

BMJ Open Efficacy and safety of human-derived neural stem cell in patients with ischaemic stroke: study protocol for a randomised controlled trial

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ABSTRACT

Introduction Stroke is the most common cause of neurological disability in adults worldwide. Neural stem cell (NSC) transplantation has shown promising results as a treatment for stroke in animal experiments. The pilot investigation of stem cells in stroke phase 1 and phase 2 trials showed that transplantation of the highest dose (20 million cells) was well tolerated. Preliminary clinical benefits have also been observed. However, the trials were open-label and had a small sample size. Furthermore, human NSCs (hNSCs) were intracerebrally implanted, and some serious adverse events were considered to be related to the surgical procedure. Therefore, we plan to conduct a double-blinded, randomised controlled trial to test the safety and efficacy of intranasal injection of hNSCs.

Methods and analysis This single-centre, randomised, double-blinded, parallel-controlled trial will be conducted in China. Sixty patients with ischaemic stroke who met the qualification criteria will be randomly divided into two groups: the NSCs and control groups. All participants will receive intranasal administration of hNSCs or placebo for 4 consecutive weeks. Patients will be followed up at baseline and at 4, 12, 24 and 48 weeks after intervention. The primary outcome is the National Institutes of Health Stroke Scale score (4, 12, 24 weeks after intervention). Secondary outcomes include the modified Rankin scale, Barthel index, Mini-Mental State Examination score (4, 12, 24 weeks after intervention) and cranial MRI changes (24 and 48 weeks after intervention). All adverse events will be recorded during the study period.

Ethics and dissemination The study protocol was approved by the Ethics Committee of Ren Ji Hospital (2018-009). All subjects will provide informed consent. The results will be accessible in peer-reviewed publications and will be presented at academic conferences.

Trial registration ChiCTR1900022741; Chinese Clinical Trial Registry.

INTRODUCTION

Stroke is the most common cause of neurological disabilities in adults worldwide. Every year, more than 795 000 people in the USA have a stroke. About 610 000 of these are first or new strokes. About 87% of all strokes

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study is a prospective, randomised controlled trial.
- ⇒ Participants with ischaemic stroke 3–24 months before screening will be recruited.
- ⇒ Participants will receive intranasal injection of neural stem cells or placebo for 4 consecutive weeks.
- ⇒ The study's small sample size and single-centre design may affect its generalisability.
- ⇒ No restriction on participant's rehabilitation exercise may affect the efficacy assessment.

are ischaemic strokes.¹ China faces great challenges from stroke. In 2018, the death rate from cerebrovascular diseases in China was 149.49 per 100 000, accounting for 1.57 million deaths.² Disability after stroke causes a serious social and economic burden. Methods to effectively prevent stroke and improve functional recovery in patients with stroke are urgently needed.

Rehabilitative approaches aid functional recovery and brain reorganisation, but the effect diminishes over time, which indicates that neurological recovery is time limited.³ Owing to the limitations of endogenous repair, cell-based exogenous therapies have been investigated. Neural stem cells (NSCs) are ideal candidates for treating stroke and neurodegenerative diseases.^{4–5} Intracerebral delivery of NSCs, the preferred route in animals, significantly improves sensorimotor function in rats. NSCs achieved functional recovery of the injured brain mainly by cell replacement (neuron and glia regeneration), nutritional effects (paracrine effects) and immune-regulation (neuro-protective) effects.^{6–9} NSCs were also reported to promote vascular remodelling in brain lesions.¹⁰

To date, many modified stem cell preparations have been developed, and preclinical evidence supports their long-term safety.

