STEM CELLS

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A Long-Term Follow-Up Study of Intravenous Autologous Mesenchymal Stem Cell Transplantation in Patients With Ischemic Stroke

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Key Words. Stroke • Mesenchymal stem cells • Stem cells • Neurogenesis • Clinical trials

ABSTRACT

We previously evaluated the short-term follow-up preliminary data of mesenchymal stem cells (MSCs) transplantation in patients with ischemic stroke. The present study was conducted to evaluate the long-term safety and efficacy of i.v. MSCs transplantation in a larger population. To accomplish this, we performed an open-label, observer-blinded clinical trial of 85 patients with severe middle cerebral artery territory infarct. Patients were randomly allocated to one of two groups, those who received i.v. autologous ex vivo cultured MSCs (MSC group) or those who did not (control group), and followed for up to 5 years. Mortality of any cause, long-term side effects, and new-onset comorbidities were monitored. Of the 52 patients who were finally included in this study, 16 were the MSC group and 36 were the control group. Four (25%) patients in the MSC group and 21 (58.3%) in the control group died during the follow-up period, and the cumulative surviving portion at 260 weeks was 0.72 in the MSC group and 0.34 in the control group (log-rank; $p = .058$). Significant side effects were not observed following MSC treatment. The occurrence of comorbidities including seizures and recurrent vascular episodes did not differ between groups. When compared with the control group, the follow-up modified Rankin Scale (mRS) score was decreased, whereas the number of patients with a mRS of 0–3 increased in the MSC group ($p = .046$). Clinical improvement in the MSC group was associated with serum levels of stromal cell-derived factor-1 and the degree of involvement of the subventricular region of the lateral ventricle. Intravenous autologous MSCs transplantation was safe for stroke patients during long-term follow-up. This therapy may improve recovery after stroke depending on the specific characteristics of the patients. STEM CELLS *2010;28:1099–1106*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Ischemic stroke is associated with high mortality and severe morbidity, and many stroke survivors are left with permanent neurological disability. Although rehabilitation therapy is important for maximization of functional recovery after stroke, once neurological deficits are fixed there are few options for recovery. It has been suggested that any therapy that leads to even slight recovery might be helpful [1]. Cell therapy could provide trophic support or cell replacement to the infarcted area. Various cell types are candidates for cell therapy in ischemic stroke (from embryonic stem cells to neuronal cell lines), and clinical trials of neuronal differentiated tumor cell lines [2], neural progenitor cells from primordial porcine striatum [3], and autologous bone marrow-derived mesenchymal stem cells (MSCs) [4] have been conducted. The use of MSCs is attractive in that autologous cells may allow immune reactions to be avoided. MSCs are multipotent and can transdifferentiate into neural cells [5, 6]. Brain samples taken from women who received bone marrow transplants from male donors showed nerve cells containing Y chromosomes, suggesting that MSCs function in the brain [6]. Moreover, MSCs secrete cytokines, growth and trophic factors, which activate mechanisms that lead to improved neurological functions such as neurogenesis, angiogenesis, and synaptogenesis [7–11].

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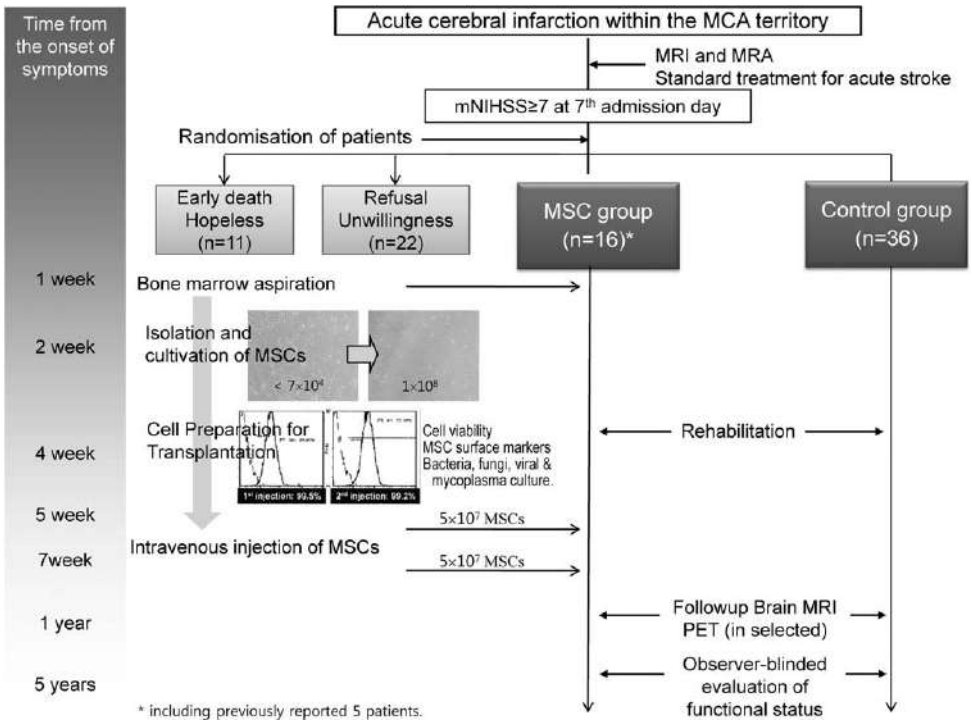

Figure 1. Study protocol.

Preclinical studies of brain metabolic profiling have shown that i.v. MSCs infusion normalized ischemia-induced changes in free fatty acid levels [12].

