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FULL-LENGTH ARTICLE
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The effects of intravenous infusion of autologous mesenchymal stromal cells in patients with subacute middle cerebral artery infarct: a phase 2 randomized controlled trial on safety, tolerability and efficacy

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ABSTRACT

Background aims: Mesenchymal stromal cells (MSCs) are characterized by paracrine and immunomodulatory functions capable of changing the microenvironment of damaged brain tissue toward a more regenerative and less inflammatory milieu. The authors conducted a phase 2, single-center, assessor-blinded randomized controlled trial to investigate the safety and efficacy of intravenous autologous bone marrow-derived MSCs (BMMSCs) in patients with subacute middle cerebral artery (MCA) infarct.

Methods: Patients aged 30–75 years who had severe ischemic stroke (National Institutes of Health Stroke Scale [NIHSS] score of 10–35) involving the MCA territory were recruited within 2 months of stroke onset. Using permuted block randomization, patients were assigned to receive 2 million BMMSCs per kilogram of body weight (treatment group) or standard medical care (control group). The primary outcomes were the NIHSS, modified Rankin Scale (mRS), Barthel Index (BI) and total infarct volume on brain magnetic resonance imaging (MRI) at 12 months. All outcome assessments were performed by blinded assessors. Per protocol, analyses were performed for between-group comparisons.

Results: Seventeen patients were recruited. Nine were assigned to the treatment group, and eight were controls. All patients were severely disabled following their MCA infarct (median mRS = 4.0 [4.0–5.0], BI = 5.0 [5.0–25.0], NIHSS = 16.0 [11.5–21.0]). The baseline infarct volume on the MRI was larger in the treatment group (median, 71.7 [30.5–101.7] mL versus 26.7 [12.9–75.3] mL, $P = 0.10$). There were no between-group differences in median NIHSS score (7.0 versus 6.0, $P = 0.96$), mRS (2.0 versus 3.0, $P = 0.38$) or BI (95.0 versus 67.5, $P = 0.33$) at 12 months. At 12 months, there was significant improvement in absolute change in median infarct volume, but not in total infarct volume, from baseline in the treatment group ($P = 0.027$). No treatment-related adverse effects occurred in the BMMSC group.

Conclusions: Intravenous infusion of BMMSCs in patients with subacute MCA infarct was safe and well tolerated. Although there was no neurological recovery or functional outcome improvement at 12 months, there was improvement in absolute change in median infarct volume in the treatment group. Larger, well-designed studies are warranted to confirm this and the efficacy of BMMSCs in ischemic stroke.

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Introduction

Stroke is the second leading cause of death after ischemic heart disease, accounting for 6.5 million deaths annually around the world [1]. Although thrombolytic and endovascular therapies have led to

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significant improvement in stroke outcomes, only 10–15% of patients presenting with stroke receive reperfusion therapies, which are not always successful [2–4]. Large-vessel infarcts, particularly those involving the middle cerebral artery (MCA), can often lead to severe, permanent neurological deficits, with a high mortality rate and poor functional outcome [5,6]. There is a need for novel treatment strategies aimed at enhancing neuronal plasticity or neuronal regeneration to improve neurological recovery after stroke.

In recent years, mesenchymal stromal cells (MSCs) have gained increasing interest as a promising neuroregenerative therapy. Infused MSCs are capable of promoting cell migration, angiogenesis, immunomodulation, neuroprotection and neural circuit reconstruction [7]. MSCs increase stromal-derived factor 1α to promote the migration of neuroblasts to the ischemic tissue, while reducing axon-inhibitory proteoglycans, to enable axonal sprouting and regeneration [8]. These paracrine actions of MSCs have led to neurotrophic effects and improved neurological function in stroke animal models [9–11] and *in vitro* studies [12]. MSCs enhance angiogenesis in the ischemic tissues via mitochondrial transport, assist neurotrophic factor secretion from neuron cells and suppress neural cell apoptosis in the affected ischemic area [13–15]. Liu *et al.* [16] showed that the administration of human MSCs in an animal model of distal MCA occlusion decreased the infarct area and improved neurological function. In view of these promising results from both *in vitro* and *in vivo* studies, the authors conducted a phase 2 clinical study over 12 months to evaluate the safety and efficacy of intravenous infusion of autologous bone marrow-derived MSCs (BMMSCs) in stroke disability, functional recovery and infarct volume in patients with subacute MCA infarct.

Methods

Study design

This was a phase 2, single-center, assessor-blinded randomized controlled study conducted at Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia, from May 2012 to March 2017. Ethical approval was obtained from the institution's medical research and ethics committee (project code FF-115-2011). The trial was registered on ClinicalTrials.gov (identifier no. NCT01461720). Written informed consent was obtained from each patient or their next of kin (if they lacked capacity because of aphasia or other stroke-related reasons) before they entered the study. The study was performed in accordance with the Declaration of Helsinki.

