Intramyocardial Bone Marrow Cell Injection: Clinical and Functional Effects in Ischemic Heart Disease

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Chapter 5

Effect of Intramyocardial Bone Marrow-derived Mononuclear Cell Injection on Cardiac Sympathetic Innervation in Patients with Chronic Myocardial Ischemia

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ABSTRACT

Purpose: Intramyocardial bone marrow cell injection has been associated with improvements in myocardial perfusion and left ventricular function. The current substudy of a randomized, placebo-controlled, double-blinded study, investigated the effect of intramyocardial bone marrow cell injection on myocardial sympathetic innervation in patients with chronic myocardial ischemia.

Methods: In a total of 16 patients (64±8 years, 13 men), early and late iodine-123 metaiodobenzylguanidine (MIBG) imaging was performed before and 3 months after intramyocardial bone marrow cell injection.

Results: No improvements were observed in global early H/M ratio (P=0.40), late H/M ratio (P=0.43) and cardiac washout rate (P=0.98). However, late 123-I MIBG SPECT defect score showed a trend to improvement in the bone marrow cell group (from 31.0±7.1 to 28.1±14.9) as compared to the placebo group (from 33.6±8.5 to 34.5±9.8, P=0.055 between groups). This trend was mainly driven by a substantial improvement in 3 bone marrow cell-treated patients, which all had diabetes and severe MIBG defects. In these patients, the extent and severity of MIBG defects improved substantially independent of myocardial perfusion and cell injection sites.

Conclusion: The present study does not demonstrate improvements in global cardiac sympathetic nerve innervation after intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia. However, regional analysis of sympathetic nerve innervation reveals improvements in 3 diabetic patients independent of myocardial perfusion, suggestive of a therapeutic effect on diabetic cardiac sympathetic dysinnervation.

INTRODUCTION

Bone marrow cell therapy is currently being investigated as a novel therapeutic option for patients with severe refractory angina pectoris due to chronic myocardial ischemia. Recently, 2 randomized, placebo controlled, double-blinded studies demonstrated that intramyocardial bone marrow cell injection is associated with improvements in myocardial perfusion and anginal complaints[1, 2].

In line with these clinical observations, pre-clinical studies showed that bone marrow cells promote neovascularization in ischemic myocardium[3]. More recently, experimental studies using models of diabetic neuropathy suggested that bone marrow cell transplantation may also have beneficial effects on neural function. In particular, bone marrow cells may increase neural blood flow both by incorporation in the vasa nervorum and through paracrine effects, resulting in improved nerve function[4-6].

Myocardial sympathetic innervation, as assessed by iodine-123 metaiodobenzylguanidine (MIBG) imaging, has been established as an important prognostic factor in patients with ischemic heart failure[7-10]. Furthermore, in patients with diabetes, abnormalities in cardiac sympathetic innervation have been described which are related to long-term prognosis in these patients[11, 12]. Therefore, a potential effect of bone marrow cell injection on myocardial sympathetic innervation may be of prognostic importance in patients with ischemic heart disease and/or diabetes.

To date, the effects of bone marrow cell therapy on cardiac sympathetic innervation have not been investigated in the clinical setting. The current substudy of a randomized, placebo-controlled, double-blinded study investigated whether intramyocardial bone marrow cell injection is associated with improvements in myocardial sympathetic innervation in patients with chronic myocardial ischemia.

METHODS

Patient Selection and Study Protocol

The detailed study protocol of this randomized, double-blinded, placebo-controlled trial has been earlier described[1]. In brief, patients with severe coronary artery disease were eligible for inclusion if they had refractory angina (Canadian Cardiovascular Society (CCS) class III or IV despite optimal medical therapy) and stress-inducible ischemia on

technetium-99m tetrofosmin single photon emission computed tomography (SPECT). Bone marrow was aspirated from the iliac crest in all patients. After mononuclear cell isolation, patients were randomized in a 1:1 ratio to receive intramyocardial injection of either $100x10^6$ autologous bone marrow-derived mononuclear cells or placebo solution (NaCl 0.9% with 0.5% human albumin). During cardiac catheterization, a 3-dimensional electromechanical map of the left ventricle (LV) was obtained using the NOGA system (BDS, Cordis, California, USA). This electromechanical map was visually matched with the SPECT scan as previously described[1] to identify myocardial regions with stress-inducible ischemia. Subsequently, 8-10 intramyocardial injections were targeted at these ischemic regions. The protocol was approved by the institutional ethics committee and complied with the declaration of Helsinki. The study was registered at the Dutch trial registry (www.trialregister.nl, no. NTR400/ISRCTN58194927).

