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Autologous bone marrow mononuclear cell infusion and hyperbaric oxygen therapy in type 2 diabetes mellitus: an open-label, randomized controlled clinical trial

ZHIXIAN WU*, JINQUAN CAI*, JIN CHEN, LIANGHU HUANG, WEIZHEN WU, FANG LUO, CHENGUANG WU, LIANMING LIAO & JIANMING TAN

Organ Transplant Institute, Fuzhou General Hospital, Xiamen University, Fuzhou, Peoples Republic of China

Abstract

Background aims. The use of bone marrow mononuclear cells (BM-MNCs) has achieved great outcomes in clinical practice. We aim to evaluate the efficacy and safety of autologous BM-MNC infusion and hyperbaric oxygen therapy (HOT) in type 2 diabetes mellitus. *Methods.* This single-center, randomized, open-label, controlled clinical trial with a factorial design included two phases. The patients received standard medical therapy in the run-in phase; in the treatment phase, patients with glycated hemoglobin of 7.5–9.5% were randomly assigned into four groups and underwent BM-MNC infusion along with HOT (BM-MNC+HOT group), BM-MNC infusion (BM-MNC group), HOT (HOT group) and standard medical therapy (control group), respectively. The area under the curve of C-peptide was recorded as a primary end point. Our research is registered at ClinicalTrials.gov (NCT00767260). *Results.* A total of 80 patients completed the follow-up. At 12 months after treatment, the area under the curve of C-peptide (ng/mL per 180 min) of the BM-MNC+HOT group and the BM-MNC group were significantly improved (34.0% and 43.8% from the baseline, respectively). The changes were both significant compared with that in the control group, but no remarkable change was observed in the HOT group. Treatment-related adverse events were mild, including transient abdominal pain (n = 5) and punctual hemorrhage (n = 3). *Conclusions.* BM-MNC infusion for type 2 diabetes mellitus improves islet function and metabolic control, with mild adverse effects. HOT does not synergize with BM-MNC infusion.

Key Words: *bone marrow, hyperbaric oxygen therapy, mononuclear cells, type 2 diabetes mellitus*

Introduction

The development of type 2 diabetes mellitus (T2DM) is characterized by the progressive deterioration of glycemic control and glycated hemoglobin (HbA_{1c}) level, which could lead to variable complications and is related to gradual degeneration of islet β cells (1). One of the primary mechanisms causing the β-cell injuries proved to be related to chronic inflammation (2). Recently, stem cells—especially bone marrow–derived cells—has become a new methodology for such degeneration diseases because of its regenerative potentials, anti-inflammatory effects and other promising features (3). The stem cell treatment in diabetic animal models has shown inspiring outcomes in terms of restoring islet function and improving diabetic control (4). The mechanisms may be relevant to the migration of stem cells to the inflammatory location and promotion of autologous stem cell re-differentiation in the pancreas (4). The results of this research have been translated to clinical practice. Estrada *et al.* (5) conducted a preliminary trial with the use of autologous bone marrow mononuclear cells (BM-MNCs) combined with hyperbaric oxygen therapy (HOT) to treat 25 patients with T2DM. The results showed remarkable improvement in metabolic control and reduction of insulin requirement. However, the evidence was insufficient because of the lack of a control group and randomization, and its sample was limited. Moreover, the respective roles of BM-MNCs and HOT were not identified. Our study aimed to conduct a randomized, open-label, controlled trial with a factorial design to investigate the effect of BM-MNCs and HOT on T2DM as well as their interactions. Pancreatic arterial intervention was used for infusion to increase the cell concentration.

*These authors contributed equally to this work.
Correspondence: **Jianming Tan**, MD, PhD, Organ Transplant Institute, Fuzhou General Hospital, Xiamen University, Fuzhou, 350025, PRC. E-mail: doctortjm@yahoo.com

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Methods

Subjects and eligibility

Patients with T2DM were recruited according to the American Diabetes Association diagnosis criteria (2008). Inclusion criteria were age 40–65 years; body mass index <35 kg/m^2; onset at ≥35 years of age; diabetes history ≥2 years and ≤15 years; HbA_{1c} ≥7.5 and ≤12%; c-peptide level ≥0.3 and ≤2.0 ng/mL; and daily total insulin dose <1.0 IU/d per kg. Exclusion criteria were pancreatitis, liver cirrhosis, hemorrhagic disease, abdominal aneurysm; chronic systematic inflammation (C-reactive [CRP] protein >3.0 mmol/L); liver enzymes >2× upper limit of normal; severe coronary artery disease (myocardial infarction within the past 6 months or active angina); heart failure stages III–IV; pregnancy or lack of approved contraception; untreated proliferative diabetic retinopathy; and any life-threatening condition. All patients gave signed informed consent, and the study was approved by the institute review board.

