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RAPID COMMUNICATION

Safety of Intravenous Autologous Bone Marrow-Derived Mesenchymal Cell Transplantation in 5 Patients With Reduced Left Ventricular Ejection Fraction

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Background: Although intracardiac injection or intracoronary delivery of mesenchymal stem cells (MSCs) has been reported, there have been few studies on the intravenous injection of MSCs, particularly in Japan.

Methods and Results: Five patients with left ventricular ejection fraction (LVEF) ≤45% received 1.0×10^8 MSCs intravenously. The procedure did not induce significant changes in vital signs. One patient had an elevated body temperature after 1 day, but recovered spontaneously. Laboratory tests remained normal for 1 month after cell delivery. Computed tomography was performed after 1–2 years, and there was no evidence of malignancy.

Conclusions: In this pilot study of patients with reduced LVEF, intravenous MSC delivery had no adverse effects.

Key Words: Heart failure; Mesenchymal cell; Regeneration therapy

Heart failure is a life-threatening progressive disease, and although optimal pharmacological and non-pharmacological therapies are recommended in clinical guidelines, the prognosis of heart failure remains unacceptably poor. Regeneration therapy is a promising option in this regard. Mesenchymal stem cells (MSCs), cardiac progenitor cells, bone marrow mononuclear cells, skeletal myoblasts, and the recently described induced pluripotent stem cells are candidate cells for transplantation.[1–5] Cell delivery routes vary and may entail transendocardial or intramyocardial heart injection, transcoronary injection, cell sheets, and intravenous injections. However, no consensus has been reached regarding cell types and methods of cell delivery.

Not only do MSCs have the ability to self-replicate and the potential to differentiate into cardiomyocytes, but they also have complicated biological effects, including (but not limited to) paracrine anti-inflammatory effects, antifibrotic effects, and neovascularization.[6,7] Nagaya et al reported that, in a rat model of acute myocardial infarction, intravenous injection of bone marrow-derived MSCs markedly reduced the size of the myocardial infarct and improved left ventricular (LV) function,[8] whereas in a rat model of cardiomyopathy, intramyocardial injection of MSCs improved cardiac function.[9] This group also reported on the safety of intracardiac injection of autologous MSCs in human chronic heart failure.[10] A recent meta-analysis showed that MSC therapy with various delivery routes improved LV ejection fraction (LVEF) in patients with heart failure.[2,3] However, there are few reports on the intravenous delivery of MSCs in humans.[11–13]

Although intravenous cell delivery is easy and non-invasive, concerns include fatal arrhythmias, shock, infections, or thrombotic disorders. Although a meta-analysis of MSC administration to patients with various diseases has not shown serious side effects,[1] MSC administration sometimes induces fever, which may trigger acute exacerbation of heart failure. Moreover, the safe cell dose for Japanese patients is unknown. To the best of our knowledge, this report is the first of the intravenous administration of MSCs to Japanese patients with chronic heart failure.

Methods

Patients

This pilot study enrolled patients with reduced LVEF. Patients were eligible for inclusion in the study if they were ambulatory with an LVEF ≤45% on baseline echocardiog-

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raphy, were aged ≥20 years, had B-type natriuretic peptide (BNP) concentrations >50 pg/mL and had New York Heart Association (NYHA) Class II/III symptoms at screening. Baseline computed tomography (CT) did not show any tumors in the chest or abdominal cavity in any of the patients. Patients had been receiving stable, tolerated, guideline-directed medical therapy for ≥6 months prior to the MSC isolation procedure.

Patients were excluded from the study if they had had cardiovascular events or had undergone surgery or invasive catheter therapy 6 months prior to cell isolation and if they had preoperative severe valvular, pulmonary, hepatic or renal failure, and a history of malignant disease. For the safety of cell incubation, patients who had previously been infected with hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and syphilis were also excluded from the study.

Cell Isolation and Incubation

Bone marrow aspirate was obtained from the iliac crest of the patient to accumulate MSCs. The procedure was performed in a clean operating room under local anesthesia with lidocaine.

Under Good Gene, Cellular, and Tissue-based Products Manufacturing practices, MSCs were expanded ex vivo in a cell-processing center (Japan Tissue Engineering Co. Ltd, Gamagori, Japan).