Our preliminary data revealed that i.v. infusion of autologous MSCs was feasible and safe during the short-term follow-up period [4]. Although functional outcomes were improved significantly early after MSCs treatment, the degrees of improvement declined until 1-year after follow-up. In addition, there has been concern regarding the use of fetal calf serum or fetal bovine serum (FBS) in cell culture [13]. Thus, long-term follow-up is needed to evaluate functional outcomes and possible harmful effects of xenogeneic components. In our preliminary study, only five patients were followed for relatively short periods. In addition, individual patients may have different responses to the treatment. Therefore, patient selection is an important matter for enhancing the efficacy of MSC therapy.

Thus, the present study was conducted to evaluate the long-term safety and functional efficacy of i.v. autologous ex vivo cultured MSCs infusion in patients with acute ischemic stroke. In addition, we evaluated factors associated with the response to MSC therapy.

Patients and Methods

Study Design

This was a randomized open-labeled, observer-blinded clinical trial of patients with ischemic stroke who had severe and persistent deficits and received i.v. ex vivo cultured autologous MSCs. The clinical trial protocol and consent form were approved by the Korean Food and Drug Administration and the Local Institutional Review Board for Human Investigation. We obtained written informed consent from all patients and/or their first-degree relatives. The study protocol is shown in Figure 1.

Patient Enrollment and Randomization

Consecutive patients were selected from July 2003 to December 2005. Patients between 30 and 75 years of age were included in this study only when they met the following conditions: (a) they had been observed within 7 days of the onset of symptoms; (b) they had relevant lesions within the middle cerebral artery territory as assessed using diffusion-weighted imaging (DWI); (c) they had severe persistent neurologic deficit (modified NIH Stroke Scale [mNIHSS] [14] $\geq$7 points) on the seventh admission day with stable course; and [4] they had no hematologic disorders or bone marrow suppression. Selection of candidates on the seventh admission day was based on our previous data; 748 patients with acute (<72 hours after onset) nonlacunar infarction within the middle cerebral artery territory were followed-up for 6 months [15]. This was because a marked improvement or worsening was frequently seen within 7 days of admission ($\sim$20%), and mNIHSS scores of at least six on day 7 after admission accurately forecasted a high probability of poor long-term outcome after middle cerebral infarctions.

We excluded patients who met any of the following conditions: (a) lacunar infarction; (b) premorbid dependence; (c) hematologic cause of stroke; (d) presence of severe medical illness; (e) presence of severe febrile illness; (f) hepatic or renal dysfunction; (g) positive response of penicillin skin test; and (h) unwillingness to participate. Patients were randomly allocated to one of two groups, the MSC or control group, by use of a randomization table. The randomized allocation to groups was performed on the seventh day of admission by a blinded, independent coordinator. After the initial random allocation of patients to treatment groups, experimental procedures were not blinded. Neither bone marrow aspiration nor sham infusion was performed in the control patients.

Preparation and Transplantation of MSCs

The same methods were used for bone marrow aspiration, isolation of MSCs, cell culture, cell preparation, and i.v. infusion [4]. Briefly, 5 ml of bone marrow were aspirated on the posterior iliac crest of patients in the MSC group. The aspiration was performed

within 1 week after randomization to the MSC group. Bone marrow mononuclear cells were separated by Ficoll density centrifugation. Mononuclear cells were cultured in a 175 cm^2 flask (Falcon, Franklin Lakes, NJ, http://www.bd.com) with low-glucose Dulbecco's modified Eagle's medium (Gibco-BRL, Grand Island, NY, http://www.invitrogen.com) containing 10% FBS (Hyclone, Irvine, CA, http://www.hyclone.com), and 1% penicillin–streptomycin (Sigma, St. Louis, MO, http://www.sigma-aldrich.com) in a humidified incubator at 37°C under 5% CO_2. Nonadherent cells were removed when the medium was exchanged at the fifth day. When the primary MSCs had expanded to 80% confluence, they were harvested and subcultured. Thus, autologous MSCs were culture-expanded to reach 1×10^8 cells per patient, the human dose equivalent to the dose that was effective in a rat model of stroke (1×10^5 to 3×10^6 cells per rat) based on mean body weight [4]. Approximately 4 weeks after bone marrow aspiration (median, 32.5 days; range, 18.8–37.0), 5×10^7 autologous MSCs were transplanted into patients through i.v. infusion with 100 ml of saline and the same amount of cells were again transplanted approximately 2 weeks after initial boosting. We used GMP (Good Manufacturing Practice) conditions (FCB-Pharmicell Corporation, Seongnam, South Korea, http://www.fcbpharmicell.com) and clinical grade reagents for preparation of the cells.

Cell viability was determined by trypan blue staining at the end of the harvest and before infusion, and the viability was greater than 95% for every infusate at both time points. Cell culture was tested weekly for sterility and there was no evidence of bacterial, fungal, viral, or mycoplasmal contamination in any of the flask tests. Because stem cells are highly likely to be differentiated, the surface expression of SH-2 (Src homology, CD105) and SH-4 on culture-expanded MSCs were measured in each patient before i.v. transplantation using flow cytometry (FACScan; Becton-Dickinson, Rutherford, NJ, http://www.bd.com), as previously described [4]. Each harvest of MSCs revealed a homogenous population of cells with high side and forward scatter and high-expression levels of SH-2 antigens (>90% of cells) in all patients.

Measurement of Safety and Efficacy

All patients were evaluated according to a protocol that included demographic data, medical history, vascular risk factors, laboratory tests, and stroke scales as in our previous study [4]. The patients were followed serially for safety and efficacy at 1 (randomization), 4–5 (first boosting), 7–9 (second boosting), and 14 weeks after admission, and every 3 months thereafter. Study evaluations consisted of thorough physical and neurological examinations and evaluations of adverse effects.

