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Autologous Endothelial Progenitor Cells Transplantation for Acute Ischemic Stroke: A 4-Year Follow-Up Study

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Key Words. Endothelial progenitor cells • Bone marrow stromal cells • Stroke • Transplantation • Clinical trial

ABSTRACT

Transplantation of endothelial progenitor cells (EPCs) is a proven safe and effective method for treatment of cerebral ischemia in animal experiments. However, safety and efficacy need to be determined in clinical trials. We performed a two-center, randomized, placebo-controlled phase I/IIa trial with blinded outcome assessment on 18 patients with acute cerebral infarct within the middle cerebral artery territory, and followed for up to 4 years. Autologous ex vivo expanded EPCs were injected intravenously in the EPC group, and patients who received saline or autologous bone marrow stromal cells served as control groups. Mortality of any cause, adverse events, and new-onset comorbidities were monitored. Changes in neurological deficits were assessed at different time points. We found no toxicity events or infusional or allergic reactions in any treated group. Three patients in the placebo group died during the 4-year follow-up. We found that the EPC group had fewer serious adverse events compared with the placebo-controlled group, although there were no statistical differences in mortality among the three groups. Furthermore, there was no significant difference in neurological or functional improvement observed among the three groups, except for the Scandinavia Stroke Scale score at 3 months between the EPC group and placebo-controlled group. Autologous transplantation of EPCs appears to improve long-term safety in acute cerebral infarct patients, supporting the feasibility of this novel method for treatment of ischemic stroke (ClinicalTrials.gov: NCT01468064). STEM CELLS TRANSLATIONAL MEDICINE 2019;8:14–21

SIGNIFICANCE STATEMENT

This study suggests that autologous transplantation of endothelial progenitor cells improved long-term safety in patients with acute cerebral infarct, supporting the feasibility of this novel method for treatment of ischemic stroke, and warrants a larger phase II trial.

INTRODUCTION

Stroke is associated with persistent disability and is a leading cause of death [1]. Recent studies in People's Republic of China suggested a mortality of 3.3%–5.2% in 2 weeks and 9%–9.6% in 3 months after symptom onset. The likelihood of mortality or disability increased to 34.5%–37.1% a year after onset [2]. Despite the enormous health impact of stroke, the only approved treatments for acute ischemic stroke, thrombolytic therapies and endovascular recanalization techniques, have a narrow time-window and only benefit a small subset of patients [3]. Because few neuroprotective reagents have been successfully translated "from bench to bedside" in the past several decades, neurorestoration has become a new research hot spot in stroke treatment [4], of which stem cell transplantation has been shown by both animal research and clinical trials to open a novel avenue to promote tissue and function repair after stroke [5, 6].

In addition to hematopoietic stem cells and bone marrow stromal cells (BMSCs), endothelial progenitor cells (EPCs) are another type of stem cell derived from bone marrow that can proliferate and differentiate into mature vascular endothelial cells [7]. In the normal central nervous system, EPCs are distinct from endothelial cells, but play an active role in vascular structures [8]. After stroke, circulating EPCs can be recruited and participate in cerebral neovascularization [9]; however, a critical limitation for the therapeutic application of EPCs is their inherently low number in circulation, which is

even smaller in patients with higher cardiovascular risk or aging [10], demonstrating further that transplantation of ex vivo expanded EPCs is an attractive approach to promote restoration after stroke.

In fact, the safety and efficacy of EPC transplantation for stroke have been supported by evidence from numerous laboratories [11–14]. The major mechanism underlying this EPC-mediated functional recovery may be that EPCs promote endothelial repair and angiogenesis, thus enhancing the neuroplastic effect and resulting in better neurological outcomes [15]. Because EPCs can be readily obtained from bone marrow and used for autologous transplantation, virtually eliminating those ethical and immunological considerations, they are believed to be a good graft source for clinical applications. However, no clinical trial for evaluating EPC transfusion in treating ischemic stroke was reported until recently.

Identifying the potential therapeutic effects of EPCs in ischemic stroke will be a challenging but incredibly important breakthrough in neurology that may bring promise for patients with ischemic stroke [15]. In this trial, we test the safety and feasibility of autologous transplantation of ex vivo expanded EPCs in patients with acute ischemic stroke. BMSCs are also derived from bone marrow, and have been proved to be safe and effective for treatment of ischemic stroke by animal research and preliminary clinical trials [16–18]. Therefore, autologous transplantation of BMSCs and placebo treatment were employed as controls in this study.

