

Study Protocol with SAP

Efficacy Study of Yi-Zhi-An-Shen Granules For Older Adults With
Amnesic Mild Cognitive Impairment: a Randomized, Double-blind,
Placebo-controlled Study

Protocol approved by Medical Ethics Review Board of
Teaching Hospital of Chengdu University of TCM

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1. Objectives

The primary objective of this study is to evaluate the efficacy and safety of Yi-Zhi-An-Shen Granules (YZASG) in optimizing cognitive performance over time in elderly individuals with amnesic mild cognitive impairment (aMCI). Secondly, the investigators intend to assess whether YZASG can improve sleep quality among aMCI patients. Finally, participants' fecal genomic DNA will be extracted to analyze the gut microbial differences between individuals with aMCI and ones with normal cognition.

2. Design

This study is a randomized, double-blind, placebo-controlled trial with 16-week intervention and 18-month follow-up assessment. The design met the principles of the Declaration of *Helsinki*, and was approved by the Ethics Committee of Teaching Hospital of Chengdu University of Traditional Chinese Medicine. Then signed informed consents will be obtained from each participant.

The enrolled participants will be randomly assigned into the YZAS group or the placebo group with the allocation ratio of 1:1 using the statistical package. The unique code will be assigned to each newly enrolled participant and preserved in the trial management board. A statistician expert will act as the coder and be shielded from subject recruitment.

3. Methods

3.1 Participants

The investigators anticipate recruiting 80 participants from Teaching hospital of Chengdu University of Traditional Chinese Medicine, community, and nursing homes. All patients will undergo standard medical examination and neuropsychological testing to ensure correct diagnosis of aMCI.

The inclusion criteria are (1) subject has assigned informed consent to participate in the study and continues to give willing consent for participation; (2) age from 60 to 85 years with a diagnosis of aMCI; (3) educational level of at least 6 years; (4) availability of a "study partner" who can assist in completing rating scales for the duration of the study; (5) cognitive complaints reported by the subject and confirmed by the "study partner"; (6) clinical Dementia Rating (CDR) global score of 0.5, and memory item score of 0.5; (7) mini-mental state examination (MMSE) score of 24-30; (8) Diagnostic and Statistical Manual of Mental Disorders, Version 5 (DSM-V) criteria of dementia not fulfilled

The exclusion criteria are as follows: (1) has been previously enrolled in this study and received the investigational product; (2) has received an investigational product within 30 days prior to screening; (3) has received disease-modifying therapy; (4) has a known allergy to the study drug or any of its constituents; (5) has a history of alcohol abuse or alcohol dependency in the 3 years prior to study entry, or is an alcoholic or drug addict, as determined by the investigator; (6) has ongoing clinically significant (as judged by the investigator), metabolic or any other disease that could currently cause impaired memory (e.g., untreated thyroid disease, vitamin or other nutritional deficiencies, chronic kidney, or liver disease); (7) memory impairment that can be attributed to a disease or condition other than an early phase neurodegenerative syndrome; (8) has a parkinsonian movement disorder; (9) use of psychoactive medications that would affect the subject's ability to reliably perform neurocognitive testing or create uncertainty in distinguishing between the effects of the psychoactive medication and the subject's underlying cognitive impairment (e.g., benzodiazepines, sedatives, antipsychotics); (10) with history of major recurrent depressive disorder (DSM-V) within the last 5 years prior to screening; (11) has a brain tumor or other

intracranial lesion, a disturbance of cerebral spinal fluid circulation (e.g., normal pressure hydrocephalus), and/or a significant history of head trauma or brain surgery; (12) has signs of major cerebrovascular disease, with score of modified Hachinski Ischemia Score (mHIS) at more than 4, or as verified by medical history and/or brain MRI or CT; (13) has severe visual or hearing impairments that cannot cooperate with examinations; (14) has severe digestive system diseases; (15) has received antibiotics within 60 days prior to screening.

Handling of withdrawal and dropout: (1) Voluntarily withdrawal, (2) loss of follow-up, (3) poor compliance and presence of severe adverse effects, (4) revealing and uncovering blind in urgency, (5) misdiagnosis, (6) using forbidden drugs or treatments in the course of the trial, (7) taking no medication during the trial, (8) no evaluable records after medication. Reasons for withdrawing participants will be recorded in CRFs, and the last data will be included in data analysis.

3.2 Interventions

Besides receiving the same basic treatment, including health education, moderate aerobic exercise (30-60 minutes per day) and general nutritional support, participants assigned to the YZAS group will take Yi-Zhi-An-Shen granule, which is composed of *YiZhiRen* (*Alpinia oxyphylla* Miq.) - 5g, *SanQi* (*Panax notoginseng*) - 3g, *ChuanXiong* (*Ligusticum chuanxiong hort*) - 10g, *ZhiZi* (*Gardenia jasminoides Ellis*) - 10g, *YuJin* (*Curcuma longa L.*) - 10g, *DanZhuYe* (*Lophatherum gracile*) - 10g, while ones in the placebo group will take placebo made from starch which appears the same shape, color, smell, taste, texture package and Lot Number. Participants will be instructed to dissolve granules into 100ml of boiled water and to take the solution orally between 30°C to 37 °C three times daily for 16 weeks. Each granule is prepared by Sichuan Neo-green Pharmaceutical Technology Development Co., Ltd, Sichuan China according to the standards of Good Manufactory Practice (GMP).

80% to 120% of drug usages are eligible for protocol plan. The package, drug name, function and indication, usage and dosage, storage condition, valid period and name

of the manufacturer will be marked and a tag indicating 'trial use' will be attached. Drugs must be kept in the appropriate temperature in a dry, cool and shady place. Drug administrators should return unused drugs to estimate participant compliance and record these in the CRFs.

