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Jilin Province Health Science and Technology Capacity Improvement Project Task Book

Project Leader Category: Key Laboratory (Key Specialty)

Discipline: Internal Medicine of Traditional Chinese Medicine

Project Title: Study on the proteomics effect of Chuanhong stroke capsule on patients with acute cerebral infarction

Applicant: Ding Yingyue

Undertaking unit: Changchun University of Traditional Chinese Medicine

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Jilin Provincial Health Commission

Research programme

(i) Proposed research methodology, technical programme and feasibility analysis.

1 Research Methodology and Technical Programme

1.1 Research methods

In this project, 4D Label-free proteomics technology is applied, and the signal ratio (Ratio) of serum proteins in the treatment and control groups is used to measure the difference in their expression in different states, and the significance test T-test p value is calculated from the results of multiple repetitions of the experiment. when the Ratio is greater than the specified threshold and the p value is less than 0.05, the corresponding proteins can be considered to have significant differences in expression. the difference in expression of the corresponding protein can be considered significant. Then, the identified serum proteins were subjected to functional module analysis, clustering and then functional enrichment. The results of the analysis were more intuitive and detailed, and the functional correlation of the differentially expressed proteins in the comparative groups was found, and the expected related pathways were uncovered. Finally, PRM technology is applied to validate the candidate proteins and confirm their reproducibility and reliability as clinical biomarkers.

1.2 Diagnostic criteria

1.2.1 Diagnostic criteria for TCM

According to the Technical Guidelines for Clinical Research on New Chinese Medicines for Stroke issued by the State Food and Drug Administration in 2015, the diagnostic points of stroke disease and its disease categories are as follows:

Disease diagnosis:

(1) The main symptoms are sudden fainting, unconsciousness, distorted mouth and eyes, poor or unspoken speech, numbness of the paraplegic body, or only hemiplegia, distorted mouth and eyes, and unfavourable speech without fainting;

(2) Acute onset and rapid development, similar to the characteristics of ‘wind’ in nature;

(3) Symptoms and signs last for more than 24 hours;

(4) Most often occurs in people over 40 years of age.

Diagnosis of disease categories:

(1) Middle meridian: meets the diagnostic criteria of Chinese medicine for stroke, but there is no mental disorder;

(2) Middle viscera: those who meet the diagnostic criteria for stroke in Chinese medicine, but have delirious disorder.

1.2.2 Western medicine diagnostic criteria

According to the Chinese Guidelines for Diagnosis and Treatment of Acute Ischaemic Stroke 2018 issued by the Cerebrovascular Group of the Neurology Division of the Chinese Medical Association in 2018, the diagnostic points of acute ischaemic stroke (acute cerebral infarction) are as follows:

(1) Acute onset;

(2) Focal neurological deficits, and in a few cases, comprehensive neurological deficits;

(3) Symptoms or signs lasting for an unlimited period of time, or more than 24 hours;

(4) Exclusion of non-vascular causes;

(5) Brain CT/MRI to exclude cerebral haemorrhage.

1.3 Inclusion criteria

Inclusion criteria

(1) Meet the diagnostic criteria for ischemic stroke in the Chinese Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke 2018.

(2) Comply with the diagnostic criteria for ischemic stroke in TCM.

(3) Within 2 weeks of acute onset of ischaemic stroke.

(4) 4≤NIHSS score <15.

(5) Age 40-80 years old.

Exclusion criteria

(1) Diagnosis of haemorrhage or other pathological brain diseases based on head CT or MRI;

(2) Allergy, allergy to the test drug or its related flavour or ingredients;

(3) Age <40 years or >80 years;

(4) Severe hepatic or renal impairment.