Trial flow

Patients who met the inclusion criteria were enrolled with a run-in phase of 4 months when standard medical therapy (SMT) was administered, which included subcutaneous insulin injection and oral administration of metformin (use of β-cell–stimulating medication, α-glycosidase inhibitor and insulin sensitizers were prohibited) to reach optimal glycemic control (HbA_{1c} ≤7.0%) without hypoglycemia. Other measures included intensified nutritional and lifestyle counseling, diabetes education and fingertip blood glucose monitoring (at least once per day). Patients with HbA_{1c} ≥7.5% and ≤9.5% at the end of the run-in phase were randomly assigned into four groups: the BM-MNC+HOT group, BM-MNC group, HOT group and the SMT (control) group. The BM-MNC+HOT group underwent pancreatic intra-arterial infusion of BM-MNC and 20 sessions of HOT before and after the infusion; the BM-MNC and HOT groups underwent the cell infusion and HOT, respectively; the above treatments were based on the SMT, whereas the control group continued to receive SMT. During HOT, patients were in a hyperbaric pressure chamber (Multiplace Hypermed-Med, model 302, South Yarra, Australia) for 1 h, with each session conducted at a target pressure of 2.0 atmospheres, breathing 100% pure oxygen through a facial mask. This trial was conducted from January 2010 to March 2012.

BM-MNC production

Under local anesthesia with 2% lidocaine, BM was aspired from both iliac crests to obtain a minimum of 300 mL and a maximum (target) of 375 mL (5), which was mixed with 20,000 U of heparin and preserved in the primary bag of a Quadruple Collection Bag (Terumo Medical Products Co Ltd, China). The primary bag was placed upside down and centrifuged (Beckman, J-26, Pasadena, CA, USA) at 2000*g* for 15 min. The bottom-layer red cells were gravitated into the second bag and discarded; the median layer buffy coat was collected in the third bag, and the upper layer plasma and fat were discarded. The buffy coat was washed and resuspended in isotonic normal saline in the third bag, which was approximately 500 mL in volume. The bag was centrifuged again at 2000*g* for 5 min to remove residual plasma and fat from the buffy coat; it was then sampled for complete blood cell count (Peroxidase method, ADVIA2120, Siemens, Munich, Germany). After the procedure, BM-MNCs were transported for immediate transplantation.

Infusion procedure

The angiography procedure was carried out as reported by Wu et al. (6). Briefly, the dorsal pancreatic artery or its substitute was identified. When the artery was cannulated, BM-MNCs were infused in 10 min.

Laboratory assessment of end points

The primary end point was C-peptide area under the curve ($AUC_{C\text{-}pep}$) of the oral glucose tolerance test (OGTT, 7 points). OGTT was performed at fasting status >12 h from the last insulin injection before and 12 months after treatment. The blood samples for C-peptide and serum insulin levels were collected at OGTT time points −10, −5, 30, 60, 90, 120 and 180 min. The $AUC_{C\text{-}Pep}$ was calculated by means of the trapezoidal method.

Secondary end points were safety, HbA_{1c}, exogenous insulin requirement (daily dose per kg), fasting blood glucose (FBG), fasting C-peptide and serum insulin AUC (AUC_{Ins}) of OGTT. Blood samples were collected at fasting status before and every 3 months after treatment for FBG (hexokinase method, AU2700, Olympus, Tokyo, Japan), HbA1c (high-performance liquid chromatography assay, Variant II, Bio-Rad, Hercules, CA, USA) and C-peptide (chemiluminescent immunoassay, Advia Centaur XP, Siemens, Munich, Germany).

Safety parameters included close observation on amylase (short-term post-intervention), infectious diseases (such as upper respiratory tract infection), CRP, white blood cell counts, hemoglobin, serum creatinine and alanine transaminase at 3-month intervals.