At baseline, 3 and 6 months follow-up, quality of life was measured using the Seattle Angina Questionnaire, clinical status was assessed according to the CCS classification, and exercise capacity was evaluated with the use of a bicycle exercise test. Furthermore, technetium-99m tetrofosmin SPECT was performed at baseline and 3 months after injection, to assess myocardial perfusion. Left ventricular (LV) function was evaluated using magnetic resonance imaging (MRI) at baseline and 3 months follow-up. The results of these evaluations have been published previously[1].

In a subset of patients included in this trial, 123-I-metaiodobenzylguanidine (123-I MIBG) imaging was performed at baseline and 3 months follow-up. Since this technique became available during the inclusion period of the study, 123-I MIBG imaging was available for the last 16 patients of the study cohort, after approval by the institutional ethics committee of an amendment to the study protocol allowing the use of 123-I MIBG imaging. All patients provided written informed consent.

123-I MIBG imaging

Uptake of free iodine-123 by the thyroid gland was blocked by pre-treatment of patients with 120 mg of sodium iodide, which was orally administered 1 hour before intravenous injection of 185 MBq 123-I MIBG (Adreview, General Electric Healthcare, USA). 123-I MIBG planar and SPECT imaging was performed in supine position. Following acquisition of a 10 min planar image from an anterior thoracic view and stored in a 256 x 256 matrix., 10 to 15 min after tracer administration, a SPECT study (step and shoot mode, 90 projections, imaging time 30 min) was performed using a dual-head camera

system (GCA-7200, Toshiba Corp.,Tokyo, Japan) equipped with low-energy, parallel-hole, high-resolution collimators.

For SPECT studies, a 128 x 128 matrix was used, and a 15 % energy peak was centered around the 159-keV energy peak of 123-I MIBG. Planar and SPECT imaging were repeated after 3 to 4 h of tracer administration. Heart-to-mediastinum (H/M) ratio was calculated from the planar images: the regions of interest were placed over the entire heart respectively and the upper mediastinum (7 x 7 pixels)[13]. Processing of 123-I MIBG SPECT studies was performed using filtered back-projection, yielding standard long and short-axis reconstructions, perpendicular to the heart axis.

The H/M ratio was computed from planar images by dividing the mean counts per pixel within the myocardium by the mean counts per pixel within the mediastinum. The H/M ratio was calculated both for early and late planar imaging. Cardiac washout rate was calculated by dividing the difference between the early and delayed H/M ratio by the early H/M ratio and was expressed as a percentage. No background correction was performed in this study.

The early and late 123-I MIBG SPECT defect scores were calculated by evaluation of segmental 123-I MIBG tracer uptake score using the 17-segment model according to the American Heart Association/American College of Cardiology recommendations[14]. Each myocardial segment was scored according to the following tracer uptake scale: 1 = normal tracer uptake; 2 = mildly reduced tracer uptake; 3 = severely reduced tracer uptake; and 4 = no tracer uptake. Subsequently, summation of segmental tracer uptake scores yielded the 123-I MIBG SPECT defect score, ranging from 17 to 68[15]. The 123-I MIBG SPECT defect score was calculated for early and delayed SPECT imaging. Two blinded and independent observers analyzed the data. Both observers scored all study segments, and disagreements were resolved by consensus. Myocardial perfusion SPECT imagines and 123-I MIBG SPECT images were evaluated in separate sessions.

Myocardial perfusion assessed by SPECT imaging

Technetium-99m tetrofosmin SPECT imaging was performed as previously described[1] In brief, pharmacological stress consisting of adenosine infusion (0.14 mg/kg/min for 6 minutes) was used, and 500 MBq Tc-99m tetrofosmin was injected intravenously after 3.5 minutes of adenosine infusion. On the second day, resting images were obtained after a second injection of 500 MBq Tc-99m tetrofosmin. For the analysis of myocardial stress and rest perfusion, long- and short-axis projections perpendicular to the heart axis were

reconstructed. The short-axis slices were displayed in polar map format, adjusted for peak myocardial activity (100%).

