World Journal of
Stem Cells

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Contents

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AIM AND SCOPE

World Journal of Stem Cells (*World J Stem Cells*, *WJSC*, online ISSN 1948-0210, DOI: 10.4252), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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INDEXING/ABSTRACTING

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FLYLEAF

Randomized Controlled Trial

Phase Ⅰ/Ⅱ randomized controlled trial of autologous bone marrow-derived mesenchymal stem cell therapy for chronic stroke

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Author contributions: Tsang KS conducted experiments, analyzed data and wrote the manuscript; Ng CPS coordinated the trial and performed statistical analysis of data; Zhu XL and Wong GKC consulted and administered cells and placebos to patients; Lu G reviewed data; Ahuja AT analyzed radio-imaging; Wong KSL and Ng HK contributed intellectual content; Poon WS designed, supervised and monitored the trial, secured grant support, interpreted data and revised the manuscript.

Institutional review board statement: The randomized, controlled, double-blind, phase Ⅰ/Ⅱ clinical trial was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee of Hong Kong Hospital Authority in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonisation - Good Clinical Practice.

Clinical trial registration statement: The clinical trial CREC #2006.425-T was registered at http://crec.cuhk.edu.hk.

Informed consent statement: Written informed consent was obtained from all subjects - in the case of vegetative state, from their next of kin.

Conflict-of-interest statement: All authors declared that no conflict of interest exists.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Abstract

AIM

To examine the safety and efficacy of mesenchymal stem cell (MSC) therapy for intracerebral haemorrhage with neurological dysfunctions for a year.

METHODS

MSC were *ex vivo* expanded from 29 mL (17-42 mL) autologous bone marrow. Patients were randomized to have two intravenous injections of autologous MSC or placebos in four weeks apart. Neurological functions and clinical outcomes were monitored before treatment and at 12th, 16th, 24th, 36th and 60th week upon completion of the treatment.

RESULTS

A mean of 4.57×10^7 (range: 1.43×10^7-8.40×10^7) MSC per infusion was administered accounting to 8.54×10^5 (2.65×10^5-1.45×10^6) per kilogram body weight in two occasions. There was neither adverse event at time of administration nor sign of *de novo* tumour development among patients after monitoring for a year post MSC therapy. Neuro-restoration and clinical improvement in terms of modified Barthel index, functional independence measure and extended Glasgow Outcome Scale were evident among patients having MSC therapy compared to patients receiving placebos.

CONCLUSION

Intravenous administration of autologous bone marrow-derived MSC is safe and has the potential of improving neurological functions in chronic stroke patients with severe disability.

Key words: Stroke; Intracerebral haemorrhage; Central nervous system; Mesenchymal stem cells; Cell therapy

Core tip: Contemporary treatments are ineffective in restoring lost neurological functions after stroke. Many stroke patients were noted to have lesions close to the sub-ventricular zone. The likely beneficial effects of mesenchymal stem cell (MSC) treatment might correlate with the spatial lesion, not part of the sub-ventricular zone where endogenous neurogenesis persists during adulthood, and indirect chaperon effects of MSC promote endogenous neuro-regeneration. We administered MSC intravenously to patients having severe neurological disability and presenting stable baseline scores one year after the onset of intracerebral haemorrhage to eliminate confounding attributes to the observation of MSC-mediated neurological recovery.

## INTRODUCTION

Stroke is a common neurological disorder and is a leading cause of death. More than six million cases of stroke are reported in the world annually[1]. Approximately 50% of the patients died and most of the survivors are left with various degree of neurological dysfunction. There is no effective treatment for restoring the neurological function of the patients to date. Recent studies in animal models of intra-cerebral haemorrhage demonstrated active neurogenesis in the sub-ventricular zone leading to new neurons of phenotypes of their dead counterparts[2,3]. Nevertheless, a majority of newly formed neurons die during the early weeks after stroke, and successful replacement only accounts for a small portion of the mature dead neurons[4]. The feasibility of using a variety of cell types including neural stem cells, embryonic stem cells, umbilical cord blood cells and mesenchymal stem cells (MSCs) to enhance re-innervation has been demonstrated in animal models[5-8]. The breakthrough opens cellular therapy for stroke. In clinical setting a reliable and accessible cell source is requisite. Bone marrow-derived MSCs, which were noted to generate trophic factors, growth stimulants, signalling regulators and cytokines, might help promote neuro-regeneration and neuro-restoration after stroke *via* neurogenesis, angiogenesis and synaptogenesis[9]. Cellular therapy employing large numbers of *ex-vivo* expanded viable MSC might be a potential treatment modality to patients after stroke.

