Olfactory ensheathing cells in chronic ischemic stroke: A phase 2, double-blind, randomized, controlled trial

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Olfactory ensheathing cells in chronic ischemic stroke: A phase 2, double-blind, randomized, controlled trial

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ABSTRACT

Olfactory ensheathing cells (OECs) have shown promising results for patients with neurologic diseases in non-double-blind, placebo control studies. Thirty patients with a unilateral ischemic stroke of more than a year were enrolled in a phase 2, multicenter, randomized, double-blind, and placebo-controlled cell therapy trial with a subsequent 12-month follow-up. The primary therapeutic objective has shown that after 12 months, there were significant differences in National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS) and Barthel Index (BI) assessment scores among the OEC group, Schwann cell group and placebo medium group at one-year follow-up. The second therapeutic objective found that there were significant differences in NIHSS, mRS, and BI assessment scores when comparing the endpoint data with the baseline data in the OEC group. There was neither hypersensitivity reaction nor adverse event. The results of this multicenter, randomized, double-blind, and placebo-controlled study indicate that injecting OECs into the olfactory sub-mucosa have neurorestorative effects, which can improve the quality of life for patients with chronic ischemic strokes without serious side effects.

KEYWORDS
olfactory ensheathing cells; Schwann cells; chronic ischemic stroke; cell therapy; randomized control trial

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1 Introduction

Stroke is the leading cause of adult disability worldwide. Patients who have had an acute ischemic stroke experience neurologic deficit and suffer from functional disabilities even after medical intervention, care, and rehabilitation [1]. The ability of the neurorestorative effects of some cells have been investigated, and they have shown promising results in patients with acute, sub-acute, and chronic stroke [1–5]. These cells are transplanted into various sites in the central nervous system (CNS), including the parenchyma of the brain, cerebrospinal fluid (CSF; ventricle, and subarachnoid space), vesicle (intravenous or intra-arterial), and areas above combination [1, 2]. However, the majority of these clinical cell therapy studies were small or single center, phase 1 or/and 2, randomized, and none of these studies have been double-blind and placebo-controlled, or retrospective [2].

Despite the small number of studies, there have been randomized, double-blind, placebo-controlled clinical trials of cell therapies for stroke [6–8]. Prasad et al. in 2014 reported a multicenter, randomized trial with blinded outcome assessment in which intravenous autologous bone marrow mononuclear cell therapy for sub-acute ischemic stroke did not show any beneficial effects [6]. Hess et al. in 2017 reported that a randomized, double-blind, placebo-controlled, phase 2 trial of multi-potent adult progenitor cells derived from the bone marrow for patients with acute ischemic stroke failed to show any evidence of neurologic recovery at day 90 in both the cell therapy and placebo groups [7]. Savitz et al. in 2019 reported a multicenter, randomized, blinded assessment, sham-controlled trial of autologous bone marrow-derived ALD-401 cells infused through the internal carotid artery of patients recovering from an ischemic stroke did not show any difference in the primary therapeutic objective between the groups [8]. Higher level and evidence-based studies of cell therapy for other CNS diseases and trauma have also shown negative results to date [9–12].

Our team has been engaged in clinical research of olfactory ensheathing cells (OEC) therapy for CNS diseases and damage since 2001. OECs have shown promising effects for patients with chronic ischemic stroke in non-double-blind or retrospective clinical studies [3, 13]. Previously, a double-blind, placebo-controlled clinical cell therapy trial through surgery was not approved by our hospital as the procedure of cell or placebo medium transplantation had the potential operational risks and harm [3, 14, 15].

Danielyan et al. first reported that cells following transnasal delivery in mice and rats could (1) migrate into the olfactory bulb and then to other parts of the brain, and (2) enter the CSF and move along the surface of the cortex before entering the brain parenchyma [16]. In this study, injecting cells into the olfactory mucosa was performed via a minimally invasive procedure and complied with ethical guidelines [17]. This study was conducted in accordance with the Chinese government's regulations on carrying out clinical studies of cell therapy (National Health Commission, 2015 Document No. 71).