The first in-human trial, a pilot investigation of stem cells in stroke (PISCES) phase I dose-escalation trial, was reported in 2016. PISCES is a phase I, open-label, single-centre, dose-escalation trial of the intracerebral stereotactic implantation of CTX-DP, a human NSC line. Men aged ≥ 60 years with stable disability (National Institutes of Health Stroke Scale (NIHSS) score ≥ 6 and modified Rankin Scale (mRS) score 2–4) were implanted with single doses of 2, 5, 10 or 20 million cells by stereotactic ipsilateral putamen injection 6–60 months after ischaemic stroke. Clinical and brain imaging data were collected over a period of 2 years. The primary endpoint was safety (adverse events (AEs) and neurological changes). Thirteen male patients were recruited, of whom 11 underwent CTX-DP treatment. No immunological or cell-related AEs were observed. Other AEs were related to the procedure or comorbidities. At 2 years, improvement in the NIHSS score ranged from 0 to 5 (median, 2) points.¹¹

The PISCES study offers preliminary data on the feasibility, tolerability and cell-related safety of stereotactic intracerebral injection of human NSCs (hNSCs) in patients with chronic ischaemic stroke. The PISCES phase II study was also completed as a multicentre, single-arm, open-label study in adults aged >40 years with significant upper-limb motor deficits. Twenty-three patients underwent cell implantation at eight hospitals. Transient procedural AEs were observed; however, no cell-related AEs occurred up to 12 months of follow-up.¹² In addition to the PISCES study, the results of another trial have been published. NSI-566, a stable, primary adherent NSC line derived from a single human fetal spinal cord, was evaluated for feasibility and safety in the treatment of stroke. Three cohorts ($n=3$ per cohort) were transplanted with single intracerebral injections of 1.2×10^7 , 2.4×10^7 or 7.2×10^7 cells. The study demonstrated that transplantation of the highest dose (7.2×10^7 cells) was well tolerated and exerted preliminary clinical benefits. MRI results indicated new neural tissue formation in the stem cell implantation area.¹³

However, all the above studies were open-labelled and had a small sample size. Thus, the exploratory efficacy data should be regarded with extreme caution because of the open-label design. Therefore, some potential AEs with low incidence might not be identified. Furthermore, the hNSCs were implanted intracerebrally and some AEs were considered to be related to the surgical procedure. Therefore, we plan to conduct a double-blind, randomised controlled trial to further test the safety and efficacy of hNSCs in the treatment of ischaemic stroke. In contrast to previous studies, hNSCs will be administered intranasally. Intranasal injections of stem cells have been used to treat animal models of stroke, MS and other diseases.^{14–16} Studies have shown that nasal administration of NSCs is safe, and that NSCs can migrate to lesions in the CNS. In this trial, we will further explore the safety and efficacy of intranasal injection of hNSCs in the treatment of ischaemic stroke.

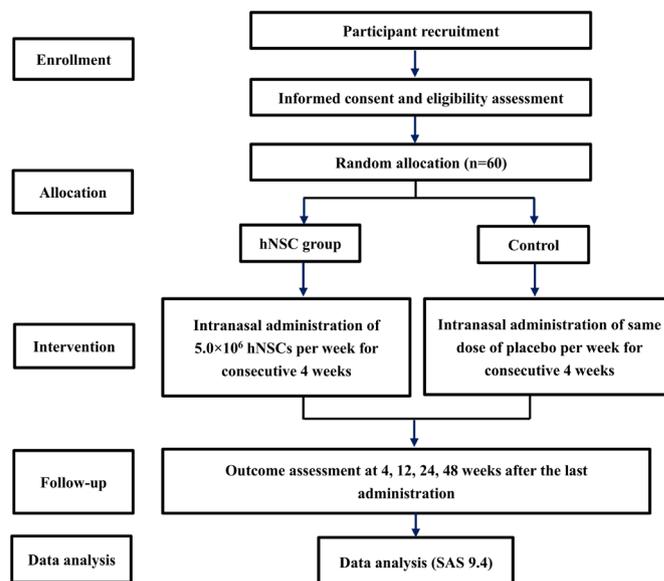


Figure 1 Flow diagram of the study. hNSCs, human neural stem cell.

