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ORIGINAL ARTICLE

Clinical Trials Study

Safety evaluation of human umbilical cord-mesenchymal stem cells in type 2 diabetes mellitus treatment: A phase 2 clinical trial

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Abstract

BACKGROUND

Progressive pancreatic β cell dysfunction is a fundamental aspect of the pathology underlying type 2 diabetes mellitus (T2DM). Recently, mesenchymal stem cell (MSC) transplantation has emerged as a new therapeutic method due to its ability to promote the regeneration of pancreatic β cells. However, current studies have focused on its efficacy, and there are few clinical studies on its safety.

AIM

To evaluate the safety of human umbilical cord (hUC)-MSC infusion in T2DM treatment.

METHODS

An open-label and randomized phase 2 clinical trial was designed to evaluate the safety of hUC-MSC transplantation in T2DM in a Class A hospital. Ten patients in the placebo group received acellular saline intravenously once per week for 3 wk. Twenty-four patients in the hUC-MSC group received hUC-MSCs (1×10^6 cells/kg) intravenously once per week for 3 wk. Diabetic clinical symptoms and signs, laboratory findings, and imaging findings were evaluated weekly for the 1st mo and then at weeks 12 and 24 post-treatment.

RESULTS

No serious adverse events were observed during the 24-wk follow-up. Four

patients (16.7%) in the hUC-MSC group experienced transient fever, which occurred within 24 h after the second or third infusion; this did not occur in any patients in the placebo group. One patient from the hUC-MSC group experienced hypoglycemic attacks within 1 mo after transplantation. Significantly lower lymphocyte levels (weeks 2 and 3) and thrombin coagulation time (week 2) were observed in the hUC-MSC group compared to those in the placebo group (all $P < 0.05$). Significantly higher platelet levels (week 3), immunoglobulin levels (weeks 1, 2, 3, and 4), fibrinogen levels (weeks 2 and 3), D-dimer levels (weeks 1, 2, 3, 4, 12, and 24), and neutrophil-to-lymphocyte ratios (weeks 2 and 3) were observed in the hUC-MSC group compared to those in the placebo group (all $P < 0.05$). There were no significant differences between the two groups for tumor markers (alpha-fetoprotein, carcinoembryonic antigen, and carbohydrate antigen 199) or blood fat. No liver damage or other side effects were observed on chest X-ray.

CONCLUSION

Our study suggested that hUC-MSC transplantation has good tolerance and high safety in the treatment of T2DM. It can improve human immunity and inhibit lymphocytes. Coagulation function should be monitored vigilantly for abnormalities.

Key Words: Type 2 diabetes mellitus; Cell transplantation; Human umbilical cord-mesenchymal stem cells; Safety; Lymphocytes; Immunity

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Core Tip: Diabetes mellitus is a major public health problem worldwide. Type 2 diabetes mellitus is regarded as a chronic progressive disease that arises from an impairment in the insulin-sensing mechanisms culminating in insulin resistance. Our article focused on the safety of human umbilical cord mesenchymal stem cell infusion for treating type 2 diabetes mellitus. The results suggested that human umbilical cord mesenchymal stem cell treatment can impact human immunity and inhibit lymphocytes. We should pay attention to its influence on coagulation.

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INTRODUCTION

Diabetes mellitus (DM) is a major public health problem worldwide. Type 2 DM (T2DM) is the most common type of diabetes, with adults accounting for 90% of diagnoses[1]. T2DM is regarded as a chronic progressive disease that arises from an impairment in the insulin-sensing mechanisms culminating in insulin resistance. Long-term chronic hyperglycemia can cause multisystem complications, including cardiovascular and cerebrovascular diseases, retinopathy, nephropathy, diabetic foot, *etc*. Although novel medications and diet therapies continue to be developed, none have provided full protection against deterioration of β cell function[2,3]. Meanwhile, many side effects like hypoglycemia, gastrointestinal adverse reactions, heart failure, and atypical fracture have increased with drug treatment[4].

In recent years, mesenchymal stem cell (MSC) therapy has been studied extensively as a novel therapeutic option for diabetes[5,6]. Among the different types of MSCs, those from the human umbilical cord (hUC) have been widely applied in the treatment of different diseases[7]. The hUC-MSCs are a group of more primitive cells derived from neonates and express original stem cell-specific surface markers such as embryonic stem cell stage-specific surface antigen 4 and tumor rejection antigen 1-60. Compared with MSCs derived from other tissues such as bone marrow and fat, the hUC-MSCs have a more abundant content, stronger proliferation ability, and lower immunogenicity[8]. Moreover, hUC-MSCs can be sampled conveniently without damage to the health of the donor, and they do not present any ethical challenges. As such, they are attractive and preferred for clinical applications.