2 Materials and methods

2.1 Study design

This study was a phase 2, multicenter, randomized, double-blind, placebo-controlled trial. The objectives of the study were to determine if OECs had neurorestorative effects in patients with an ischemic stroke in the chronic phase (over 12 months), and whether OEC transplantation into the olfactory mucosa...
via injection is safe. Schwann cells (SCs) were the treatment control while the culture medium was the placebo control in this study [18]. The study was conducted in accordance with the Declaration of Helsinki, and ethics approval was obtained from the ethics committees of participating hospitals. This study has been registered in the Chinese Clinical Trial Registry (ChiCTR1800014476).

2.2 Participants

The study recruited 30 participants (23 males and 7 females) aged 40 to 70 years old (average 60.93 years). The participants were determined using an inclusion criterion of having a chronic ischemic stroke, and their medical records were provided by the participating hospitals. The participants were eligible for enrollment in the study if they (1) had a unilateral stroke due to carotid artery ischemia 12 months or more prior to the time of enrollment, and their National Institutes of Health Stroke Scale (NIHSS) scores were between 4 to 25; (2) brain magnetic resonance imaging (MRI) showed no other neurologic disease except ischemic stroke; (3) were fully conscious, cooperative with checkups, with complete self-control capacity, and were willing and able to participate in the study's follow-up and rehabilitation therapy (physical, occupational, speech/language, or cognitive rehabilitation therapy as needed) for 12 months. Participants were excluded if they (1) were comatose or initially had mild symptoms with rapid degeneration or progressive stroke; (2) had blood glucose $<2.7$ mmol/L or $>22.2$ mmol/L, or blood pressure $>150/90$ mmHg despite appropriate treatment; (3) had heart, lung, liver, or kidney failure, severe anemia, other severe conditions, and/or mental illness; (4) were unable to complete the 12-month follow-up; and (5) if they were enrolled in other clinical trials or did not sign the informed consent.

Participants were withdrawn from this study if they (1) formally withdrew their signed informed consent; (2) had complications unrelated to the study which may affect the results; (3) used unconventional drugs (such as neurotropic factors, etc.); (4) had adverse reactions not related to the study; (5) had other serious medical conditions; (6) failed to adhere to the rehabilitation schedule; (7) failed to complete the follow-ups, or (8) died from unrelated causes. Data of drop-outs were not included in the final statistical analysis.

A written informed consent was obtained either from the enrolled participant or a legal representative of the participant after agreeing to participate in this study.

2.3 Cell preparation and procedure

OEC preparation: OECs were derived from the olfactory mucosa of an aborted fetus (received approval and signed donation consent form) and cultured in Dulbecco’s modified Eagle’s medium (DMEM/F12; Gibco) with the neurotrophic factors. Cells were allowed to proliferate and differentiate according to the methods of the patent (China patent ZL 201510516055.2. 2018.02.09) until the volume was appropriate for clinical transplantation.

SC preparation: SCs were isolated from human fetal sciatic nerve (received approval and signed donation consent form). The specimens were sliced, dissolved in trypsin (Invitrogen, Carlsbad, CA, USA), and allowed to settle into a monolap suspension. These cells were cultured in DMEM/F12 with 15% fetal bovine serum (FBS, Hyclone) and harvested using trypsin after 7 days [3].

OECs and SCs were visualized by immunostaining p75 (over 85%) and S100 (over 90%) [3]. All cells used in this study were cultured and prepared by the Third Medical Center and General Hospital of PLA and Beijing Hongtianji

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Neuroscience Academy, China. Transplantation was carried out in the procedure room of an otolaryngology clinic by an otolaryngologist. Participants were instructed to lay supine while a local mucosal anesthetic was administered in the olfactory area 10–15 minutes before the procedure. A 0.3 mL cell culture medium with or without cells was injected into the olfactory sub-mucosa of the nasal septum between the superior and middle turbinates (Figs. 1–3). The injected cells would migrate into the olfactory bulb and into other areas of the brain. They would then enter the CSF, moving along the surface of the cortex before entering the brain parenchyma [16]. Each participant in the OEC group was injected with 5×10⁶ OECs (in 0.3 mL medium) on each side of the septum (total 10×10⁶). Each participant in the SC group was injected with 5×10⁶ SCs (in 0.3 mL medium) on each side of the septum, (total was 10×10⁶). Each participant in the placebo group was injected with cell culture medium (0.3 mL) on each side of the septum.

Fig. 1 A otolaryngologist was preparing to do injection.

Fig. 2 Before (A) and after (B) cell culture medium injecting into sub-mucosa, local tissue looks plump.

