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Bone marrow mesenchymal stem cell and mononuclear cell combination therapy in patients with type 2 diabetes mellitus: a randomized controlled study with 8-year follow-up

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Abstract

Background To investigate the long-term effects of combining bone marrow mesenchymal stem cells (MSCs) with mononuclear cells (MCs) in the treatment of type 2 diabetes mellitus (T2DM).

Methods T2DM patients were divided into the combination group (Dual MSC + MC, $n = 33$), the mononuclear cell group (MC-Only, $n = 32$) and the control group (Control, $n = 31$). All groups were treated with insulin and metformin. The Dual MSC + MC group additionally received MSC and MC infusion and the MC-Only group additionally received MC infusion. The patients were followed up for 8 years. The primary endpoint was the C-peptide area under the curve (C-p AUC) at 1 year. This study was registered with clinicaltrial.gov (NCT01719640).

Results A total of 97 patients were included and 89 completed the follow-up. The area under the curve of C-peptide of the Dual MSC + MC group and the MC-Only group was significantly increased (50.6% and 32.8%, respectively) at 1 year. After eight years of follow-up, the incidence of macrovascular complications was 13.8% ($p = 0.009$) in the Dual MSC + MC group and 21.4% ($p = 0.061$) in the MC-Only group, while it was 44.8% in the Control group. The incidence of diabetic peripheral neuropathy (DPN) was 10.3% ($p = 0.0015$) in the Dual MSC + MC group, 17.9% ($p = 0.015$) in the MC-Only group, and 48.3% in the Control group.

Conclusions The combination of MSC and MC therapy can reduce the incidence of chronic diabetes complications and improves metabolic control with mild side effects in T2DM patients.

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Keywords Type 2 diabetes mellitus, Mesenchymal stem cells, Mononuclear cells, Bone marrow, Autologous, Diabetes complications

Background

Diabetes mellitus is a significant chronic disease affecting millions of people worldwide, with the population projected to reach 1.8 billion by 2030. Type 2 diabetes mellitus (T2DM) accounts for 90% of these cases [1] and is associated with chronic complications, including macrovascular and microvascular complications, which remain the main cause of mortality [2]. While conventional hypoglycemic agents barely aim at improving islet function, regenerative approaches such as stem cell therapy has shown great promise [3–5]. Studies have demonstrated the potential of stem cells including tissue repair, regeneration and immunomodulation, with recent clinical trials showing improved metabolic control and islet function in T2DM [3, 6]. However, insulin independence has been scarce among patients, and modifications to cell regimens are necessary. Various cell classes including bone marrow (BM) mesenchymal stem cells (MSC), bone marrow mononuclear cells (MC) and umbilical cord mesenchymal stem cells have been employed in different studies. Based on our experience [7], the combination of different classes of cells is also feasible. Furthermore, the improvement in chronic diabetes complications by stem cell therapy was only reported in animal experiments [8–10]. Given the promising preliminary findings on the individual effects of MSCs and MCs in treating T2DM, this study aims to explore the long-term impacts of their combined use. By assessing the outcomes over an 8-year period, we seek to determine whether the combination of MSCs and MCs can offer superior benefits in improving metabolic control and reducing chronic complications compared to MCs alone or standard therapy.

Methods

Patient selection

Type 2 diabetes mellitus was diagnosed based on the 2008 criteria of American Diabetes Association. The inclusion criteria were age ≥ 40 and ≤ 65 years, body mass index (BMI) ≤ 35 kg/m^2, onset age ≥ 35 years, history ≥ 3 and ≤ 15 years, glycated hemoglobin (HbA1c) ≥ 7.5 and ≤ 12%, fasting c-peptide ≥ 0.3 and ≤ 2.0 ng/mL, medication with stable doses of exogenous insulin and metformin over the previous 3 months. The rationale for the high limit of BMI and c-peptide level was to mitigate the possibility of marked insulin resistance compromising islet function. The exclusion criteria were evidence of renal dysfunction (serum creatinine > 1.5 mg/dl (males) and 1.4 mg/dl (females)), proteinuria > 300 mg/day, diabetic retinopathy, chronic pancreatitis, liver cirrhosis, hemorrhagic disease, abdominal aneurysm, chronic systematic inflammation (C-reactive protein [CRP] > 3.0 mmol/L), liver enzymes > 1.5 × upper limit of normal, severe coronary artery disease (myocardial infarction within the past 6 months or active angina), heart failure stages III ~ IV, poorly controlled hypertension and hyperlipidemia, pregnancy or lack of approved contraception, and malignancy.

Study flow

Patients eligible to initial criteria underwent a 4-month run-in phase of standard medical treatment (SMT), which included insulin injection, metformin (2 g per day, orally), exercise (2–3 km two times a week), and healthy diet as counseled by an endocrinologist. At the end of run-in phase, patients were screened for randomization criteria, which included HbA1c ≥ 7.5 and ≤ 9.5%, insulin daily dose ≤ 1.0 units/day/kg, controlled blood pressure (systolic BP < 140 mmHg or diastolic BP < 90 mmHg) and controlled hyperlipidemia (fasting low density lipoprotein cholesterol > 130 mg/dL; and/or fasting triglycerides > 200 mg/dL). Then, eligible patients were randomized into the Dual MSC+MC group, the MC-Only group and the Control group. BM collection and MSC culture were performed for the Dual MSC+MC group. When the number of MSCs were reached approximately 2×10^6/kg (total numbers varied based on patient body weight), the patients were admitted to the center and underwent BM collection again to harvest mass volume. The MC-Only group only underwent mass BM collection. The two groups were infused intraarterially with MSCs (1×10^6/kg) plus MCs or MCs alone, respectively. The Dual MSC+MC group received another dose of MSCs (1×10^6/kg) intravenously at 1 week. Exogenous insulin and metformin were maintained for all three groups for one year. After 1 year, the patients were referred to primary physicians. Before the 8-year follow-up, the patients were reached by phone and subjected to SMT again for 3 months to achieve stable insulin (fluctuated within 10%) and metformin (2 g per day) doses.