MATERIALS AND METHODS

Study Design

This study is a two-center, single-blinded, randomized, parallel, placebo-controlled trial that was approved by the Ethics Committee of Zhujiang Hospital and registered with clinicaltrials.gov (NCT01468064). The institutional review board of each participating center approved the treatment protocol before initiation of enrollment. All patients or their legally authorized representatives gave written informed consent. Patients were randomly assigned using layered segment randomization in a 1:1:1 ratio to three groups, and randomization codes were revealed only to the technician responsible for preparing the cells or placebo material. The overall trial profile is shown in Figure 1.

Patients

Patients with acute ischemic infarction (within 7 days from symptom onset) who had severe neurological deficits were enrolled from the Zhujiang Hospital of Southern Medical University and Guangdong General Hospital's Nanhai Hospital. Our inclusion criteria were (a) patients aged 18–80 years, within 7 days of onset of symptoms; (b) ischemic lesion within the middle cerebral artery territory as assessed using diffusion-weighted imaging; (c) a National Institutes of Health Stroke Scale (NIHSS) score ≥7 at day 7 after onset; and (d) signed informed consent. The exclusion criteria were (a) Lacunar syndrome; (b) diagnosis other than ischemic stroke (e.g., intracranial hemorrhage or intracranial tumor); (c) hematological causes of stroke; (d) severe respiratory, hepatic, or renal disorders; (e) presence of severe febrile illness or viral diseases; (f) malignant diseases; (g) presence of autoimmune diseases; (h) positive response of penicillin skin test, or multiple drug allergies; and (i) breast-feeding or pregnant women.

Patients meeting all of the inclusion and none of the exclusion criteria were approached for consent to participate in the study. All patients received conventional treatment after stroke, and underwent a bone marrow aspiration procedure on the 7th day after symptom onset. Patients were blinded to the grouping. EPCs and BMSCs were expanded ex vivo and then infused intravenously. The placebo-controlled group did not receive cells transfusion but underwent a bone marrow aspiration procedure and saline transfusion. Neurological assessment, neuroimaging, death of any cause, adverse events, and new onset comorbidities were monitored.

### Cell Preparation and Administration

Bone marrow (50 mL) was aspirated from the iliac crest under local anesthesia on the 7th day after symptom onset in each group. Bone marrow mononuclear cells were isolated by centrifugation through a density gradient (Ficoll-Paque Plus, Pharmacia, Guangzhou, Guangdong Province, China). Cell preparation was performed under good manufacturing practices. For preparation of EPCs, mononuclear cells were seeded on fibronectin (354008, BD Pharmingen)-coated plastic dishes (1×10^5 cells/cm^2) and cultivated in EC basal medium-2 (EBM-2, CC-3162, Lonza) with EGM-2 MV SingleQuots (CC-4176, Lonza). BMSCs were prepared and cultured as described previously [16, 17]. Nonadherent cells were discarded by removing the medium 4 days later. Once cells reached 80% confluence, they were detached with 0.05% trypsin/0.01% EDTA and sub-cultured. Cell morphology was observed under a Type CK2 phase-contrast microscope (Olympus).

Approximately 3–4 weeks after bone marrow aspiration, autologous BMSCs and EPCs were culture-expanded to reach 3.125×10^6 cells/kg body weight per patient. Among these, 2.5×10^6 cells/kg body weight of autologous EPCs or BMSCs were transplanted into patients intravenously with 250 mL of saline containing 5% autologous serum. The rest of the cells (0.625×10^6 cells/kg body weight) were further expanded for two passages, and thus, another 2.5×10^6 cells/kg body weight of EPCs or BMSCs were again transplanted approximately 1 week after initial boosting. Namely, each cell-treated patient received a total of 5×10^6 cells/kg body weight of autologous EPCs or BMSCs. This cell amount is equivalent to the dose that was effective in animal studies (1×10^5 to 3×10^6 cells/rat) [6] and in a previous human study (1×10^8 cells/patient) [16, 17]. Placebo-controlled patients received only saline containing 5% autologous serum.