Eligibility criteria

The study recruited patients with subacute MCA infarct aged 30–75 years who were admitted with stroke to the Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia Medical Centre. The criteria for study eligibility included recent stroke, with onset ranging from 1 week to 2 months, later revised to within 2 months. Other inclusion criteria were (i) did not receive or failed intravenous thrombolytic therapy, (ii) National Institutes of Health Stroke Scale (NIHSS) score between 10 and 35 and (iii) evidence of unilateral MCA infarct on brain magnetic resonance imaging (MRI) or computed tomography scan.

Patients were excluded if they (i) were medically unfit, with worsening consciousness; (ii) had a brain tumor or other space-occupying lesion on brain MRI; (iii) had transient ischemic attacks or lacunar infarct; (iv) had suffered from infections, malignancy and/or primary hematological disorders; (v) had renal impairment, with serum creatinine $\geq$200 μmol/L or creatinine clearance $<$30 mL/min; (vi) had liver impairment, with serum aspartate transaminase and alanine transaminase four times greater than the normal upper limit; (vii) had other comorbidities that would be deemed contraindications to BMMSC transplantation therapy or bone marrow aspiration (BMA); (viii) had other comorbidities that affected the ability to obtain adequate stem cells, such as chronic debilitating diseases, frailty or known osteoporosis; or (ix) had contraindications to brain MRI.

Randomization and allocation concealment

Eligible patients were randomized using the permuted block randomization method at a ratio of 1:1 to receive autologous BMMSC infusion with standard medical care or standard medical care only. Allocation concealment was done using opaque sealed envelopes that contained the letter “A” for the treatment group or “B” for the control group.

Sample size calculation

Sample size was calculated based on a previous study where the mean difference of the Barthel Index (BI) between the treatment and control groups was 22 [17]. With α = 0.05 and a power of 80%, and accounting for 20% loss to follow-up, the estimated sample size was 25 patients per group, with a total of 50 patients.

Study protocol amendments

Two amendments were made to the initial protocol to facilitate recruitment. First, the patient eligibility criteria were revised to include patients with stroke occurring within 2 months rather than stroke onset within 1 week to 2 months. Second, a non-randomized design was planned from March 2015 onward to facilitate patients' preference for a BMA. However, there were no new recruitments beyond this period, and the study was terminated because of lack of funding and poor recruitment rate.

Intervention

Patients in the treatment group received standard medical care and culture-expanded autologous BMMSCs intravenously at a cell dosage of 2 million cells per kilogram of body weight (2×10^6 cells/kg) at a median concentration of 0.7 million cells per milliliter (0.6–0.75). The patients in the control group were provided with standard medical care, which included treatment to prevent recurrence, optimal control of risk factors and post-stroke follow-up rehabilitative therapies.

BMMSC culture, storage and quality control

Stem cell procurement and processing were conducted in a certified Good Manufacturing Practice laboratory in compliance with the Malaysia Guidelines for Stem Cell Research and Therapy [18]. The isolation and culturing methods of BMMSCs have been established and previously described [19,20]. Briefly, a minimum of 40 mL of bone marrow aspirate was aspirated from patients' posterior superior iliac crest under local anaesthesia. BMMSCs were isolated based on density gradient centrifugation and adherence to a plastic surface. BMMSCs were cultured with proprietary BMMSC culture medium and maintained in a 5% carbon dioxide incubator at 37°C. After 3 days, the non-adherent cells were discarded. Culture medium was replaced every 3–4 days until the cells reached confluency. The adherent cells were harvested and subcultured to expand the cell population until the required number of cells was achieved. Upon achieving the required number of cells, the BMMSCs were harvested and cryopreserved in 90% human serum (CELLect, catalogue no. 2930149; MP Biomedicals, Santa Ana, CA, USA) and 10% dimethyl sulfoxide (CryoSure, catalogue no. WAK-DMSO-70; Wak-Chemie Medical GmbH, Steinbach, Germany).

For quality control purposes, patients' BMMSCs were randomly selected and characterized by immunophenotyping and checked for

their differentiation abilities according to the method described previously [21]. The methods and results of the immunophenotyping and differentiation assays are provided in supplementary figure A. The expanded BMMSCs were checked for viability and sterility from bacterial, fungal, endotoxin and mycoplasma contamination before being released for transplantation. Once the transplantation date was confirmed, the cryopreserved BMMSCs were transported to the medical center with a cryoShipper (MVE, catalogue no. SC 2/1V; Chart Industries, Ball Ground, Georgia, USA).

