Int J Clin Exp Med 2017;10(2):3059-3068
www.ijcem.com /ISSN:1940-5901/IJCEM0041850

Original Article

Analysis of the long-term effect of bone marrow mononuclear cell transplantation for the treatment of cerebral infarction

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Received July 7, 2016; Accepted November 18, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: To explore the long-term clinical effects and safety of bone marrow mononuclear cell transplantation for the treatment of cerebral infarction. Patients with cerebral infarction, hospitalized in the Second Department of Neurology at the China-Japan Friendship Hospital of Jilin University from March to November 2008, who had been diagnosed between 3 weeks and 3 months prior, and whose NIHSS score was between 5 and 30, were included in the study. Bone marrow mononuclear cells were collected the treatment group using the density gradient method. A cell suspension containing 1×10^7 bone marrow mononuclear cells was then injected into the subarachnoid space, and the outcome monitored. Neurological function, amongst other measures, were tested before transplantation, in the 3rd, 6th, 12th months of the first postoperative year, as well as in the 2nd, 3rd, 4th, 5th, 6th and 7th years after transplantation. Some mononuclear cells were amplified ex vivo; flow cytometry was used to test the markers CD90 and CD105, and immunofluorescence was used to test Nestin. Fever was observed in members of the treatment group in the initial phase after transplantation; however, this abated with time. NIH Stroke Scale scores in the 3 years after transplantation for the control and treatment groups were not significantly different ($P > 0.05$). In the 4th year after transplantation, the NIH Stroke Scale score of the treatment group was significantly lower than that of the control group ($P < 0.05$). Differences between the Barthel index, modified Rankin, and modified Functional Independence Measure scores were not significant ($P > 0.05$) in the 4th year after transplantation; however, they were significant in the 5th year ($P < 0.05$). Twelve months after transplantation, the difference between Fugl-Meyer scores was not significant ($P > 0.05$); however, the difference was significant ($P < 0.05$) in the 2nd year after transplantation. Of the mononuclear cells tested, 93.77% expressed CD90 and 60.63% expressed CD105. After ex vivo expansion, most of the bone marrow mononuclear cells were positive for Nestin. Autologous bone marrow mononuclear cell transplantation for the treatment of cerebral infarction is feasible and safe, without long-term adverse effects. It can improve neural function injury after cerebral ischemia.

Keywords: Bone marrow, mononuclear cell, transplantation, cerebral infarction

Introduction

Cerebral stroke is the third leading cause of death in the Chinese population, with ischemic stroke accounting for about 70% of these cases. Although ~80% of stroke patients survive, only 10% of survivors recover normal neural function. The remaining 70% have nervous system sequelae, including paralysis, aphasia and blindness-all of which are seriously detrimental to the health and quality of life of the patients, and are a heavy financial burden on families and society. The effectiveness of current treatments for the acute phase of stroke is limited, with the exception of thrombolytic therapy. Despite its proven benefits, the narrow therapeutic time window and the risk of bleeding mean that only 3% of patients with cerebral stroke are able to receive treatment [1]. In the subacute and chronic phases of stroke, rehabilitation training is considered to be the only effective method of treatment. As a result, cerebral ischemic stroke has become a pressing problem that urgently requires a solution.

Cerebral ischemic stroke, also called cerebral infarction, is caused by the blockage of a cerebral artery, leading to focal ischemia, loss of neurons and glial cells, and a resultant impairment in neural function [2]. It was previously

thought that once mature, nerve cells cannot reproduce and renew; however, with the rapid development of neuroscience in the latter part of the 20th century came the discovery of a population of cells with the ability to self-renew and differentiate [3]. These stem cells can produce new neurons and glial cells within a defined microenvironment and appropriate conditions, move to the functional area, continuously produce changes in plasticity, and connect with other neurons to produce neural function [4]. Based on this discovery, exogenous cells with the ability to self-review, orientate and differentiate, as well as the ability to recover the injury, are being explored as a potential strategy in the treatment of cerebral ischemic stroke [5].

The attractiveness of stem cell-based therapies lying in the potential ability of these cells was replaced dead or injured cells within a damaged neural network. To date, research in this area has mainly focused on neural, embryonic, and mesenchymal stem cells. Bone marrow mononuclear cells (BM-MNCs) are the primary cells able to be separated and extracted from the bone marrow of the thigh or shin-be it human or animal. A BM-MNC belongs to the "stem cell" group and is the general name given to cells with a single nucleus in bone marrow; this group includes bone marrow hematopoietic stem cells, mesenchymal stem cells, mononuclear cells, lymphocytes, endothelial progenitor cells and stromal cells. Mesenchymal stem cells account for about 0.001-0.01% [6], and hematopoietic stem cells about 0.0001%, of the total population of BM-MNCs [7]. These cells are easily extracted (in ~1.5-3 h) without being cultured, and are able to be self-transplanted without ethical issues [8]. These advantages make BM-MNCs ideal for research into stem cell transplantation strategies, as well as the best seed cell for the treatment of cerebral ischemic stroke.