METHODS

Study design, setting and approval

This study is a single-site, double-blinded, randomised controlled trial. Random treatment allocation will be done at the individual level. The trial protocol is illustrated in [figure 1](#). Sixty patients will be recruited at Ren Ji Hospital, affiliated with the Shanghai Jiaotong University School of Medicine, Shanghai, China. The study has been approved by the Ethics Committee of Ren Ji Hospital. The approved trial protocol was registered in the Chinese Clinical Trial Registry on 24 April, 2019. The current status of this trial is recruiting.

Inclusion criteria

(1) Aged 18–80 years old; (2) with ischaemic stroke 3–24 months before screening, and stroke was caused by stenosis or embolism of the unilateral anterior and/or middle cerebral artery; (3) hemiplegia (NIHSS score ≥ 6 and at least one limb exits motor dysfunction); (4) stable neurological deficits for at least 2 months before screening (a variety of NIHSS scores no greater than 2); (5) mRS scores of 2–4; (6) minimum infarct diameter of 1 cm on MRI; (7) informed consent signed by the patient or guardian and (8) completion of the follow-up.

Exclusion criteria

(1) Two or more stroke attacks (transient ischaemic attack was not included); (2) infarct diameter larger than 8 cm; (3) experiencing acute coronary events 3 months before or after stroke; (4) mini-mental state examination (MMSE) score is smaller than 24; (5) a history of seizures or current use of antiepileptics; (6) a history of cell therapy (blood transfusion was not included); (7) gastrointestinal bleeding or other severe haemorrhagic diseases within 1 month before screening; (8) surgical history 1 week before screening; (9) abnormal coagulation (prothrombin time, activated partial thromboplastin

time, fibrinogen, international normalised ratio exceeding or below 50% of the institute's normal limit), severe liver and kidney diseases (total bilirubin, aspartate transaminase, alanine transaminase, creatinine exceeding 50% of the institute's normal limit), mental disorders, malignant tumour or history of malignant tumour, primary or secondary immunodeficiency; (10) pregnant or lactating women; (11) involved in other trials within 3 months before screening and (12) any other factors that could influence the study results or lead to study termination, as deemed by the investigators.

Baseline assessment

Detailed demographic data such as age, sex, race and education will be collected. The following results will also be collected: (1) vital signs and physical examination; (2) laboratory test or examination: routine blood, liver and kidney function, blood coagulation detection, tumour marker detection, ECG, chest radiograph, heart colour Doppler ultrasound, abdominal ultrasound examination and cranial MRI examination and (3) neurological evaluation: NIHSS, Barthel index, mRS and MMSE.

Randomisation, allocation concealment

Randomisation sequence was created using SAS V.9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. The randomisation list will be sealed in sequentially numbered opaque envelopes. The envelopes will be stored in a double-locked cabinet and will only be opened by the practitioner to assign participants to the NSC or control group after obtaining informed consent and performing eligibility screening.

Blinding

This trial used a double-blinded design. hNSCs will be diluted with saline and appear nearly colourless with minimal visual difference compared with placebo (saline only). The participants, investigator and statistician will be blinded to the treatment allocation. A specific statistical researcher will prepare emergency letters containing random numbers and treatment assignments. Only emergencies when actual intervention is necessary for further management could allow code breaks.

Interventions

All participants will receive intranasal administration of agents for 4 consecutive weeks (two sets of agents once a week one set for each side of the nose). One set of agents contained 2.5×10^6 hNSCs diluted in 100 μ L saline or saline only. The administration of hNSCs or placebo is add-on and does not change the participants' original therapy. hNSCs will be supplied by Shanghai Angecon Biotechnology Corporation and subjected to quality testing at the China National Institutes for Food and Drug Control. hNSCs were isolated from donated placentas and cultured without modifications.