Final BMMSC preparation and infusion

On the day of the BMMSC infusion, patients underwent physical examination, and their vital signs were measured to ensure that they were medically stable for the procedure. Prior to receiving the BMMSC infusion, patients received an intravenous infusion of 250 mL of 0.9% normal saline over 1–2 h. While receiving the normal saline, the cryopreserved BMMSCs were thawed, washed and resuspended in 200 mL of 0.9% normal saline. Once the normal saline infusion was completed, the prepared BMMSCs were administered intravenously over 1 h at a dose of $2.05 \pm 0.20 \times 10^6$ BMMSCs per kg using an infusion pump at a rate of 3.33 mL/min or 200 mL/h. Once the BMMSC infusion was completed, another 50 mL of 0.9% normal saline was infused to ensure that all BMMSCs were fully delivered while also maintaining vein patency. Vital signs (blood pressure, temperature, heart rate and oxygen saturation levels) were monitored every 10 min throughout the procedure and 30 min following completion of the cell infusion.

Assessments, monitoring and follow-up

Clinical assessments

Stroke severity, functional impairment and stroke disability outcome were assessed using the NIHSS, BI and modified Rankin Scale (mRS), respectively. For patients who died during the trial, a default score of −5 for BI, 42 for NIHSS and 6 for mRS was assigned, as commonly practiced in stroke clinical trials [22,23]. Clinical assessments were conducted at baseline and at 6 weeks (or 2 weeks post-BMMSC infusion in the treatment group), 3 months, 6 months, 9 months and 12 months post-randomization.

Radiological assessment

MRI was performed at baseline and 12 months. MRI images were evaluated for the presence and size of ischemic lesions, including gliotic and adjacent encephalomalacic changes. Infarct size was measured on diffusion-weighted or T2 FLAIR MRI sequences using the semi-automated segmentation function of ITK-SNAP 3.6.0 software [24]. At 12 months, total infarct volume was defined as a combination of gliotic changes and adjacent encephalomalacia. The authors also calculated absolute total infarct volume changes, expressed in milliliters, and relative changes, expressed as a percentage, in relation to the baseline volume.

Primary outcome

The primary outcome assessments included between-group comparisons of the NIHSS, mRS and BI scores and brain infarct volume on MRI at 12 months. To minimize bias, the clinical assessments were performed by a trained physiotherapist who was not involved in patient care and was blinded to treatment allocation. Similarly, the MRI images were reviewed and reported by a neuroradiologist who was blinded to patients' treatment assignments.

Adverse events and safety outcomes

Adverse events were evaluated at all time points. For patients in the treatment group, any adverse events occurring during the infusion procedure were recorded. Biochemical parameters such as renal profile, liver function tests and full blood count were evaluated at 12 months.

Statistical analysis

All data are presented in mean (standard deviation), median (interquartile range [IQR]) and number (%) formats. Statistics used are Mann–Whitney U test, Student's *t*-test and chi-square test for median, mean and categorical comparisons, respectively. Because of the small sample size, the data were deemed to be not normally distributed. Group comparisons of outcomes were made using nonparametric tests (Mann–Whitney U tests) and chi-square tests. Friedman and Wilcoxon signed-rank tests were used for intragroup comparisons of outcomes across follow-ups. $P < 0.05$ was considered significant, and analyses were performed using SPSS Statistics 26 (IBM, Armonk, NY, USA).

Results

Patient recruitment

A total of 17 patients were recruited. Nine patients received the BMMSC treatment, whereas eight received standard medical care in the control group. In the treatment group, one patient was lost to follow-up as a result of being unreachable from 6 months onward, whereas another patient withdrew from the study because of an acute coronary event, which occurred after the BMA procedure but before receiving the BMMSC infusion (Figure 1). Two patients (one from each group) died before the 12-month assessment.

Baseline characteristics

The baseline characteristics of the treatment and control groups were comparable, with the exception of a higher number of male subjects in the treatment group (P= 0.008). The baseline mRS, BI and NIHSS scores were comparable between the groups (Table 1). The baseline infarct volume was larger in the treatment group compared

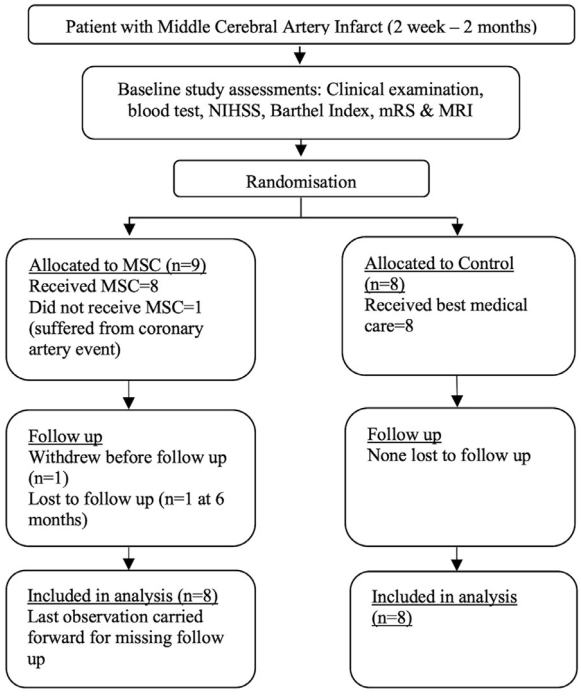

Figure 1. CONSORT diagram. A total of 17 patients were recruited into this study, nine of whom were in the treatment group and eight of whom were in the control group. One patient from the treatment group was lost to follow-up at 6 months, resulting in the data collected at the last observation being carried forward and used as data for the 12-month follow-up. One patient in the treatment group, who withdrew before receiving treatment and follow-up assessment, was not included in the final analysis.