In animal experiments, Goel [9] transplanted BM-MNCs directly into the broken ends of the sciatic nerve and found medullation and axonal regeneration to improve, and degeneration to be prevented. Hu et al [10] discovered that transplanted BM-MNCs could rebuild the left lobe and improve its function in cases of old and chronic myocardial infarction. Among the published research on cerebral ischemic stroke, evidence to date indicates that BM-MNC transplantation can reduce the volume of cerebral infarction and improve neural function [11]. In vitro techniques to separate, purify, culture, passage, build systems, and induce orientation and differentiation of stem cells provide the basis for the in vivo treatment of cerebral infarction.

Although there has been much research into the use of BM-MNCs in the treatment of cerebral infarction, long-term clinical tests and follow-up studies are lacking. The current research attempts to stimulate the reparative capacity of BM-MNCs, replace necrotic cells, improve nerve function damage, and improve patient symptoms and prognosis.

Materials and methods

General information

As approved by the China-Japan Friendship Hospital Ethics Committee of Jilin University, patients with cerebral infarction, who were hospitalized in the Second Department of Neurology within the hospital from March to November 2008, were included in the present study. All patients were required to meet the diagnostic criteria laid down by the Fourth National Conference of the Chinese Medical Association on cerebrovascular disease. All patients included in the study were diagnosed with cerebral infarction by MRI were; those who suffered malignancy, serious cardiopulmonary insufficiency, or liver or kidney dysfunction were excluded from the study, as were patients who refuse to be treated. Having received medical treatment, patients with acute cerebral infarction were in a stable condition; however, they were left with sequelae to varying degrees. The patients' conditions were classified as light (NIH Stroke Scale (NIHSS) score < 4 points), medium (NIHSS score ≥ 4 to < 31 points), or severe (NIHSS score ≥ 31 points). In total, 20 patients with medium cerebral infarction met the inclusion criteria, and were subsequently randomly divided into treatment group and control group, with 10 cases of each group (See **Table 1**).

Treatment scheme

The control group received routine medical treatment, including anti-platelet aggregation (aspirin 10 mg/d or clopidogrel hydrogen sulfate 75 mg/d), statins (rosuvastatin 10 mg/d). The treatment group received routine medical treatment and BM-MNC transplantation. Pa-

Table 1. Comparison of general characteristics

Item	Control group	Treatment group	P
Age (years)	53.10 ± 13.068	50.80 ± 17.428	0.743
NIHSS score	10.70 ± 3.713	12.30 ± 3.945	0.363
Time of entering group	59.90 ± 20.851	57.10 ± 32.586	0.822
Sex			
Male	6	9	
Female	4	1	
Lesion site			
Anterior circulation	7	6	
Posterior circulation	3	4	

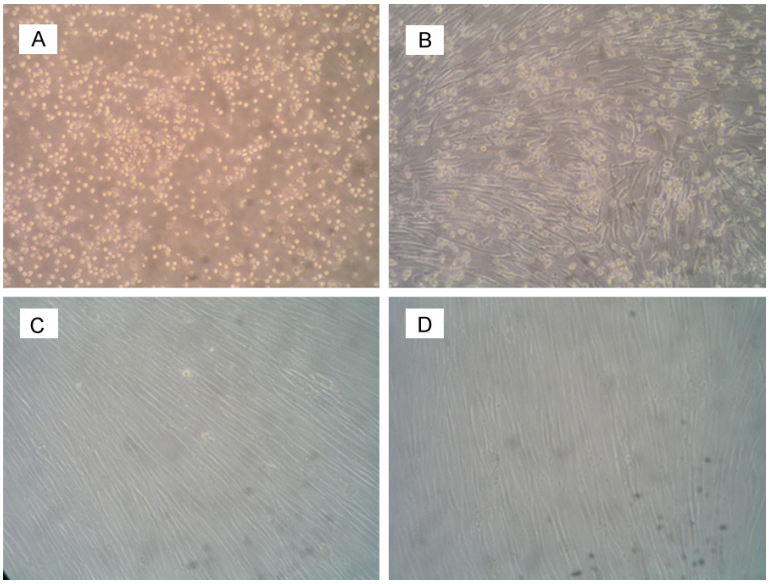


Figure 1. Growth of BM-MSCS under light microscope (×10). The 3rd (A) and 11th (B) days of the primary culture of bone marrow mononuclear cells. (C) The 7th day of the first generation of bone marrow mononuclear cells. (D) The 5th day of the second passage of bone marrow mononuclear cells.