The nasal delivery process of the control group was the same as that of the NSC group: (1) The nasal cavity will be cleaned, and a nasal endoscope will be used to

observe and clean the nasal cavity. If one side of the nasal cavity is obviously obstructed, the operation will immediately stopped to clean the opposite side. (2) Intranasal administration: participants will be instructed to adopt the supine position and a pillow will be placed on the back of the participant's shoulder. A special delivery tube will be used to inject hNSCs/saline into the surface of the middle turbinate using a nasal endoscope. The participant will be then instructed to rotate his/her head slowly. Five minutes later, the same operation will be performed in the other nasal cavity. If one side of the nasal cavity is obviously obstructed, two sets of agents will be injected into the same side, 5 min apart. (3) Observation: the participant will be instructed to remain lying down on the pillow in the original position for 30 min after the administration. The pillow will then be removed, and the participant lay down in the same position for another 30 min.

Outcomes

The overall schedule of the trial is shown in [table 1](#). The primary outcome is the NIHSS score (at weeks 7, 15 and 27). The secondary outcomes are (1) mRS score (at weeks 7, 15 and 21), (2) Barthel index (at weeks 7, 15 and 27), (3) MMSE score (at weeks 7, 15 and 27) and (4) cranial MRI changes (at weeks 27 and 51).

Safety assessment

The potential AEs may include (1) AEs during injection: local complications (dizziness, headache, bleeding, nerve damage, nasal congestion, nasal pain); allergic reactions (fever, tachycardia, dyspnoea); systemic complications (infection including encephalitis, embolism); (2) long-term AEs: the following indicators are obviously abnormal: tumour markers, haematuria routine, liver and kidney function, blood coagulation test, ECG, chest radiograph, heart colour Doppler ultrasound, abdominal ultrasound, cranial MRI examination or other proven events including tumour formation and nerve injury.

Any serious clinical AEs or abnormal laboratory values, which occur during the study, must be reported to the institutional academic committee and ethics committee within 24 hours after the investigator is informed of the event, and subsequently reported to the local Health Commission and the National Health Commission.

Management of AE and SAE

To prevent and promptly deal with any possible AEs, we will:

- ▶ Strictly screen patients and follow the inclusion and exclusion criteria.
- ▶ Perform comprehensive quality inspection before reagent administration.
- ▶ Clean the nasal cavity, and if the patient has a cold, nasal congestion, etc., wait for the patient to recover before administration.
- ▶ Perform all operations in clinics or wards with first-aid equipment, with operators who are all properly trained.

reviewed the PISCES I study, in which the NIHSS score improved by a median of 2.0 point in subjects who underwent stereotactic injection of hNSCs. We assumed a 1.0-point improvement in this trial. The NIHSS scores (6 months) in the control group and NSCs group were estimated as 8.0 (SD=1.2) and 7.0 (SD=1.2), respectively. The sample size per group was calculated to be 24 using a two-sided 0.05 significance level and 80% statistical power. The total sample size was 60 (30 per group), indicating a lower drop rate.

Statistical analysis

An independent statistical expert blinded to the group allocation will implement the statistical analysis. Patients who are enrolled in this study and receive nasal administration at least once, with at least one post-administration evaluation, will be included in the full analysis set (FAS). The FAS analysis set will be used for statistical analysis. Subjects who withdraw after receiving the treatment, or subjects who were excluded during treatment will be included; however, we will not use data from illegible participants, subjects who have not received any intervention, or subjects who have not undergone any outcome measurement. Participants who are administered the hNSCs at least once will be included in the safety set. SAS V.9.4 will be used for statistical analysis. Demographic characteristics, general conditions and baseline conditions (before treatment) will be compared.

For the primary outcomes, NIHSS score at weeks 7, 15 and 27, the rank sum test will be used for comparison between two groups. For the secondary outcomes, the Barthel index and MMSE score will be analysed using a two-sample T-test or Mann-Whitney U-test. The mRS score will be analysed using the rank sum test. For cranial MRI changes, a radiological expert will analyse the images and calculate the estimated infarct volume.

ETHICS AND DISSEMINATION

Any protocol amendments will be reapproved by the Ethics Committee of Ren Ji Hospital, revised at the Chinese Clinical Trial Registry and reflected in the participants' informed consent form. A signed consent form reflecting the modification will be acquired from all participants. The results of this trial will be reported in the relevant academic journals and conferences.