Table 1
Clinical characteristics and other comorbidities in stroke patients during baseline assessment.

Parameter	All	Treatment group	Control group	p
Number of patients, n (%)	17 (100%)	9 (52.9%)	8 (47.1%)	
Age, years, mean (SD)	59.0 (14.0)	54.6 (13.2)	64.0 (13.9)	0.17
Male, n (%)	10 (58.8%)	8 (88.9%)	2 (25.0%)	0.008
Risk factors, n (%)				
Diabetes mellitus	8 (47.1%)	4 (44.4%)	4 (50.0%)	0.82
Hypertension	12 (70.6%)	5 (55.6%)	7 (87.5%)	0.15
Hyperlipidemia	12 (70.6%)	7 (77.8%)	5 (62.5%)	0.49
Ischemic heart disease	3 (17.6%)	2 (22.2%)	1 (12.5%)	0.60
Current smoker	4 (23.5%)	3 (42.9%)	1 (12.5%)	0.089
Family history of stroke	4 (23.5%)	2 (22.2%)	2 (25.0%)	0.89
Blood tests				
Creatinine, μmol/L, mean (SD)	72.8 (20.7)	71.5 (22.2)	70.9 (20.9)	0.73
White blood cells, x10^9L, mean (SD)	11.4 (4.4)	9.9 (4.6)	13.3 (4.3)	0.11
Haemoglobin, g/dL, mean (SD)	13.2 (2.3)	13.2 (2.5)	13.2 (2.4)	0.99
Platelets, x10^9/L, mean (SD)	255.3 (79.2)	263.7 (81.2)	245.9 (81.3)	0.66
HbA1c, %, mean (SD)	6.2 (0.8)	6.7 (1.1)	6.0 (0.1)	0.55
Albumin, g/L, mean (SD)	36.9 (3.7)	39.7 (3.0)	34.8 (1.0)	0.079
Vital signs				
Systolic blood pressure, mmHg, mean (SD)	133.4 (17.5)	130.9 (22.5)	136.3 (10.1)	0.55
Diastolic blood pressure, mmHg, mean (SD)	79.2 (17.5)	78.6 (22.7)	80.0 (10.4)	0.87
Heart rate, beats/min, mean (SD)	76.3 (18.3)	74.7 (16.7)	78.7 (21.9)	0.69
Stroke assessments				
Modified Rankin Scale, median [IQR]	4.0 [4.0, 5.0]	4.0 [4.0, 5.0]	4.5 [4.0, 5.0]	0.61
Barthel Index, median [IQR]	5.0 [5.0, 25.0]	10.0 [5.0, 27.5]	5.0 [0, 27.5]	0.14
NIHSS, median [IQR]	16.0 [11.5, 21.0]	16.0 [14.5, 21.0]	15.0 [10.0, 21.5]	0.48
Radiological features				
Infarct volume, mL, mean (SD)[a]	55.0 (38.7)	68.2 (41.0)	38.0 (30.1)	0.13
Infarct volume, mL, median [IQR][a]	43.9 [18.8, 81.3]	71.7 [30.5, 101.7]	26.7 [12.9, 75.3]	0.10
Left hemisphere, n (%)	12 (70.6%)	8 (88.9%)	4 (50%)	0.079
Haemorrhagic transformation, n (%)	3 (17.6%)	3 (33.3%)	0 (0%)	0.72
Treatment details				
Dose of BMMSC, million cells, median [IQR]	-	140 [123.75, 153.60]	-	-
Concentration of BMMSCs, million cells per mL, median [IQR]	-	0.7[0.6,0.75]	-	-
Onset to enrolment time, days, median [IQR]	8.0 [4.0, 15.0]	13.0 [8.0, 36.5]	4.5 [2.5, 9.0]	0.012
Onset to BMMSC infusion, days, median [IQR]	-	63.0 [61.0, 122.0]	-	-
Enrolment to BMMSCs infusion, days, median [IQR]	-	48.0 [38.0, 73.0]	-	-

BMMSC= bone-marrow derived mesenchymal stromal cells; HbA1c = Glycosylated Haemoglobin; IQR= interquartile range; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale. [a]Based on diffusion-weighted MRI

Table 2
Primary, radiological and safety outcomes at 12 months.