Similar to the 123-I MIBG SPECT analysis, the myocardium was divided into 17 segments according to the AHA/ACC recommendations[14]. Quantitative analysis of myocardial perfusion was performed using Quantitative Gated SPECT software (QGS software, Cedars-Sinai Medical Center, Los Angeles, California) and segmental tracer activity was categorized on a 4-point scale: 1=tracer activity >75%; 2=tracer activity 50-75%; 3=tracer activity 25-50%; 4=tracer activity <25%. Perfusion defects on stress images were considered present when tracer activity was <75% of maximum. Segments with reduced perfusion on stress images were classified as ischemic when significant fill-in (>10%) of perfusion defects was observed on the resting images and as infarcted when no significant fill-in was observed on the resting images. Summed stress score was calculated by summation of the patients' segmental scores at stress and summed rest score was calculated by summation of the patients' segmental rest scores rest score (both values range 17 to 68). The 123-I MIBG/perfusion mismatch score was calculated by subtracting the rest perfusion defect score from the late 123-I MIBG SPECT defect score[15].

Exercise testing

A symptom-limited bicycle exercise test was performed with a 20 Watt starting load and 10 Watt increment per minute. A 12-lead electrocardiogram was recorded before, during, and after the test. All antianginal medication was continued and patients were encouraged to perform as much exercise as possible. Test endpoints were angina, dyspnea, physical exhaustion, or significant decrease in blood pressure (>10 mmHg). A study coordinator unaware of the patients' group assignment monitored the exercise tests.

Statistical analysis

Data are reported as mean±SD. Comparison of continuous data was performed using the Student t-test and categorical variables were compared using the chi-square test or Fischer's exact test. Repeated measures analysis of variance (ANOVA) was used to study the relation between the allocated treatment and (changes in) continuous data at baseline and 3 months follow-up. Correlations were determined using the Pearson correlation coefficient. A P-value <0.05 was considered significant. All statistical analyses were performed with SPSS software (version 16.0, SPSS).

RESULTS

The present study comprised 16 patients (64±8 years, 13 men). A total of 8 patients were randomized to the bone marrow cell group and 8 to the placebo group. The baseline characteristics of the patients are listed in Table 1. During the study period, the type and dose of medications remained unchanged. Furthermore, no significant changes in systolic blood pressure were observed, as evidenced by a mean blood pressure of 121±18 mmHg at baseline and 123±15 mmHg at 3 months in the bone marrow cell group (P=0.61), compared with 116±14 mmHg vs. 113±18 mmHg in the placebo group (P=0.70). Similarly, renal function as measured by creatinine level was stable both in the bone marrow group (83±15 μmol/L at baseline and 84±12 μmol/L after 3 months follow-up, P=0.80) and in the placebo group (80±16 μmol/L vs. 82±11 μmol/L, P=0.92).

Table 1. Baseline characteristics of the study population

	Bone marrow cell group (n=8)		Placebo group (n=8)		P-value
Age, years	64±10		63±8		0.75
Gender (Male)	7	(88%)	6	(75%%)	1.00
Cardiovascular risk factors					
Smoking	2	(25%)	2	(25%)	1.00
Hypertension	3	(38%)	3	(38%)	1.00
Diabetes	5	(63%)	3	(38%)	0.61
Dyslipidemia	2	(25%)	4	(50%)	0.61
Coronary artery disease in family	4	(50%)	2	(25%)	0.61
Current Medication					
Nitrates	7	(88%)	5	(63%)	0.57
Beta-blockers	8	(100%)	8	(100%)	1.00
Calcium channel blockers	6	(75%)	3	(38%)	0.31
Statins	8	(100%)	8	(100%)	1.00
ACE inhibitors	7	(88%)	7	(88%)	1.00
History					
Prior Myocardial infarction	3	(38%)	7	(88%)	0.12
Prior CABG	8	(100%)	5	(63%)	0.20
Prior PCI	5	(63%)	4	(50%)	1.00

All analyses were performed according to the intention-to-treat principle and complete case analysis was performed for all paired tests. Procedural data and clinical outcome data of the total study cohort have been previously reported, as well as the results of myocardial perfusion imaging and MRI[1].