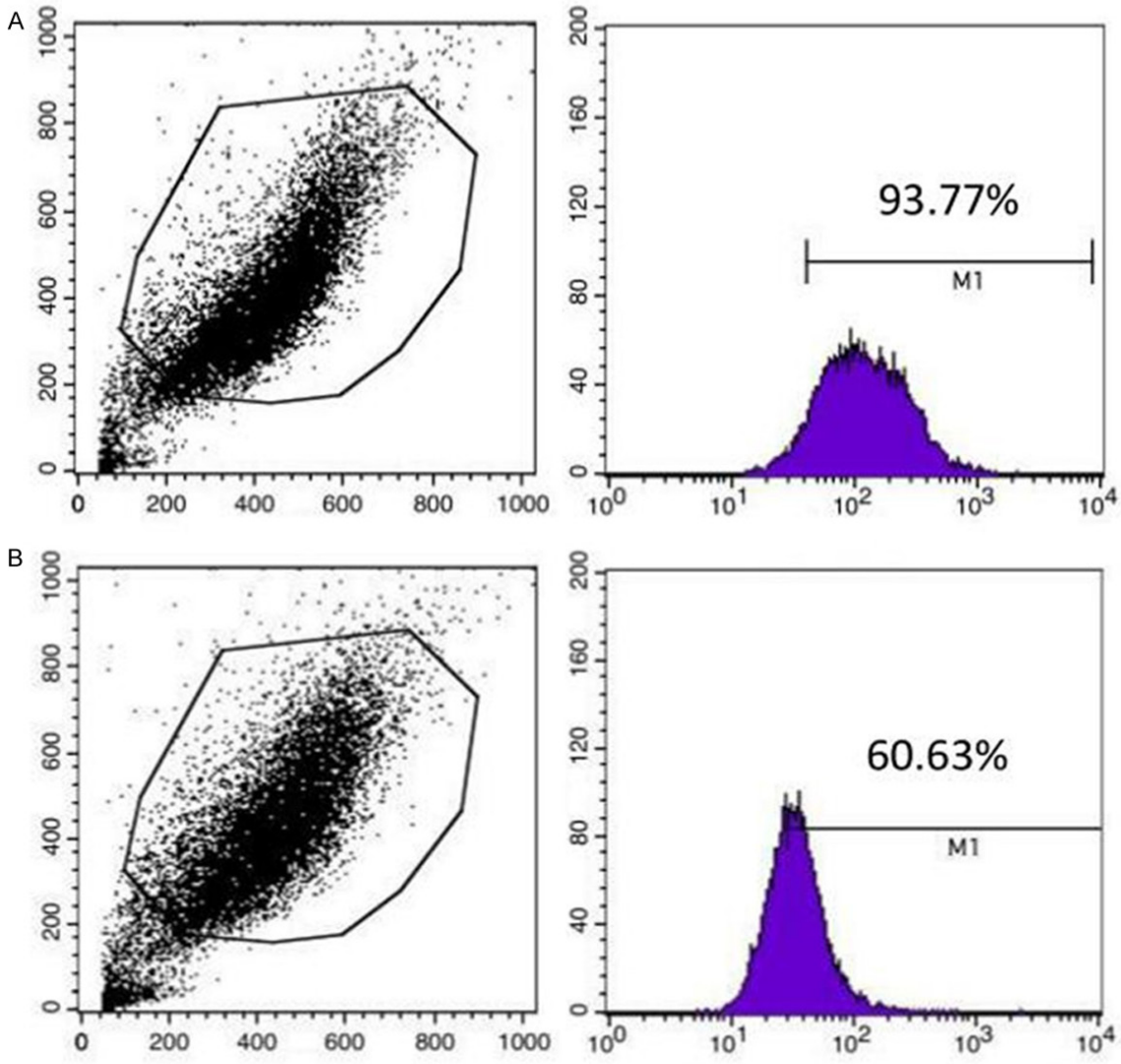
tients or family members signed informed consent, having finished the preoperative routine examination (blood routine, coagulation routine, routine urine test, blood sugar, blood fat, blood ions, liver function, kidney function, immune routine, electrocardiogram and chest X-ray). Under general anesthesia, patients underwent a bone biopsy operation, and about 160-200 mL of bone marrow was extracted from the bilateral anterior superior iliac spine.

Experimental scheme

Separation and culture of BM-MNCs: A cell treatment kit, referring to bone marrow, peripheral blood, and cord blood with nuclear cell separation medium, produced by China Aviation (Ningxia) biological Co. Ltd, was used to separate BM-MNCs using the density-gradient method. Flow cytometry was used count the number of total cells within the resulting cell suspension.

BM-MNC transplantation

Two milliliters (~10^7 cells) of the BM-MNC suspension was collected and injected into the patient's subarachnoid space by lumbar puncture. The cell suspension (0.5 mL; 1.0×10^7 cells/mL) was placed into a 37°C incubator with a humidified atmosphere of 5% CO_2. After 24 h, the cell culture medium was replaced with fresh medium, and this process was repeated every 3 days until the confluence reached 80-90%. When changing the culture media, 5 mL of trypsin was added to the culture flask. The flask was returned to the 37°C incubator for 5 min, before being removed, shaken, and the cells were subsequently observed under a microscope. When the cells were observed to have rounded, trypsin was added to the mesencult culture medium to slow the thawing process. The solution was transferred to a centrifuge tube, before saline was added and the tube shaken repeatedly to quench the reaction. After the solution had settled, indicated by the bubbles having disappeared, the supernatant was removed and the solution was again shaken (1500 rpm/5 min). Fresh mesencult medium (5 mL) was added, bringing the cell number to 5×10^5 mL. The cells were then transferred into a cell culture flask, and allowed to culture for 7-9 days. During this time, cells were passaged when they neared confluence at a ratio of 1:2. The culture medium was replaced once it had yellowed. Once the cells reached basic fusion,



Bone marrow mononuclear cell marker detection

Surface markers	CD90	CD105
Score (x ± s)	88.58 ± 4.33	53.37 ± 4.82

Figure 2. Markers CD90 (A) and CD105 (B) on the cell surface of bone marrow mononuclear cells.