In these cases, the total cell culture time was 33.75 ± 2.75 days (range: 31–37 days) in the BMSC group and 23.60 ± 3.78 days (range: 18–27 days) in the EPC group. And if the cells that reached the first 80% confluence were defined as passage 0, the total population doublings were 5.52 ± 0.22 in the BMSC group and 5.62 ± 0.31 in the EPC group, respectively.

Cell Phenotypes and In Vitro Tube Formation Assay

Cell viability was determined by trypan blue staining at the end of the harvest, and was >98% for every infusion. Cell cultures were tested weekly for sterility, and there was no evidence of bacterial, viral, fungal, or mycoplasmal contamination in any of the flasks. Cell phenotypes were determined by flow cytometry. To identify EPCs, cell binding of UEA-1 lectin and
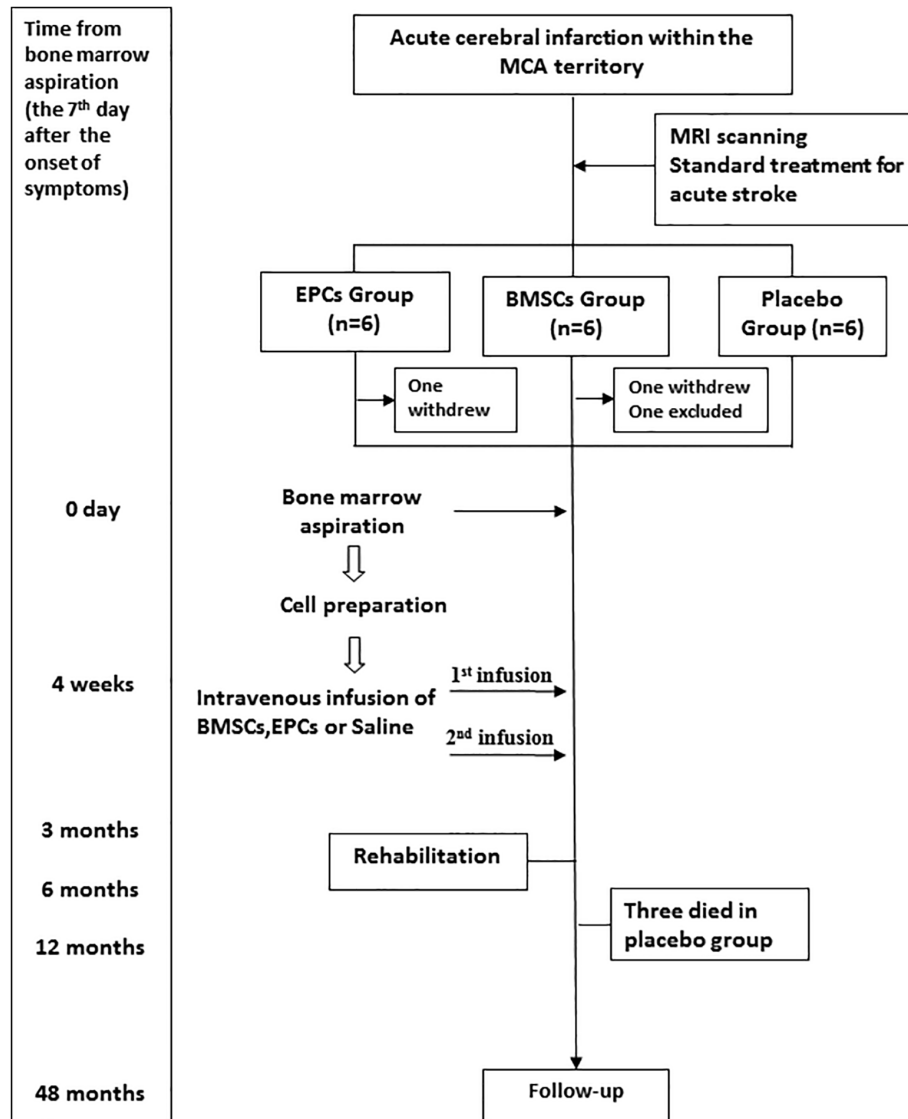
Figure 1. Study profile. Abbreviations: BMSCs, bone marrow stromal cells; EPCs, endothelial progenitor cells; MCA, middle cerebral artery.

endocytosis of acetylated low-density lipoprotein (Dil-Ac-LDL), two characteristic features of endothelial lineage cells, were detected as previously described [12].