Fig. 3 Diagram of cell injection and migration. After cell being injected into sub-mucosa of olfactory area, they migrated into olfactory bulb and then to lesion area.
2.4 Outcome

Assessments were conducted by physicians at each participating hospital in this study. Prior to the study, all physicians received training for consistency and standardization of assessments. Participants in all groups were assessed by study personnel before treatment (baseline) and at intervals of 1 month, 3 months, 6 months, and 12 months (endpoint). The NIHSS, modified Rankin Scale (mRS), and Barthel Index (BI) were also included in the assessment during each follow-up. Participants were allowed to contact their physicians if they had any adverse events. Inflammatory biomarkers together with heart, pulmonary, renal, and hepatic functions were taken at baseline and endpoint. MRI brain scans were done at baseline and end point.

The primary objective was to determine whether the change in assessment scores between the groups was statistically significant. The secondary objective was to determine whether there was a significant difference in assessment scores between the baseline and end point in the OEC group.

The study would be discontinued if the adverse events were serious enough to result in either worsening of neurologic functions or endangering the lives of enrolled patients.

2.5 Sample size

Our previous studies have shown a significant difference in neurologic functional improvement scores; self-assessment of 10 patients before and after treatment [3], and comparison between OEC treatment and control treatments groups with 6 and 8 [15] patients, respectively. Thus, we recruited 30 participants for the study and they were randomly assigned and divided into 3 groups of 10 participants per group.

2.6 Randomization

Through a computer-generated process run by a designated staff member in the cell processing facility, the 30 participants were randomly assigned in a natural order and in a 1:1:1 ratio to receive either OECs (1/3), SCs (1/3) or placebo (medium) (1/3). This designated staff member was non-blind to patient treatment assignments and followed the treatment assignment sequence list to prepare and dispense the investigational product. This staff member had no further involvement with the participants for the rest of the study.

2.7 Blinding

Participants received a matching treatment according to the generated sequence. The injected media, with or without cells, have similar color, and appearance. Participants, investigators, and all study personnel (including otolaryngologists, neurologists or rehabilitation physicians) were blinded to the treatment assignment.

2.8 Statistical analysis

Participants’ data were statistically analyzed. All continuous variables were first examined by Shapiro-Wilk test to determine the normal distribution. For normal continuous variables, we used mean ± standard deviation to describe variable characteristics, and then Levene’s test was used to assess the assumption of homogeneity of variances for these variables. One-way ANOVA was performed to assess significant differences of the above variables in different groups. For non-normal variables, results were expressed as median and interquartile range. Kruskal-Wallis test was performed to assess any significant differences among the groups. Dunn-Bonferroni post hoc test was used for all pairwise comparisons. Chi-Square was conducted to assess statistical differences in categorical variables. Wilcoxon signed-rank test was used for evaluating the
intervention effect in different intervention periods as compared to the baseline. P values less than 0.05 were considered statistically significant with two-tailed tests.

Primary and secondary objectives were analyzed in participants who completed follow-ups. Safety outcomes were analyzed in the intention-to-treat population, which comprised all participants who were randomly assigned to receive cells or placebo treatment.

Statistical analyses were carried out by the Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Recruiting and randomly assigning patients

The study began to recruit participants on April of 2017, and participants enrolled in the study had follow-ups during a 12-month period. Thirty participants were recruited from 571 patients (350 from 960 Hospital of Chinese PLA, 160 from 981 Hospital of Chinese PLA, 23 from Civil Aviation Guangzhou Hospital and 38 from Taian Central Hospital, China). They were equally divided and randomly assigned into three groups (OEC, SC, placebo). One participant in each group failed to finish the study (Fig. 4).

3.2 Baseline characteristics

Demographic and clinical information of baseline outcomes for all patients who received OECs, SCs, or placebo treatment was summarized in Table 1. The groups were well matched for age, history of stroke, median NIHSS scores, median mRS scores, and median BI scores.