5 mL of 0.25% trypsin was added to suspend the cells.

BM-MSC surface marker identification

BM-MNCs at the 2nd passage were removed from the plate via scraping, and distributed evenly between into two Eppendorf® tubes. The cells were centrifuged, the medium removed and 100 μL of 1% BSA (dissolved in 1% sodium azide) added, before 0.5 μL of CD90 antibody was added to one tube and CD105 fluorescent antibody to the other, and the tubes kept on ice for 30 min. Cells were then centrifuged, washed once with PBS, and 600 μL of 10 g/L paraformaldehyde was added to each tube. Following fixation, cells were transferred into a flow cytometry tube for immediate testing.

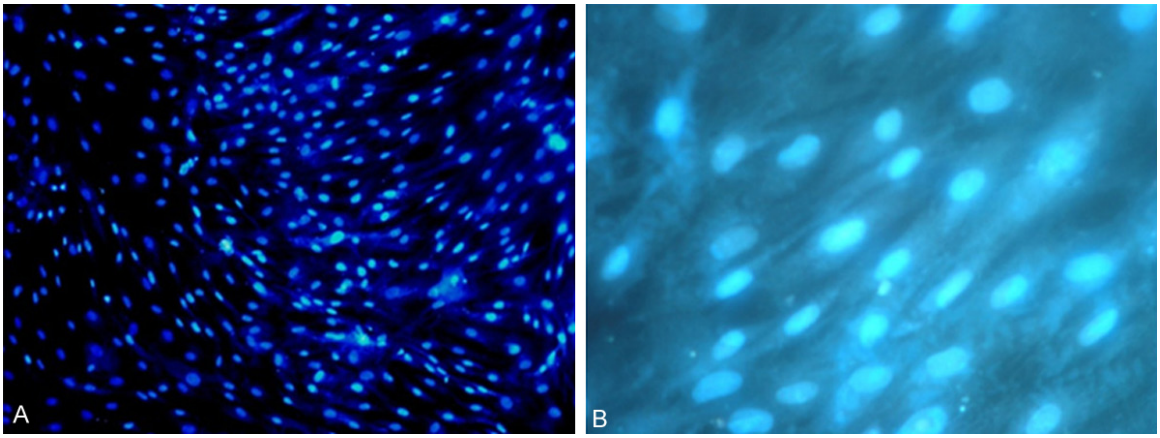


Figure 3. Nestin expression in bone marrow mononuclear cells (A: ×10; B: ×40).

Table 2. Comparison of NIHSS scores after treatment

Item	Control group	Treatment group	P
3rd month	10.30 ± 3.234	9.8 ± 4.131	0.767
6th month	8.20 ± 3.490	9.40 ± 3.806	0.472
12th month	6.50 ± 3.342	8.80 ± 3.706	0.162
2nd year	5.70 ± 3.199	8.60 ± 3.688	0.077
3rd year	5.50 ± 3.100	8.60 ± 3.688	0.057
4th year	5.30 ± 3.020	8.60 ± 3.688	0.043
5th year	4.38 ± 1.598	7.13 ± 1.959	0.009
6th year	4.33 ± 1.506	6.33 ± 1.211	0.031
7th year	4.33 ± 1.506	6.33 ± 1.211	0.031

Detection of induced Nestin expression in BM-MSCs

Cells at the 2nd passage were seeded into culture dishes 40 mm in diameter, and differentiated once the confluence reached 80-90%. At this time, the medium was discarded, the cell surface washed with PBS, and the preinduction medium added (DMEM/10% volume fraction of FBS/10 μg/LbFG). Twenty-four hours later, induction medium (DMEM/2% DMSO/6 mmol/L BME) was added and the cells allowed to incubate for 4 h. After induction, cells were washed with PBS, fixed (up to 30 min with 40 g/L paraformaldehyde), and washed again with PBS. The primary antibodies Nestin (mAb; 1:100), NF-M (mA; working fluid), and MAP-2 (mAb; working fluid) were then added separately, and the cultures left overnight at 4°C. Secondary antibodies (goat anti-rabbit and goat anti-mouse IgG) were added separately and left to incubate at 37°C for 30 min. The color reaction was performed using a DBA kit.

Recording adverse reactions

During the 3 days after transplantation, body temperature, respiration, pulse, and blood pressure were closely monitored. Symptoms of neurological function deficit, and the presence of headache, nausea and vomiting, as well as pain from puncture site were noted and assessed as adverse reactions.

Therapeutic evaluation

Neurological function, performance in activities of daily living (as measured by the Barthel index), degree of disability or dependence during daily activities (as measured by the modified Rankin Scale), functional independence (as measured by the modified Functional Independence Measure; FIM), and motor function (as measured by the Fugl-Meyer motor scale) were measured before transplantation, as well as in the 3rd, 6th and 12th months of the first postoperative year, and every year thereafter, from the 2nd to the 7th postoperative year.