An earlier study demonstrated the feasibility and safety of infusion of autologous MSC in patients nine weeks after stroke onset[10]. In the present study we conducted a phase I/II randomized controlled trial of autologous bone marrow-derived MSC therapy in patients one year after onset of stroke with the aim to study the long-term safety and functional efficacy of intravenous administration of MSC.

MATERIALS AND METHODS

Study design

This study is a randomized, controlled, double-blind, phase I/II clinical trial (CREC #2006.425-T) and was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee of Hong Kong Hospital Authority in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonisation - Good Clinical Practice. Inclusion criteria are that patient had the onset of stroke for one year ago with stable National Institutes of Health Stroke Scale scores $\geq$ 7 and Glasgow Outcome Scale score of severe disability and vegetative state at one year after onset of stroke[11,12]. Exclusion criteria are lacunar syndrome, malignant diseases, severe co-morbidity, hepatic/renal dysfunction and unwillingness to participate. Patients who presented stable baseline scores indicating severe neurological disability were recruited to the study. Informed consent was obtained from all subjects - in the case of vegetative state, from their next of kin. Eligible patients were randomly assigned to the
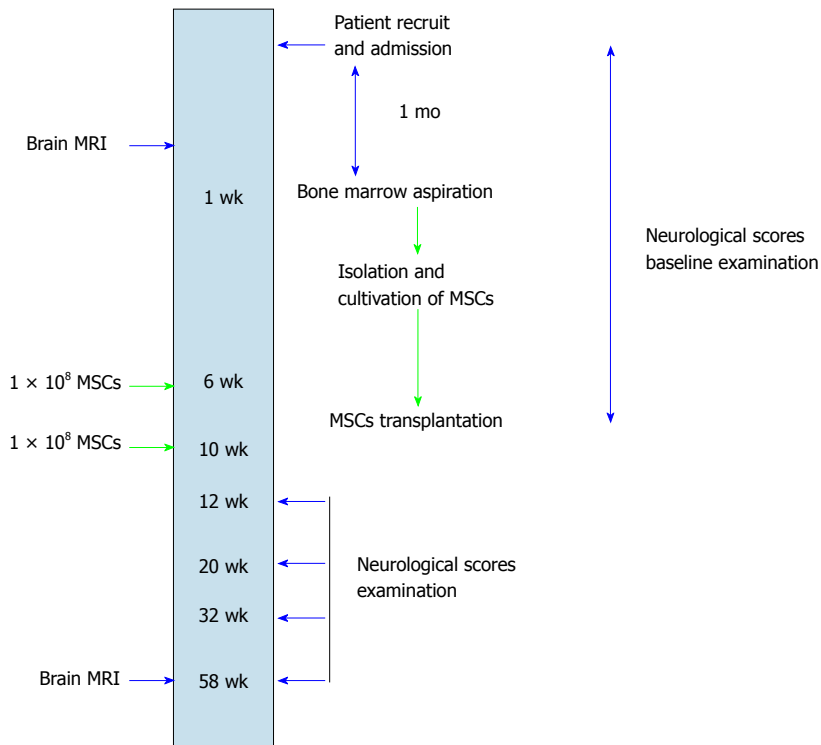
treatment group and control group for autologous MSC therapy (Figure 1). The study protocol was developed according to the guidelines of Consolidated Standards of Reporting Trials available on-line at http://www.consort-statement.org/.

Radio-imaging
Computed tomography (CT) scan was conducted at onset of intracerebral haemorrhage. Haematoma volume in mL was computed by using the formula: ½ × maximal height (cm) × width (cm) × anterior-posterior diameter (cm). Magnetic resonance imaging (MRI, 1.5 Tesla) of the brain were performed on the day before the first injection of either MSC or placebos. A follow-up procedure was conducted on patients at 60th week upon completion of the study (Figure 1).

Ex vivo expansion and infusion of MSC
The procedure of MSC expansion described by Le Blanc *et al*[13] was adopted with minor modifications. In brief, bone marrow aspirates from the superior iliac crest of patients under local anaesthesia were anti-coagulated in 10 IU/mL preservative-free heparin (DBL, Hospira, Melbourne, Australia). Mononuclear cells were enriched by using density-gradient centrifugation in ficoll-hypaque with specific gravity of 1.077 g/mL (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and cultured in low-glucose Dulbecco modified eagles' medium (Invitrogen, Life Technologies, Carlsbad, CA, United States) supplemented with 10% foetal bovine serum (Invitrogen). MSC cultures of approximately 80% in confluence were passaged using 0.05% trypsin (Invitrogen) and *ex vivo* expanded by subcultures in 175 cm² flasks.