The primary outcome measure was the long-term safety profile. Mortality of any cause and serious adverse effects were the primary adverse events monitored. In addition, we monitored long-term adverse effects that were possibly related to MSC treatment and any immediate reactions after MSC treatment. Long-term adverse effects possibly related to MSC treatment included tumor formation (physical examination, plain x-ray, and follow-up magnetic resonance imaging [MRI] at 1-year after treatment), aberrant connections (newly diagnosed seizure or arrhythmia), and zoonoses from the use of FBS (myoclonus, rapidly progressing dementia, or ataxia). The immediate reaction included allergic reactions (tachycardia, fever, skin eruption, leukocytosis), local complications (hematoma or local infection at the site of bone marrow aspiration), vascular obstruction (tachypnea, oliguria, or peripheral vascular insufficiency), and systemic complications (systemic infections, increased aspartate aminotransferase and alanine aminotransferase, or blood nitrogen/creatinine levels).

The secondary outcome measures were the difference in functional outcomes measured by the modified Rankin Scale (mRS) at the last visit. To accomplish this, during the period of July 2008 to October 2008, the outcome was evaluated separately by one of the authors who were blinded to clinical information. This measurement was performed in all patients through office interview or careful telephone interview in patients who could not visit the outpatient clinic.

Measurement of Stromal Cells Derived Factor-1α Level in Plasma

Preclinical studies suggested that stromal cells derived factor-1α (SDF-1α, CXCL 12) protein expression was associated with MSC homing and that it was upregulated in the infarcted hemisphere within 24 hours and maintained for at least 1 month after stroke [16, 17]. The serum levels of expression of this protein were evaluated to determine if they were associated with the patient's response to MSC treatment. Peripheral blood samples were drawn from patients in the MSC group at the time of the first infusions of MSCs. The plasma levels of the SDF-1α were then determined utilizing enzyme-linked immunosorbent assays (R&D Systems Inc., Minneapolis, MN, http://www.rndsystems.com).

Measurement of Subventricular Zone Involvement

Preclinical and clinical studies have reported that hypoxic-ischemic insult induces activation of neurogenesis from inactive neural progenictor cells [18–20] and that MSCs promote this process [7, 11, 21, 22]. Middle cerebral artery territory infarction often involves the lateral border of the ipsilateral lateral ventricle (the subventricular zone [SVZ]), where endogenous neural progenitor/stem cells are located. Thus, we investigated the relationship between the degree of damage of SVZ and the degree of improvement in both the control and MSC groups during the follow-up period. To accomplish this, we measured the degree of involvement of the ipsilateral SVZ on the initial DWI at two axial levels: (a) the upper thalamus and head of caudate nucleus and (b) the corona radiate (7-mm-upper level). Based on the median value of the SVZ involvement, we divided patients into a "less SVZ involvement" and "more SVZ involvement" group (Fig. 5). The hippocampus, another brain region in which endogenous neural progenitor/stem cells are located, was not considered in the analysis as this structure is usually not involved in patients with middle cerebral artery territory infarction.

Statistics

Mean differences between groups were compared by a *t* test. Additionally, a Chi-square and Mann–Whitney *U* test were used to compare nonparametric values. Mortality was analyzed by a Kaplan-Meier survival curve for each group and compared by the log-rank test. Survival time was defined as the time from admission to death or the end of the study. Cox's proportional hazard models were used to investigate independent associations between mortality and the groups. Confounders included age, sex, mNIHSS score on the seventh day of admission, and initial infarct volume on DWI. Individual changes in the mRS between the seventh day of admission and the day of last evaluation were analyzed by Wilcoxon signed rank test for ordinal data and McNemar's test for binary data (good [mRS 0–3] vs. poor [mRS 4–6] functional status). The correlation between SDF-1α and clinical outcomes, such as mRS and the Barthel index (BI), was analyzed by Spearman's correlation analysis. The correlation between the degree of SVZ involvement and the degree of improvement (the reduction in the mRS between the seventh day admission and last evaluation) was analyzed by the Wilcoxon signed rank test. A $p < .05$ was taken to indicate statistical significance. All statistical analyses were conducted using SPSS 15.0 (Chicago, IL).

Results

Baseline Characteristics of Patients

Of 85 patients with acute ischemic stroke initially randomized, 52 were finally included in the study; 11 patients died early or underwent hopeless discharge within 4 weeks after randomization (before the first boosting of MSCs); and 22

Table 1. Baseline characteristics of patients

	Control group ($n = 36$)	MSC group ($n = 16$)	p
Age	64.9 ± 14.5	64.0 ± 11.6	.819
Male gender	26 (72.2)	8 (50.0)	.120
Hypertension	21 (58.3)	11 (68.8)	.476
Diabetes	11 (30.6)	4 (25.0)	.683
Cardiac problems	14 (38.9)	8 (50.0)	.454
Smoking	18 (50.0)	6 (37.5)	.404
Dyslipidemia	8 (22.2)	1 (6.3)	.160
Prior cerebrovascular events	4 (11.1)	2 (12.5)	.885
Stroke subtype			.906
Atherosclerosis	17 (47.2)	7 (43.8)	
Cardioembolic origin	12 (33.3)	6 (37.5)	
Others	1 (2.8)	0 (0)	
Unknown	6 (16.7)	3 (18.8)	
mNIHSS on 7th admission day	10.17 ± 3.67	10.63 ± 3.03	.669
mRS on 7th admission day	4.4 ± 0.9	4.8 ± 0.5	.142
Left hemisphere lesion	24 (66.7)	9 (56.3)	.645
Intrainfarct hemorrhage	1 (2.7)	2 (12.5)	—[a]
Infarct volume (ml)	90.1 ± 86.8	115.7 ± 95.2	.358
tPA application	12 (33.3)	6 (37.5)	.771
Craniectomy	1 (2.7)	2 (12.5)	—[a]
Rehabilitation[a]	14 (38.9)	6 (37.5)	.924
Mean follow-up period (wk)	110.3 ± 87.4	129.6 ± 91.8	.364

Values in parentheses represent percentage.
[a]Conventional rehabilitation that include neurodevelopmental therapy for more than 2 weeks.