Statistical analyses

Statistical analyses were performed using the SPSS software (version 18.0). Differences between experimental groups were analyzed using Student's t-tests. *P* values of less than or equal to 0.05 were considered statistically significant.

Results

General information: treatment and control groups

The general information of treatment and control groups was shown in the **Table 1**.

Table 3. Comparison of Barthel index scores before and after treatment ($\bar{x} \pm s$, score)

Item	Control group	Treatment group	P
Before transplantation	15.00 ± 8.498	14.50 ± 13.006	0.92
3rd month	26.00 ± 11.738	22.50 ± 12.304	0.523
6th month	29 ± 12.867	26 ± 16.799	0.66
12th month	41.5 ± 17.646	37.5 ± 15.855	0.60
2nd year	47 ± 24.060	51.5 ± 26.146	0.694
3rd year	53 ± 20.028	56.5 ± 23.339	0.723
4th year	55 ± 18.409	60 ± 24.267	0.61
5th year	60 ± 18.708	78.33 ± 12.990	0.030
6th year	63.33 ± 18.886	87.5 ± 7.583	0.024
7th year	63.33 ± 18.886	87.5 ± 7.583	0.024

Table 4. Comparison of Modified Rankin Scale scores before and after treatment ($\bar{x} \pm s$, score)

Item	Control group	Treatment group	P
Before transplantation	4.10 ± 0.994	4.60 ± 0.699	0.212
3rd month	4.00 ± 0.816	4.50 ± 0.707	0.161
6th month	3.90 ± 1.101	4.00 ± 0.816	0.82
12th month	3.40 ± 0.966	3.60 ± 0.699	0.603
2nd year	3.10 ± 1.101	3.00 ± 1.333	0.857
3rd year	2.60 ± 1.265	2.80 ± 1.317	0.733
4th year	2.50 ± 1.269	2.30 ± 1.160	0.717
5th year	2.44 ± 1.236	1.33 ± 0.50	0.030
6th year	2.33 ± 1.033	1.17 ± 0.408	0.039
7th year	2.33 ± 0.816	1.17 ± 0.408	0.016

BM-MSC isolation and culture

After in vitro culture in cell medium for 2 to 4 h, the cells adhered to the plate were observed to be mononuclear, with a rounded shape and relatively little cytoplasm. Twenty-four hours later, almost all cells had adhered and were polygonal fusiform-shaped. After 3 days, the cells began to expand and increase in size, with some cell bodies forming irregular shapes and others forming clusters and proliferating. The passaged cells gradually formed fusiform cells of the same size, with an enlarged karyoplasm. With time, the cell density was observed to increase, and the cells oriented along the long axis, growing in a whirlpool-like shape (**Figure 1**).

Surface antigen expression of BM-MSCs

Flow cytometry results (**Figure 2**) showed that the positive rate of BM-MNCs expressing CD90 was 88.58% ± 4.33%, with the highest rate observed at 93.77%. The positive rate of BM-MNCs expressing CD105 was 53.37% ± 4.82%, with the highest rate recorded at 60.63%.

Nestin expression in BM-MSCs

One day after being induced, partial cell expression of Nestin was observed in scattered BM-MNC clusters (**Figure 3**). Nestin-positive granules were observed in the cytoplasm. These cells were ovoid in shape, as was the nucleusin. The nucleus in retained less stain than the cytoplasm, which was strongly positive for the stain. The nucleocytoplasmic ratio was high.

Reports of adverse reactions

Two patients were reported to be feverish on the second day after the operation, with temperature of 37.8-38.3°C. Their temperatures returned to normal on the third postoperative day, after using physical methods to lower body temperature. There were no obvious changes in blood pressure, respiration and pulse. Symptoms of postoperative neurological functional impairment were not detected, nor were any cases of headache, nausea or vomiting. All patients reported pain at the puncture site, which did not influence daily life. One patient within the treatment group died of intrapulmonary infection 5 years after transplantation. One patient within the control group, who had a large area of infarction, died 3 years after the commencement of the study.

Therapeutic evaluation

The NIHSS scores for both groups were not significantly different in the 3rd, 6th and 12th month of the first year, or in the 2nd or 3rd year after transplantation ($P > 0.05$). The NIHSS scores of the treatment group were significantly lower than those of the control group in the 4th, 5th, 6th and 7th year after transplantation ($P < 0.05$). See **Table 2**.

Barthel index scores before and after treatment

The Barthel index scores were not significantly different between the groups in the 3rd, 6th and

Table 5. Comparison of functional independence measure scores before and after treatment ($\bar{x}$ ± s, score)

Item	Control group	Treatment group	P
Before transplantation	40 ± 7.703	39.50 ± 12.488	0.915
3rd month	38.80 ± 12.488	43.50 ± 12.030	0.403
6th month	42.10 ± 13.634	48.20 ± 14.741	0.349
12th month	51.50 ± 9.443	59 ± 13.784	0.173
2nd year	61.50 ± 24.537	68.56 ± 24.153	0.528
3rd year	61.20 ± 9.647	71.90 ± 22.595	0.193
4th year	61.30 ± 14.885	73.90 ± 22.028	0.154
5th year	61.56 ± 11.918	78.44 ± 20.107	0.046
6th year	61.83 ± 13.934	78.67 ± 11.690	0.047
7th year	61.83 ± 13.934	78.67 ± 11.690	0.047