Approximately 40 mL of the donor-derived bone marrow was used for the culture expansion of MSCs in flasks containing culture medium (α-minimum essential medium with 15% fetal bovine serum and 20 μg/mL gentamicin, supplemented with 10 ng/mL Basic fibroblast growth factor). All culture steps were performed at +37°C and in humidified atmospheric oxygen with 5% CO_2. The adherent cells became nearly confluent after 11–14 days and were passaged using a trypsin-like enzyme solution into another flask. After several days, the passaged cells were collected using the trypsin-like enzyme solution and used for transplantation.

As we reported previously,[14] we analyzed the surface antigens of the cultured bone marrow-derived cells using a FACSCalibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA). These cells were positive for CD13, CD44, and CD90, and negative for CD34, CD45, and HLA-DR, which is indicative of MSCs.

MSCs (1.0×10^8 cells) were administered intravenously to patients over a period of 60 min, and patients stayed at least overnight in hospital.

Study Endpoints

The primary endpoint was safety assessment. Patients were placed on electrocardiographic monitoring and monitored for vital signs (blood pressure, heart rate, and oxygen saturation) for 1 h during cell delivery. Routine laboratory blood tests (blood cell counts, aspartate aminotransferase, alanine aminotransferase, and creatinine) were performed on Day 0 (day of transplantation), Day 1, and then after 1 and 6 months, with a change of >50% from baseline considered significant. CT was performed 1–2 years after the procedure to address the safety concerns regarding the induction of malignant disease.

The secondary endpoint was long-term efficacy. To assess cardiac effects, we evaluated LVEF and LV volume using echocardiography with the modified Simpson's method before and then 6 months after the procedure. We also measured plasma BNP, serum cardiac troponin I, and peak oxygen uptake (peak $\dot{V}O_2$) on cardiopulmonary exercise testing as surrogate markers of heart failure. Non-cardiac effects evaluated were body composition (bioelectrical impedance data were obtained using an InBody 720-Biospace [Tokyo, Japan]) and pulse wave velocity (PWV).

Table 1. Clinical Characteristics

Age (years)	70.0±4.0
Body mass index (kg/m^2)	21.4±1.2
No. males/females	5/0
LVEF (%)	29.2±7.6
Etiology of heart failure	
Ischemic disease	2 (40)
Postoperative valve disease	1 (20)
Dilated cardiomyopathy	2 (40)
Medications	
β-blockers	5 (100)
ACEI/ARB	3 (60)
Aldosterone antagonist	4 (80)
Loop diuretic	5 (100)

Data are presented as the mean±SD or as n (%). ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker; LVEF, left ventricular ejection fraction.

Ethical Considerations

All study procedures were performed in compliance with the principles outlined in the Declaration of Helsinki and the institutional guidelines of Hyogo Prefectural Amagasaki General Medical Center. The study was registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (ID: 000025233) and was approved by the Ethics Committee of the Hyogo Prefectural Amagasaki General Medical Center (27-49) and Gamagori Municipal Hospital Specified Certified Regenerative Medicine Committee (356). Written informed consent was obtained from all patients, and the clinical study was conducted in compliance with the guidelines of the Ministry of Health, Labour and Welfare of Japan.

Statistical Analysis

Data are expressed as the mean±SD. Normally distributed continuous variables were analyzed using t-tests, whereas continuous variables with a skewed distribution were analyzed using the Mann-Whitney U test. Categorical variables are presented as numbers or percentages. Statistical analyses were performed using JMP® version 10.0 (SAS Institute, Cary, NC, USA).

Results

Patient Enrollment

The clinical study was initiated in January 2017, and 5 patients (**Table 1**) were enrolled for the intravenous administration of 1.0×10^8 MSCs. Patients received stable, tolerated, guideline-directed medical therapy for ≥6 months after MSC delivery.