Fig. 4 Study flow diagram.
Table 1  Baseline characteristics.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Placebo group (n = 9)</th>
<th>OEC group (n = 9)</th>
<th>SC group (n = 9)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years mean ± SD</td>
<td>63.56 ± 4.36</td>
<td>60.67 ± 4.53</td>
<td>56.89 ± 7.72</td>
<td>0.066</td>
</tr>
<tr>
<td>Sex, males number (percentage)</td>
<td>5 (55.56%)</td>
<td>7 (77.78%)</td>
<td>8 (88.89%)</td>
<td>0.299</td>
</tr>
<tr>
<td>History of stroke in months median (IQR)</td>
<td>21.00 (13.00, 35.00)</td>
<td>27.00 (14.50, 52.00)</td>
<td>28.00 (18.50, 39.00)</td>
<td>0.643</td>
</tr>
<tr>
<td>NIHSS score median (IQR)</td>
<td>5.00 (4.00, 6.50)</td>
<td>4.00 (4.00, 6.00)</td>
<td>5.00 (4.00, 11.50)</td>
<td>0.878</td>
</tr>
<tr>
<td>mRS score median (IQR)</td>
<td>3.00 (2.00, 4.00)</td>
<td>3.00 (2.50, 4.00)</td>
<td>2.00 (1.50, 3.00)</td>
<td>0.243</td>
</tr>
<tr>
<td>BI score median (IQR)</td>
<td>85.00 (90.00, 90.00)</td>
<td>65.00 (47.50, 82.50)</td>
<td>90.00 (55.00, 92.50)</td>
<td>0.222</td>
</tr>
</tbody>
</table>

* Differences among control, OEC group and SC group were determined by one-way ANOVA for age comparison in years. Chi-square test was used in sex comparison among three groups. Kruskal-Wallis tests were used in non-normal variables including history of stroke, NIHSS score, mRS score and BI score. IQR, interquartile range. NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; BI, Barthel Index.

3.3 Primary objective results

Twenty-seven participants in three groups who finished the study were assessed for the primary objective. Data analysis showed a significant difference between baseline-to-end point comparison and pairwise comparison, (between OEC group with SC group or placebo group) in NIHSS, mRS, and BI assessments at 12 months; however, there was no significant difference between the SC group and placebo group. Table 2 summarized those results. A significant difference was already noted at 3 months (Table 3) which was more pronounced at 6 months (Table 4) and 12 months.

Table 2  Comparison of changes in assessment scores at 12 months after treatment.

<table>
<thead>
<tr>
<th>Assessment scores</th>
<th>Placebo group (n = 9)</th>
<th>OEC group (n = 9)</th>
<th>SC group (n = 9)</th>
<th>Test statistic</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>−3.00 (−4.50, −0.50)</td>
<td>−1.00 (−2.00, 0.00)</td>
<td>8.842</td>
<td>0.012</td>
</tr>
<tr>
<td>mRS median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>−1.00 (−1.50, 0.00)</td>
<td>0.00 (0.00, 0.00)</td>
<td>6.693</td>
<td>0.035</td>
</tr>
<tr>
<td>BI median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>15.00 (2.50, 22.00)</td>
<td>0.00 (0.00, 5.00)</td>
<td>10.509</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* Differences among placebo group, OEC group and SC group were determined by Kruskal-Wallis Dunn-Bonferroni post hoc test was used for all pairwise comparisons. * Compared with placebo group; P < 0.01; † compared with OEC group, P < 0.05.

Table 3  Comparison of changes in assessment scores at 3 months after treatment.

<table>
<thead>
<tr>
<th>Assessment scores</th>
<th>Placebo group (n = 9)</th>
<th>OEC group (n = 9)</th>
<th>SC group (n = 9)</th>
<th>Kruskal-Wallis test statistic</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>−1.00 (−2.50, 0.00)</td>
<td>0.00 (−0.50, 0.00)</td>
<td>4.953</td>
<td>0.084</td>
</tr>
<tr>
<td>mRS median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.00 (−0.50, 0.00)</td>
<td>0.00 (−0.50, 0.00)</td>
<td>0.473</td>
<td>0.789</td>
</tr>
<tr>
<td>BI median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>5.00 (0.00, 10.00)</td>
<td>0.00 (0.00, 0.00)</td>
<td>9.296</td>
<td>0.010</td>
</tr>
</tbody>
</table>

* Differences among placebo, OEC group and SC group were determined by Kruskal-Wallis Dunn-Bonferroni post hoc test was used for all pairwise comparisons. * Compared with OEC group, P < 0.05. No difference was found between placebo group and OEC or SC group.
### Table 4: Comparison of changes in assessment scores at 6 months after treatment.