Table 2 displays the results of myocardial perfusion imaging, LV function assessment by MRI, and exercise testing. The improvement in myocardial perfusion in bone-marrow cell treated patients in the current study is in line with the observations in the entire study cohort. In addition, changes in parameters of systolic function were in line with the results of the total group. In particular, LV ejection fraction showed a modest but significant increase in the bone marrow cell-treated patients, but not in placebo-treated patients. Furthermore, changes in exercise capacity were also comparable with the documented improvement in the entire study cohort, although the increase in the bone marrow cell group was not significant in this relatively small subgroup.

Global 123-I MIBG uptake parameters

Paired data of 123-I MIBG SPECT imaging were available in all 16 patients. In both the bone marrow cell group and the placebo group, the early and late H/M ratio remained unchanged at 3 months follow-up (Table 2). Consequently, no significant change in the cardiac washout rate was observed in both groups.

The early 123-I MIBG SPECT defect score remained unchanged in both groups. Interestingly, however, the late 123-I MIBG SPECT defect score tended to improve in the bone marrow cell group (from 31.0±7.1 at baseline to 28.1±14.9 after 3 months follow-up, P=0.084, Table 2). In the placebo group, the late 123-I MIBG SPECT defect score remained unchanged (33.6±8.5 vs. 34.5±9.8, P=0.44). When the 2 groups were compared, a trend towards a greater improvement in the bone marrow cell group was observed (-2.9±4.0 vs. 0.9±3.0, P=0.055). As a result, the 123-I MIBG/perfusion mismatch score showed a similar trend towards improvement of bone marrow cell-treated patients as compared to placebo-treated patients (P=0.052, Table 2).

As demonstrated in Figure 1, the trend towards an improved late 123-I MIBG SPECT defect score in the bone marrow cell group was mainly driven by a substantial decrease (≥5 points) in the late defect score in 3 patients. Of interest, all these 3 patients had diabetes (1 insulin dependent, 2 non insulin dependent) and a high late 123-I MIBG SPECT defect score at baseline.

Table 2: Global parameters of cardiac sympathetic innervation, myocardial perfusion, and systolic function

	Bone marrow cell group (n=8)				Placebo group (n=8)				P-value
	Baseline	3 months	Change	P-value	Baseline	3 months	Change	P-value	
123-I MIBG SPECT									
Early H/M ratio	1.74±0.21	1.79±0.18	0.05±0.16	0.43	1.89±0.32	1.87±0.23	-0.02±0.17	0.35	0.40
Late H/M ratio	1.71±0.28	1.71±0.25	0.00±0.17	0.98	1.83±0.32	1.77±0.27	-0.06±0.10	0.09	0.43
Cardiac washout rate (%)	1.9±12.6	4.3±22.1	2.4±7.1	0.37	3.1±10.0	5.4±4.6	2.3±7.7	0.21	0.98
Early MIBG defect score	24.5±6.4	23.6±7.6	-0.9±2.8	0.40	27.8±7.5	29.5±8.9	1.7±4.3	0.29	0.17
Late MIBG defect score	31.0±7.1	28.1±14.9	-2.9±4.0	0.084	33.6±8.5	34.5±9.8	0.9±3.0	0.44	0.055
MIBG/perfusion mismatch score	11.4±7.9	8.5±7.6	-2.9±4.0	0.084	12.9±8.0	14.4±9.6	1.5±4.2	0.34	0.052
Perfusion SPECT									
Summed Stress Score	24.0±7.0	21.2±7.5	-2.8±1.0	<0.001	24.3±5.9	22.6±6.0	-1.7±2.4	0.026	0.28
Summed Rest Score	19.6±6.6	19.6±6.6	0±0	1.00	20.3±5.3	19.7±4.7	0.6±1.4	0.12	0.26
LV volumes by MRI									
LV end-diastolic volume (ml)	166±43	171±55	5±15	0.18	179±61	173±57	-6±17	0.21	0.11
LV end-systolic volume (ml)	75±44	72±50	-3±9	0.18	92±47	87±45	-5±12	0.05	0.35
LV ejection fraction (%)	57±13	61±12	4±4	0.016	51±10	52±10	1±4	0.21	0.049
Exercise testing									
Maximal achieved workload (W)	104±22	113±32	9±14	0.21	113±34	110±29	-3±10	0.52	0.15