On the day of infusion, MSC cultures were enzymatically segregated and dislodged from culture flasks by trypsin digestion, washed with phosphate-buffered saline, sieved to remove cell aggregates *via* 40-μm filter and re-suspended in 10 mL 5% normal human albumin (Hong Kong Red Cross Blood Transfusion Service) for intravenous injection in five to ten minutes. Another booster bolus of autologous MSC was prepared and administered to patient four weeks thereafter. A placebo of an equal volume of 5% normal human albumin was administered to patients being allotted to the control group. Cultures and cell processing were conducted under conditions meeting the requirements of good manufacturing practices.

Characterisation of MSC
Immunophenotyping of MSC by flow cytometry was reported elsewhere[14]. Unless stated otherwise, fluorescence-conjugated monoclonal antibodies from Beckman Coulter were used. They were IgG1-FITC, IgG1-PE, HLA-DR-FITC, CD45-FITC, CD3-FITC, CD19-PE, CD16-FITC, CD33-FITC, CD38-FITC, CD34-PE, CD133-PE (Miltenyi Biotec GmbH, Germany), CD29-PE, CD44-FITC, CD73-FITC, CD90-PE, CD105-PE (Serotec, United Kingdom) and CD166-PE were used. At least 10000 events were acquired and signals were analysed by using the Coulter Epic XL MCL flow cytometer (Coulter, Miami, FL, United States).

Procedural details of immunofluorescence staining were described previously[15]. IgM anti-stage-specific embryonic antigen-4 (SSEA-4, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, United States), IgG2b anti-octamer-binding transcription factor-4 (Oct-4; 1:100, Santa Cruz Biotechnology), IgG1 anti-Nestin (1:400;

BD Biosciences, San Francisco, CA, United States) were employed.

Cell viability was evaluated by using trypan blue dye exclusion test. Sterility check against microbial contamination was conducted at each MSC passage.

Neurological functional assessments

Patient safety and efficacy of cell therapy were evaluated during the first and second MSC infusion and thereafter at 16th, 24th, 36th and 60th week of the study (Figure 1). The safety of intravenous infusion of either MSC or placebos was assessed in terms of the development of immediate or delayed adverse reactions. Immediate reactions included allergic responses (tachycardia, fever, skin eruption and leucocytosis), local complications (hematoma, local infection at the injection site), vascular obstructions (tachypnea, oliguria, peripheral vascular insufficiency, recurrence of stroke), and systemic complications (systemic infections, increased aspartate aminotransferase, alanine aminotransferase and/or blood urea nitrogen/creatinine levels). Presence of delayed adverse reaction of tumour formation was evaluated by physical examination of skin and oral mucosa and followed up with magnetic resonance imaging (MRI), if necessary. Modified Barthel Index and Functional Independence Measure were monitored by a neurologist being blinded to group allocation and radiological data[16,17]. Scores of Extended Glasgow Outcome Scale were also used to track the progress of disability of patients over time[12]. Stroke scale scores, vascular risk factors, medical history and demographic details were recorded.

Statistical analysis

Means, ranges and standard deviations of continuous variables of years of age, kilogram in body weight, milliliter of hematoma and bone marrow, percentages of cell counts and viability were calculated. Assuming that data were normally distributed, non-parametric Wilcoxon's rank sum test was used to compare variables derived from the treatment and control groups in the study. Paired *t*-test with one-sided testing was used to analyse scores of modified Barthel Index, Functional Independence Measure and Extended Glasgow Outcome Scale of patients at time of assessments. Fisher's exact test was applied to examine the incidence of clinical neurological improvement between the treatment and control groups of patients. Differences between groups were regarded as significant if $P \leqslant 0.05$.

RESULTS

Patient characteristics

We conducted a double-blind, randomized, controlled phase Ⅰ/Ⅱ trial to examine the safety and efficacy of autologous MSC therapy in a small cohort of nine patients (four females and five males) with a mean age of 52 years (range: 41-59 years) who had undergone intracerebral haemorrhage (ICH) for a year. CT scan at time of the onset demonstrated cerebral haematoma of 52 mL (12-75 mL) located in the basal ganglion region of the brains of the nine patients in the study cohort. The sizes of the lesion areas were comparable between the treatment and control groups.

MSC were *ex vivo* expanded from a mean volume of 29 mL (17-42 mL) autologous bone marrow. Patients were randomized to have two intravenous injections of autologous MSC (treatment group of MSC: *n* = 5 or control group of placebos: *n* = 4) four weeks apart. The body weight of patients in the treatment group were statistically lighter than those of the control group [treatment *vs* control; 54.2 kg (42-60 kg) *vs* 67.2 kg (64-72.7 kg), *P* = 0.03], however the years of age of both groups were comparable [treatment *vs* control; 53.4 (48-56) *vs* 51.5 (41-59); *P* = 0.64]. There was no difference between the severities of disability in terms of neurologic scores of patients assigned to both groups (data not shown).