Bone marrow collection

The patients were tested for the infection of hepatitis B, hepatitis C and human immunodeficiency viruses, and syphilis before the operation. The patient was placed in prone position, and bone marrow was aspired from both posterior superior iliac spine under local anesthesia with 2% lidocaine. The syringes were pre-treated with heparin saline to prevent clotting and loss of BM. For MSC culture, 100mL BM was collected. For MC separation, a minimum of 350 ml and a maximum of 380 ml of bone

marrow were collected and mixed with 20,000 units of heparin and preservative in a Quadruple Collection Bag (Terumo Medical Corporation, Changchun, China).

MSC culture

The heparinized BM was mixed with an equal volume of low glucose Dulbecco's Modified Eagle Medium (LG-DMEM) and centrifuged at 1000 g for 5 min. The fat and supernatant were discarded. After resuspended with LG-DMEM, an equal volume of Percoll (1.073 g/mL, Sigma-Aldrich, St Louis, MO) was gently added and centrifuged at 2000 g for 25 min. The mononuclear layer was separated and resuspended with LG-DMEM and plated to 75 cm^2 flask in a 37 ℃ humidified 5% CO2 incubator. The medium was renewed every 48–72 h, and nonadherent cells were discarded. After incubation for 5–7days and reaching 80% confluence, cells were harvested with 0.25% trypsin and 0.02% EDTA, replated at a density of $0.5–1\times10^6$ cells in flasks and incubated. The cells of passage 5 were suspended in phosphate-buffered saline for infusion. As per standard practice at our center and in accordance with 2006 International Society for Cellular Therapy criteria, we performed cell surface marker analysis (Coulter EPICS XL Flow Cytometer acquisition report) and showed that UC-MSCs were positive for CD29, CD73, CD90, and CD105 and negative for CD34 and CD45. Batch testing for bacteria, mycoplasma, fungi, and endotoxin were performed before release for infusion. The cells can only be infused into the patient when all tests are negative, to prevent the risk of pancreatic injury.

MC production

The Quadruple Collection Bag was placed upside down the centrifuge (Beckman, J-26) and centrifuged at 1000 g for 10 min. The red cells were gravitated into the second bag from the primary bag and then discarded, the buffy coat and some plasma were collected in the third bag, and the remaining plasma and fat discarded with the primary bag. The buffy coat was washed and resuspended in isotonic normal saline in the third bag, which a volume of approximately 500 ml. The bag was centrifuged again at 2000 g for 15 min to separate the resident plasma and fat from the buffy coat. After the procedure the MCs were transported for immediate infusion.

Intraarterial infusion

This invasive procedure is performed by a senior surgeon of interventional therapy at the center for all patients. Patients underwent angiography of the celiac axis and splenic artery. In all subjects a large vessel consistent with the dorsal pancreatic artery was identified and confirmed to feed the pancreatic body and tail with digital subtraction angiography. After the artery was cannulated, MSCs and/or MCs, which were dispensed in several 20mL syringes, were infused slowly. Previous studies at our center have confirmed that the procedure was safe [7, 11].

Endpoints and follow-up

The primary endpoint was the C-peptide area under the curve (C-p AUC) at 1 year. Secondary endpoints included serum insulin AUC, glycated hemoglobin (HbA1c), fasting blood glucose (FBG), exogenous insulin requirement (daily dose per kg), and fasting C-peptide at 1 year as well as the incidence of macrovascular and microvascular complications assessed over an 8-year period. Macrovascular complications included myocardial infarction (MI), angina, stroke and amputation, and microvascular complications included diabetes nephropathy (DN), and diabetes retinopathy (DRP, both non-proliferative DRP and proliferative). Other secondary endpoints included the incidence of diabetic peripheral neuropathy (DPN), uncontrolled hypertension, hyperlipidemia, and safety.

C-p-AUC and insulin AUC were determined by the oral glucose tolerance test (OGTT). The OGTT was performed at fasting status>12 h from the last insulin injection at admission, 6 months, 1 year and 8 years. Blood samples for the detection of serum C-p and insulin levels were collected at -10, -5, 30, 60, 90, 120 and 180 min in OGTT. The C-p-AUC and insulin AUC was calculated using the trapezoidal method. Blood samples were collected at fasting status at admission and 3, 6, and 9 months and 1 year post-infusion 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).