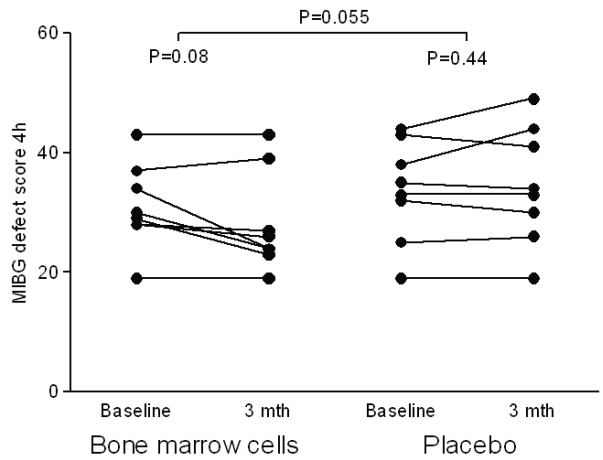

Figure 1. Late 123-I MIBG SPECT defect score in bone marrow cell-treated patients as compared to placebo-treated patients. In the bone marrow cell group, a trend to improvement was observed which was mainly determined by a substantial decrease (≥5 points) in the late defect score in 3 patients. When compared to the placebo group, there was a trend towards a greater improvement in the bone marrow cell group (-2.9±4.0 vs. 0.9±3.0, P=0.055). Square data markers with error bars are the mean (SD) percent LV ejection fraction for each group.

Segmental analysis

In bone marrow cell-treated patients, late 123-I MIBG uptake improved in 26 of 136 myocardial segments, whereas late 123-I MIBG uptake improved in 10 of 136 segments in the placebo-treated patients (P=0.007).

On a segmental level, the improvement in late 123-I MIBG uptake was not related to baseline myocardial perfusion (Δsegmental late 123-I MIBG vs. baseline segmental stress score R=0.01, P=0.90), improvements in myocardial perfusion (Δsegmental late 123-I MIBG uptake vs. Δsegmental stress score R=0.05, P=0.42) or the cell injection sites (injected vs. non-injected segments, p=0.80). Interestingly, it must be noted that 18 of 26 segments with improved late 123-I MIBG uptake were present in the abovementioned 3 diabetic patients with a high late 123-I MIBG SPECT defect score. Figure 2 shows an example of a diabetic patient in which late regional 123-I MIBG uptake in the inferior wall increased substantially 3 months after bone marrow cell injection, independent of myocardial perfusion.

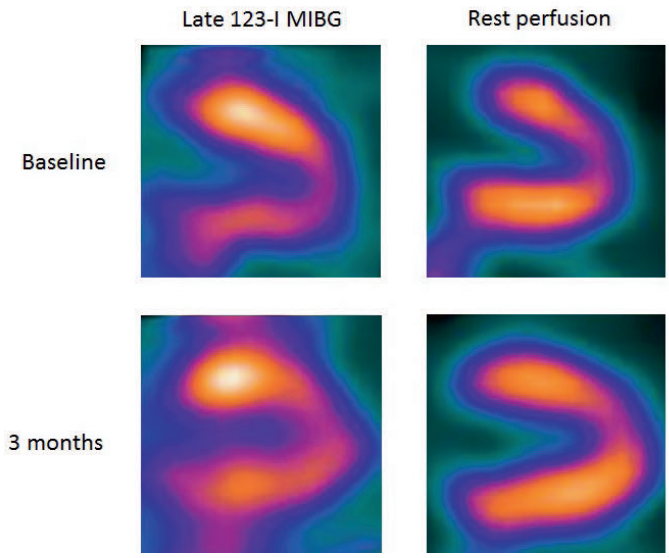

Figure 2. An example of a diabetic patient with decreased sympathetic nerve activity in the inferior wall, in the absence of perfusion abnormalities in that region. After bone marrow cell injection, marked improvement of late 123-I MIBG uptake is observed in the inferior wall, whereas myocardial perfusion remains normal.

