Study on the Gut Microbial Mechanism of Negative Symptoms of Schizophrenia

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1.Subjects

Recruit 30 outpatient/inpatient schizophrenia patients (dominant negative symptoms) in Shanghai Mental Health Center (research group). At the same time, 15 cases of normal healthy people (control group) with similar eating habits and ages in the same region were matched.

Inclusion criteria:

1）18-45 years old, Han nationality, no gender limit;

2）Meet the diagnostic criteria of "Schizophrenia" in the American Diagnostic 3）Standards for Mental Disorders (DSM-V);

3) The first attack, no antipsychotic drugs have been used in the past six months;

4) The PANSS negative symptom subscale has at least 3 items with 4 points and above or at least two items with 5 points and above, and the positive symptoms score is less than 19 points;

5) The negative symptom score is at least 6 points or more higher than the positive symptom score;

6) The cultural, social and educational background is sufficient to understand informed consent and research content.

Exclusion criteria:

1）Combined with DSM-V diagnosis other than schizophrenia;

2)G6≥4 points in PANSS, or CDSS≥6 points;

3)SAS Side Reaction Scale> 3 points;

4) Organic diseases of the central nervous system;

5) In the past two months, there were people who were dependent or abused on alcohol or other substances, which caused significant impairment of social and cognitive function;

6) In the past year, there have been major life events such as widowhood;

7) Serious suicide attempts;

8) The current patient's severe and unstable physical disease;

9) Pregnant and lactating women;

10) Have a history of antibiotic use of more than 3 days within 3 months;

11) Use probiotics (lactic acid products, etc.) for more than 3 days within 3 months;

12)Type 1 diabetes and severe diabetes complications;

13) Digestive system diseases such as gastrointestinal inflammation, acute or chronic hepatitis;

14)Severe organ diseases, such as cancer, coronary heart disease, myocardial infarction, cerebral hemorrhage;

15)Have infectious diseases, such as tuberculosis, AIDS;

16)Drug treatment of cholecystitis, peptic ulcer, urinary tract infection, acute nephritis, cystitis or hyperthyroidism.

Withdrawal criteria: Anyone who meets any of the following conditions at any time will terminate the study and complete all the evaluations originally scheduled for the 8th weekend:

① Those who did not meet the requirements of the group were found after joining the group;

②The patient or his legal guardian decides to withdraw from the study;

③Serious adverse reactions occur, and the patients cannot tolerate them;

④Serious physical diseases that may endanger the patient's life occur during the study, and the treatment should be terminated regardless of whether it is related to the study medication;

⑤Those who do not use the medicine according to the study protocol, and have a follow-up period of 7 consecutive days or more than 7 days cumulatively;

⑥ Combined use of prohibited drugs or other treatment methods (including ECT, laser, cerebral circulation, brain function, etc.);

⑦Other circumstances where the main researcher thinks it is necessary to terminate the research;

⑧ Lost to follow-up.

2. Calculation of sample size

Using (G*Power software to calculate, a 20% reduction rate for amisulpride treatment of negative symptoms is taken as the effective response, the first type of error α is 0.05, the second type of error β is 0.2, bilateral Test, the test power 1-β is 0.8. With reference to the previous literature using amisulpride to treat negative symptoms of schizophrenia, the statistical effect (effect size) is 0.98. This study conservatively estimates that the effect value is 0.8 for sample size calculation. After calculation, The required sample size is 26. According to 10% dropout, about 30 samples from the study group and 15 samples from the healthy control group are matched.

3.Methods

This study included patients with schizophrenia with dominant negative symptoms as the study group; the control group included normal healthy people with similar eating habits and ages in the same region.

1) Drug intervention method of the study group: The study group was given amisulpride tablets first. The initial dose was 50 mg/d. The doctor titrated the dose to the therapeutic amount within 1 to 2 weeks according to the patient's condition. The maximum dose was 300 mg/d, every day. clothes. Observe for 8 weeks. Instructions for the patient's use of other drugs: ①It is forbidden to use other antipsychotics, anti-anxiety drugs, other antidepressants or drugs that act on the

central nervous system (such as nootropics, drugs that improve cerebral circulation or brain metabolism, etc.) during the study and treatment); ②In principle, it is not advisable to combine other medications. For clinical needs (insomnia), non-benzodiazepine sleep aids (such as zopiclone, zolpidem, etc.) can be used first; short-term use of benzodiazepines is allowed , But avoid the use of drugs with long half-life such as clonazepam. Lorazepam or sulrazepam can be used for the purpose of improving sleep (single dose at night before going to bed), and should be stopped in time according to clinical conditions Use; ③Allow the use of drugs to deal with adverse reactions, symptomatic or physical diseases (no pharmacological conflict with current medications), but the drug dose should be fixed as much as possible during the study period; ④All combined medications must be recorded in detail.