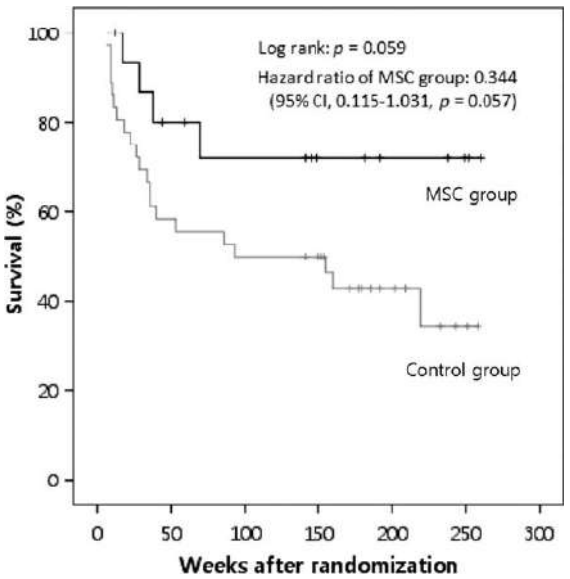

Figure 2. Kaplan-Meier survival curve showing that mortality is lower in the MSC group than in the control group. Abbreviation: MSC, mesenchymal stem cell.

were unwilling to participate. Sixteen patients received i.v. autologous MSC transplantation (MSC group) and 36 did not (control group).

The baseline characteristics are summarized in Table 1. The age at onset, gender, vascular risk factors, etiology, and initial clinical indices did not differ significantly among groups. The mean mNIHSS score and distribution of mRS on the seventh day of admission did not differ among groups.

Mortality of Any Causes and Serious Adverse Effects

After patient follow-up for an average of 117.8 weeks (control group: 110.3 ± 87.4 weeks and MSC group: 129.6 ± 91.8 weeks), 21 patients in the control group and four patients of the MSC group were dead, and the cumulative surviving proportion at 260 weeks after randomization was 0.34 and 0.72 in the control group and the MSC group, respectively (log-rank test: $p = .058$; Fig. 2). Hazard ratios were calculated with the Cox hazard proportional model, which was adjusted for baseline age, sex, mNIHSS score on the seventh admission day, and initial stroke volume on DWI. The hazard ratio in the MSC group was 0.344 when compared with the control group (95% CI, 0.115–1.031; $p = .057$).

None of the patients in the MSC group had malignant tumor. In one patient in the MSC group, a small mass was observed on the lateral malleolus of the left ankle 18 months after MSC transplantation. This mass was removed by excision and shown to be eccrine poroma, which is a benign tumor. Follow-up brain MRI including gadolinium-enhancement was performed in all patients after 12 months of MSC treatment, and the results revealed no structural changes (including tumor formation) relative to baseline. Five patients developed seizure after stroke in the control group, whereas this was observed in three patients in the MSC group. Newly diagnosed arrhythmia during the follow-up period was not observed in any patients.

Recurrent vascular episode was observed in three patients in the control group (one with ischemic stroke and two with coronary heart disease), and in four patients in the MSC group (two with ischemic stroke, one with coronary heart disease, and one with peripheral artery occlusive disease). Among patients in the MSC group, one had recurrent ischemic stroke 7 months after the index stroke. This patient could not take anticoagulants even though she had atrial fibrillation because her stroke was accompanied by hemorrhage after use of tissue plasminogen activator. Another patient who had right cerebral infarct and was subsequently able to walk independently with a cane complained of right leg weakness at approximately 4 years after MSC treatment. These new symptoms lasted for 10 days and improved after hydration during hospitalization. However, no new lesion was observed upon brain MRI.

Table 2. New onset morbidities

Disease entities	Control group ($n = 36$)	MSC group ($n = 16$)
Early reactions		
Vascular obstruction		
Recurrent stroke[a]	1	2[b]
Myocardial infarction or angina[a]	2	1[b]
Peripheral artery occlusive disease	0	1[b]
Local complications	0	0
Systemic complications		
Infection (pneumonia, urinary tract infection)[a]	9	3
Acute renal failure[a]	1	0
Liver enzyme elevation	2	1
Allergic reactions	0	0
Long-term adverse effects		
Tumor formation		
Systemic cancer[a]	1	0
Brain tumor	0	0
Mass, benign	1	1[c]
Aberrant connections		
Seizure	5	3
Arrhythmia	0	0
Neuropyschological illness[d]	7	6

[a]Major causes of death.
[b]No relationship between the onset of recurrent stroke and time of administration of mesenchymal stem cells.
[c]Eccrine poroma.
[d]Including newly developed cognitive impairments, depression, and psychiatric features.

Zoonoses and Other Adverse Effects

Depressive mood and psychiatric features were observed in both the control and the MSC group. However, none of the patients in the MSC group showed myoclonus, rapidly progressing dementia, or ataxia, suggesting a prion disease.

Other possible adverse effects or comorbidities of the control and MSC group were evaluated, but no differences were observed in the frequencies of newly onset morbidity between groups (Table 2). No new neurological deficits were immediately identified after MSC treatment. One patient had a fever when MSCs were infused and therefore did not receive a second booster. There was no evidence of septicemia in this patient, but urinary tract infection was found.