Clinical treatment standardization during global trial procedure

During hospitalization or at home, fingertip blood glucose monitoring was performed before meals, 2 h after meals or at bedtime for 1−2 times per day. Insulin titration was based on hemoglucose before meals and 2 h after meals, with target levels of <110 mg/dL (6.1 mmol/L) and <140 mg/dL (7.8 mmol/L), respectively. If the patient presented clinical symptoms of hypoglycemia and/or blood glucose levels <5.0 mmol/L (90 mg/dL), the dose of insulin was decreased. The patients were periodically counseled on healthy diet and exercise by an endocrinologist to avoid clinical care discrepancy or nonadherence. All changes in insulin doses were ordered by the endocrinologist of the team (Z.W.).

Quality of life

The summary scores for the physical and mental quality-of-life (QOL score) components of the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) (range, 0−100, with higher scores indicating better health status) were assessed by the study physician, who was unaware of the group assignment.

Statistical analysis

A computer-generated block randomization was used to assign each subject to one of the four groups. Statistical analysis was performed with the use of SPSS for Windows (version 10.1; SPSS, Chicago, IL, USA). Data were presented as mean ± standard deviation. One-way analysis of variance was used to compare means at the baseline between groups. A factorial variance analysis was used to examine the interaction effect of BM-MNCs and HOT and differences between groups. Tests resulting in a value of $P < 0.05$ were considered to be statistically significant. Power and sample size considerations assume a 30% increase of C-peptide $AUC_{C\text{-}Pep}$ at 12 months after treatment from an average 300 ng/mL per 180 min of Chinese patients with T2DM. The Student's t test of independence considered four independent groups with 20 patients per group, with adequate power to detect this assumed difference (type I error = 0.05 and 90% power).

Results

Characteristics of patients

A flow chart of patient selection is shown in Figure 1. The demographic and baseline data are displayed in Table I. The four groups were well matched in terms of the baseline characteristics. No significant differences were observed in age, sex, weight, blood pressure, waist circumference, $AUC_{C\text{-}pep}$, HbA_{1c}, FBG, insulin dose, and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), as well as the quantities of infused BM-MNCs and other biochemical parameters.

Therapeutic efficacy

At 12 months, $AUC_{C\text{-}Pep}$ (ng/mL per 180 min) increased 34.0% in the BM-MNC+HOT group (from 339.9 ± 103.2 to 455.4 ± 122.1) and 43.8% in the BM-MNC group (from 326.1 ± 79.2 to 469.0 ± 102.5) from the baseline, whereas it decreased 12.6% in the HOT group (from 341.0 ± 117.4 to 298.1 ± 132.5) (Figure 2A). These changes in the first two groups were significant compared with that in the control group (from 335.0 ± 128.4 to 303.5 ± 112.6, $P < 0.01$ versus control, respectively) but not the HOT group. There were no interactions between BM-MNC infusion, and HOT ($P > 0.05$). AUC_{Ins} (mmol/L per 180 min) increased markedly in the BM-MNC+HOT group (from 3585.8 ± 637.0 to 4542.1 ± 681.0) and in the BM-MNC group (from 3571.1 ± 446.1 to 4667.9 ± 880.2), whereas it was stable in the HOT group (from 3617.9 ± 479.6 to 3643.7 ± 536.5) and the control group (from 3632.0 ± 450.4 to 3565.7 ± 522.7) (Figure 2B). The statistical results were similar to those of $AUC_{C\text{-}Pep}$.

HbA_{1c} (%) decreased from the enrollment to randomization without significance ($P > 0.05$ for all) in the four groups after SMT during the run-in phase. It was reduced significantly (changes from randomization versus control, $P < 0.01$) both in the BM-MNC+HOT and the BM-MNC groups at 3, 6, 9 and 12 months but stable in the HOT and the control groups (Figure 3A). HbA_{1c} decreased 1.2 in the BM-MNC+HOT group and 1.1 in the BM-MNC group at 12 months (randomization versus 12 months: 8.6 ± 0.6 versus 7.4 ± 0.8; 8.5 ± 0.7 versus 7.4 ± 0.9, respectively) but remained stable in the HOT and the control groups (randomization versus 12 months: 8.5 ± 0.6 versus 8.4 ± 0.8; 8.6 ± 0.5 versus 8.6 ± 0.6, respectively) (Figure 3A).