Tube formation assay was carried out to estimate the in vitro angiogenic capacity of cells. Briefly, 3×10^4 cells were seeded onto 96-well plates coated with growth factor-reduced matrigel (70 μL/well; BD Biocoat) and cultured in EBM-2 (Lonza) at 37°C. After incubation for 8 hours, tubes in each well were visualized under an inverted microscope, photographed at ×10 magnification, and analyzed using the Angiogenesis Analyzer for ImageJ software.

Measurements of Safety and Efficacy

All patients were evaluated according to a protocol that included demographic data, medical history, vascular risk factors, laboratory examination, radiological data, and functional scales. The primary endpoint was safety, including adverse events, neurological deterioration (defined as four points or more increase on the NIHSS), and mortality. Patients were

followed up at 3, 6, 12, and 48 months after bone marrow aspiration for clinical and functional evaluation. Blood examination of patients including liver, heart, renal function, glycometabolism, lipid metabolism, electrolyte, magnetic resonance scanning, and electrocardiogram were recorded. Death of any cause and adverse effects caused by this study were the primary adverse events monitored. A treatment-emergent adverse event was defined as any event not present before the initiation of treatment or any event already present that worsened in either intensity or frequency after exposure to study treatment [18].

The secondary endpoints were the difference in neurological or functional outcomes evaluated by the global impairment scales, NIHSS and Scandinavia Stroke Scale (SSS), and the functional scales, Barthel Index (BI) and modified Rankin Scale (mRS). These assessments were evaluated at 0, 3, 6, 12, and 48 months. The assessments were independently conducted by experimental neurologists blind to patient grouping. All assessments of patients were performed through inpatient or

Table 1. Baseline characteristics of modified intent-to-treat population

Characteristics	EPCs (n = 5), n (%)	BMSCs (n = 5), n (%)	Placebo (n = 6), n (%)	p value
Age, years	50.80 ± 14.11	49.40 ± 10.85	52.83 ± 14.95	.915
Male	4 (80.0)	4 (80.0)	5 (83.3)	.986
Married	5 (100.0)	5 (100.0)	6 (100.0)	
Body mass index, kg/m^2	25.57 ± 3.83	23.08 ± 3.66	22.33 ± 1.73	.278
Allergic	1 (20.0)	0 (0.0)	0 (0.0)	.309
Time of cells culture	40.80 ± 11.43	27.00 ± 7.17		.342
Risk factors				
Smoking	2 (40.0)	2 (40.0)	2 (33.3)	.965
Alcohol use	1 (20.0)	1 (20.0)	0 (0.0))	.504
Hypertension	3 (60.0)	4 (80.0)	1 (16.7)	.09
Dyslipidemia	0 (0.0)	0 (0.0)	0 (0.0)	
Diabetes	0 (0.0)	0 (0.0)	0 (0.0)	
Coronary artery disease	0 (0.0)	0 (0.0)	0 (0.0)	
Atrial fibrillation	2 (40.0)	1 (0.0)	1 (0.0)	.641
COPD	0 (0.0)	0 (0.0)	0 (0.0)	
TIA	0 (0.0)	0 (0.0)	0 (0.0)	
Stroke mechanisms				
Atherosclerotic	3 (60.0)	4 (80.0)	5 (83.3)	.641
Cardioembolic	2 (40.0)	1 (20.0)	1 (16.7)	.641
Cryptogenic	0 (0.0)	0 (0.0)	0 (0.0)	
Treatment				
Thrombolytics	0 (0.0)	0 (0.0)	0 (0.0)	
Drugs	AP(3); AC(2)	AP(3); AC(2)	AP(4); AC(2)	
mRS on 7th day	3.40 ± 0.89	4.00 ± 1.22	3.67 ± 0.82	.636
NIHSS on 7th day	12.20 ± 4.92	17.60 ± 5.59	15.50 ± 3.02	.632
Barthel index on 7th day	39.00 ± 24.60	27.00 ± 30.74	25.00 ± 20.00	.203
SSS on 7th day	28.00 ± 17.38	18.20 ± 13.50	19.33 ± 10.39	.483

Abbreviations: AC, anticoagulant; AP, antiplatelet agent; BMSCs, bone marrow stromal cells; bpm, beats per minute; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; EPCs, endothelial progenitor cells; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood; SSS, Scandinavia Stroke Scale; TIA, transient ischemic attack.

outpatient visits to the hospital, or for those who could not visit the hospital, by careful telephone interview.