Table 6. Comparison of simplified Fugl-Meyer motor scale scores before and after treatment ($\bar{x}$ ± s, score)

Item	Control group	Treatment group	P
Before transplantation	14.40 ± 5.948	12.60 ± 6.484	0.526
3rd month	22.30 ± 8.070	23.40 ± 14.065	0.833
6th month	29.20 ± 12.136	39.20 ± 23.261	0.249
12th month	42.60 ± 16.801	57.20 ± 29.996	0.2
2nd year	44.50 ± 26.828	67.20 ± 26.865	0.039
3rd year	49.20 ± 17.819	72.90 ± 21.916	0.017
4th year	54.10 ± 18.205	73.40 ± 22.227	0.048
5th year	57.11 ± 18.395	76.78 ± 20.675	0.049
6th year	57.83 ± 11.374	78.67 ± 19.169	0.045
7th year	57.83 ± 11.374	78.67 ± 19.169	0.045

12th month of the first year after transplantation, nor in the 2nd, 3rd or 4th year after transplantation (P > 0.05). In the 5th, 6th and 7th year after transplantation, the Barthel index scores of the treatment group were higher than those of control group (P < 0.05). See **Table 3**.

Modified Rankin Scale scores before and after treatment

The Modified Rankin Scale scores before treatment, in the 3rd, 6th and 12th month of the first year, as well as in the 2nd, 3rd and 4th year after transplantation were not significantly different between the groups (P > 0.05). In the 5th, 6th and 7th year after transplantation, the scores of the treatment group were significantly lower than those of control group (P < 0.05). See **Table 4**.

Functional independence measure scores before and after treatment

FIM scores before treatment, in the 3rd, 6th and 12th month of the first year, and in the 2nd, 3rd and 4th year after transplantation were not significantly different between the groups (P > 0.05). Modified FIM scores in the 5th, 6th and 7th year after transplantation were higher in the treatment group than in the control group (P < 0.05). See **Table 5**.

Simplified Fugl-Meyer motor scale scores before and after treatment

The simplified Fugl-Meyer motor scale scores before treatment and in the 3rd, 6th and 12th month after transplantation were not significantly different between the group (P > 0.05); however, in the 2nd, 3rd, 4th, 5th, 6th and 7th year after transplantation, the scores of the treatment group were higher than those of the control group (P < 0.05). See **Table 6**.

Discussion

Cerebral ischemic stroke is a neurological disorder caused by tissue necrosis and cell death in an area of infarction that results from limited blood supply. In clinical settings, the NIHSS is often used to evaluate the severity of the disease. The test includes 15 items designed to assess of nerve function and symptoms of neurological disorders that may result from cerebral artery lesions. A 'light stroke' NIHSS score (≤3 points) refers to minor symptoms and good prognosis; whereas, a 'severe cerebral stroke' NIHSS score (> 31 points) indicates a large infarct or one involving multiple brain lobes, hemorrhage of the digestive tract associated with stress ulcer, pneumonia, urinary tract infection, lower extremity deep vein thrombosis, and/or other complications [12]. Light stroke patients display no obvious sequelae after the acute period of conventional drug treatment, while severe cerebral stroke patients suffer more complications and cannot tolerate general anesthesia. For these reasons, the present study focused on patients with 'moderate stroke', as classified by an NIHSS score of 5-30 points.

Ischemic brain injury can lead to the death of brain cells and chronic injury of the nervous system. Treatment in the acute phase after stroke focuses on protecting and rescuing injured neurons from death; whereas, treat-

ment in the chronic phase focuses on replacing nerve cells lost as a result of injury and restoring the associated loss of neurological function. Stem cell transplantation has potential in the treatment of brain injury after stroke, as endogenous multi-potential stem cells can be differentiated into various neurons and glial cells in response to various stimulating factors.

Stem cells include neural, embryonic and mesenchymal stem cells. BM-MNCs are a heterogeneous cell population consisting of not only hematopoietic and mesenchymal stem cells, but also neural stem cells capable of differentiation. BM-MNCs are easy to obtain - they are easier to isolate and prepare than pure hematopoietic or mesenchymal stem cells. Kucia et al [13]. It is reported the existence of a kind of neural tissue-committed stem cell (NTCSC), which can be directed to the injured neurological tissue and subsequently repair the wound and promote tissue regeneration. It does so by lessening the injury and apoptosis of tissue. They propose that 0.02% of BM-MNCs are involved in repairing the wound after entering the injured area.