FBG (mg/dL) also decreased from enrollment to randomization without significance ($P > 0.05$) in the four groups. It was significantly (changes from randomization versus control) reduced in the BM-MNC+HOT and the BM-MNC groups at 6, 9 and 12 months (Figure 3B) (randomization versus 12 months: 152.8 ± 21.0 versus 124.5 ± 22.0; 152.3 ± 25.7 versus 121.4 ± 21.8, respectively). There were mild decreases in the HOT and the control groups (randomization versus 12 months: 157.3 ± 32.4

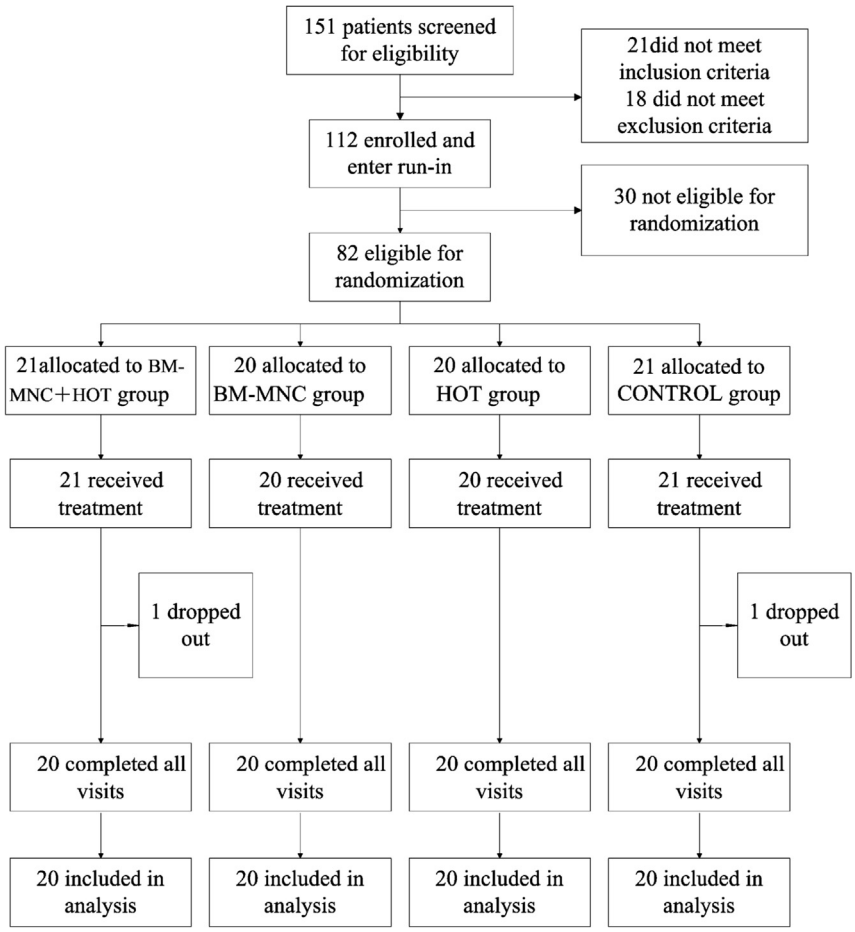

Figure 1. Screening, randomization and completion of 1-year evaluations.

versus 153.9 ± 27.3; 155.3 ± 24.8 versus 154.4 ± 28.9, respectively).

After the run-in phase, the insulin dose (IU/d per kg) increased slightly ($P > 0.05$) from enrollment to randomization in the four groups. It decreased significantly (changes from randomization versus control, $P < 0.01$) in the BM-MNC+HOT and the BM-MNC groups at 3, 6, 9 and 12 months (randomization versus 12 months: 0.49 ± 0.15 versus 0.30 ± 0.16; 0.49 ± 0.18 versus 0.34 ± 0.22, respectively), whereas it remained practically unchanged in the HOT and the control groups

Table I. Demographic and baseline data of the patients.