Functional Outcomes

Observer-blinded evaluation of mRS was performed separately at a median of 3.5 years after stroke in the control group (interquartile range, 3.0–4.5 years; full range, 2.7–4.9 years) and 3.2 years after stroke in the MSC group (interquartile range, 1.5–4.7 years; full range, 0.3–5.0 years; Fig. 3). Within-group comparison was performed using the Wilcoxon signed rank test, which took mRS at the seventh admission day from the score at the last evaluation in each group. In the control group, 13 of 36 patients had a negative rank, which indicates an improved functional outcome for each patient, whereas 21 patients had a positive rank (i.e., a worsened outcome; $z = -0.412$, $p = .681$). In the MSC group, 11 of 16 patients had a negative rank, whereas only four patients had a positive rank. There tended to be more patients with improved outcome than with worsened outcome in the MSC group ($z = -1.955$, $p = .051$). Additionally, the proportion of patients with mRS 0–3 increased in the MSC group (McNemar test, $p = .046$), but not in the control group ($p = .257$).

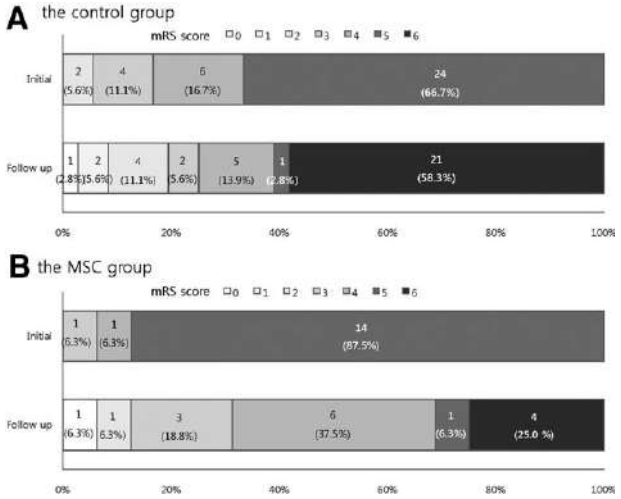

Figure 3. Proportion of patients in the control and MSC group according to the mRS at day 7 of admission and last evaluation (median, 3.5 years; interquartile range, 2.9–4.6 years). Abbreviations: mRS, modified Rankin Scale; MSC, mesenchymal stem cell.

Factors Associated With the Response to MSC Treatment: Biomarker and Neuroimaging Findings

Correlation of biomarker and functional outcomes was verified by the SDF-1α level in patients within the MSC group ($n = 9$). Serum levels of SDF-1α at the time of MSC treatment were significantly correlated with both mRS and BI at 1 year after MSC treatment (Fig. 4). Spearman's correlation analysis revealed that the SDF-1α level was positively correlated with BI and negatively correlated with the mRS score (SDF-1α vs. BI, $r = 0.882$, $p = .002$; SDF-1α vs. mRS, $r = -0.814$, $p = .008$).

DWI data showed that the degree of SVZ involvement was varied among patients (median, 0.285; interquartile range, 0.098–0.485; and full range, 0–1). Functional improvement was consistently observed in the MSC group, especially when the SVZ was less infarcted (Wilcoxon signed rank test, $n = 5$, $z = -2.060$, $p = .039$ for MSC-less group; Fig. 5D). However, functional improvement was not consistently observed in other groups (Wilcoxon signed rank test, $n = 11$, $z = -0.942$, $p = .346$ for MSC-more group; $n = 21$, $z = -0.350$, $p = .726$ for control-less group; and $n = 15$, $z = -0.263$, $p = .793$ for control-more group). The infarct volume upon initial MRI was not different among groups ($p = .430$).

Discussion

Our preliminary short-term follow-up data revealed that i.v. application of autologous MSCs is a feasible and safe therapy that may improve functional recovery in stroke patients [4]. However, a long-term follow-up study is necessary for the following reasons. First, there have been emerging concerns regarding the use of xenogeneic FBS during ex vivo cultivation of MSCs [13, 23, 24]. In addition to the risk of zoonoses, Spees et al. raised the possibility that repeated administration of MSCs contaminated by xenogeneic proteins leads to a risk of immunologic rejection of the injected cells and may also lead to more serious complications such as autoimmune reactions against one's own stem cells [13]. Second, our preliminary data showed that functional improvement occurred shortly after

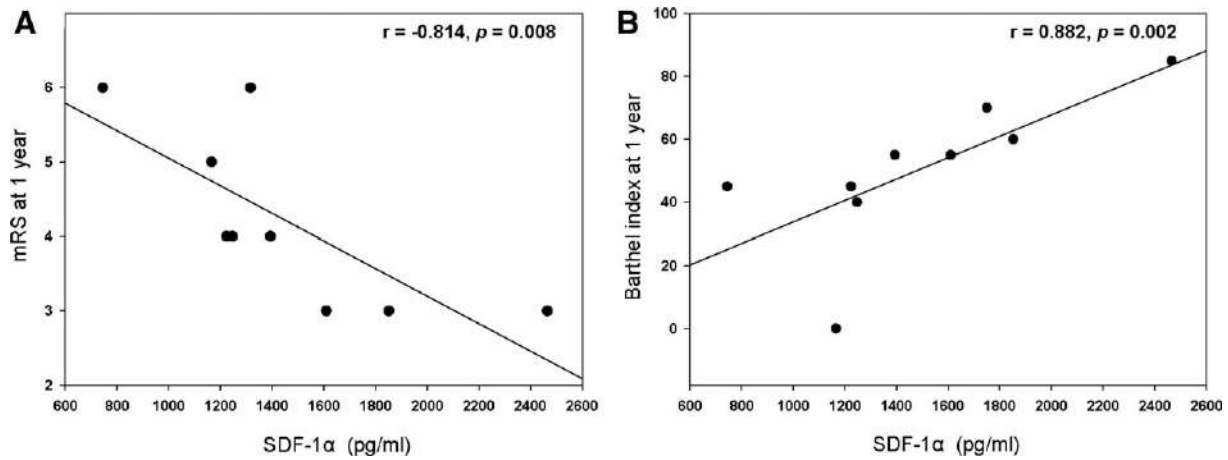

Figure 4. Correlation between plasma SDF-1α level and clinical outcomes; **(A)** mRS score and **(B)** Barthel index. Abbreviations: mRS, modified Rankin Scale; SDF-1α, stromal cells derived factor-1α.