Statistics

Continuous variables were expressed as the mean ± standard deviation. In addition to descriptive statistics, differences of indicators at baseline between groups were analyzed by one-way analysis of variance or Kruskal-Wallis test as appropriate. The incidence of serious adverse events (SAEs) and mortality were analyzed by chi-square test. Covariance analysis was used to calculate the difference of neurological outcomes of different groups at different time points. Data analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Differences were deemed statistically significant at $p < .05$.

RESULTS

Patient Characteristics

Eighteen patients were recruited and randomly allocated into one of three groups: the BMSC group (n = 6), EPC group (n = 6), and placebo control group (n = 6). Two patients, one from each of the BMSC and EPC groups, declined to receive bone marrow aspiration after enrollment and were excluded from the study. The baseline characteristics of the modified intent-to-treat population are shown in Table 1. We found no significant differences between each of the three groups at baseline regarding age, gender, and body mass index, as well as NIHSS, SSS, BI, or mRS score ($p > .05$).

Cell Characteristics

As shown in supplemental online Figure 1, the human bone marrow-derived EPCs expressed HLA-ABC, CD105, CD90, CD44, and CD29; moderately expressed HLA-DR, KDR, and CD54; and negatively expressed CD34, CD31, CD45, or CD144. These cells were also shown to endocytose Dil-Ac-LDL and bind to UEA-1, consistent with endothelial lineage cells. As shown in supplemental online Figure 2, the BMSCs expressed HLA-ABC, CD105, CD90, CD44, and CD29. They moderately expressed HLA-DR and did not express KDR, CD34, CD31, CD45, or CD144. These cells did not endocytose Dil-Ac-LDL or bind to FITC-UEA-1 lectin. Moreover, the EPCs formed extensive capillary-like structures in the tube formation assay, indicating strong angiogenic ability. But the BMSCs did not form extensive capillary-like structures on matrigel. Quantitatively compared with the EPCs, the BMSCs showed shorter total segment length, indicating lower angiogenic ability than that of the EPCs (supplemental online Fig. 3).

Table 2. List of SAEs

No.	Gender	Age, years	Group	SAE	Diagnostic date	Treatments	Relationship with transplantation	Outcome	Death time
2	Male	37	Placebo	Seizure	11th month	Oral drugs	No	Survival	
3	Male	45	Placebo	Seizure	11th month	Oral drugs	No	Survival	
4	Male	32	BMSCs	Deep vein thrombosis	6th month	Thrombolysis	No	Survival	
11	Male	69	Placebo	Parkinson's syndrome	10th month	Oral drugs	No	Dead	22nd month
16	Male	74	Placebo	Arrhythmia	3rd month	Hospitalized	No	Dead	4th month
1	Female	47	Placebo	Recurrent stroke	42nd month	Hospitalized	No	Dead	42nd month
11	Male	69	Placebo	Cancer	20th month	Hospitalized	No	Dead	22nd month

Abbreviations: BMSCs, bone marrow stromal cells; SAE, serious adverse events.

Safety Evaluation

One patient in the BMSC group was diagnosed with intestinal tumors after bone marrow aspiration and before cell infusion. Because the tumor was considered unlikely to be related to the bone marrow aspiration in such a short time, this patient, who died 14 months later, was also excluded from the safety assessment population and the per-protocol population. No treatment-emergent adverse events relating to bone marrow aspiration or cell transplantation, such as fever, allergy, increased headache, nausea, constipation, phlebitis, local infection or hematoma, or neurological deterioration, were found in any of the three groups. Profiles of the five patients in the EPC group are shown in supplemental online Table 1. Two patients from this group have complications with atrial fibrillation, which was treated in one patient (patient 10) 6 months after EPC treatment with no recurrence, but remained in the other patient (patient no. 12).