3.3 Outcomes

3.3.1 Primary outcome

Cognitive decline is measured using the Chinese version of the Alzheimer Disease Assessment Scale-Cognitive subscale (ADAS-cog₁₁). The total possible score is 70. A score increase indicates greater severity of impairment. The specific hypothesis is that the change from baseline to endpoint will be significantly less than that for placebo.

The ADAS-cog will be assessed at baseline (before intervention), at 16 weeks (the end of intervention) and 6, 12, and 18 months after intervention.

3.3.2 Secondary outcome

Secondary measures includes the MMSE, MoCA (Changsha Version), CDR, PSQI, ADLs, and gut microbiome.

- The MMSE is an 11-question measure that tests five areas of cognitive function (orientation, registration, attention and calculation, recall, and language). The maximum score is 30 and a score below 24 is considered abnormal for dementia screening.
- The MoCA (Changsha Version) will also be used to evaluate general cognitive function, as it contains visuospatial processing and organizational capability which can make up for the shortcoming of the MMSE. The total score for it is 30, with a higher number indicating a more intact cognitive function. Meanwhile, the MoCA has been shown to be a promising tool to detect MCI and early AD.
- The CDR will be used as an assistant evaluation for patients' dementia severity, which scored 0-3, with higher scores indicating more severity. It is a semi-structured interview performed with the patient and caregiver (informant),

characterizing six domains of cognitive and functional performance. The CDR sum of boxes (CDR-SB) scored 0-18, will also be applied to assess patients' cognitive status, with higher scores indicating worse functioning.

- The Pittsburgh Sleep Quality Index (PSQI) will be used to assess participants' comprehensive quality of sleep, which involves sleep quality, sleep duration, sleep efficiency, sleep disorders, daytime dysfunction, sleeping aids, etc. The total score for it is 21, with a higher score indicating a worse sleep quality.
- ADLs will be assessed including basic activities of daily living (BADL) and instrumental activities of daily living (IADL). An individual's BADL will be evaluated mainly by the subjects' performance from the perspectives of bathing, dressing, grooming, initiation, toileting and feeding, with six items and a sum of scores ranging from 0 (normal) to 24 (complete dependence on others). And modified Lawton Instrumental Activities of Daily Living Scale will be used to measure the IADL of a subject, with eight items and a sum of scores ranging from 0 (normal) to 32 (complete dependence on others).

These clinical tests will be administrated by a trained, certified clinician or rater experienced in the assessment of patients with cognitive deficits. And the rater who will conduct the CDR for a patient cannot complete any other rating scales for the same patient, and will be blinded to the results of all other neuropsychological scales. All the questionnaires will be assessed at baseline (before intervention), at 8 and 16 weeks (during intervention) and 6, 12, and 18 months after intervention.

- Fecal genomic DNA will be extracted from frozen stools using QIAamp DNA mini stool kit (Qiagen, Hiden, Germany), obtained from the patients with aMCI and 15-20 participants with normal cognition at baseline. After PCR amplification, DNA fragments will be sequenced on an Illumina HiSeq 2500 instrument and an Illumina HiSeq X instrument for 16SrDNA and metagenomics analyses respectively at Biomarker Technologies Co, Ltd (Beijing, China) to analyze the differences in gut microbiome between patients with aMCI and individuals with normal cognition.

When it is available, the investigators will also assess the change in gut microbiome between the treatment group and the placebo group after the intervention of YZASG in the same way.

3.3.3 Other outcomes

To assess the safety of YZASG compared to placebo in subjects with aMCI, the investigators will record the incidence and severity of treatment-emergent adverse events (TEAEs), and clinically important changes in safety assessment results (including vital signs, weight, clinical laboratory tests, physical and neurological exams, ECGs, and MRIs).

4. Statistical considerations

4.1 Sample size

Using pre-intervention and post-intervention scores obtained from Miao *et al.* ^[1], 33 patients are needed for inclusion in the treatment group to achieve a statistical power of 90%. Allowing for a maximum dropout rate of 20%, the number of subjects in the treatment group has been set to 40 patients with aMCI. With the allocation ratio of 1:1, 40 subjects will be randomized to the placebo group.

4.2 Statistic analysis

Analysis will be conducted by a statistician of the National Clinical Trial Center of Chinese Medicine (Chengdu, China), who will be blinded to the whole trial and using SAS 9.2 software (Cary, NC, USA) and SPSS 19.0 software (IBM, NY, USA).

Unless otherwise specified, all mechanism, efficacy and safety analyses will be strictly conducted according to the intention-to-treat (ITT) principle and the full analysis set (FAS) population, the per-protocol set (PPS) population and the safety set (SS) population. Missing values will be replaced by the last observation carried forward (LOCF) method. ADAS-cog (including its monomial item) changes from baseline and the secondary outcomes will be assessed using an analysis of covariance with treatment groups as factors and baseline values as covariates. Standardized mean

differences will be used to express effect sizes in SD. The baseline homogeneity of the baseline characteristics between the two groups will be analyzed with Fisher's exact test, or χ^2 test for categorical measures and with the t test or Wilcoxon rank-sum test for continuous measures. The statistical significance is defined as a one-sided P-value of <0.05 and 95% confidence interval.

For the gut microbiome analysis, sequenced data will be interpreted using the bioinformatics tools programmed in the Ion Reporter software. QIIME algorithms will determine the bacterial diversity within a sample (α diversity) and among all the samples (β diversity). Additionally, multivariate data analysis with principal component analysis on the diversity indexes and comparisons of genus and species level data will be performed to reveal differences in the microbial composition between individuals with normal cognition and ones with aMCI. The Benjamini–Hochberg false discovery rate adjustment will be used to account for the number of taxa tested in each comparison.