Parameter	Treatment group	Control group	p value
Death	1 (12.5%)	1 (12.5%)	1.00
Stroke assessments			
Modified Rankin Scale 3-6, n (%)	3 (37.5%)	6 (75.0%)	0.13
Modified Rankin Scale, median [IQR]	2.0 [1.5, 3.0]	3.0 [1.5, 4.0]	0.38
Modified Rankin Scale, % change, median [IQR][a]	50.0 [28.8, 68.8]	25.0 [20.0, 66.3]	0.31
Barthel Index, median [IQR]	95.0 [85.0, 95.0]	67.5 [0, 97.5]	0.33
Barthel Index, % change, median [IQR][a]	658 [119, 1563]	1200 [143, 1500]	0.77
NIHSS, median [IQR]	7.0 [2.5, 9.5]	6.0 [1.5, 21.0]	0.96
NIHSS, % change, median [IQR][a]	62.8 [24.4, 85.8]	45.3 [13.3, 94.6]	0.75
Radiological outcomes			
Total Infarct volume, mL, median [IQR][b]	56.91 [14.28, 68.40]	19.53 [12.62, 67.83]	0.62
Total infarct volume, mL, mean (SD)[b]	44.43 (28.37)	33.33 (33.89)	0.61
Absolute change in infarct volume, mL, median [IQR]	-11.88 [-21.12, -2.13]	4.45 [-1.65,10.59]	0.027
Absolute change in infarct volume, mL, mean (SD)	-16.23 (9.34)	4.46 (6.46)	0.040
Relative change in infarct volume, %, median [IQR]	-29.0% [-46.3%, -2.7%]	6.8% [-13.1%, 99.2%]	0.050
Gliosis volume, mL, median [IQR]	39.97 [11.45, 58.46]	18.27 [12.17, 47.02]	0.81
Encephalomalacia volume, mL, median [IQR]	5.65 [0.05, 16.15]	1.27 [0.19, 21.07]	0.90
Safety outcomes, n (%)			
Serious adverse events, total	2 (22.2%)	2 (25.0%)	0.91
Cardiovascular	2 (22.2%)	0 (0)	-
Renal	0 (0)	1 (12.5%)	-
Sepsis	0 (0)	1 (12.5%)	-
Serious adverse reaction	0 (0)	-	-
Pain at BMA site	0 (0)	-	-
Allergic reaction to BMMSCs	0 (0)	-	-
Malignancy	0 (0)	-	-
Brain tumour[c]	0 (0)	-	-
Safety blood tests			
Creatinine, μmol/L, mean (SD)	79.5 (23.7)	48.0 (8.5)	0.16
White blood cells, x10^9L, mean (SD)	7.8 (2.8)	5.7 (0.8)	0.38
Haemoglobin, g/dL, mean (SD)	13.5 (0.4)	12.4 (0.9)	0.34
Platelets, x10^9/L, mean (SD)	194.3 (63.9)	266.0 (67.9)	0.27
HbA1c, %, mean (SD)	5.9 (0.4)	5.5 (0.3)	0.35
Albumin, g/L, mean (SD)	43.8 (1.7)	41.0 (1.4)	0.13

NIHSS score > 20 = severe stroke and score < 5: minor stroke; Barthel Index score < 40 = requires constant care and score > 85 = nearly complete independence; and mRS score 0 = no symptoms and score 5 = severe disability.
BMMSC= bone-marrow derived mesenchymal stromal cells; HbA1c = Glycosylated Haemoglobin; IQR= interquartile range; SD= standard deviation
[a] Refers to % change compared to baseline scores. [b]Total infarct volume = gliosis (hyperintensity on T2 FLAIR) + intra/peri-lesional encephalomalacia (hypointensity of T2 FLAIR). [c]Screened by MRI at 12 months.

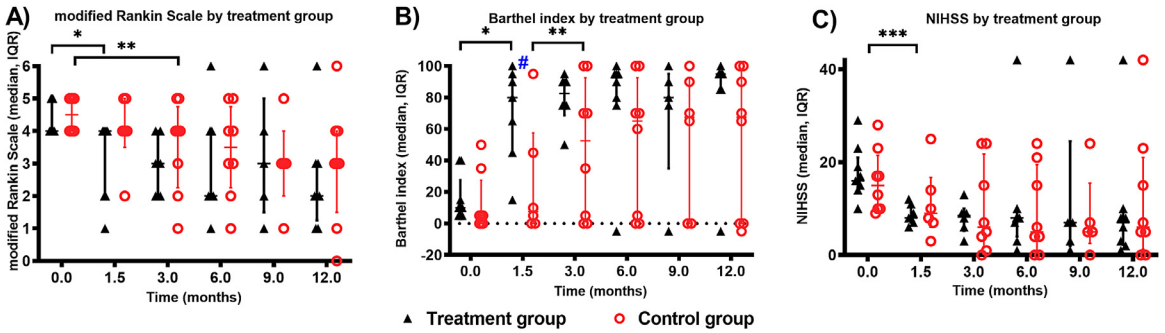

Figure 2. Scatterplots of (A) mRS, (B) BI and (C) NIHSS for the treatment and control groups. There was a significant intergroup difference for the BI at 1.5 months (6 weeks) as indicated with # (Mann–Whitney U test, $P = 0.045$). Meanwhile for within-group comparison, significant differences were observed in *treatment, **control and ***both groups for all three measurements at early assessements (Wilcoxon signed rank test, $P<0.05$). (Color version of figure is available online).