The SAEs during the 4-year follow-up are shown in Table 2. No new infectious diseases associated with transplantation occurred in our cohorts. Epilepsy occurred in two patients from the placebo group at the 11th and 12th month, respectively, and they received in-hospital treatments with antiepileptic drugs. A right lower limb deep vein thrombosis was found in one patient in the BMSC group at the 6th month, who received thrombolysis. A different patient in the placebo group was diagnosed with Parkinson syndrome at the 10th month and received anti-Parkinson treatment. This patient ultimately died at the 22nd month because of colorectal cancer. Two other patients in the placebo group died from arrhythmia and recurrent stroke at the 4th and 42nd month, respectively. In contrast, all patients in BMSC and EPC groups survived. No event was judged to be attributable to stem cell transplantation. No statistical difference of mortality was found between the arms (Table 3; $p = .06$); however, the EPC group had lower incidence of SAE compared with the placebo group (Table 4; $p = .02$).

Preliminary Efficacy

Changes of the neurological and functional outcomes evaluated by NIHSS, SSS, BI, and mRS are shown in Figure 2. We found that the NIHSS score of the EPC group at baseline was 12.20 ± 4.92 (95% confidence interval [CI]: 6.09–18.31), which decreased to 5.60 ± 1.54 (95% CI: 5.40–10.22) at 3 months and 3.00 ± 2.35 (95% CI: 0.09–5.91) at 48 months. However, no significant differences were found among the three groups at each time point ($p > .05$). The SSS score of the EPC group at baseline was 28.00 ± 17.38 (95% CI: 27.89–49.58), and it gradually increased to 47.20 ± 2.96 (95% CI: 38.71–47.93) at 48 months. Except for the 3-month time point, at which there was a significant difference of SSS score between the EPC group and the placebo group ($p < .05$), no statistical differences were observed between arms at any other time point ($p > .05$). In addition, the BI score of the EPC group at baseline was 39.00 ± 24.60 (95% CI: 8.46–69.54), which increased to 84.00 ± 6.78 (95% CI: 59.41–92.02) at 3 months and 94.00 ± 8.94 (95% CI: 82.89–105.11) at 48 months; however, there were also no significant differences between arms ($p > .05$). The mRS score of the EPC group at baseline was 3.40 ± 0.89 (95% CI: 2.29–4.51). It decreased to 2.00 ± 0.45 (95% CI: 1.57–3.00) at 3 months and to 1.40 ± 1.14 (95% CI: −0.16 to 2.82) at 48 months, but no significant differences were found between arms ($p > .05$).

DISCUSSION

This study is hitherto the first clinical trial to test the safety and exploratory efficacy of bone marrow-derived EPCs on patients with ischemic strokes. Our preliminary data indicate

**Table 3.** Mortality of different groups

Group	Dead, n (%)	Survival, n (%)	Total, n (%)	Chi-square	p value
EPCs	0 (0.0)	5 (100.0)	5 (100.0)	5.63	.06
BMSCs	0 (0.0)	4 (100.0)	4 (100.0)		
Placebo	3 (50.0)	3 (50.0)	6 (100.0)		

Abbreviations: BMSCs, bone marrow stromal cells; EPCs, endothelial progenitor cells.

Table 4. Comparison of incidence of SAEs

Group	None, n (%)	SAE, n (%)	Total, n (%)	Chi-square statistic	p value
EPCs	5 (100.0)	0 (0.0)	5 (100.0)	8.40	.02
BMSCs	3 (75.0)	1 (25.0)	4 (100.0)		
Placebo	1 (16.7)	5 (83.3)	6 (100.0)		