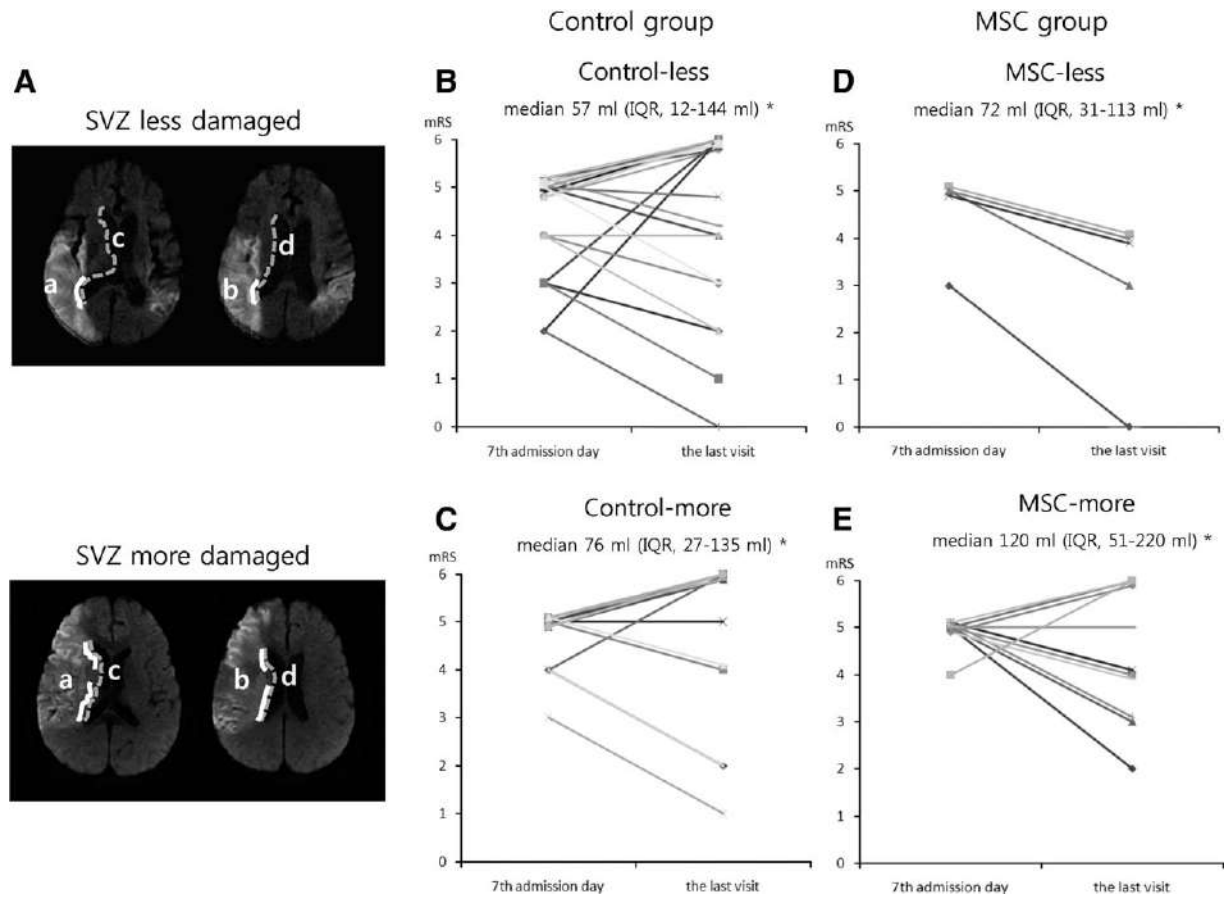

Figure 5. Association of intactness of the SVZ with effects of MSC transplantation. **(A)**: Examples of diffusion-weighted images of patients with the more and less SVZ involvement. The SVZ involvement = [length of infarcted SVZ ($a + b$)/total length of SVZ ($b + d$)]. Only the ipsilateral side was considered. **(B–E)**: The changes in the mRS scores according to the involvement of the SVZ. Improvement in functional outcome was consistently observed only in the MSC group when the SVZ was less damaged **(D)**. There was no significant change of mRS in other groups (**B**, **C**, and **E**). The symbol "*" indicates the diffusion-weighted imaging lesion volume upon admission was not different among groups. Abbreviations: IQR, interquartile range; MSC, mesenchymal stem cell; SVZ, subventricular zone.

cell therapy and diminished with time [4]. Thus, it is necessary to evaluate the long-term functional outcome to clarify the possible beneficial effects of MSC transplantation.

The results of the present study showed that i.v. application of ex vivo culture expanded MSCs is safe based on 5 years of follow-up. The mortality rate in the MSC group was lower than that in the control group, and there was no difference in comorbidities during the follow-up period. None of the patients in the MSC group showed typical features of zoonoses. Although additional follow-up of our patients may be needed, bovine spongiform encephalopathy has a relatively short latent period when compared with Creutzfeldt-Jakcob disease [25]. We have

also evaluated other serious adverse effects that may be associated with MSC therapy. There has been concern about arrhythmia following bone marrow transplantation. Supraventricular tachyarrhythmia was reported in patients who received hematopoietic stem cell transplantation [26]. Similarly, seizure may be caused by aberrant innervation from newly formed neural circuits. In addition, vascular occlusion can be caused by MSCs at the time of infusion via occlusion of the arteries of the brain or other organs [27] or by restenosis [28]. In the present study, the frequency of arrhythmia or seizure by the formation of aberrant pathway or vascular events did not different between groups.