Safety of Cell Delivery

Blood pressure, heart rate, and oxygen saturation remained stable (**Table 2A**), and there were no new-onset arrhyth-

Table 2. (A) Vital Signs Before and After Cell Delivery, (B) Results of Routine Laboratory Tests

A	Baseline	End of cell infusion	P value	
Systolic blood pressure (mmHg)	115.4±22.5	107.2±12.6	0.92	
Diastolic blood pressure (mmHg)	74.4±8.0	74.2±8.5	0.56	
Heart rate (beats/min)	73.8±7.6	70.2±7.3	0.98	
SpO_2 (%)	97.8±0.8	97.8±0.4	0.50	
B	**Day 0 (cell delivery)**	**1 day**	**1 month**	**6 months**
White blood cells (/μL)	4,860±545	5,120±756	5,460±740	5,260±1,031
Red blood cells ($\times10^4$/μL)	419.4±53.8	400.4±67.7	427.6±56.7	411.4±63.2
Platelets ($\times10^3$/μL)	170.2±31.9	146.6±20.2	173.6±48.3	154.6±18.3
AST (U/L)	23.2±4.0	19.4±2.0	19.6±2.5	24.4±8.0
ALT (U/L)	15.6±7.4	13.0±6.7	14.8±8.1	17.8±9.0
LDH (U/L)	227.4±75.9	211.2±87.0	202.0±69.2	Not measured
Creatinine (mg/dL)	1.4±0.5	1.3±0.5	1.4±0.5	1.5±0.6

(A) Unless indicated otherwise, data are presented as the mean±SD. (B) Data are presented as the mean±SD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

Table 3. Changes in Parameters From Before to 6 Months After Cell Implantation

	Screening	6 months	P value
LVEF (%)	29.2±7.6	28.4±9.8	0.5
LVEDV (mL)	180.4±50.8	186.0±25.6	0.5
LVESV (mL)	130.4±47.5	135.0±34.2	0.5
BNP (pg/mL)	330.6±290.6	302.8±324.9	0.59
Troponin I (pg/mL)[A]	47.3±20.4	42.6±15.5	0.63
Peak $\dot{V}O_2$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	17.4±4.8	17.3±4.6	0.56
Body weight (kg)	61.2±9.9	62.5±11.0	0.22
Skeletal muscle mass	24.9±4.1	25.1±4.2	0.31
Body fat mass	15.2±4.6	16.0±4.3	0.41
Pulse wave velocity (cm/s)			
Right	1,631.6±534.6	1,431.0±276.0	0.84
Left	1,621.8±547.1	1,433.0±299.1	0.78

Unless indicated otherwise, data are presented as the mean±SD. [A]Two patients had troponin I concentrations <10 pg/mL (both before and after cell delivery). Therefore, data are shown for 3 patients. LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.

mias or shock during cell delivery. No abnormalities suggestive of hemolysis, thrombocytopenia, liver dysfunction, and renal dysfunction were found on routine laboratory tests (**Table 2B**).

Long-Term Follow-up

After 6 months of observation, no changes were observed in LVEF, LV diastolic and systolic volume on echocardiography, BNP and troponin I concentrations, and peak $\dot{V}O_2$ on the exercise test (**Table 3**; **Figure**). CT scans did not show any tumors in the chest or abdominal cavity.

Discussion

Although many human heart failure studies have been conducted worldwide with numerous types of cells, very few have been conducted in Japan, and these studies involved myoblast sheets,[4] intramuscular injection of MSCs,[10] and, recently, intravenous Muse cells.[5] Moreover, there is limited experience with the intravenous administration of cells in patients with heart failure, mainly because of safety concerns regarding the entrapment of donor cells in the pulmonary circulation and concerns regarding their therapeutic efficacy in the context of low cardiac engraftment. In this preliminary study, a single dose of intravenous autologous 1.0×10^8 MSCs had no adverse effects and was well tolerated in patients with reduced LVEF. However, the administration of MSCs had no effect on the heart failure.