Abbreviations: BMSCs, bone marrow stromal cells; EPCs, endothelial progenitor cells; SAE, serious adverse events.
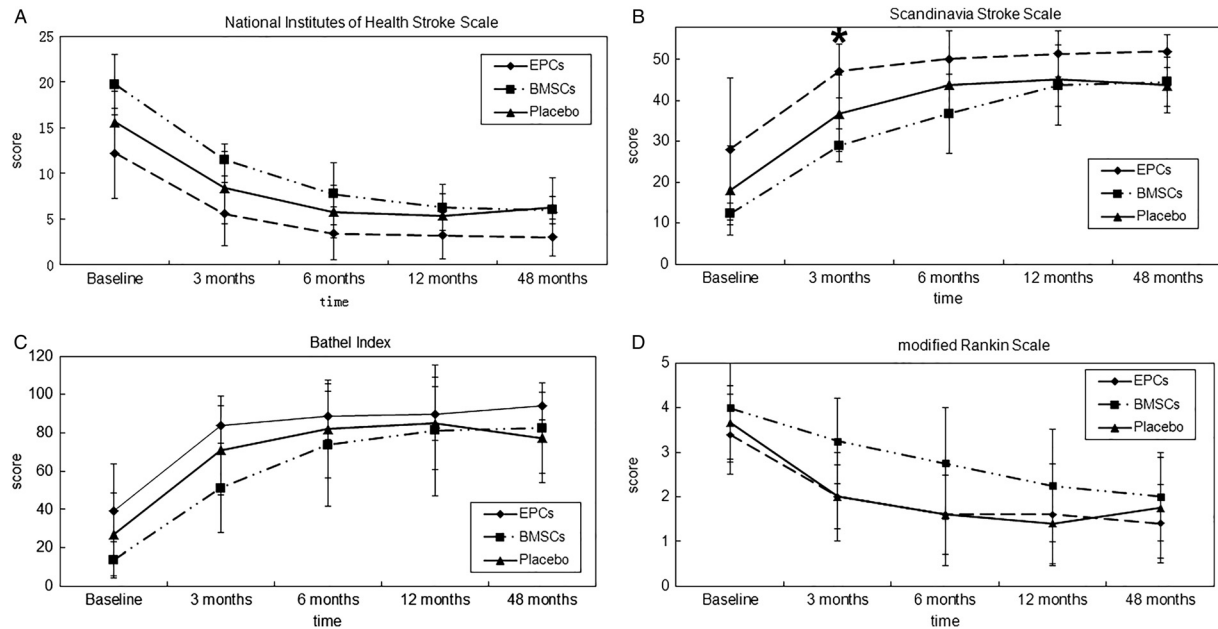
Figure 2. Change of neurological or functional outcome from baseline to 48 months. Gradual improvement of neurological function measured by NIHSS **(A)**, SSS **(B)**, BI **(C)**, and mRS **(D)** could be observed in the EPCs group. However, no significant difference of the scores at each time point could be observed between arms, except for the SSS score at 3-month time point between the EPCs group and the placebo-controlled group. *$p < .05$. Abbreviations: BMSCs, bone marrow stromal cells; EPCs, endothelial progenitor cells; SSS, Scandinavia Stroke Scale .

that intravenous transplantation of ex vivo expanded autologous EPCs is safe. There were no treatment-emergent adverse events or delayed toxicity related to intravenous infusion of EPCs during the therapeutic window or within the 4-year follow-up period. In addition, neurological function after transplantation of EPCs assessed by NIHSS, SSS, BI, and mRS showed different levels of amelioration compared with baseline assessment, indicating that EPC transplantation at a minimum did not aggravate existing neurological deficits. Furthermore, we also evaluated other SAEs that may be associated with EPC therapy. Arrhythmias, especially supraventricular arrhythmias, were reported to develop in some patients receiving hematopoietic cell transplantation [19, 20]. Similarly, seizure may be caused by aberrant innervation from newly formed neural circuits [17]. In this study, we found one case of newly occurred arrhythmia and two cases of seizure in the placebo group. In contrast, one case originally complicated with atrial fibrillation in the EPC group was treated 6 months later with no further recurrence. This cure cannot be entirely attributed to EPC treatment, but the treatment did not induce arrhythmia or seizure in our cases. Deep vein thrombosis was found in one patient in the BMSC group at the 6th month, but it is difficult to determine its relationship with BMSC transplantation.

Because of the rare number of EPCs or BMSCs in bone marrow mononuclear cells, a further consideration regarding therapeutic application of these two types of stem cells is the need for ex vivo expansion to obtain a sufficient number of cells, and ex vivo expansion always raises concerns that it may cause cell karyotype instability and increase tumorigenicity. However, in our long-term study, we observed no increased tumorigenicity after autologous EPC or BMSC treatment. This safety indicator was also confirmed by many animal studies and other trials using expanded bone marrow-derived stem cells for treatment of ischemic stroke, including allogeneic or gene-modified cells [17, 18, 21–23]. Direct use of bone marrow mononuclear cells was once thought to be effective for stroke treatment [24, 25]; however, a recent multicenter, randomized, controlled study has disproved this approach for the treatment of stroke [26], indicating that ex vivo purification and expansion may be necessary for acquiring therapeutic efficacy.