with the control group (median, 71.7 [30.5–101.7] mL versus 26.7 [12.9–75.3] mL), but this was not statistically significant ($P= 0.10$). The time from stroke onset to study enrollment was significantly shorter in the control group, with a median of 4.5 days, compared with the treatment group, with a median of 13.0 days ($P = 0.012$). The individualized baseline characteristics of all patients enrolled in this study are provided in supplementary table B.

Primary outcomes

Clinical assessments

There were no significant between-group differences in the NIHSS, mRS and BI scores at 12 months (Table 2). The authors performed additional analyses to compare the outcome measurements at 6 weeks, 3 months, 6 months and 9 months. At 6 weeks, the BI was significantly higher in the treatment group compared with the controls (median, 80.0 [45–95] versus 7.5 [0–57.5], $P = 0.045$) (Figure 2B). No significant between-group differences in BI score were observed at 3 months, 6 months and 9 months (Figure 2; also see supplementary table C).

Post-hoc analysis

When the trial was designed, the 6-week assessment was standardized at 2 weeks post-MSC infusion, as the authors had anticipated that the BMMSCs would be ready for infusion at 4 weeks from enrollment. However, in the actual trial, the time to receiving the BMMSC infusion was unexpectedly prolonged by more than 4 weeks (because of delays in achieving optimal cell concentration), resulting in a lag in the 6-week assessments, with a median duration of BMMSC infusion from enrollment of 48 days (IQR, 38–73). To adjust for this time lag in the 6-week assessments between groups, the authors conducted a post-hoc time-adjusted analysis, which was done by merging the 6-week and 3-month assessments into one 3-month assessment by accounting for only the assessment performed nearest to the 3-month time point. The time from baseline to the adjusted 3-month follow-up was 94 (87–99.3) days for the treatment group and 89.5 (73.5–93.5) days for the control group, which the authors felt was more comparable. In the time-adjusted analysis, there were no differences in the mRS, BI and NIHSS scores between the two groups at any of the follow-up time points (Figure 3; also see supplementary table C).

Within-group analysis

In the original analysis, the mRS score improved significantly in the treatment group at the 6-week follow-up compared with baseline (4.0 [4.0–5.0] to 4.0 [2.0–4.0], $P= 0.041$) (Figure 2A). The BI score improved significantly at 6 weeks (80.0 [45.0–95.0] to 10 [5.0–27.5], $P= 0.018$) (Figure 2B) compared with baseline. In the control group, there was significant improvement in the mRS score (4.5 [4.0–5.0] to 4.0 [2.25–3.75], $P= 0.039$) (Figure 2A) and BI score (52.5 [1.25–92.5] versus 5.0 [5.0–27.5], $P= 0.042$) (Figure 2B) at 3 months compared with baseline. In addition, the NIHSS scores were significantly lower in both groups at the 6-week follow-up (Figure 2C). In the time-adjusted analysis, improvement in BI, mRS and NIHSS scores occurred at 3 months in both groups (Figure 3). The results for the within-group comparisons of the mRS, BI and NIHSS measurements for the interval assessments (6-week, 3-month, 6-month and 9-month assessments) are available in supplementary table C.

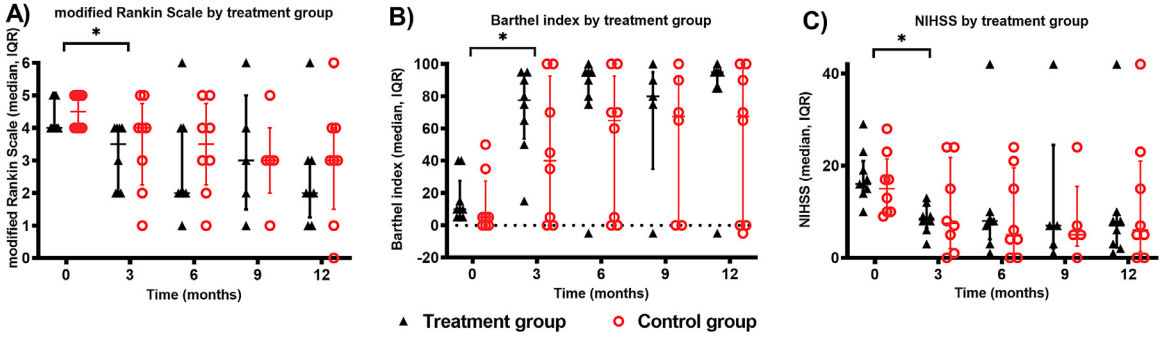

Figure 3. Scatterplots of (A) mRS, (B) BI and (C) NIHSS for the treatment and control groups in a post-hoc time-adjusted analysis. The original 1.5-month (6-week) and 3-month scores were merged into one 3-month assessment by accounting for only the assessment performed nearest to the 3-month time point. There was no significant intergroup difference at any time point. Meanwhile for within-group comparison, there were significant differences at 3-month time point for all three measurements. (Color version of figure is available online).