Patient and public involvement

The patients and public were not involved in planning and design of this study.

DISCUSSION

This is the first RCT study of intranasal administration of hNSCs in the treatment of ischaemic stroke. The nasal administration technique adopted in this study has been proven to be safe in animal experiments, and operators

will also receive professional training to ensure the safety of administration.^{14 15 17}

The previously published PISCES I and II studies indicated that intracranial injection carries certain risks, including intracranial haemorrhage, epilepsy, infection, etc.^{11 12} Those side effects are sometimes fatal. It is for this reason that we decide to take the intranasal route. In this study, we will inject hNSCs at a low dose. Only after obtaining safety data will we begin to try different doses of hNSCs.

Stem cell research in China is currently performed only under strict supervision. A registration and recording system has been established. For each patient enrolled, relevant information must be submitted to the national medical research registration and management system. The Clinical Research Center of Ren Ji Hospital will also ensure compliance with national policies and protect the rights of participants. The primary concern of this study is safety. In addition to systemic risks such as tumour formation and abnormal immune response, we will also observe AEs related to nasal administration, such as nasal congestion, nasal pain, headache and bleeding. We also purchased insurance for every participant. In addition, we will continue the long-term follow-up protocol after the end of follow-up.

We will recruit participants with ischaemic stroke 3–24 months before screening. The purpose of setting this time range is to evaluate the effect of hNSCs on patients whose physical rehabilitation level is stable. According to the previous study, nasally administered cells mainly migrated to the olfactory bulb, hippocampus, thalamus and cortex; therefore, we recruit patients with ischaemic stroke caused by pathologies in the carotid artery system.¹⁸ Patients with ischaemic stroke caused by the vertebrobasilar artery will be excluded because the migration route of hNSCs to brainstem and cerebellum may be more difficult. Participants who experienced more than one stroke will also be excluded.

Considering the possibility that hNSCs may aggravate stroke, NIHSS score is selected as the primary endpoint of this study. The NIHSS not only provides a quantitative measure of stroke-related neurologic deficit but can also help to determine the appropriate treatment and predict patient outcomes. Of course, the mRS score and Barthel index will also be recorded at every follow-up visit to assess disability.

One disadvantage of this study is that there is no restriction on the rehabilitation exercise of participants during the study period. Rehabilitation exercises are essential for poststroke recovery. The speed and level of function recovery are largely determined by rehabilitation training. In this trial, we will not control the participant's secondary prevention therapy of stroke. However, since this study is a randomised controlled placebo study, the theoretical level of rehabilitation exercise in the two groups may be consistent.

Another limitation of this study is the efficacy of intranasal administration is unknown. Although animal

experiments have demonstrated that intranasal delivery of NSCs results in their appearance in the CNS, it is difficult to observe whether NSCs migrate and plant in the human CNS. Cell labelling and imaging using various imaging modalities such as positron emission tomography, single-cell MRI may help to achieve cell tracking in vivo, but it is not allowed in human beings by the ethic committee.^{19 20}

Overall, this pilot study will explore the safety and effectiveness of intranasal administration of hNSCs for ischaemic stroke. Although the small sample size and single-centre design may affect its generalisability, we expect that the result of this study will provide clinical evidence for the use of hNSCs.

Contributors (1) Conception and design: CX and KW; (2) administrative support: YG; (3) provision of study materials or patients: XJ and LW; (4) collection and assembly of data: JP, KW and YW; (5) data analysis and interpretation: CX and SP; (6) manuscript writing: all authors; (7) final approval of manuscript: all authors.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement The datasets generated and/or analysed during the current study will be available upon request from the principle investigator. Individual participant data that underlie the results reported in final report will become available for share, after deidentification. Researchers should provide a methodologically sound proposal to get data access. To gain access, data requestors will need to sign a data access agreement. Further, informed consent may be considered according to the study aims. The shared data will only be allowed to be used by the applicant for scientific studies. No commercial activities are allowed.

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