To determine the exploratory efficacy of EPCs on patients with ischemic stroke, we compared the neurological and functional outcomes measured by NIHSS, SSS, BI, and mRS among the three groups. No significant differences in outcomes were observed among the three groups, except for the SSS score at the 3-month time point between the EPC and placebo groups. Therefore, it is still not possible to conclude that EPCs or BMSCs improved the neurological or functional outcomes. However, the lower incidence of SAEs in the EPC group not only confirmed safety of the treatment but also suggests a certain degree of efficacy. It improved the quality of life and benefited patient survival. It is also worth noting that all patients in the EPC group survived after long-term follow-up, whereas three patients in the placebo group died, although no statistical difference of mortality was found between arms. Taken together, our finding indicates that transplantation of EPCs tended to ameliorate clinical outcome and improve prognosis of patients with stroke.

In fact, improvement of neurological function is important but not exclusive for stroke treatment. As most stroke occurs based on other factors, such as hypertension, atherosclerosis, or diabetes, we consider stroke more a systemic disease, rather than local. In this trial, the route for stem cell transplantation was intravenous systematic infusion. Although there is a concern that only a limited number of cells can reach and reside in the

peri-infarct area, and thus participate in injury repair, intravenous infusion has several unique advantages over stereotactic local injection. First, because many clinical trials have shown that autologous EPC transplantation may confer beneficial effects on several diseases, such as myocardial infarction [27, 28], liver cirrhosis [29], limb ischemia [30, 31], and idiopathic pulmonary arterial hypertension [32, 33], we speculate that many other tissues or organs in addition to the brain may benefit from systematic EPC treatment, and these positive effects all contribute toward the improved prognosis of patients in the EPC group. More attention to the functional improvement of other organs from EPC treatment is necessary in further trials. Second, intravenous infusion is simple and noninvasive, and there were no adverse events related to the procedure in our study. In contrast, SAEs were found in 4 of 11 patients in a study of stereotactic injection of CTX0E03 (an immortalized human neural stem-cell line) for treatment of chronic ischemic stroke [34]. A similar outcome was found in another chronic ischemic stroke study, in which the stereotactic injection of SB623 (a type of modified BMSCs) resulted in all 18 patients experiencing at least one treatment-emergent adverse event, and 6 patients experienced six serious treatment-emergent adverse events [18]. Third, as mentioned previously, because of surgical and anesthesia risks, it is challenging to design placebo controls for stereotactic injection of stem cells in phase II trials. The surgical procedure raises ethical concerns and may be unacceptable to patients [35], an issue that does not exist for intravenous infusion.

Conclusion

Our results regarding safety, the primary endpoint of our study, suggest that autologous transplantation of EPCs improved long-term safety in patients with acute cerebral infarct, supporting the feasibility of this novel method for treatment of ischemic stroke. However, the study had limitations, such as a lack of patient-centered quality of life outcomes. Moreover, because of the small size of the cohorts involved, this trial was also limited in that we could neither identify the neurological or functional benefits of EPCs on ischemic stroke nor determine the pros and cons between EPCs and BMSCs for treatment of stroke, and thus, a larger phase II trial is warranted.

Data Availability

Please contact author for data requests.

Author Contributions

Z.C. and X.J. conceived and designed the trial. Z.C., Y.G., S.T., Z.L., and H.X. contributed to patient enrollment and follow-up. Y.G., J.F., C.H., and P.H. contributed to the functional assessments. P.C., K.W., J.F., and Y.G. contributed to data analysis. J.F. contributed to the immunofluorescence and flow cytometry detection. J.F. and Z.C. wrote the manuscript with significant input from X.J. and Y.K. All authors have read and approved the final manuscript.

Disclosure of Potential Conflicts of Interest

X.J. received research funding from the government of People's Republic of China. The other authors indicated no potential conflicts of interest.

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