MSC autograft

Ex vivo expanded MSC at a mean of 4 passages (1-8) were used for infusion. MSC up to passage-8 displayed longitudinal, bi-polar, spindle-shaped and fibroblast-like morphology. Immunofluorescence staining demonstrated expressions of embryonic stem cell marker SSEA-4, transcription factor Oct-4 and neural stem cell marker Nestin; suggesting the pluripotency and neurogenesis of MSC (Figure 2). Flow cytometry demonstrated that they were immunophenotypically positive for CD29, CD44, CD73, CD90, CD105 and CD166 (Figure 3A), but negative for haematopoietic stem cell markers (CD34 and CD133), myeloid progenitor cell markers (CD33 and CD38), leucocyte markers (HLA-DR and CD45), T-cell marker CD3, NK cell marker CD16 and B-cell marker CD19 (Figure 3B).

MSC infusions

Table 1 shows numbers and doses of MSC in 11 episodes of infusion into five patients (three females and two males). A mean of 4.57×10^7 (1.43×10^7-8.40×10^7) MSC per infusion was administered accounting to 8.54×10^5 (2.65×10^5-1.45×10^6) per kilogram body weight in two occasions except Patient NSCT02 underwent three infusions. Infused cells were immunophenotypically homogenous; HLA-DR-, CD45-, CD3-, CD19-, CD16-, CD33-, CD38-, CD34- and CD133-positive cells were less than 1% on average, whereas CD29-, CD44-, CD73-, CD90-, CD105- and CD166-positive cells were more than 96% (Supplementary Tables 1 and 2). Cell viability was 94.4% (88.5%-99.0%). There was no microbial growth as evident by aerobic and anaerobic cultures of 11 infusates. The control group of four patients (one female and three males) received placebos in an identical manner. No adverse reaction of acute infusion-related toxicity, transient fever, complication in organs or infection was experienced by both groups of patients at time of and a day following MSC administration. There was no sign of tumour development among patients in
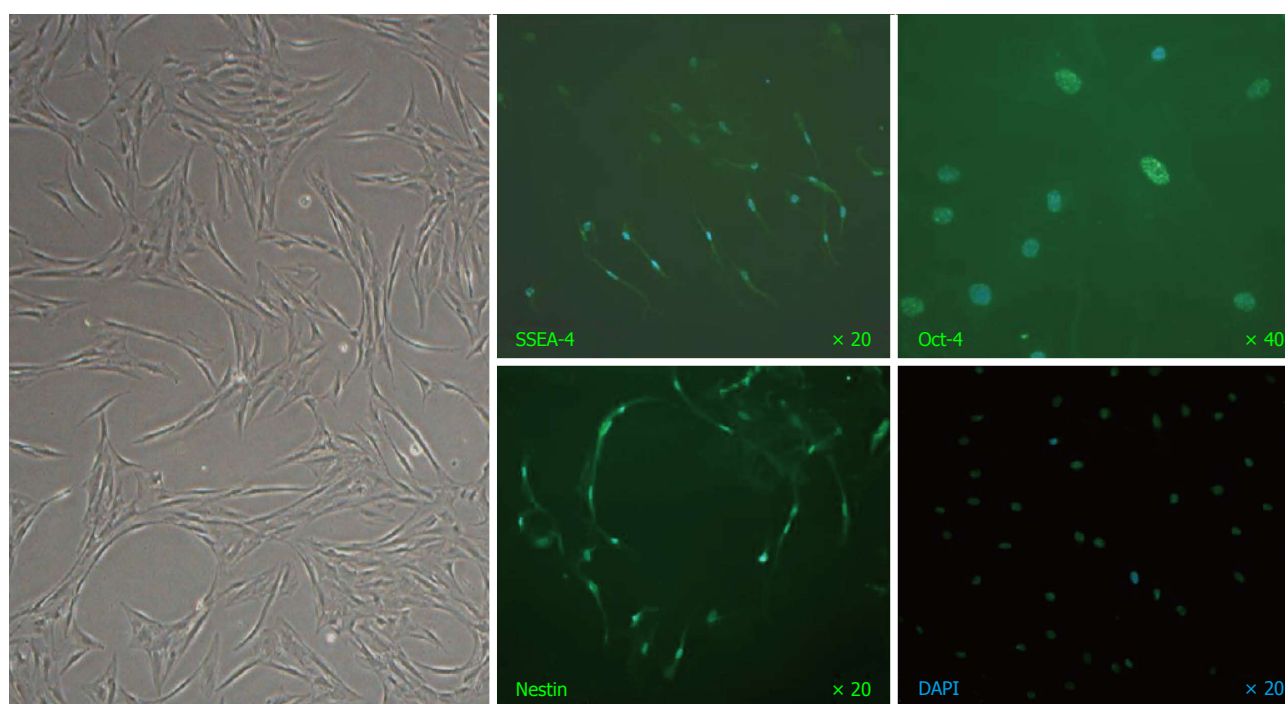
Figure 2 A representative image of mesenchymal stem cells at passage-8 captured under phase-contrast microscopy (left panel) and immunofluorescence staining of stage-specific embryonic antigen SSEA-4, transcription factor Oct-4 and neural stem cell marker Nestin (green fluorescence) with nuclei counterstained by DAPI (blue fluorescence).