MSCs

MSC regeneration studies in patients with heart failure have been designed based on the assumption that the benefits of stem cell therapy accrue only from activities derived from cells engrafted in the dysfunctional myocardium. These activities may reflect cells differentiating into cardiac myocytes, stimulating resident cardiac stem cells to expand and to form a greater number of functioning myocytes, or broad paracrine and systemic activities that allow, for example, favorable cardiac remodeling, enhancement of angiogenesis, and decreased apoptosis.[6,7] Indeed, a meta-

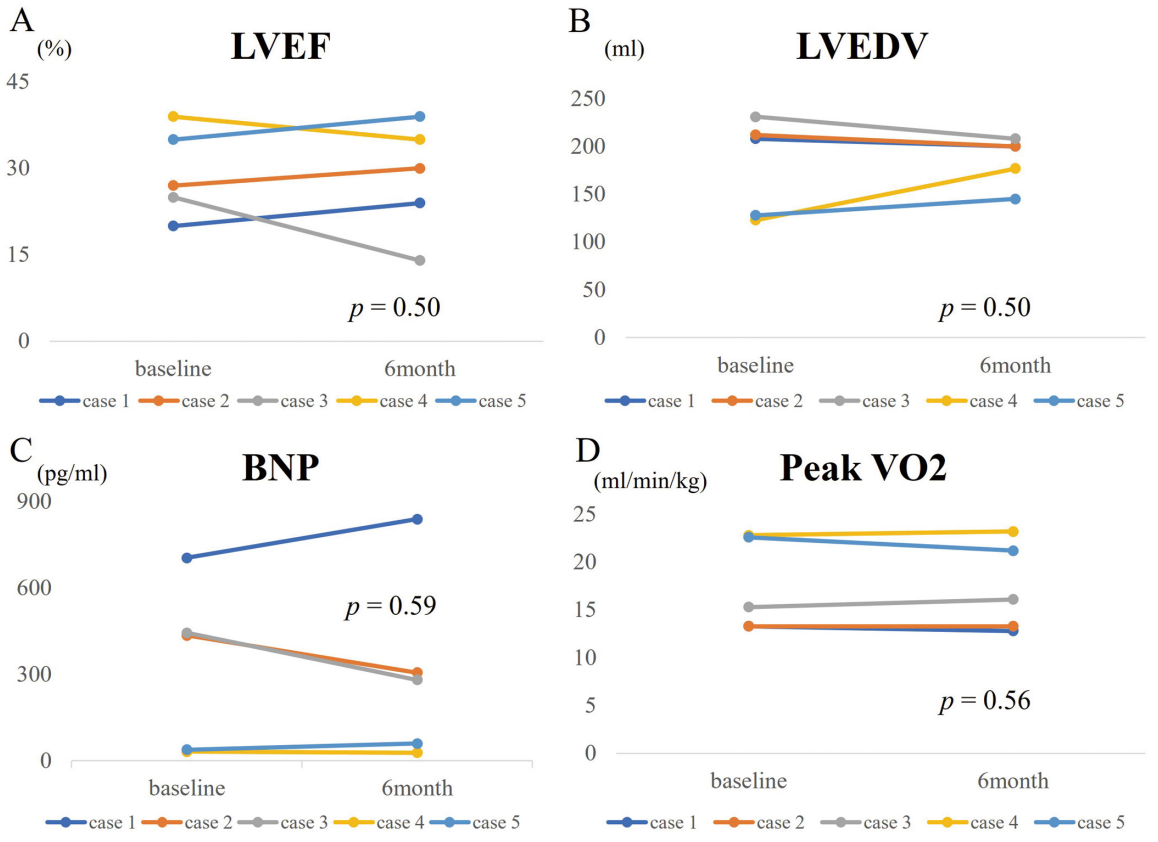

Figure. Changes in (**A**) left ventricular ejection fraction (LVEF), (**B**) left ventricular end-diastolic volume (LVEDV), (**C**) B-type natriuretic peptide (BNP), and (**D**) peak $\dot{V}O_2$ from baseline to 6 months after cell delivery.

analysis of MSC studies with various delivery routes showed an improvement in LVEF and decreased LV volume in patients with heart failure.[2,3]

There are 3 routes for cell administration: intravenous infusion, intracoronary injection, and intramyocardial injection. However, intramyocardial cell delivery may result in mechanical myocardial injury or create isolated clusters of injected cells with the potential to introduce myocardial heterogeneity and a risk of arrhythmia. Given the large cell size, intracoronary injection of cells carries a risk of coronary embolization, and ischemic electrocardiographic changes have been reported. Moreover, both procedures require a catheter or surgical technique. The advantages of intravenous administration include technical ease, less invasive nature, and lower costs. However, cell engraftment into the myocardium with intravenous delivery is potentially poor as a result of uptake by lung, hepatic, and splenic tissue. Nevertheless, animal models have shown that MSCs could be engrafted into the myocardium,[8] and there are also animal and human studies that have investigated intravenous administration of MSCs with heart failure.[8,11–13]