	BM-MNC+HOT	BM-MNC	HOT	Control	*P* value
Age (y)	57.4 ± 5.7	56.4 ± 5.9	54.9 ± 6.2	54.9 ± 6.3	0.49
Men	12/20	12/20	10/20	11/20	0.61
Weight (kg)	67.2 ± 9.0	68.9 ± 9.1	65.4 ± 13.6	68.5 ± 12.0	0.95
Body mass index (kg/m^2)	24.5 ± 1.7	24.5 ± 2.2	23.9 ± 3.3	24.5 ± 2.8	0.87
Waist circumstance (cm)	87.3 ± 11.2	85.7 ± 14.1	84.2 ± 10.4	86.8 ± 12.6	0.71
Diabetic duration (y)	10.0 ± 3.3	9.8 ± 3.4	11.5 ± 3.0	9.5 ± 3.0	0.25
Systolic pressure (mm Hg)	124.7 ± 10.0	119.0 ± 11.6	118.5 ± 13.2	122.4 ± 9.8	0.59
Diastolic pressure (mm Hg)	74.5 ± 8.9	66.5 ± 8.9	69.4 ± 12.1	72.5 ± 9.0	0.70
$AUC_{C\text{-}Pep}$	339.9 ± 103.2	326.1 ± 79.2	341.0 ± 117.4	335.0 ± 128.4	0.91
HbA1c (%)	8.6 ± 0.6	8.5 ± 0.7	8.5 ± 0.6	8.6 ± 0.5	0.86
FBG (mg/dL)	152.8 ± 21.0	152.3 ± 25.7	157.3 ± 32.4	155.3 ± 24.8	0.92
Insulin (IU/d per kg)	0.49 ± 0.15	0.49 ± 0.18	0.51 ± 0.16	0.52 ± 0.15	0.91
HOMA-IR	4.6 ± 1.2	5.2 ± 1.5	4.9 ± 1.5	4.3 ± 1.3	0.82
CRP (mmol/L)	1.7 ± 1.1	1.3 ± 0.8	1. 4 ± 0.7	1.5 ± 1.0	0.46
QOL (SF-36) score	67.4 ± 19.6	62.8 ± 14.2	65.3 ± 14.9	69.4 ± 15.6	0.52
BM-MNCs (10^6)	3641.2 ± 1585.4	4012.5 ± 1431.9	/	/	

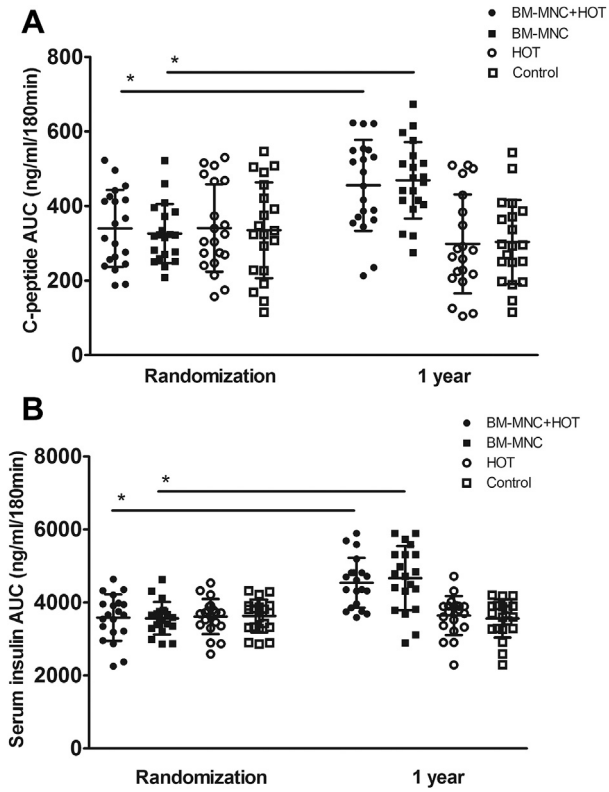

Figure 2. $AUC_{C\text{-}Pep}$ increased markedly in the BM-MNC+HOT and the BM-MNC groups at 12 months, whereas it decreased slightly in the HOT and the control groups (A). Similar results were observed in AUC_{Ins} (B). *$P < 0.01$, changes from baseline versus control.

(randomization versus 12 months: 0.51 ±0.16 versus 0.52 ± 0.17; 0.52 ± 0.15 versus 0.45 ± 0.18, respectively, $P > 0.05$) (Figure 3C). Three cases achieved insulin independence, one of which was in the BM-MNC+HOT group and two in the BM-MNC group.