Bone marrow mesenchymal cells are capable of self-renewal, are highly proliferative and are able to differentiate into multiple cell types. Neural stem cells can differentiate into neurons, astrocytes and oligodendrocytes, and neural precursor cells have a great capacity for self-renewal. Neural stem cells act as a reserve - their primary function thought to be in the protection of injured neurons and glial cells or to provide a supply when cells die [14]. Both bone marrow mesenchymal stem cells in BM-MNCs and neural stem cells capable of differentiation are reported to be involved in neural repair. BM-MNCs can differentiate into neural cells; specifically, in vitro culture of BM-MNCs, conducted by Lilin [15], confirmed the existence of neural stem cells (labeled with Nestin), which were able to differentiate into nerve cells and be mobilized to the injured area under pathological conditions, such as cerebral infarction. An experiment conducted by Wislet [16] showed that mesenchymal stem cells from the adult rat, cultured in vivo, could express Nestin; furthermore, they demonstrated that only Nestin-positive stromal cells could form a neurosphere and differentiate into glial fibrillary acidic protein (GFAP)-positive cells when co-cultured with neural stem cells. Thus, in adult rat stromal cells, the expression of Nestin is a precursor to differentiation into neurons. It is therefore possible that high expression of Nestin may indicate bone marrow mesenchymal stem cells are likely to differentiation into neuron-like cells after transplantation.

Studies in recent years [17] have shown that transplanted bone marrow mesenchymal stem cells can survive in vivo after brain injury and move towards ischemic foci, trigger endogenous precursor cells to differentiate into neurons and glial cells, and promote the repair of neurological function. In this study, following stem cell transplantation, Nestin-positive cells shifted towards the infarct during the second week after perfusion. Based on the above research, most BM-MNCs appear to be positive for Nestin after in vitro culture and passaging. Most blast cells expressing neural stem cells exist in BM-MNCs, which is consistent with the findings reported by Kucia [13].

Nestin is the main intermediate filament protein of embryonic central nervous system progenitor cells. Due to the expression of Nestin in nerve and glial cells, as well as their precursor cells, it is unsurprising that Nestin is expressed in the developing and injured brain, as well as the spinal cord. Nestin can therefore be used to identify the early characteristics of neural stem cells [18]; Nestin-positive cells are considered to be neural precursor cells, which are required for self-renewal of neural stem cells [19].

Mesenchymal stem cells have different immunophenotypes. On the one hand, mesenchymal stem cells are known to be negative for the hematopoietic stem cell markers CD45, CD34, and the endothelial marker CD31; on the other hand, they are known to be positive for the markers CD105, CD73, CD44, CD90, CD54, CD29, CD47, CD146, CD49a, CD164 [20]. As no specific markers have yet been identified, positive expression of CD14, CD34 and CD45 is currently considered to be the difference between hematopoietic and mesenchymal stem cells. To solve this problem, the International Society for Cellular Therapy has published identification standards; specifically, they state that to be classified as mesenchymal stem cells, they must be positive for at least CD105, CD73, CD90 and negative for CD34, CD45, CD14, CDIIb, CD19 and HLA-DR [21]. Although CD73 is acknowledged as a mesenchymal stem cell surface marker, it has been

recently shown that in cases where a tumor is present, CD73 may promote tumor growth by either inhibiting the protective immune response of tumor cells or interfering with chemotherapeutic drug-induced apoptosis of the tumor [22]. As a result, this experiment only identified the surface antigens CD90 and CD105, but not CD73, to be mesenchymal stem cell markers.

Our laboratory has performed basic research, using animal models, to accurately identify the appropriate transplantation time for BM-MNCs. We have found that on the 1st, 7th, 14th and 30th day [23-25] after cerebral infarction, neurological function is improved after transplantation. As discussed, cerebral infarction has three phases: acute, subacute and chronic. Although transplanted cells have been reported to survive all phases, the survival rate in the acute phase is lower than in the subacute and chronic phases [26]. The selection of transplantation time is closely related to the pathological changes of cerebral infarction. Most scholars think we should consider not only the influence of inflammatory factors, oxygen free radicals and toxic neurotransmitters on transplanted cells, but also the impact of chronic phase scars on growth and integration of the transplanted cells. During the treatment of chronic cerebral stroke using BM-MNCs, after 1 month, rodent models showed significant recovery, and animals who had suffered cerebral infarction benefited from multiple methods of transplantations [27]. When stroke was induced, mononuclear cells were transplanted between the 3rd week and 3rd month. The study demonstrated that during different stages of treatment, transplanted cells play different roles.

The NIHSS score is a good indicator of recovery speed and extent of neurological dysfunction at different stages post-stroke. In the present study, there were no significant differences between the control and treatment groups in the first 3 years after transplantation; however, in the 4th year NIHSS scores were significantly different. No significant differences in Barthel index, modified Rankin or modified FIM scores were observed after transplantation in the first 4 years, but significant changes were observed in the 3 years to follow. The Fugl-Meyer score did not differ significantly between the groups before treatment, in the 3rd, 6th and 12th months of the first year, and in the 2nd year after transplantation. The patients improved a lot on specific sports. Although improvements in neurological function were not obvious in the early stages after transplantation in the treatment group, when compared to the control group, the long-term effects were clear; that is to say, transplanted mononuclear cells improved prognosis 2, 3 and 4 years later. The specific mechanism of this delayed efficacy is unknown; however, it is supposed that after the BM-MNCs have produced new neurons and glial cells, they require time to contact with other neurons, also need to testify in further vitro tests.