1.4 Intervention measures and treatment course

Treatment group: basic treatment + Chuanhong Zhongfeng Capsules

Control group: basic treatment (refer to China Acute Ischaemic Stroke Diagnosis and Treatment Guidelines 2018)

Treatment course: 14±2 days

1.5 Specific experimental steps

(1) Extraction of sample whole protein: extract sample whole protein using protein lysate, protease/modification inhibitor, ultrasonic crushing, high-speed centrifugation;

(2) Protein extraction quality control: use SDS-PAGE, BCA, Bradford and other methods to do preliminary analysis of the extracted sample proteins, in order to assess the parallelism between the samples, the presence of high abundance of proteins, the presence of protein degradation phenomenon, the protein concentration to meet the subsequent experiments and so on;

(3) Proteolysis: After the extracted proteins are deformed and reduced, trypsin is used to enzymatically digest the proteins to obtain peptide samples;

(4) Enrichment of modified peptide fragments: enrichment of modified peptide fragments in peptide samples with antibodies specific for the purpose of modification;

(5) Separation of peptide fractions: Using high performance liquid chromatography (HPLC) or enriching peptides obtained by enzymatic digestion or modified antibodies, peptide fractions are separated according to their abundance, in order to reduce the complexity of the sample and improve the sensitivity of mass spectrometry detection;

(6) Liquid-mass spectrometry analysis: The enriched peptide samples were analysed by Orbitrap Exploris 480 liquid-mass spectrometer to obtain raw data containing primary and secondary spectra;

(7) Spectrogram search data analysis: peptide samples containing modified peptides were identified and quantitatively analysed using Maxquant, Mascot and Proteome Discoverer software;

(8) Bioinformatics analysis.

2 Feasibility analysis:

(1) Novel topic selection: This project adopts 4D Label-free proteomics technology and PRM technology to elucidate for the first time the mechanism of action and potential targets of Chuanhong Zhongfeng Capsule in the treatment of acute cerebral infarction, to provide a prospective basis for the precise medical treatment of this disease, and to provide a research direction for the prevention and treatment of cerebral infarction by traditional Chinese medicine compound. Therefore, this topic is feasible in terms of topic selection and theory.

(2) Sufficient preliminary work: Ren Jixue, a national medical master, found that Chuanhong Stroke Capsule was effective in the treatment of stroke, reduced the disability rate, and improved the long-term prognosis of the patients in the National 8th Five-Year Plan ‘Clinical and Experimental Research on Treatment of Stroke Disease with Traditional Chinese Medicine’. The project leader is conducting research on the proteomics technology of intracranial atherosclerotic stenosis in patients with acute cerebral infarction, and has a good understanding of proteomics technology. The project has a solid working foundation, which can ensure the smooth progress of the subsequent experiments.

(3) Reasonable research team: the project leader has been committed to clinical and basic research on acute cerebral infarction and has accumulated rich experience. The research team consists of 3 senior titles, 8 intermediate titles, 1 doctoral student and 1 master's student. The team members have the experience of hosting the National Natural Project and provincial and ministerial level projects, and are familiar with the domestic and international development of acute cerebral infarction and proteomics research; they have published many high-level articles, and are capable of completing the research of this project.

(4) Complete experimental conditions: This project will be carried out by PTM BIO. PTM BIO is committed to providing top proteomics services and high-quality antibody

reagents for global researchers and industrial customers. With the scientific background of ‘proteomics - precision medicine’, the company has formed an innovative business model integrating R&D, production and service, and has now built a proteomics, protein modification genomics technology platform, bioinformatics analysis platform, protein modification antibody development platform, high-efficiency rabbit monoclonal antibody development platform and big data platform. The company has established a proteomics and protein modification genomics technology platform, a bioinformatics analysis platform, a protein modification antibody development platform, a high-efficiency rabbit monoclonal antibody development platform and a big data platform. The company has instruments such as Orbitrap Exploris 480 liquid-mass spectrometer and software such as Maxquant, Mascot and Proteome Discoverer. The above research conditions and equipment can meet the needs of the experiment.

3.Innovative points

This study is the first to investigate the mechanism of action and potential targets of Chuanhong Zhongfeng Capsule in the treatment of acute cerebral infarction by using 4D Label-free proteomics technology and PRM technology.

(III) Annual programme progress and expected goals.

(‘Starting and ending years’ are counted from the date of application, and the completion period is generally no more than 3 years).

First year/annual plan: collect patients for clinical enrolment and improve the study protocol.

Expected goal: Improve the study protocol and complete the collection of patient blood samples.

Year 2/annual plan: complete QC and total protein extraction, start mass spectrometry and raw letter analysis.

Expected goal: Proteomics data analysis.

Third year/annual plan: conduct PRM validation, article writing and report project summary report.

Expected goal: complete 1 SCI and 1 core journal paper.