Myocardial infarction (MI), angina, stroke and amputation were recorded based on medical history from primary care and local hospitals. According to the third generic definition of myocardial infarction, MI and angina can be recognized by clinical features, including electrocardiographic findings, elevated values of biochemical markers of myocardial necrosis, and by imaging, or may be defined by pathology. The diagnosis of stroke may be based on: acute onset, focal neurological deficits, and in a few cases, total neurological deficits, symptoms and signs lasting for more than a few hours, and brain CT or MR to exclude cerebral hemorrhage and other lesions with responsible infarct lesions. These were confirmed by physical examination, electrocardiograph and magnetic resonance imaging at the center. Diabetes nephropathy (DN), and diabetes retinopathy (DRP, both non-proliferative DRP and proliferative) were assessed at the center. When microalbuminuria exceeded 30 mg in a 24-hour urine collection (Afinion AS100; Alere Medical Sales Co Ltd, Shanghai, China), the presence of DN

was considered. The diagnosis of DN was confirmed in two out of three samples collected over a 3- to 6-month period. DRP was diagnosed using the International Clinical Diabetic Retinopathy classification scale. An ophthalmologist examined patients' retinas and photographed, and an independent ophthalmologist reviewed the images and confirmed the diagnosis.

DPN was defined by a neurothesiometer reading > 25 V and/or positive monofilament test in two or more sites in either foot and/or Michigan Neuropathy Screening Instrument questionnaire score [12]. According to previous studies [7, 11], Safety included amylase level (an indicator for post-intervention pancreatitis), incidence of infectious diseases (such as upper respiratory tract infection), CRP, white blood cell counts, hemoglobin, serum creatinine level and alanine transaminase at 3-month intervals.

Insulin titration was based on targets of fasting blood glucose levels of 110 mg/dL (6.1 mmol/L) and 2 h after meals 180 mg/dL (10.0 mmol/L), respectively. The dose of insulin was reduced by 1–2 IU if patients presented clinical findings of hypoglycemia and/or blood glucose levels less than 4.9 mmol/L (90 mg/dL). Metformin doses were maintained unless the patients became insulin-independent. All changes in doses were ordered by the endocrinologist of the team (Z W).

Statistical analysis

A computer-generated block randomization was used to assign each subject to one of the three groups. Statistical analysis was performed using software GraphPad Prism 9.0. Data were presented as mean ± standard deviation. Power and sample size considerations assume a 30% increase of C-p-AUC at 1 year post-infusion from an average of 300 ng/mL per 180 min in Chinese patients with T2DM. The ANOVA considered three independent groups with 20 patients per group, providing adequate power to detect this assumed difference (type I error = 0.05 and 90% power). The Chi-square test and two-factor repeated-measures ANOVA were used for three-group numeration data comparison, and three-group repeated-measures comparison, respectively. A value of $P < 0.05$ were considered to be statistically significant. For numeration data, per-protocol (PP) and intention-to-treat (ITT) analyses were used. PP analysis included only those patients who had completed the follow-up schedule of the study. On the other hand, the ITT analysis included all patients who had finished 1-year follow-up, even those who had dropped out of the 8-year follow-up.

Results

Demographic data and baseline characteristics

A flow chart of patient selection, randomization and follow-up is shown in Fig. 1. (Fig. 1 Flowchart of the study screening, randomization, and completion. Out of 132 patients screened, 96 were eligible and randomized. One patient in the Dual MSC+MC group experienced MSC culture failure due to cell growth arrest and was excluded. A total of 96 patients were enrolled, 95 received scheduled treatments and 86 completed the follow-up. The Dual MSC+MC group had 33 patients enrolled, 32 patients treated and three lost to follow-up. The MC-Only group had 32 patients enrolled, 32 patients treated and four dropouts. The Control group had 31 patients enrolled and treated, and two dropouts. One patient in the Dual MSC+MC group was excluded due to MSC culture failure caused by cell growth arrest. The demographic data and baseline characteristics were listed in Table 1 and no significant differences in baseline variables were observed between three groups. Furthermore, there were no differences in lipids, blood pressure, or C-reactive protein between the three groups of patients (data not shown).

Therapeutic benefits

Metabolic control

At 1 year, the C-peptide AUC increased 50.6% (vs. Control, $p < 0.001$) in the Dual MSC+MC group and 32.8% (vs. Control, $p < 0.001$) in the MC-Only group, respectively, while it decreased by 5.9% in the Control group. The increase in the Dual MSC+MC group was significantly higher than in the MC-Only group ($p < 0.001$). At 8 years, the increment were 19.2% in the Dual MSC+MC group and 5.9% in the MC-Only group, both remaining higher than the −30.5% decrease in the Control group (vs. either group, $p < 0.001$, Fig. 2A).

The insulin AUC showed a 41.2% increase in the Dual MSC+MC group (vs. Control, $p < 0.001$) and a 23.0% increase in the MC-Only group (vs. Control, $p = 0.004$) at 1 year, compared to a 2.0% decrease in the Control group. The Dual MSC+MC group's increase was also significantly higher than the MC-Only group ($p = 0.005$). At 8 years, the increment was 13.6% in the Dual MSC+MC group and 6.5% in the MC-Only group, while the Control group experienced a 15.5% decrease (vs. either group, $p < 0.001$, Fig. 2B).