Table 1 Characteristics of intracerebral haemorrhage patients and infuses for autologous bone marrow-derived mesenchymal stem cell therapy								
Patients	Sex/age in years	Location of haemorrhage	CT readout (cm)	Hemorrhage volume (mL)	MSC passage numbers	Viability (%)	MSC ($\times 10^7$)	MSC/kg ($\times 10^5$)
UPN02	F/50	Basal ganglia, left	$4.4 \times 5.8 \times 5.9$	75	4, 7	96.8	3.1	5.44
					3-5	92.1	3.2	5.61
					3-6	92.6	3.1	5.44
UPN08	F/48	Basal ganglia, left	$4.0 \times 2.0 \times 3.0$	12	3, 4	95.8	1.43	2.65
					3, 4	96.5	1.43	2.65
UPN09	M/56	Basal ganglia, right	$6.5 \times 5.0 \times 4.0$	65	3-8	98.5	7.6	13.1
					2-6	96.8	4	6.84
UPN10	F/55	Frontal lobe, right	$5.6 \times 4.5 \times 5.0$	63	1-6	88.5	5.5	13.1
					1-6	98	5.6	12.4
UPN11	M/55	Basal ganglia, left	$2.4 \times 5.0 \times 3.5$	21	1-6	90.5	5.4	9
					1-7	99	8.4	14.5
UPN05	F/59	Basal ganglia, left	$6.0 \times 4.0 \times 4.0$	48				
UPN06	M/56	Basal ganglia, frontoparietal temporal lobe, right	$7.3 \times 3.5 \times 5.0$	64				
UPN07	M/41	Basal ganglia, right	$4.3 \times 6.4 \times 5.0$	69				
UPN12	M/50	Basal ganglia, putaminal, right	NA	55				

MSC: Mesenchymal stem cell; CT: Computed tomography; NA: Not available.

the study cohort having monitored for a year.

Functional outcomes

Neurological functions and clinical outcomes were monitored before and at 12th, 16th, 24th, 36th and 60th week upon completion of the treatment. In terms of the scores of modified Barthel Index and Functional Independence Measure, the magnitudes of physical and cognitive disability were comparable between the treatment and control groups. Improvements of motor disability and cognitive impairment were observed over the course of a year among patients undergoing MSC therapy (Tables 2 and 3). Similar progresses were not apparent in the control group receiving placebos (Supplementary Tables 3 and 5). Scores of Extended Glasgow Outcome Scale demonstrated a trend of improvement of clinical outcomes of patients at 24th, 36th and 60th week upon completion of the MSC therapy (Table 4). Evident clinical improvement in patients of both groups were comparable (Patients with higher