Before and after the run-in phase, fasting C-peptide (ng/mL) remained stable in the four groups; it was elevated in the BM-MNC+HOT group at 3, 6, 9 and 12 months (changes from randomization versus control, $P < 0.01$ for all) and the BM-MNC group at 6, 9 and 12 months ($P < 0.01$ for all) (randomization versus 12 months: 1.0 ± 0.3 versus 1.5 ± 0.5; 1.2 ± 0.4 versus 1.7 ± 0.3, respectively) (Figure 3D). However, it remained stable in the HOT and the control groups (randomization versus 12 months: 1.1 ± 0.4 versus 1.0 ± 0.4; 1.1 ± 0.4 versus 1.0 ± 0.4, respectively) (Figure 3D).

Quality of life

At 12 months, the QOL (SF-36) score was improved in the BM-MNC+HOT and the BM-MNC groups (baseline versus 12 months: 67.4 ± 19.6 versus 78.3 ± 11.8; 62.8 ± 14.2 versus 77.1 ± 13.2, respectively, changes from randomization versus control, $P < 0.01$ for both). It remained practically unchanged in the HOT and control groups (baseline versus 12 months: 65.3 ± 14.9 versus 67.5 ± 12.8; 69.4 ± 15.6 versus 65.3 ± 14.7, respectively).

Adverse events

Five cases of abdominal pain during pancreatic arterial intervention were recorded, of which three were in the BM-MNC+HOT group and two in the BM-MNC group. The symptoms were mild and transient, and the patients recovered without drug intervention. Three cases of arterial puncture-site hemorrhage were observed after the patients were sent back to the wards, two of which were in the BM-MNC+HOT group and one in the BM-MNC group. Two were mild and required no treatments, whereas one resulted in hematoma formation and was handled after pressing. No remarkable changes in amylase, CRP, white blood cell counts, Hb, serum creatinine and alanine transaminase were observed (data not shown).

Discussion

The rationale for age ≥45 years is that the likelihood of all patients having true T2DM is higher and that at ≤65 years there is higher probability for regenerative potential than in an older age (7,8). The rationale for the presence of the established C-peptide levels is that there is still the possibility for regeneration and at the same time the possibility to detect a significant change, if that is induced by the therapy (9,10). The rationale for ≥2-year (but ≤15) T2DM duration is that it is likely that most patients will be treated with insulin or insulin and other oral agents by that time but still would have some β-cell reserve for regeneration (11,12). To limit body mass index to <35 and CRP to <3.0 mmol/L is to limit the effect of extreme insulin resistance that would limit detection of improvement in β-cell function (13).

The collection of bone marrow stem cells can be achieved by two methods: bone marrow aspiration and the leukapheresis method (14). Our study adopted the bone marrow aspiration method because of its handy operating techniques and large quantity of leukocytes harvested. There has been no evidence indicating the proper volume of bone marrow or the specific type of bone marrow cells for the therapeutic effect we need; therefore, the maximum volume of bone marrow was required, and the entire buffy coat was preserved. The biggest feeding artery of the pancreas was selected as the infusion path of stem cells, with the purpose of infusing the stem cells to