HbA1c levels improved significantly in both the Dual MSC+MC group (from 8.6 ± 1.1% to 6.7 ± 1.3%, vs. Control, $p < 0.001$)) and the MC-Only group (from 8.7 ± 1.2% to 7.3 ± 1.2%, vs. Control, $p < 0.001$) at 1 year, whereas the Control group remained stable (from 8.7 ± 1.0% to 8.4 ± 1.2%). By 8 years, HbA1c levels had risen in the Dual MSC+MC group (8.5 ± 1.6%) and the MC-Only group (8.8 ± 1.5%), but both were still better than the Control

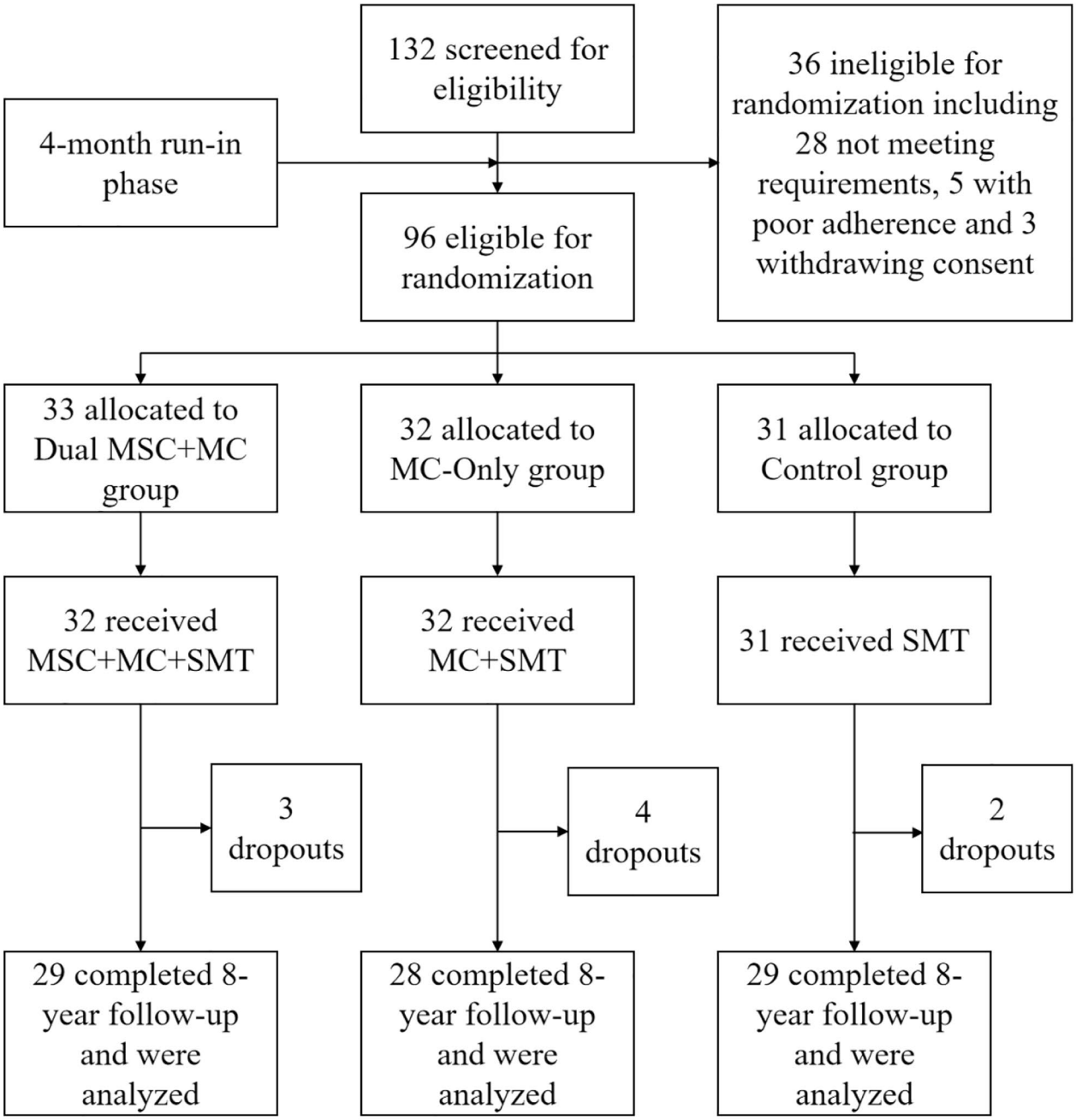

Fig. 1 Flowchart of the study screening, randomization, and completion. Out of 132 patients screened, 96 were eligible and randomized. One patient in the Dual MSC + MC group experienced MSC culture failure due to cell growth arrest and was excluded

group, which saw a marked deterioration (9.7±1.6%, vs. either group, $p<0.001$, Fig. 2C).

FBG levels decreased significantly in the Dual MSC+MC group (from 158.3±32.4 mg/dL to 122.3±25.8 mg/dL, vs. Control, $p<0.001$) and in the MC-Only group (from 162.4±29.6 mg/dL to 131.5±27.3 mg/dL, vs. Control, $p<0.001$) at 1 year. The Control group saw no significant change (155.8±33.0 mg/dL vs. 158.7±34.3 mg/dL, $p=0.90$). At 8 years, FBG levels increased in the Dual MSC+MC group (159.6±34.6 mg/dL) and MC-Only group (164.7±32.2 mg/dL), but both were still significantly lower than the Control group (187.1±36.5 mg/dL, vs. Dual MSC+MC, $p<0.001$; vs. MC-Only, $p=0.039$, Fig. 2D).