A

B
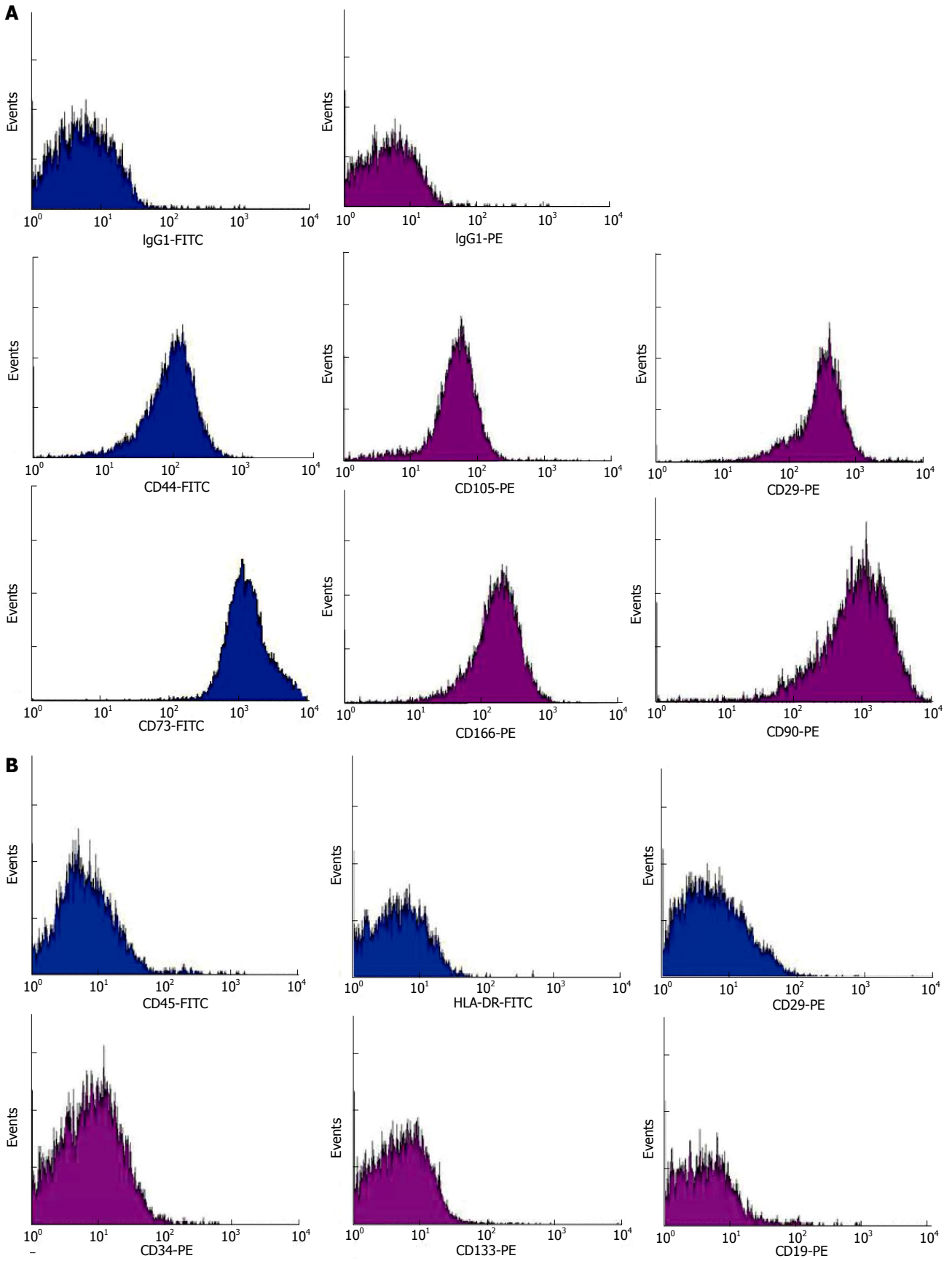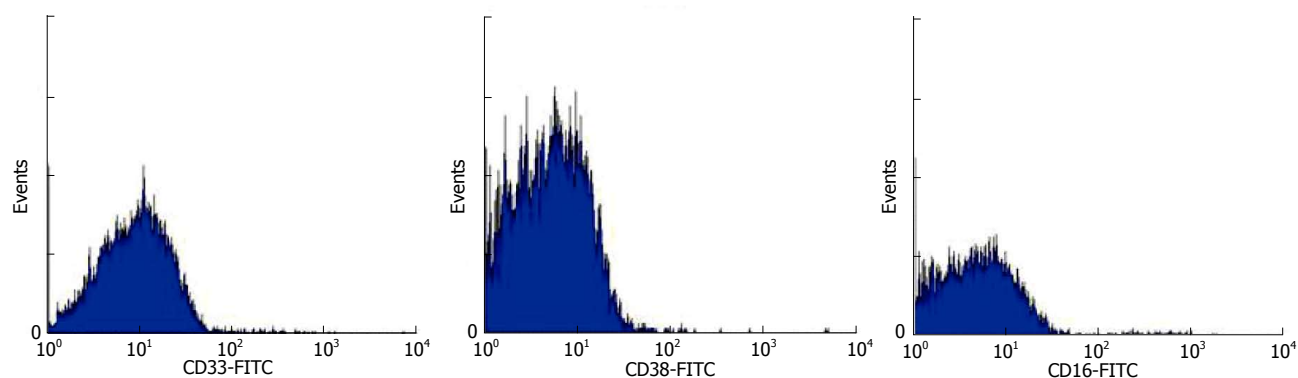
Figure 3 Representative histograms derived from infusates by flow cytometric analyses of mesenchymal stem cell markers CD29, CD44, CD73, CD90, CD105 and CD166 (A) and haemic markers HLA-DR, CD45, CD3, CD19, CD16, CD33, CD38, CD34 and CD133 (B). FITC conjugation in blue and PE conjugation in purple.