DISCUSSION

The aim of the present study was to investigate the effect of intramyocardial bone marrow cell injection on myocardial sympathetic innervation in patients with chronic myocardial ischemia by evaluating global and regional 123-I MIBG uptake. The results of the current study do not demonstrate improvement in global parameters of 123-I MIBG uptake. However, regional analysis revealed a trend towards improved 123-I MIBG uptake in bone marrow cell-treated patients compared with placebo-treated patients, which was predominantly determined by a substantial improvement in regional 123-I MIBG uptake in 3 patients with diabetes mellitus.

The current study is a substudy of a randomized, placebo-controlled, double blinded trial which demonstrated an improved myocardial perfusion in patients with chronic myocardial ischemia. Experimental studies suggested that an increase in myocardial perfusion may improve myocardial sympathetic nerve function in ischemic myocardium[16]. Furthermore, direct effects of bone marrow cell injection on neural function have been observed in preclinical studies using models of diabetic neuropathy. In particular, restoration of motor and sensory nerve conduction velocities was observed after

injection of bone marrow-derived mononuclear cells[6], endothelial progenitor cells[4] and mesenchymal stem cells[5]. These functional improvements may have resulted from cell engraftment along the vasa nervorum, increased levels of angiogenic and neurotrophic factors, and increased number of vasa nervorum resulting in enhanced nerve blood flow. Based on these pre-clinical findings, we hypothesized that intramyocardial bone marrow cell injection may improve sympathetic innervation, as assessed by 123-I MIBG SPECT imaging. 123-I MIBG is a guanetidine analogue which is taken up into presynaptic cardiac nerves, sharing the same neuronal transport and storage mechanisms as norepinephrine. Global or regional impairments of myocardial MIBG uptake reflect injury or functional alteration of the sympathetic system. Based on previous pre-clinical and clinical studies myocardial sympathetic innervation as assessed by 123-I MIBG imaging has been established as an important prognostic factor in patients with ischemic heart failure [7-10, 17, 18]. In addition, in patients with diabetes, 123-I MIBG imaging has revealed abnormalities in cardiac sympathetic innervation in myocardial regions with normal perfusion[11, 12], and the extent and severity of these abnormalities has been identified as an independent predictor of long-term survival in these patients[12].

The current study is the first to assess the effect of cardiac bone marrow cell transplantation on myocardial sympathetic innervation. Although myocardial perfusion improved in bone marrow cell-treated patients, no improvement in global myocardial sympathetic innervation could be demonstrated. Interestingly however, in this relatively small study population there was a clear trend towards an improved regional sympathetic innervation in the bone marrow cell group. Although this improvement was not significant, it is obvious that this trend is mainly determined by a substantial improvement in regional sympathetic innervation in 3 bone marrow cell-treated patients, as shown in figure 1. On a segmental basis, no significant relation was found between the improvements in regional sympathetic nerve innervation and baseline myocardial perfusion, the improvement in myocardial perfusion, or the cell injection sites. Of note, a common characteristic of the 3 patients with a substantial improvement in regional sympathetic nerve innervation was the presence of diabetes. Furthermore, these patients had a high late 123-I MIBG SPECT defect score suggesting substantial abnormalities in cardiac innervation, as described in earlier studies in patients with diabetes[12, 19]. Consequently, it may be hypothesized that the documented improvements in sympathetic innervation in the bone marrow cell group are more likely resulting from a direct therapeutic effect of the injected cells on diabetically induced cardiac dysinnervation, than from restoration of myocardial innervation secondary to improved myocardial perfusion.

The present study has several limitations. First, because the study population is small and the observed improvements were predominantly driven by 3 subjects, only preliminary conclusions can be drawn from the current data. Second, it should be mentioned that cardiac sympathetic nerve function is dynamic and may be influenced by several factors such as transient myocardial ischemia and plasma norepinephrine levels[20]. Thus, 123-I MIBG SPECT imaging provides a snapshot view of sympathetic innervation. Since plasma levels of endocrine factors such as norepinephrine were not measured in the present study, a bias resulting from subtle variations in cardiac sympathetic nerve function cannot be excluded.

In conclusion, the present study does not show improvement in global cardiac sympathetic innervation after intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia. However, regional analysis of sympathetic nerve innervation reveals improvements in 3 diabetic patients independent of myocardial perfusion, suggestive of improvement in diabetically induced cardiac dysinnervation. Additional investigations are necessary to confirm these findings and further elucidate the mechanism by which bone marrow cells may improve cardiac sympathetic innervation.

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