Insulin dosage significantly decreased in the Dual MSC+MC group (-57.4%, from 0.68±0.21 IU/day/kg to 0.29±0.08 IU/day/kg, vs. Control, $p<0.001$) and the MC-Only group (-42.2%, from 0.64±0.19 IU/day/

Table 1 Demographic data and baseline characteristics of the three groups

	Dual MSC + MC (*n* = 29)	MC-Only(*n* = 28)	Control(*n* = 29)	*p*
Age (y)	52.8 ± 4.5	53.2 ± 6.1	54.6 ± 5.0	0.39
Men	18/29	19/28	19/29	0.21
Weight (kg)	75.6 ± 7.2	73.4 ± 8.3	76.0 ± 7.8	0.40
Weight at 8 years(kg)	79.1 ± 11.0	75.5 ± 8.7	78.0 ± 8.2	0.34
BMI (kg/m^2)	25.2 ± 2.1	25.6 ± 3.2	25.3 ± 2.6	0.84
Waist circumstance (cm)	95.8 ± 14.2	94.4 ± 12.9	94.7 ± 13.8	0.92
Duration of DM (y)	8.9 ± 4.2	8.6 ± 5.3	9.1 ± 4.8	0.92
C-p-AUC (ng/mL/180min)	343.2 ± 46.7	332.8 ± 39.8	350.3 ± 44.5	0.32
HbA1c (%)	8.6 ± 1.1	8.7 ± 1.2	8.7 ± 1.0	0.92
FBG (mg/dL)	158.3 ± 32.4	162.4 ± 29.6	155.8 ± 38.0	0.76
Insulin dose (IU/day/kg)	0.68 ± 0.21	0.64 ± 0.19	0.71 ± 0.23	0.46
Fasting C-p(ng/mL)	1.2 ± 0.32	1.3 ± 0.37	1.3 ± 0.34	0.45
HOMA-IR	5.1 ± 1.3	4.9 ± 1.2	5.4 ± 1.6	0.37
MCs(10^6/kg)	512.6 ± 124.8	496.1 ± 135.3	N/A	0.64
MSCs(10^6/kg)	1.3 ± 0.21	N/A	N/A	

kg to 0.37 ± 0.11 IU/day/kg, vs. Control, $p < 0.001$) at 1 year, while remaining stable in the Control group (from 0.71 ± 0.23 IU/day/kg to 0.73 ± 0.22 IU/day/kg). Three patients in the Dual MSC + MC group achieved insulin independence, unlike in the MC-Only group. At 8 years, the insulin doses returned to baseline levels in both treatment groups, while the Control group saw a significant increase (0.81 ± 0.24 IU/day/kg, vs. either group, $p < 0.001$, Fig. 2E).

Fasting C-peptide levels significantly increased in the Dual MSC + MC group (from 1.2 ± 0.32 ng/mL to 1.7 ± 0.41 ng/mL, vs. Control, $p < 0.01$) and the MC-Only group (from 1.3 ± 0.37 ng/mL to 1.6 ± 0.41 ng/mL, vs. Control, $p = 0.049$) at 1 year, while remaining stable in the Control group. At 8 years, levels decreased in both treatment groups but were still higher than the Control group (0.9 ± 0.27 ng/mL, vs. Dual MSC + MC, $p < 0.001$; vs. MC-Only, $p = 0.049$, Fig. 2F).

Chronic diabetes complications

At 8 years, the incidence of macrovascular complications was significantly lower in the Dual MSC + MC group (13.8%, 4/29) compared to the Control group (44.8%, 13/29, $p = 0.009$), while the MC-Only group showed a lower incidence (21.4%, 6/28) but without statistical significance ($p = 0.061$, Fig. 3A). For microvascular complications, the Dual MSC + MC group had a significantly lower incidence was (6.9%, 2/29) compared to the Control group (31.0%, 13/29, $p = 0.0445$), with the MC-Only group at 14.3% (4/28, vs. Control, $p = 0.234$, Fig. 3A). The incidence of DPN was 10.3% in the Dual MSC + MC group (vs. Control, $p = 0.0015$) and 17.9% in the MC-Only group (vs. Control, $p = 0.015$), both lower than in the Control group (48.3%, Fig. 3A).

Among macrovascular complications, the incidence of MI was 3.5% in the Dual MSC + MC group (vs. Control, $p = 0.11$) and 7.1% in the MC-Only group (vs. Control, $p = 0.28$) with no significant difference from Control group (20.7%, Fig. 3B). Angina incidence was significantly lower in the Dual MSC + MC group (10.3%, vs. Control, $p = 0.028$) compared to the Control group (34.5%,), and lower in the MC-Only group (14.3%, vs. Control, $p = 0.077$, Fig. 3B). Stroke incidence showed no significant differences between groups (6.9% for the Dual MSC + MC, 7.1% for the MC-Only, 13.8% for the Control group, Fig. 3B). Similarly, amputation rates were not significantly different between either the Dual MSC + MC group (3.5%, 1/29) and Control group (3.5% for Dual MSC + MC, 7.1% for MC-Only, 10.3% for Control, Fig. 3B).

For microvascular complications, DN incidence were not significantly different among groups (6.9% for Dual MSC + MC, 10.7% for MC-Only, 20.7% for Control, Fig. 3C). DRP incidence showed no significant differences (6.9% for Dual MSC + MC, 14.3% for MC-Only, 24.1% for Control, Fig. 3C). The same trend was observed for proliferative DRP (3.5% for Dual MSC + MC, 3.6% for MC-Only, 10.3% for Control, Fig. 3C).