Safety

A recent systematic review, including various diseases, did not detect an association between MSC regeneration therapy and the risk of acute infusion toxicity, organ system complications, infection, death, or malignancy.[1] However, patients with heart failure are very vulnerable to slight changes in vital signs or condition that may lead to severe deterioration. Therefore, careful observations and safety endpoints are very important in addition to efficacy endpoints.

Hare et al performed a double-blind placebo-controlled dose-ranging (0.5, 1.6, and 5×10^6 cells/kg) intravenous allogeneic MSC trial in patients with myocardial infarction;[11] Butler et al conducted a single-blind placebo-controlled trial of intravenous allogeneic MSCs (1.5×10^6 cells/kg) for non-ischemic cardiomyopathy patients;[12] and, in RIMECARD (Randomized Clinical Trial of Intravenous Infusion Umbilical Cord Mesenchymal Stem Cells on Cardiopathy), Bartolucci et al intravenously infused umbilical cord-derived MSC (1×10^6 cells/kg) in patients with heart failure.[13] Serious side effects were not been reported in these trials, which were conducted in Western countries where the body mass index (BMI) of the patients was approximately 30 kg/m^2. In contrast, the BMI of Japanese patients is lower, so a safe dose of intravenous MSCs in Japanese patients needs to be determined.

In the present pilot study, 5 patients received 1.0×10^8 MSCs intravenously. None of the patients experienced a fatal arrhythmia, shock, infections, or clinically apparent thrombotic events, which are safety concerns associated with intravenous cell therapies, although 1 patient had a fever of up to 37.6°C that spontaneously recovered. In a

meta-analysis, Lalu et al reported a significant association between MSCs and transient fever without infection.[1] Lalu et al speculated that the mechanism could be related to an acute inflammatory reaction by a subset of patients to particular preparations of MSCs.

Long-Term Efficacy

The reason why the patients in this study did not show long-term LVEF improvements is not clear. However, there are some possible reasons. The first is recipient age. Studies raise a clinically relevant issue as to whether recipient age is a crucial factor limiting the response to cell therapy, although some investigators have reported that older recipients did not have an impaired response to MSC therapy.[15] The mean age of 70.0 years in the present study was higher than that of other published reports. Second, we used autologous MSCs rather than allogeneic MSCs. It has also been hypothesized that the function of autologous MSCs could be impaired in patients with comorbidities or advanced age. Third is cell dose. In the TRIDENT study, only a high dose of allogeneic MSCs in patients with ischemic cardiomyopathy increased ejection fraction.[16] Higher doses of cells may result in better improvement. Fourth is the appropriate LV size for cell treatment. The recently conducted CHART-1 study showed a benefit of a reduction in cardiac events in a subset of patients with advanced LV enlargement (baseline LV end-diastolic volume between 200 and 370 mL).[17] Therefore, patients with a relatively small LV size may be excluded.

Future of Intravenous MSC Therapy and Study Limitations

Although the main effects of MSCs is cardiac, MSCs may improve "non-cardiac systemic effects" independently of the patient's heart condition, perhaps mediated via anti-inflammatory properties or angiogenesis.[6,7] In addition, intravenous cell delivery may be a suitable delivery method for "repeated regeneration therapy".

A limitation of this study is the small number of patients. However, this sample size is typical of Japanese early phase studies of regeneration therapy for heart failure.[5,10] Moreover, we did not measure serum fibrinogen, D-dimer, and fibrinogen degradation products (FDP), which focus on pulmonary embolisms. However, our patients showed stable vital signs and no increases in lactate dehydrogenase (LDH) concentrations; LDH is often increased in patients with clinically significant pulmonary embolism.[18]

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Disclosures

The authors have no conflicts of interest to declare.

IRB Information

This study was approved by the Ethics Committee of the Hyogo Prefectural Amagasaki General Medical Center (27-49) and Gamagori Municipal Hospital Specified Certified Regenerative Medicine Committee (356).

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