<table>
<thead>
<tr>
<th>Assessment scores</th>
<th>Placebo group (n = 9)</th>
<th>OEC group (n = 9)</th>
<th>SC group (n = 9)</th>
<th>Kruskal-Wallis test statistic</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS, median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>-3.00 (-3.50, -3.50)</td>
<td>-1.00 (-1.50, 0.00)</td>
<td>9.118</td>
<td>0.010</td>
</tr>
<tr>
<td>mRS, median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>-1.00 (-1.50, 0.00)</td>
<td>0.00 (0.00, 0.00)</td>
<td>6.501</td>
<td>0.039</td>
</tr>
<tr>
<td>BI, median (IQR)</td>
<td>0.00 (0.00, 0.00)</td>
<td>15.00 (2.50, 27.50)</td>
<td>0.00 (0.00, 2.50)</td>
<td>12.241</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Differences among placebo, OEC group and SC group were determined by Kruskal-Wallis. Dunn-Bonferroni post hoc test was used for all pairwise comparisons. \* Compared with placebo group, \( P < 0.01; \dagger \) compared with OEC group, \( P < 0.05.\)

### 3.4 Secondary objective results

Nine participants in the OEC group who finished the study were assessed for the secondary objective. Data analysis show significant differences in NIHSS, mRS, and BI assessments when comparing the endpoint with the baseline. Table 5 summarizes these data. SC treatment made limited changes (Table 6), while placebo treatment did not show any changes (Table 7) in assessments when comparing the endpoint to the baseline.

### Table 5: Neurorestorative effects in OEC treatment.

<table>
<thead>
<tr>
<th>Assessment scores</th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>P value</td>
<td>Median (IQR)</td>
<td>P value</td>
</tr>
<tr>
<td>NIHSS</td>
<td>4.00 (4.00, 6.00)</td>
<td>4.00 (3.50, 6.00)</td>
<td>0.102</td>
<td>4.00 (2.50, 5.50)</td>
<td>0.042</td>
</tr>
<tr>
<td>mRS</td>
<td>3.00 (2.50, 4.00)</td>
<td>3.00 (2.00, 4.00)</td>
<td>1.00</td>
<td>3.00 (2.00, 3.50)</td>
<td>0.157</td>
</tr>
<tr>
<td>BI</td>
<td>65.00 (47.50, 82.50)</td>
<td>65.00 (50.00, 82.50)</td>
<td>0.317</td>
<td>70.00 (55.00, 85.00)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Differences between paired groups were determined Wilcoxon signed-ranks test.

### Table 6: Neurorestorative effects in SC treatment.

<table>
<thead>
<tr>
<th>Assessment scores</th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>P value</td>
<td>Median (IQR)</td>
<td>P value</td>
</tr>
<tr>
<td>NIHSS</td>
<td>5.00 (4.00, 11.50)</td>
<td>4.00 (4.00, 11.50)</td>
<td>0.317</td>
<td>4.00 (4.00, 11.50)</td>
<td>0.157</td>
</tr>
<tr>
<td>mRS</td>
<td>2.00 (1.50, 3.00)</td>
<td>2.00 (1.00, 3.00)</td>
<td>0.317</td>
<td>2.00 (1.00, 3.00)</td>
<td>0.157</td>
</tr>
<tr>
<td>BI</td>
<td>90.00 (55.00, 92.50)</td>
<td>90.00 (55.00, 92.50)</td>
<td>1.00</td>
<td>90.00 (55.00, 92.50)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Differences between paired groups were determined Wilcoxon signed-ranks test.
<table>
<thead>
<tr>
<th>Assessment scores</th>
<th>Baseline Median (IQR)</th>
<th>1 month Median (IQR)</th>
<th>P Value</th>
<th>3 months Median (IQR)</th>
<th>P Value</th>
<th>6 months Median (IQR)</th>
<th>P Value</th>
<th>One year Median (IQR)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS</td>
<td>5.00 (4.00, 6.50)</td>
<td>5.00 (4.00, 6.50)</td>
<td>0.317</td>
<td>5.00 (4.00, 6.50)</td>
<td>0.317</td>
<td>5.00 (4.00, 6.50)</td>
<td>0.317</td>
<td>5.00 (4.00, 6.50)</td>
<td>0.317</td>
</tr>
<tr>
<td>mRS</td>
<td>3.00 (2.00, 4.00)</td>
<td>1.00 (2.00, 3.50)</td>
<td>0.317</td>
<td>3.00 (2.00, 3.50)</td>
<td>0.317</td>
<td>3.00 (2.00, 3.50)</td>
<td>0.317</td>
<td>3.00 (2.00, 3.50)</td>
<td>0.317</td>
</tr>
<tr>
<td>BI</td>
<td>85.00 (50.00, 90.00)</td>
<td>85.00 (50.00, 90.00)</td>
<td>0.317</td>
<td>85.00 (50.00, 90.00)</td>
<td>0.317</td>
<td>85.00 (50.00, 90.00)</td>
<td>0.317</td>
<td>85.00 (50.00, 90.00)</td>
<td>0.317</td>
</tr>
</tbody>
</table>