Table 2 Modified Barthel indices of the treatment group (*n* = 5) having mesenchymal stem cell therapy at the 1st, 12th, 16th, 24th, 36th and 60th week upon completion of the study

Study duration (wk)	Modified barthel indices					*P* value
	UPN02	UPN08	UPN09	UPN10	UPN11	
1st	19	66	0	4	69	
12th	19	73	0	5	70	0.12
16th	30	76	0	5	77	0.03
24th	32	76	0	5	77	0.03
36th	32	78	1	6	77	0.02
60th	32	78	1	-	77	0.03

Data derived from different time points were compared to the baseline values established at the first week of the study.

Table 3 Functional independence measure of the treatment group (*n* = 5) having mesenchymal stem cell therapy at the 1st, 12th, 16th, 24th, 36th and 60th week upon completion of the study

| Study duration (wk)	Scores of functional independence measure					*P* value
	UPN02	UPN08	UPN09	UPN10	UPN11	
1st	36	72	20	83	70	
12th	35	73	21	88	74	0.07
16th	40	79	21	89	80	0.01
24th	41	85	21	89	84	0.02
36th	44	87	22	102	84	0.01
60th	42	88	22	-	85	0.03

Data derived from different time points were compared to the baseline values established at the first week of the study.

scores of Extended Glasgow Outcome Scale: MSC *vs* placebos; 3/5 *vs* 1/4; *P* = 0.52). There was no re-occurrence of ICH among patients in the study.

Radio-imaging
Comparing MRI brain before MSC injection and at completion of the study, there was no interval change in morphology.

DISCUSSION
In the study we demonstrated the safety, feasibility and improvement of neurological outcomes of intravenous administration of autologous bone marrow-derived MSC in a small cohort of nine chronic stroke patients one year after intracerebral haemorrhage through a randomized controlled double-blinded phase Ⅰ/Ⅱ clinical trial.

Despite advances in neurosurgery and contemporary medical regimes, survivors of intracerebral haemorrhage often suffer long-term to permanent severe disabilities in terms of cognitive impairment and motor dysfunction. Neurological restoration remains poor. There is an imperative to develop therapeutic

Table 4 Scores of extended Glasgow Outcome Scale of the treatment group (*n* = 5) having mesenchymal stem cell therapy at the 1st, 12th, 16th, 24th, 36th and 60th week upon completion of the study						
Study duration (wk)	**Scores of extended glasgow outcome scale**					***P* value**
	UPN02	**UPN08**	**UPN09**	**UPN10**	**UPN11**	
1st	3	4	3	3	4	
12th	3	4	3	3	4	1
16th	3	4	3	3	4	1
24th	3	4	3	3	5	0.19
36th	3	5	3	3	5	0.09
60th	4	6	3	-	5	0.05

Data derived from different time points were compared to the baseline values established at the first week of the study.

modalities to promote neurological recovery. Stem cell-based therapy has drawn a lot of attention recently and the therapeutic efficacies of various cell types were studied[18]. Some cell types are deemed difficult for a wide application. Human neural stem cells may be the prototype, however they are not easily harvestable for transplantation unless collected from aborted foetuses or during necropsy[19,20]. Embryonic stem cells are capable to give rise to all cell lineages, but the application to brain therapy is hindered by the risk of teratoma development and not to mention the ethical controversy[21]. Therapeutic potentials of human stem and progenitor cells from other sources; including bone marrow mononuclear cells, umbilical cord blood CD34+ cells, dental pulp stem cells, adipose-derived stem cells and bone marrow-derived MSC, have also been widely investigated[22-26]. Previous studies indicated that only a limited number of extraneous cells had eventually implanted and integrated into neural networks of recipients. The numbers of successfully engrafted cells were far less than those lost and died to facilitate neuro-restoration. Nonetheless, the implanted cells elicited the neurological recovery *via* indirect chaperon mechanisms of paracrine signalling of cytokines, chemokines, growth factors, trophic factors, signalling regulators and immuno-modulators, which ultimately stimulated endogenous neurogenesis, angiogenesis and synaptogenesis. It is essential to investigate MSC transplantation as a cell therapy for stroke.

Shortly after the first report on the clinical trial of MSC therapy for stroke[10], many issues arose to be resolved before MSC therapy can be safely and effectively administered to stroke patients. A plethora of clinical trials of MSC therapy for stroke, including the present study, were conducted in small cohorts of patients rendering the statistical power of safety and efficacy less valid[10,27-30]. In parallel with studies in large patient cohorts, clinical trials in small cohorts of patients would be more easily manageable and feasible to provide data for meta-analysis. The study serves the goal.

