. GRg1 and GRb1 were delivered by intraperitoneal injection once daily for 1 month, while mice in the control and AD groups received 0.2 ml saline. Spatial learning and memory were tested with the Morris water maze test with minor modifications. A circular water tank (diameter × height, 120 × 40 cm) was filled with water at 23 ± 1°C and divided into four equal quadrants. A submerged platform (diameter × height, 8 × 10 cm) painted black was centered in the fourth quadrant 1 cm below the water surface. A camera placed 2 m above the center of the tank recorded escape latencies and path length during each trial. The Morris water maze test consisted of a place navigation test and a space exploration test. The place navigation test was performed two times per day for five consecutive days. The mice were trained to find and escape onto the platform. A different starting position for each mouse was used in each trial. For each individual mouse, the position of the platform was fixed during the entire experiment. SPSS19 software was used to process the data of Morris water maze test parameters between groups by one-way analysis of variance (ANOVA) followed by Turkey multiple comparison tests. The results of the statistical analysis were expressed as mean ± standard deviation (mean ± SD). Samples were analyzed and low molecular weight metabolites were represented as the chromatographic peaks in the total ion chromatograms (TIC). The collision induced dissociation (CID) experiment was implemented to get fragmentation patterns of these potential biomarkers. Some biomarkers were identified by comparing their chromatographic retention time and MS/MS fragmentation characteristics with the available authentic references. Furthermore, full scan mass spectra of these metabolites were interpreted using available biochemical databases, such as Kyoto Encyclopedia of Genes and Genomes database (http://www.genome.jp/kegg), the Human Metabolome Database (http://www.hmdb.ca/) and so on. Meanwhile, some biomarkers were identified by the retention time and the accurate mass number of Bruker's ESI-QTOF-MS under the same liquid chromatography condition. Representative positive ion TIC chromatograms of typical brain tissue samples from control and AD mice. A pattern recognition approach using PCA, a non-supervised multivariate data analytical method, was used to reveal clustering trends in the data. In the PCA score, each point represented an individual sample; the plot of PCA scores divided different samples into blocks, suggesting different metabolic profiles. Samples from the control and AD groups were clearly divided into two classes, indicating that the AD was successfully reproduced by this model and that specific biomarkers could distinguish AD from control mice. In the PCA loading plots for AD and control mice, the distance of an ion from the origin represents its influence on PCA components. Global metabolic profiling with UPLC/MS (ultra-high-performance liquid chromatography-mass spectrometry) and principal component analysis (PCA) were performed to discover differentiating metabolites. 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