Differences between paired groups were determined Wilcoxon signed-ranks test.

3.5 Safety and adverse events

There was no hypersensitivity reactions or serious adverse events related to the study. There were no adverse events recorded during the procedure of injecting the treatment medium into the olfactory sub-mucosa. Brain MRI did not show any structural changes when comparing the end point to the baseline. No tumors were found 12 months after treatment.

4 Discussion

The OEC is distinct from other glia as it has characteristics of both SC and oligodendrocytes, and it also has the ability to secrete neurotrophic factors. OECs can migrate from the peripheral nervous system to the CNS and play a neurorestorative role by altering the micro-environment of lesions and stimulating hibernating neurons for regeneration and repair. In basic and pre-clinical researches, the neurorestorative mechanisms of OECs have been shown to include neuroprotection, neuromodulation, neuroplasticity, neurogenesis, axonal regeneration or sprouting, and, to some extent, remyelination, angiogenesis, immunomodulation, anti-inflammation, and scar/cavity formation [19, 20].

OECs support human olfactory tissue and neurons by enabling regeneration in whole life [21]. Based on previous clinical studies, OECs have shown promising neurorestorative effects for neurologic diseases and damage in a single center, randomized control trial (RCT) [22], randomized, non-double-blind studies [3, 14, 15], and retrospective studies [13].

Previous studies using SCs alone [22–25] or in conjunction with other kinds of cells [22, 26] have shown limited neurorestorative effects in patients with stroke, spinal cord injury, or other neurologic diseases. In this study, SC treatment has also shown limited neurorestorative effects when comparing the endpoint to the baseline, and there was no difference as compared to the control group. We hypothesize that the reason for the SCs limited role in neurorestoration is their restriction in migrating into the CNS [29].

At a higher level of evidence-based medicine (a multicenter, randomized, double-blind, placebo-controlled clinical trial), this study is the first to document the positive effects of OECs for patients with chronic ischemic stroke. This study has also shown that OECs appear to be the support cells with the most potential to restore damaged neurologic structures and functions in the CNS.

The limitation of this study is the relatively small sample size that may cause bias or imprecise results. A phase 3 trial may compensate for this shortcoming. Future clinical
researches of OEC therapy for stroke should be directed toward an optimal dosage, a more suitable transplantation route, appropriate therapeutic time window (our other clinical trial of OECs for sub-acute stroke is being carried out, ChiCTR1800014497 [18]), and the effects of repeated application. Additional multicenter, randomized, double-blind, placebo-controlled trials of OEC therapy should be performed for other neurologic diseases and damage.

5 Conclusions
The study demonstrates that OEC therapy is safe and can improve the quality of life for patients with chronic ischemic stroke through olfactory sub-mucosa transplantation.

Conflict of interests
All the authors claim that there is no conflict of interests except receiving salary from their hospitals or institutions. All cells used in this trial were provided by Third Medical Center of General Hospital of PLA and Beijing Hongtianji Neuroscience Academy, China.

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Author contributions
Huang HY who wrote the first draft of the manuscript was involved in study design, data interpretation, and enrollment of patients. Wang YL, Guo XL, Liu J and Zheng ZC recruited patients and were involved in data interpretation, reviewing the manuscript, editing and helping with revisions. Wang YL, Guo XL, Chen L and Mao GS were involved in study design. Liu Y, Gao WY, Xiao J, Zhou B, Liu AB and Mao GS were involved in cell culture preparing and quality control. Guo DQ, Chen WD, Liu F and Chan BM did injecting procedure. Liu YQ, Li Y, Tang ML and Chen D were involved in assessment and data collection. Wang LL and Chen L did the statistical analysis.

References
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