No significant differences were found in uncontrolled hypertension (13.8% for Dual MSC + MC, 10.7% for MC-Only, 17.2% for Control, $p > 0.05$) or uncontrolled lipidemia 20.7% for Dual MSC + MC, 25.0% for MC-Only, 31.0% for Control, $p > 0.05$) among the groups at 8 years.

Adverse events

Perioperative adverse events included intraoperative abdominal pain, hemorrhage at puncture site, fever and chill. Four cases and three cases of abdominal pain were reported in the Dual MSC + MC group and the MC-Only group, respectively, which relieved without intervention. Two patients in both the Dual MSC + MC group and the MC-Only group experienced hemorrhage at puncture

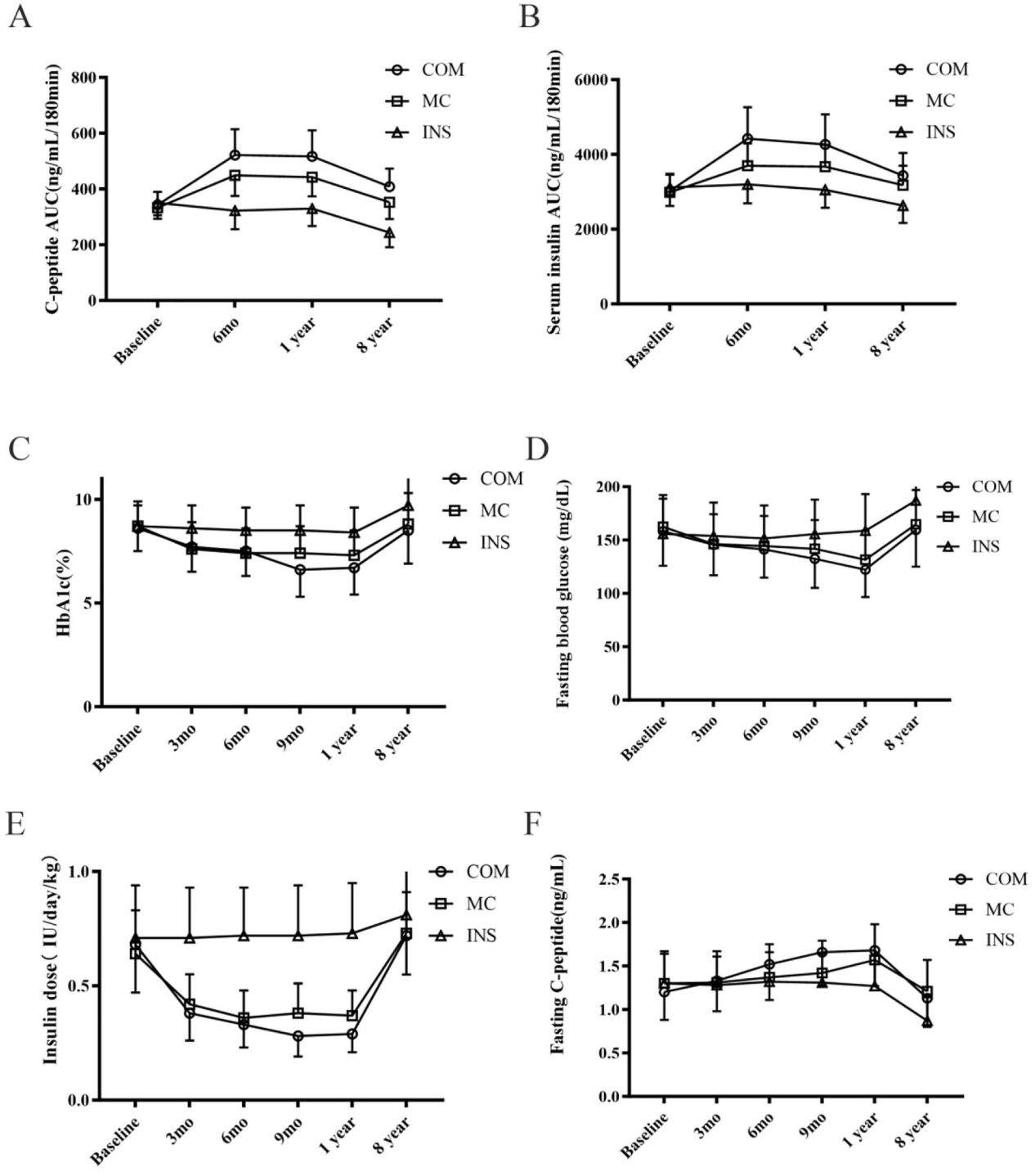

Fig. 2 The C-peptide AUC and serum insulin AUC in the Dual MSC + MC, MC-only and Control groups at the baseline, 6 months, 1 year and 8 years (2 A-B). The HbA1c, FBG, insulin dose and fasting C-p in the Dual MSC + MC, MC-only and Control groups at the baseline, 3, 6, and 9 months, 1 year and 8 years (2 C-F). * Dual MSC + MC vs. Control, $p < 0.05$; † MC-only vs. Control, $p < 0.05$

site, respectively, which were resolved with local pressure. Two patients in the Dual MSC+MC group experienced fever and chill after intraoperative infusion and venous infusion, respectively, which relieved after symptomatic treatment. No cases of postoperative acute pancreatitis occurred.