2) Stool collection and processing in the study group: ① Time points for specimen collection: during the baseline period, 2 weeks of treatment, 4 weeks, and 8 weekends. ②Specimen collection method: save 5ml of fresh fecal specimens in the morning, use a dedicated sterile specimen box, collect the specimens under the supervision of a doctor or a trained family member, and immediately notify the designated person by telephone, put the specimens in a sterile EP tube and store them in Minhang Key Laboratory of Shanghai Mental Health Center-80 degree refrigerator. ③The feces are collected and frozen in two parts. Part of it is reserved for flora analysis, using 16s amplicon sequencing technology to detect fecal DNA to understand the composition of intestinal flora, and the other part is retained.

3) Stool collection and processing in the control group: ① Time point of specimen collection: For the real world population, 5ml of fresh fecal specimens were collected at the time of enrollment, 2 weeks, 4 weeks, and 8 weeks after enrollment. ②Specimen collection method:

save 5ml of fresh fecal specimens in the early morning, use a special sterile specimen box, collect the specimens under the supervision of a doctor or trained healthy people, and immediately call the designated person, put the specimens into a sterile EP tube and store In Minhang Key Laboratory of Shanghai Mental Health Center-80 degree refrigerator. ③The feces are collected and frozen in two parts. Part of it is reserved for flora analysis, using 16s amplicon sequencing technology to detect fecal DNA to understand the composition of intestinal flora, and the other part is retained.

4) Blood specimen collection and processing: 10ml of fresh whole blood was collected at the time of enrollment, 2 weeks, 4 weeks, and 8 weeks after enrollment in the two groups. The blood specimens need to be centrifuged and processed using CBA (Flow Cytometry)) Detection of inflammatory factors (IL-1β, IL-6, IL-10, TNF-α). The blood is tested for blood sugar and blood lipids at the same time.

5) Evaluation method: ①Evaluation time points: at baseline, 2 weeks, 4 weeks, and 8 weeks of treatment, respectively. ②Assessment content: assess the negative symptom scale scores of the Positive and Negative Syndrome Scale (PANSS), Calgary Depression Scale for Schizophrenia (CDSS), and clinical efficacy scale (Clinical Depression Scale for Schizophrenia). Global impression, CGI), Treatment Emergent Symptom Scale (TESS), Simpson Angus Rating Scale (SAS), Barnes Akathisia Rating Scale (BAS), followed by evaluation The patient's negative symptoms, depressive symptoms, efficacy and adverse reactions. ③ Special evaluators will be set up during the project implementation process, and the evaluators will independently evaluate the cases according to the established time nodes, and the evaluation results (efficacy, tolerability) will be fed back to the research doctor for drug

titration selection. The assessor is responsible for the assessment and filling of each CRF. The 8-week follow-up will be completed by the evaluator. ④Research liaison: This project has set up a special research liaison who is responsible for the liaison work of this project, mastering the research number, and liaison with the evaluator and the research doctor. Follow-up method: According to the actual situation of the patient, this project can set the following 3 follow-up methods: follow-up in the hospital; follow-up at home (home visit); follow-up by telephone. Record each follow-up method in the CRF.

6) Other items: The two groups monitored Body Mass Index (BMI), abdominal circumference, heart rate, blood pressure, smoking and drinking, and psychoactive substance use before treatment and at the end of 2, 4, and 8 treatment.

7) Effectiveness evaluation index: The main evaluation index is the difference between the change of the intestinal microbial flora of the study group and the control group before and after treatment; the secondary evaluation index is the PANSS negative symptom scale score reduction rate ≥ 20% as the effective group, observe the effective group and the ineffective group The difference in the changes of the intestinal microflora of the group; the correlation between the changes of the intestinal microflora of the effective group and immune factors and negative symptoms.

4.Statistical analysis

This experiment used SPSS19.0 software for statistical analysis. The measurement data adopts the mean ± standard deviation, and the counting data adopts the rate or percentage. One-way analysis of variance was used to compare the two groups at baseline. The comparison of the study group before and after treatment used repeated measures analysis of variance. Changes in negative

symptoms, changes in immune factors and changes in intestinal flora composition were analyzed by PEARSON or SPEARMAN.