Our data demonstrated long-term beneficial effects of MSC therapy in terms of functional outcome and survival. Specifically, functional recovery was more frequently observed in the MSC group than in the control group. Differences in the mortality rate may reflect these beneficial effects of MSC therapy. Besides the short-lasting effects that occurred via trophic support of MSCs, a long-lasting effect of MSCs could also be expected [9, 10]. Enhancing neurogenesis can be a candidate explanation of the therapeutic mechanism of MSCs. Preclinical studies showed the importance of neurogenesis in an animal model of stroke and transplanted MSCs might enhance this process [7, 11, 21, 22]. Stroke-induced neurogenesis was reported to continue for up to 1 year and to occur in aged brains [19, 29]. This study compared the response of patients with damaged neurogenesis systems to MSC transplantation to that of patients with intact neurogenesis systems. To accomplish this, we indirectly estimated the association between MSC transplantation and the enhancement of neurogenesis as demonstration of neurogenesis (such as brain pathology with bromodeoxyuridine incorporation) is not feasible in clinical trials. Our neuroimaging analysis data revealed that ameliorating changes of mRS appeared when the SVZ was less involved. In other words, the effects of MSCs were unpredictable when the SVZ was severely damaged. This feature was not confounded by the infarct volume as there was no difference among groups. Because of the experimental nature of the treatment, this study was conducted on severely disabled patients who often had severe damage to the periventricular areas.

In the present study, clinical improvement was correlated with the plasma SDF-1α level at the time of MSC treatment. Previous preclinical studies revealed that the migration of transplanted MSCs into the infarcted area was associated with the SDF-1α level [16, 30, 31]. Moreover, SDF-1α/CXC chemokine receptor-4 (CXCR4) axis mediated the migration of neural progenitor cells after stroke [32]. Circulating SDF-1α may be sequestered within the vessel wall or infarcted area in patients with myocardial infarct or ischemic stroke [33], which may increase the local concentration of SDF-1α. These findings suggest that the SDF-1α level of early-stage stroke play an important role in functional recovery after MSC treatment. Thus, serum SDF-1α levels may be useful for screening candidate patients who are likely to have favorable outcome after MSC treatment. Our data raise the possible importance of selection of good candidate patients for cell therapy. Nevertheless, our data are not conclusive because of the small sample size, and they do not allow elucidation of the direct cause and effect relationships between SDF-1α levels and MSC effects. In addition, SDF-1α levels were not measured in the control group and serial measurement was not performed in the MSC group.

Limitations and Conclusions

Our results showed the long-term safety and possible beneficial effects of autologous MSCs transplantation. However, several limitations should be mentioned. First, owing to the experimental nature of treatment, relatively small numbers of MSC cases were included in this study. We stopped enrolling patients but continued follow-up due to recent concerns regarding the risk of the use of xenogeneic serum during ex vivo cultivation of MSCs. Second, this study was not double-blinded; therefore, placebo effects cannot be excluded. We could not perform bone marrow aspiration in the control group. However, observer-blinded measurement of functional outcome was performed, and patients were randomized so that most baseline characteristics were similar between groups. It has been debated whether double-blinded controlled study is necessary for evaluation of the efficacy of empirical treatment in human trials [34, 35].

Finally, further studies are needed to enhance the beneficial effects of MSC transplantation. In our study, although MSCs were administered twice with divided doses (5×10^7 cells) to shorten the time interval between randomization and the first boosting of MSCs, ex vivo cultivation took approximately 4 weeks. The time interval between the onset of stroke and the time of cell therapy may be important in terms of the efficacy of cell therapy and thus should be shortened. Earlier stem cell transplantation was associated with better motor tasks in an animal study [36]. In addition, the functional effects of MSCs including the neurogeneic potency by activating the endogeneous repair system may be decreased after ex vivo cultivation [11].

Great potential for improving the therapeutic efficacy and safety associated with MSCs transplantation exists with further preclinical and clinical trials. Recently, there have been various efforts to enhance the therapeutic beneficial effects of stem cells (including blood–brain barrier manipulation, ischemic preconditioning, and genetically modified MSCs) [37–40] and to reduce possible adverse effects of MSCs (use of culture media other than xenogeneic serum) [23, 41–43]. We hope the therapeutic effects and safety of MSCs will be improved with these efforts and suggest that the study protocol should be revised consequently.

Appendix

Stem cell Application Researches and Trials In NeuroloGy (STARTING) collaborators: Jin Soo Lee, MD; Ji Man Hong, MD, PhD; Joon Young Choi, MD; James Park; Gwang Lee, PhD; Young Hwan Ahn, MD, PhD; Phil Hyu Lee, MD, PhD; Yoon Mi Kang, MS; Hyun Soo Kim, MD, PhD; Wen Yu Li, MD, PhD; Mi Ae Lee, RN; Sook Young Woo, PhD; Gyeong Joon Moon, PhD; Oh Young Bang, MD, PhD.

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Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to report.