Autologous serum and platelet lysate were used to replace foetal calf serum in the supplement of basal culture media for MSC propagation in fear of the likelihood of zoonosis[28,31]. Likewise, animal serum-free chemically modified culture media are feasible alternatives to override the likely hurdle[27]. There were concerns of loss of stemness, change of functions, senescence and transformation of prolonged cultures of human MSC, nonetheless little report on the clinical trial of human MSC at high passages is available. Honmou *et al*[28] reported no side effect on the administration of autologous MSC at passages ⩽ 3 in stroke patients during one year of follow-up. More pronounced neurogenesis was observed in a rat stroke model receiving human MSC at earlier passage 2 than counterparts having human MSC at later passage 6[32]. Bernardo and co-workers reported that long-term *in vitro* cultures of human bone marrow-derived MSC up to passage 25 are not susceptible to malignant transformation[33]. In the study we investigated MSC at passages up to 8. There was neither morphological changes, phenotypic alterations nor growth senescence. No infusion-related toxicity and complications were experienced by patients at time and upon completion of MSC infusion. Data of the study suggest that MSC up to passage 8 are applicable to clinical use without a compromise of safety over an observation period of a year.

It is intuitive that MSC should rest precisely in the locality of interest in the brain in order to achieve the optimal therapeutic effects. Data of clinical trials of MSC therapy demonstrate that intravenous administration is a feasible approach[10,27,28,30]. However, the homing of MSC into the brain was shown to be limited and many cells were trapped into the peripheral organs especially the lungs. Alternate means of intra-arterial delivery and intracranial injection using stereotactic device were also reported to be safe and feasible in human[29,34,35]. Nonetheless, intra-arterial administration was found not superior to intravenous delivery of bone marrow mononuclear cells in a rat stroke model[36]. Both modes of cell delivery achieve comparable structural and functional outcomes in stroke animals after stem cell therapy despite the low homing efficiency.

The optimal dose of MSC applied to human is largely

unknown. The empirical cell numbers of 0.5 - 5 $\times$ 10^8 in human are extrapolated from the effective dose of 0.1 - 3 $\times$ 10^6 cells per rat in rat stroke model[10,28,37]. Cells of 5 $\times$ 10^7 were administered twice in the first report on the clinical trial of MSC therapy for stroke and better outcome in Barthel index was noted one year post-treatment[10]. Bhasin *et al*[27] transplanted a mean of 5-6 $\times$ 10^7 MSC and reported neural plasticity. Honmou *et al*[28] administered intravenously 0.6-1.6 $\times$ 10^8 cells per patient and observed reduction of lesion size by > 20% after one week. In the study a mean of 4.6 $\times$ 10^7 MSC was administered twice and improvements of motor disability and cognitive impairment were noted.

At present, available reports on clinical trials of MSC therapy suggest neuro-restoration, increase of neural plasticity and reduction of lesion volume[10,27-30,38]. Many stroke patients were noted to have lesions close to the sub-ventricular zone[39]. The likely beneficial effects of MSC treatment might correlate with the spatial lesion not part of the sub-ventricular zone where endogenous neurogenesis persists during adulthood and indirect chaperon effects of MSC promote endogenous neuro-regeneration[40]. In the study we administered MSC to patients having severe neurological disability and presenting stable baseline scores one year after the onset of intracerebral haemorrhage to eliminate confounding attributes to the observation of MSC-mediated neurological recovery.

Data of the study suggest that intravenous administration of autologous bone marrow-derived MSC is safe and facilitate the recovery of neurological functions in patients with severe disability long after the onset of intracerebral haemorrhage. Clinical neurological improvement of patients having MSC therapy was evident compared to patients receiving placebos. MSC therapy is effective independently of other treatment courses. The work may help define criteria for future phase Ⅲ studies.

ACKNOWLEDGMENTS

COMMENTS

Background
This study was initiated from the randomised Phase Ⅱ from the Korean Neurologist, suggesting efficacy. Collaborating with the authors Bone Marrow Transplant Unit, the authors were able to be using autologous bone marrow mesenchymal stem cells (MSCs) of sufficient number for two interval infusion.

Research frontiers
To generate efficacy data post-stroke is important. At present there has been no effective treatment for improving neurological deficits after any stroke illnesses.

Innovations and breakthroughs
There were more data on ischaemic stroke. For brain haemorrhage, clinical data were scarce. This study showed a trend towards improvement for intracerebral haemorrhage.

Applications
The report of this work demonstrated a trend of clinical improvement on two intravenous infusions of autologous bone marrow MSC in four weeks apart.

Terminology
The work provided supportive evidence for phase Ⅲ randomized controlled trial in the future.

Peer-review
This manuscript is worth publishing, reporting the result of the phase Ⅰ / Ⅱ clinical trial of autologous BM-MSC transplantation therapy in a small cohort of patients with chronic brain haemorrhage.

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