Within 1 year, two cases of neutropenia were observed in the Dual MSC+MC group, while none was in the

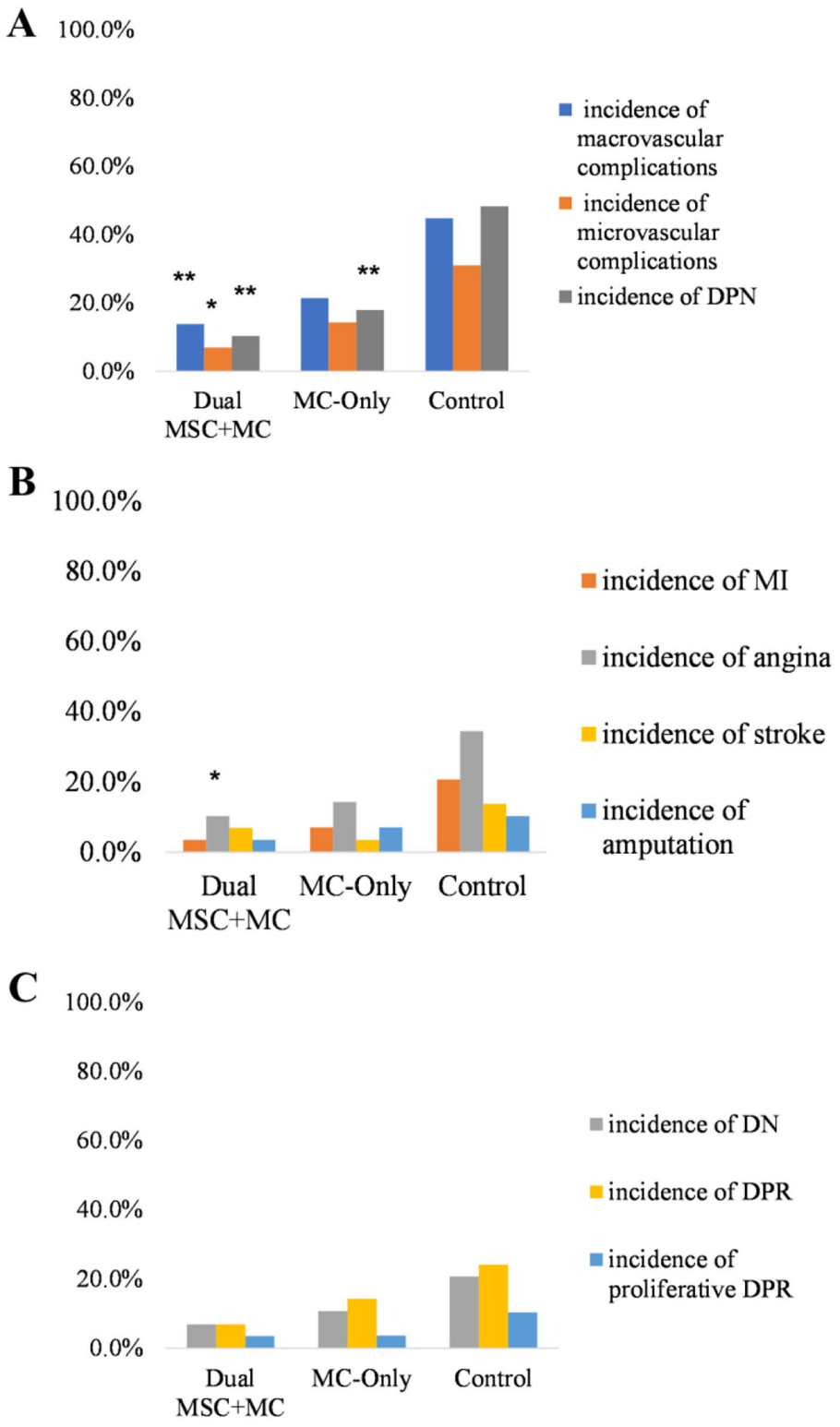

Fig. 3 The incidence of macrovascular and microvascular complications, DNP, MI, angina, stroke, amputation, DN, DRP and proliferative DRP in the Dual MSC + MC, MC-only and Control groups at 8 years. ** vs. Control, $p < 0.01$; * vs. Control, $p < 0.05$

other two groups. There were six, seven and five cases of upper respiratory tract infection in the Dual MSC+MC, MC-Only and Control groups ($p>0.05$), respectively. No episodes of severe hypoglycemia were reported within 1 year.

No death event was reported at the 8-year follow-up visit. One case of lung cancer and one case of gastric cancer occurred in the MC-Only group and the Control group, respectively. No patients in the Dual MSC+MC group developed malignancies.

Discussion

In this study, combined cell therapy was performed based on the rationale that MSCs may alleviate chronic inflammation in the pancreas [13] and promote endogenous regeneration [14], and that MCs may conduce to microenvironmental revascularization [15] and support the role of MSCs. The rational for not setting MSC group was that MC infusion alone was demonstrated to be effective in T2DM in our previous study while the efficacy of intraarterial MSC infusion alone were lack of solid evidence in trials [16]. To the best of our knowledge, few trials have explored combined cell therapy in T2DM.