Radiological assessment

There were no significant between-group differences in the total infarct volume and gliosis and encephalomalacia volumes at 12 months. The absolute change in the infarct volume (relative to baseline) was significantly lower in the treatment group compared with the control group at 12 months. The median volume reduction was −11.88 mL in the treatment group compared with an increase of 4.45 mL in the control group (P= 0.027) (Table 2). The relative change in the absolute infarct volume, expressed as a percentage, was not significant between the groups (P= 0.050) (Table 2).

Safety outcomes

There were four serious adverse events (two deaths and two major events), which were unrelated to the study treatment. Two patients died before the end of the 12-month follow-up. One patient from the treatment group, who had co-existing severe coronary artery disease, died of sudden cardiac arrest 6 months after enrollment. In addition, one patient from the control group died of septicemia secondary to pressure sores 11 months after stroke. Furthermore, one patient from each group experienced a major adverse event unrelated to the study. One patient from the treatment group had an acute coronary event and withdrew from the study before receiving the BMMSC infusion. Another patient from the control group had pneumonia and acute renal failure. There were no adverse reactions during the intravenous infusion of BMMSCs or the BMA procedure.

Discussion

The main finding in this phase 2 randomized controlled trial was that intravenous infusion of BMMSCs was safe and well tolerated. However, the study did not show significant improvement in neurological or functional outcomes at the 12-month follow-up with BMMSC infusion compared with standard medical care. Although the authors were excited by the apparent improvement in the BI score at 6 weeks in the treatment group, a post-hoc time-adjusted analysis showed no significant difference in the BI score between the two groups. The authors felt that the post-hoc time-adjusted analysis provided a more accurate time-matched comparison and, hence, reflected the true effect of the treatment.

Although the trajectory of clinical improvement was no different in the control group, it is difficult to conclude negative treatment efficacy based on the clinical parameters, as the authors' study did not achieve the required sample size. In addition, despite randomization, some of the baseline characteristics were not balanced, as there were more males in the treatment group, which is a pejorative prognostic marker for functional recovery. The male predisposition in the treatment group was entirely coincidental and may have reflected the demographics of patients admitted with MCA to the authors' center during the study period. Another possibility is that a longer follow-up period might be required to confirm efficacy. In one randomized controlled trial, Bang *et al.*[17] initially reported no significant improvement in BI scores at 12 months in patients who had received BMMSCs (n = 5) compared with controls (n = 25). Subsequently, however, the same investigators conducted a larger study (n = 52, 16 BMMSCs and 36 controls) with a longer follow-up of 5 years, which showed improvement in functional outcomes and survival in the BMMSC group compared with the controls [25].

Despite the lack of improvement in the functional and clinical outcomes, the authors were encouraged by the improvement in the radiological outcome, as there was a significant reduction in the median absolute infarct volume in the treatment group at 12 months compared with the controls. This was despite a relatively larger, though non-significant, infarct size in the treatment group at baseline. One previous case series showed a >20% reduction in MRI FLAIR lesions 6 days after BMMSC injection, which coincided with a transient improvement in NIHSS score [26]. A more recently published randomized controlled clinical trial, which evaluated intravenous autologous MSCs in 16 subacute stroke patients, showed an improvement in the motor NIHSS component, which correlated with task-related primary motor cortex recovery on functional MRI at 2 years [27]. Similar to the authors' current findings, the researchers found no improvement in BI, total NIHSS or mRS score at 2 years in the MSC group. The reduction in infarct volume in the authors' current study may have been due to modulation of the post-stroke immune response and protective effects of MSCs in minimizing ischemia-induced blood–brain barrier disruption [28,29]. In a rat stroke model, intravenously administered MSCs migrated to the ischemic area of the brain and exerted local paracrine effects by secretion and interaction between stromal cell-derived factor 1 and C-X-C chemokine receptor type 4 [30], promoting angiogenesis and neuroprotection.