REFERENCES

1 Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. Nature 2006;441:1094–1096.
2 Kondziolka D, Wechsler L, Goldstein S et al. Transplantation of cultured human neuronal cells for patients with stroke. Neurology 2000; 55:565–569.
3 Savitz SI, Dinsmore J, Wu J et al. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: A preliminary safety and feasibility study. Cerebrovasc Dis 2005;20:101–107.
4 Bang OY, Lee JS, Lee PH et al. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol 2005;57:874–882.
5 Sanchez-Ramos J, Song S, Cardozo-Pelaez F et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 2000; 164:247–256.
6 Weimann JM, Charlton CA, Brazelton TR et al. Contribution of transplanted bone marrow cells to purkinje neurons in human adult brains. Proc Natl Acad Sci USA 2003;100:2088–2093.
7 Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. Lancet Neurol 2002;1:92–100.
8 Savitz SI, Rosenbaum DM, Dinsmore JH et al. Cell transplantation for stroke. Ann Neurol 2002;52:266–275.
9 Shen LH, Li Y, Chen J et al. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. Stroke 2007;38:2150–2156.
10 Liu Z, Li Y, Zhang X et al. Contralesional axonal remodeling of the corticospinal system in adult rats after stroke and bone marrow stromal cell treatment. Stroke 2008;39:2571–2577.
11 Li WY, Choi YJ, Lee PH et al. Mesenchymal stem cells for ischemic stroke: Changes in effects after ex vivo culturing. Cell Transplant 2008;17:1045–1059.
12 Paik MJ, Li WY, Ahn YH et al. The free fatty acid metabolome in cerebral ischemia following human mesenchymal stem cell transplantation in rats. Clin Chim Acta 2009;402:25–30.
13 Spees JL, Gregory CA, Singh H et al. Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. Mol Ther 2004;9:747–756.
14 Meyer BC, Hemmen TM, Jackson CM et al. Modified national institutes of health stroke scale for use in stroke clinical trials: Prospective reliability and validity. Stroke 2002;33:1261–1266.
15 Bang OY, Park HY, Yoon JH et al. Predicting long-term outcome after subacute stroke within the middle cerebral artery territory. J Clin Neurol 2005;1:148–158.
16 Hill WD, Hess DC, Martin-Studdard A et al. Sdf-1 (cxcl12) is upregulated in the ischemic penumbra following stroke: Association with bone marrow cell homing to injury. J Neuropathol Exp Neurol 2004; 63:84–96.
17 Shen LH, Li Y, Chen J et al. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab 2007;27:6–13.
18 Zhang R, Zhang Z, Wang L et al. Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats. J Cereb Blood Flow Metab 2004; 24:441–448.
19 Kokaia Z, Thored P, Arvidsson A et al. Regulation of stroke-induced neurogenesis in adult brain–recent scientific progress. Cereb Cortex 2006;16(Suppl 1):i162–1167.
20 Jin K, Wang X, Xie L et al. Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci USA 2006;103:13198–13202.
21 Abrahams JM, Gokhan S, Flamm ES et al. De novo neurogenesis and acute stroke: Are exogenous stem cells really necessary? Neurosurgery 2004;54:150–155, discussion 155–156.
22 Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat Med 2004;10Suppl:S42–50.
23 Shahdadfar A, Fronsdal K, Haug T et al. In vitro expansion of human mesenchymal stem cells: Choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cells 2005;23:1357–1366.
24 Cobo F, Talavera P, Concha A. Diagnostic approaches for viruses and prions in stem cell banks. Virology 2006;347:1–10.
25 Will RG, Ironside JW, Zeidler M et al. A new variant of creutzfeldt-jakob disease in the UK. Lancet 1996;347:921–925.
26 Hidalgo JD, Krone R, Rich MW et al. Supraventricular tachyarrhythmias after hematopoietic stem cell transplantation: Incidence, risk factors and outcomes. Bone Marrow Transplant 2004;34:615–619.
27 Lee PH, Kim JW, Bang OY et al. Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin Pharmacol Ther 2008;83:723–730.
28 Kang HJ, Kim HS, Zhang SY et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: The magic cell randomised clinical trial. Lancet 2004;363:751–756.
29 Darsalia V, Heldmann U, Lindvall O et al. Stroke-induced neurogenesis in aged brain. Stroke 2005;36:1790–1795.
30 Ji JF, He BP, Dheen ST et al. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. Stem Cells 2004;22:415–427.
31 Wang Y, Deng Y, Zhou GQ. Sdf-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. Brain Res 2008;1195:104–112.
32 Robin AM, Zhang ZG, Wang L et al. Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. J Cereb Blood Flow Metab 2006;26:125–134.
33 Kuo LT, Chen SJ, Cherng WJ et al. Late reperfusion of a totally occluded infarct-related artery increases granulocyte-colony stimulation factor and reduces stroma-derived factor-1alpha blood levels in patients with ongoing ischemia after acute myocardial infarction. Int Heart J 2009;50:433–444.
34 Freeman TB, Vawter DE, Leaverton PE et al. Use of placebo surgery in controlled trials of a cellular-based therapy for parkinson's disease. N Engl J Med 1999;341:988–992.
35 Macklin R. The ethical problems with sham surgery in clinical research. N Engl J Med 1999;341:992–996.
36 Deacon TW, Pakzaban P, Burns LH et al. Cytoarchitectonic development, axon-glia relationships, and long distance axon growth of porcine striatal xenografts in rats. Exp Neurol 1994;130:151–167.
37 Borlongan CV, Hadman M, Sanberg CD et al. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke 2004;35: 2385–2389.
38 Kurozumi K, Nakamura K, Tamiya T et al. Bdnf gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther 2004; 9:189–197.
39 Grayson WL, Zhao F, Bunnell B et al. Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells. Biochem Biophys Res Commun 2007;358:948–953.
40 Pasha Z, Wang Y, Sheikh R et al. Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. Cardiovasc Res 2008;77:134–142.
41 Stute N, Holtz K, Bubenheim M et al. Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. Exp Hematol 2004;32:1212–1225.
42 Gregory CA, Reyes E, Whitney MJ et al. Enhanced engraftment of mesenchymal stem cells in a cutaneous wound model by culture in allogenic species-specific serum and administration in fibrin constructs. Stem Cells 2006;24:2232–2243.
43 Mannello F, Tonti GA. Concise review: No breakthroughs for human mesenchymal and embryonic stem cell culture: Conditioned medium, feeder layer, or feeder-free; medium with fetal calf serum, human serum, or enriched plasma; serum-free, serum replacement nonconditioned medium, or ad hoc formula? All that glitters is not gold! Stem Cells 2007;25:1603–1609.