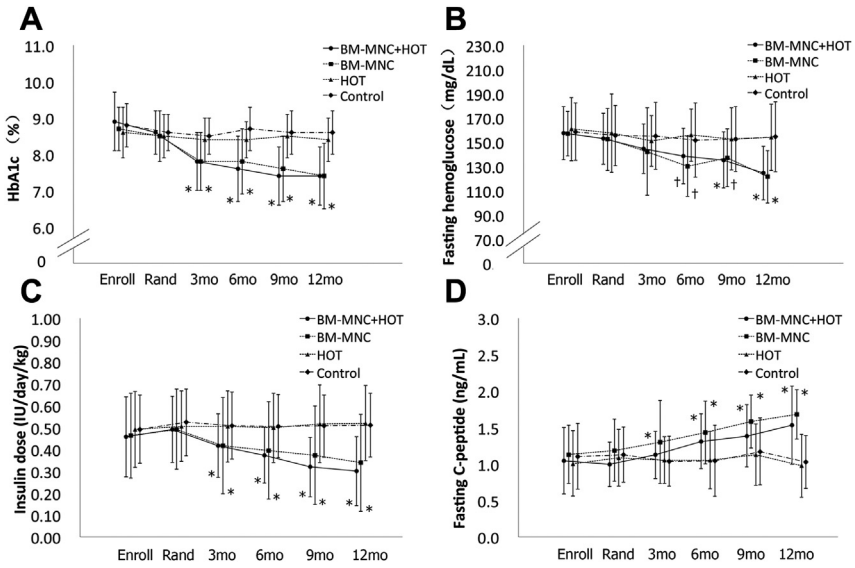

Figure 3. HbA_{1c} decreased from enrollment to randomization in the four groups after SMT during the run-in phase without significance. It was reduced significantly both in the BM-MNC+HOT and BM-MNC groups at 3, 6, 9 and 12 months but stable in the HOT and the control groups (A). FBG also decreased mildly from enrollment to randomization; it was markedly reduced in the BM-MNC+HOT and the BM-MNC groups at 6, 9 and 12 months but was stable in the HOT and the control groups (B). After the run-in phase, the insulin dose increased slightly from enrollment to randomization in all; it decreased significantly in the BM-MNC+HOT and the BM-MNC groups at 3, 6, 9 and 12 months but was stable in the HOT and the control groups (C). Before and after the run-in phase, fasting C-peptide level remained stable in the four groups; it was elevated in the BM-MNC+HOT group at 3, 6, 9 and 12 months and BM-MNC group at 6, 9 and 12 months. However, it remained stable in the HOT and the control groups. Enroll indicates enrollment; Rand, randomization $^*P < 0.01$, $\dagger P < 0.05$, changes from baseline versus control.

the entire injured units and minimizing the complications.

The buffy coat that we used in the study was a mixture of hematopoietic stem cells, bone marrow stromal cells, pluripotent precursor cells and variable mononuclear blood cells. Recent studies have indicated that *ex vivo* culture of bone marrow stem cells under specific conditions differentiated into insulin-secreting cells; transplantation to the renal capsule of hyperglycemic rats showed improvements in the FBG and intraperitoneal glucose tolerance test (15,16). Apart from the active role of stem cells in migration, restoration and paracrine function, BM-MNCs also promote angiogenesis (17). The hematopoietic stem cells and stromal stem cells are crucial to such process, which may improve the microenvironment and nutrition supplies necessary for regeneration of islets (18,19). BM-MNC treatment has achieved great outcomes in clinical practice, in terms of myocardial infarction, diabetic lower limb ischemia and so forth (20,21).

Our research clinically observed that BM-MNCs could significantly improve glycemic control, restore partial islet function and enhance patient's quality of life, indicating a promising perspective of clinical translation from the animal experimental outcomes. Compared with the study by Estrada *et al.* (5), the evidence is stronger and the results rule out the synergizing effects of HOT. Bhansali *et al.* (22) report remarkable islet function improvement and insulin dose reduction after BM-MNC treatment. Compared with our study, there are clear differences. Patients received granulocyte colony-stimulating factor injection and underwent leukapheresis to collect BM-MNC. The infusion path was a peripheral vein. Moreover, patients were enrolled with HbA1c% <7.5%.

The possible mechanism of such restoration of islet cells is unclear, which may be related to mobilization of endogenous stem cells, enhancement of local microcirculation and regeneration or re-activation of islet cells in apoptosis.

HOT can increase the concentration of carbon monoxide synthase, which causes stem cell mobilization and endothelial progenitor cell release (23,24). These cells can migrate to inflammatory locations under the effect of cytokines and chemokines. No interaction effect between HOT and BM-MNC infusion was observed. One possible explanation may be that the effects of HOT on the local pancreatic microenvironment were limited (25–27).

The observation period of the study is short, and further improvement of blood glucose as well as long-term maintenance of elevated islet function are yet to be determined. The insulin requirement reduction is limited, with only three cases achieving

insulin independence. A further modification of the treatment is needed to improve the therapeutic outcomes. Moreover, long-term adverse effects should be recorded, even though there were few short-term adverse events. Two patients were lost to follow-up. However, their demographic data were at the average levels in respective groups and the dropout rates were within an acceptable range.

In all, BM-MNC infusion can significantly improve glycemic control, restore islet cell function in progressing failure and improve patient quality of life, without severe adverse events in follow-up.

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