The optimal route of MSC delivery in ischemic stroke remains uncertain. In this study, the authors used the least invasive and safest method (intravenous) to deliver the cells. However, this method may be suboptimal in achieving successful cell migration to the infarcted area. Animal studies have shown that most intravenously infused cells are rapidly trapped in the lungs [31,32] and that less than 4% of the infused cells traverse the pulmonary microvasculature to reach the arterial circulation [33]. Pre-clinical data suggest that intra-arterial cell injection may lead to greater success in the number of cells reaching the ischemic area, as this route bypasses the filtering organs, such as the lungs, spleen and liver [34]. However, the intra-arterial route has been associated with increased risk of micro-occlusions. An MRI cell-tracking study that was able to track and visualize intra-arterially injected iron oxide nanoparticle-labeled MSCs in a rat stroke model showed that the highest engraftment correlated with reduced cerebral blood flow and led to increased mortality [35]. The propensity of MSCs to induce micro-occlusions has also been observed in several other studies, necessitating MRI cell-tracking in the development of safe transplantation protocols [36].

Although clinical studies on MSCs in stroke have yielded only modest results [17,25,27], animal studies seem to be more encouraging [9,37]. Most stroke animal models have utilized small animals, which may not be representative of stroke in humans, resulting in loss of translational efficacy when applied to human studies. In addition, most of the stroke modeling in animal studies has been transient, as all animals used in pre-clinical studies are healthy prior to stroke induction [38] and, hence, may not mimic the true disease burden in humans, where vascular comorbidities such as diabetes and hypertension are also prevalent. Large animal models may better represent human disease, although this could be expensive and may be limited to smaller sample sizes [39].

Limitations and challenges

The main limitation of this study was the small sample size. This was the result of recruitment challenges, as patients needed to undergo an invasive BMA procedure as part of the treatment. The possibility of having an invasive procedure discouraged some patients from enrolling in the study. Second, the long wait for the BMMSC culture led to a delay in the 6-week assessment for the treatment group. As such, the authors had to perform time-adjusted analyses to account for this discrepancy. Third, the authors excluded patients who had undergone reperfusion therapy for acute stroke, which reduced the pool of eligible patients for this study. Fourth, because of the small sample size, the authors were unable to perform multivariate analyses to account for the possibility of confounding effect, such as infarct volume and age, on the outcomes. A multicenter study with a stratified randomization based on prognostic factors

may lead to a larger, well-balanced study population while increasing the possibility of reaching the targeted sample size. In addition, there were more males in the treatment group, which may have adversely affected the outcome. Nevertheless, patients in the treatment group had larger infarct sizes (although non-significant) than those in the control group. This occurred by chance, as the randomization process did not employ minimization of prognostic factors. The patients were recruited after a non-enhanced computed tomography scan. MRI scans, which can measure infarct size more accurately, were performed only after enrollment. Finally, as the authors did not label the BMMSCs, we were unable to ascertain the number of MSCs that finally reached the brain and the infarcted area.

Strength and novelty

Despite the aforementioned limitations and challenges, the authors believe that this study has a number of strengths. It is one of the few randomized controlled clinical trials on autologous BMMSC transplantation in a homogeneous population of ischemic stroke patients with established subacute MCA infarct to demonstrate that intravenous autologous BMMSCs are safe, well tolerated and relatively feasible. In addition, the encouraging finding of a lower median infarct volume in the treatment group implies that MSCs administered in patients with established stroke may play a role in reducing the extent of neuronal damage through either cytoprotective or neuroregenerative mechanisms. More importantly, the authors believe that this study provides insight into the real world and practical challenges involved in conducting a phase 2 clinical trial involving autologous BMMSC transplantation, which could help other researchers working in this field.

Future direction

The role of MSCs as potential cytoprotective or neuroregenerative therapy needs to be explored using a well-designed and practically acceptable clinical trial in stroke patients who fall beyond the revascularization treatment window. Such trials should incorporate imaging-based measures of neuroinflammation using positron emission tomography [40] and MRI-based cell-labeling techniques, which enable non-invasive cell tracking following transplantation *in vivo* [41]. Future studies of MSCs in ischemic stroke could also explore other autologous sources of MSCs or utilize allogeneic MSCs to ensure greater participation. Allogeneic MSCs are reported to be safe [42] and can be obtained off the shelf, potentially improving the delivery time of MSCs to patients and making it a possible intervention of choice in the future [43,44]. Finally, studies should determine the effectiveness of different routes of MSC delivery, including those administered in a dose-dependent manner.

Conclusions

BMMSCs administered intravenously in the subacute period following MCA infarct were safe but did not improve functional outcome at 12 months. Improvements in radiological outcome were observed in the treatment group, thereby warranting further confirmation with neuroimaging markers. A larger randomized controlled trial is warranted to establish BMMSC efficacy in patients with subacute MCA infarct.

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Declaration of Competing Interest

SPC, CYW and SKC are affiliated with Cytopeutics Sdn Bhd, a joint sponsor of this study.

Author Contributions

Conception and design of the study: SPC, SKC, SFSAW and NMI. Acquisition of data: ZKL, HJT, WNNWY, ASM, RZ, MIA, NAI and NMI. Analysis and interpretation of data: ZKL, SC and NMI. Drafting or revising the manuscript: ZKL, SPC, CYW and NMI. All authors have approved the final article.

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