Though BM-MNC transplantation for the treatment of cerebral infarction has wide clinical prospects, there are still many problems to be solved, such as identifying the adequate number of cells for successful transplantation, in vivo cell tracking, survival time of cells in vivo, and recurrence of disease. More basic research and more clinical cases are needed to solve these problems and allow cell transplantation to become a viable method for clinical use.

Disclosure of conflict of interest

None.

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References

[1] Glover LE, Tajiri N, Weinbren NL, Ishikawa H, Shinozuka K, Kaneko Y, Watterson DM, Borlongan CV. A Step-up approach for cell therapy in stroke: Translational hurdles of bone marrow-derived cells. Transl Stroke Res 2012; 3: 90-8.

[2] Lindvall O and Kokaia Z. Stem cells for the treatment of neurological disorders. Nature 2006; 441: 1094-1096.

[3] Reynolds BA and Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992; 255: 1707-1710.

[4] Ming GL and Song H. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 2005; 28: 223-250.

[5] Lie DC, Song H and Colamarino SA. Neurogenesis in the adult brain: New strategies for central nervous system diseases. Annu Rev Pharmacol Toxicol 2004; 44: 399-421.

[6] Pittenger MF. Multiline age potential of adult human mesenchymal stem cells. Science 1999; 284: 143-147.
[7] Iihoshi S, Honmou O, Houkin K, Hashi K and Kocsis JD. A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. Brain Res 2004; 1007: 1-9.
[8] Battistella V, de Freitas GR and da Fonseca LM. Safety of autologous bone marrow mononuclear cell transplantation in patients with nonacute ischemic stroke. Regen Med 2011; 6: 45-52.
[9] Goel RK, Suri V and Suri A. Effect of bone marrow-derived mononuclear cells on nerve regeneration in the transection model of the rat sciatic nerve. J Clin Neurosci 2009; 16: 1211-1217.
[10] Hu S, Liu S and Zheng Z. Isolated coronary artery bypass graft combined with bone marrow mononuclear cells delivered through a graft vessel for patients with previous myocardial infarction and chronic heart failure: a single-center, randomized, double-blind, placebo-controlled clinical trial. J Am Coll Cardiol 2011; 57: 2409-2415.
[11] Savitz SI, Misra V and Kasam M. Intravenous autologous bone marrow mononuclear cell for ischemic stroke. Ann Neurol 2011; 70: 59-69.
[12] Xie SF and Yang SM. Patients with severe cerebrovascular disease hospital-acquired cases of pneumonia and related factors. Chinese Med Innov 2011; 8: 180-181.
[13] Kucia M, Zhang YP and Recal R. Cells enriched in markers of neural tissue-committed stem cells reside in the bone marrow and are mobilized into the peripheral blood following stroke. Leukemia 2006; 20: 18-28.
[14] Hung CW, Liou YJ and Lu SW. Stem cell-based neuroprotective and neurorestorative strategies. Int J Mol Sci 2010; 11: 2039-2055.
[15] Li L. Rat bone marrow neural tissue committed stem cells isolated and cultured and transplanted into rats with brain ischemia preliminary exploration. Chongqing: Chongqing Medical University 2012; 5: 120-125.
[16] Wislet-Gendibien S, Leprince P and Moonen G. Regulation of neural markers and GFAP expression by cultivated bone marrow stromal cells. J Cell Sci 2003; 116: 3295.3302.
[17] Ding J and Gao XQ. Stem cell transplantation in neonatal rat cerebral ischemia - reperfusion injury in rats. J Anat 2009; 32: 774-777.
[18] Wiese C, Rolletschek A and Kania G. Nestin expression-a property of multi-lineage progenitor cells. Cell Mol Life Sci 2004; 61: 2510-2522.
[19] Park D, Xiang AP and Mao FF. Nestin is required for the proper self-renewal of neural stem cells. Stem Cells 2010; 28: 2162-2171.
[20] Boiret N, Rapatel C and Veyrat-Masson R. Characterization of non-expanded mesenchymal Progenitor cells from normal adult human bone marrow. Exp Hematol 2005; 33: 219-225.
[21] Dominici M, Blanc KL, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Dean RJ, Keating A, Prockop DJ and Horwitz EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315-317.
[22] Stagg J, Beavis PA and Divisekera U. CD73-deficient mice are resistant to carcinogenesis. Cancer Res 2012; 72: 2190-2196.
[23] Xu C, Michael C and Alex Z. Chemokine, vascular and therapeutic effects of combination Simvastatin and BMSC treatment of stroke. Neurobiol Dis 2009; 36: 35-41.
[24] Andreiade VS, Julianada CR and Bruno DP. Therapeutic window for treatment of cortical ischemia with bone marrow-derived cells in rats. Brain Res 2009; 9: 94-104.
[25] Ye X, Chang SQ and Asim M. Delayed transplantation of human marrow stromal cell-seeded scaffolds increases transcallosal neural fiber length, angiogenesis, and hippocampal neuronal survival and improves functional outcome after traumatic brain injury in rats. Brain Res 2009; 1263: 183-191.
[26] Chen JL, Li Y and Wang L. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 2001; 32: 1005-1011.
[27] Shen LH, Li Y and Chen J. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab 2006; 27: 6-13.