It is well acknowledged that a large portion of T2DM mortality is relevant to chronic diabetes complications [1]. Recent clinical trials have preliminarily demonstrated that MSC therapy (by intravenous infusion) in T2DM markedly improved metabolic control [3, 17]. However, the effects on chronic diabetes complications in T2DM patients remain unknown [18]. The current study found that autologous bone marrow MSCs plus MCs significantly improved metabolic control and prevented chronic complications. The results showed that the treatment could reduce the risks of both macrovascular and microvascular complications, and DPN. With respect to specific chronic complications, incidence of angina was reduced. Although differences were observed in myocardial infarction, stroke, amputation, diabetic nephropathy, diabetic retinopathy, and proliferative diabetic retinopathy between groups, these changes were not statistically significant. In a small-scale randomized controlled trial on type 1 DM, the therapy with a combination of umbilical cord MSCs and MCs has shown positive outcomes of prevention of chronic diabetes complications [7]. To date, the available evidence of prevention of chronic diabetes complications largely remains in animal models. Scalinci et al. found that neuroprotective growth factors were significantly increased in DR rats injected with hMSCs [19]. In T2DM rats treated with multiple intravenous MSC infusions, Han et al. showed that DPN symptoms, motor and sensory nerve function were restored [20].

The combined cell therapy improved metabolic control in T2DM patients, including HbA1c, FBG, insulin dose and fasting c-p at 1 year. It was noted that several patients in the Dual MSC+MC group had achieved insulin independence at 1 year, suggestive of a promising prospect of the modified cell regimen. Liu et al. found that treatment with Wharton's Jelly-derived MSC improved metabolic control and beta cell function in patients with T2DM [3]. Bhansali et al. showed that both BM-MSCs and MCs resulted in sustained reduction in insulin doses, increase in C-peptide response and improvement in insulin sensitivity with MSCs in T2DM [21]. However, insulin independence was rarely seen. Although metabolic control at 8 years was significantly worse than that at 1 year, it was still significantly better than that in the Control group, suggesting that the improved islet function was degenerating in a prolonged manner. The reason for the deterioration may include the persistence of insulin resistance syndrome.

This study monitored intervention-related adverse events and short- and long-term adverse events related to MSC and MC infusion. Intervention-related adverse events, including transient abdominal pain and puncture site bleeding, were mild. The short-term adverse events of MSC infusion were mainly fever and chills, which were presumed to be unrelated to the MSCs but possibly related to the residue of conditioned medium, which might contain allergens and endotoxins. Few studies have described long-term adverse events of stem cell treatment. The long-term outcomes showed that Dual MSC+MC group did not have significantly higher tumor incidence than other groups.

The mechanisms underlying the benefit of combination therapy may include improved islet function and repair of lesioned tissues. It seemed the reduction in the risk of chronic complications could not be fully explained by the improvement in metabolic control [22]. It is accepted that MSCs may exert systemic therapeutic effects on potentially lesioned organs via the anti-inflammatory and tissue-repairing properties [23]. Although patients with clinically detectable complications were excluded in the study, as is well known, preclinical impairment to organs occurs in DM patients before diabetes complications were diagnosed [24]. Furthermore, impaired organs may experience regeneration and self-renewal due to the property of inducing and activating organ-specific stem cells by MSCs [25]. Basically, the mechanism of benefit remains unclear and further study is required to verify the speculative explanations provided.

Limitations of this study include the small sample size and the evaluation of complications was done at the 8-year time point, which was not longitudinal.

Conclusions

The results from the 8-year assessment of T2DM patients treated with combined stem cell therapy suggest that the treatment is associated with long-term improved

metabolic control and reduced incidence of chronic diabetes complications. Notably, it is safe without severe long-term concerns. These findings support further testing of this treatment in T2DM in a larger scale study. Specific chronic complications can be selected in the design to clarify whether the improvement is universal or organ-specific so as to conduct corresponding fundamental studies.

Abbreviations

T2DM	type 2 diabetes mellitus
MSCs	marrow mesenchymal stem cells
MCs	mononuclear cells
BM	bone marrow
DN	diabetic nephropathy
DRP	diabetic retinopathy
DPN	diabetes peripheral neuropathy
BMI	body mass index
C-p-AUC	c-peptide area under the curve
HbA1c	glycated hemoglobin
FBG	fasting blood glucose
C-p	c-peptide
HOMA-IR	homeostatic model assessment insulin resistance
MI	myocardial infarction

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Not applicable.

Author contributions
Zhixian Wu performed the study, collected data, analyzed data and wrote the manuscript; Shulin Huang and Shasha Li performed the study, collected and analyzed data, and revised the manuscript; Jinquan Cai and Lianghu Huang prepared the cells, Weizhen Wu performed the study, Jin Chen participated in the design of the trial, monitored and analyzed the data and co-wrote the manuscript, Jianming Tan designed the study. Jianming Tan is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All patients gave signed informed consent, and the study was approved by the institute review board (Board Name: IRB00006161 - Fuzhou General Hospital IRB #1). The study, titled "MSC and MC in Type 2 diabetes," has been registered with clinicaltrials.gov (NCT01719640) and received project approval on November 1, 2012. The study was conducted in compliance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guidelines and local regulatory requirements. Experimental bone marrow is harvested from healthy donors through a bone marrow aspiration procedure.

Consent for publication
Not applicable.

Competing interests
The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

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