Studies have suggested that hUC-MSCs can promote pancreas regeneration by improving the microenvironment and restoring β cells[9,10]. With the development of hUC-MSC treatment for diabetes and its complications[11-13], the safety of hUC-MSCs is an important concern for clinicians. In practice, the clinical application of hUC-MSCs is well-tolerated (*i.e.*, safe)[14,15]. Participants reportedly suffered from mild symptoms such as fever, dizziness, and vomiting, but no cases of tumor development or death were reported. However, the effect of hUC-MSCs on tumor development remains controversial. While some studies have shown that MSCs can promote tumor progression and metastasis[16], others have suggested that MSCs can suppress tumor proliferation and apoptosis[17]. Moreover, the safety data of hUC-MSCs in diabetes treatment are insufficient. To evaluate the safety and feasibility of hUC-MSC infusion in T2DM, we designed a phase 2 clinical trial. Importantly, this was the first clinical trial of hUC-MSC infusion for T2DM treatment approved by

the China Medical Biotech Association.

MATERIALS AND METHODS

Patients

The enrolled participants were patients admitted to Peking University Shenzhen Hospital (Shenzhen, China) for T2DM, and all provided signed informed consent. The study was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of Peking University Shenzhen Hospital [IRB Approval No. (2018) 29th]. The inclusion criteria and exclusion criteria, as previously reported[18], were applied thoroughly.

hUC-MSC preparation

The hUC-MSCs were provided by Beike Biotechnology (Shenzhen, China)[19]. The isolation process involved Wharton's jelly, a gelatinous tissue around umbilical vessels, from donated hUCs. First, the primary cells were obtained by tissue block adherent culture method, followed by inoculation with 5000 cells/cm^2 and harvesting when the fusion degree reached 85%-90%. After continuous expansion, the fourth passage of hUC-MSCs was suspended in a 10% DMSO cryopreservation solution and stored in liquid nitrogen (-196 °C)[20] (Figure 1). In their future use as a cell stock material, the samples were thawed at 37 °C, washed to remove the DMSO cryopreservation solution, and resuspended in a compound electrolyte preservation solution (containing 5% albumin) before testing of the "final frozen product" for clinical applicability and safety (Table 1). The final frozen product that passed all tests was then placed at 2-8 °C and within 1 h was transported by the specialist to the quality control department in the hospital. The quality control department then conducted a second quality test within 3 h after receiving the sample. We followed the standard that any cell fluid applied in clinical use should be tested twice.

Once the samples passed the tests they were sent to the clinical department for intravenous infusion to the patient, which occurred within 2 h after sample receipt. The total time from the recovery of cell fluid to clinical application should be less than 12 h. The contents of the cell fluid quality test include the following: Integrity of the cell fluid package; appearance of the cell fluid (including coloration and whether there are floccules or any other precipitates or foreign matter); number of cells in the cell fluid; cell viability; endotoxin presence; and Gram stain for pathogenic bacteria.

Study design

Treatment was given for a period of 16 wk, as previously reported[18], after which all patients were reassessed. The total of 34 patients who met the inclusion and exclusion criteria were randomized into two groups by random allocation software. The hUC-MSC group received an intravenous dosage of hUC-MSCs (1×10^6 cells/kg) once per week for 3 wk. The control group was given placebo, which consisted of an acellular injection of the compound electrolyte preservation solution (containing 5% albumin but lacking hUC-MSCs).

Follow-up

The follow-up visits were conducted at weeks 1, 2, 3, 4, 12, and 24 after the first infusion (Figure 2). Experience of fever, chest tightness, chest pain, dizziness, and any other clinical symptoms experienced during the treatment were recorded, along with any adverse reactions such as cardiocerebrovascular events and tumor occurrence.

All study participants were monitored by laboratory tests for routine blood parameters, liver function, renal function, blood lipids (*e.g.*, total cholesterol and triglycerides), and coagulation indexes at weeks 1, 2, and 3 after the first infusion. We also conducted tests for diabetes antibody, specific infection indexes [hepatitis B surface antigen, antibodies against hepatitis C virus, combined detection of antigen and antibody of human immunodeficiency virus (HIV), and specific antibody against *Treponema pallidu*m], and serum tumor markers [alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and carbohydrate antigen 199 (CA199)] as well as electrocardiogram, chest X-ray, and liver ultrasound at baseline and weeks 4, 12, and 24 after the first infusion.

Statistical analysis

All statistical analyses were carried out with SPSS® 25.0 software (IBM Corp, Armonk, NY, United States). Quantitative variables were summarized as median, and categorical variables were summarized numerically. Independent sample Wilcoxon test or χ^2 test was used to assess between-group differences. Differences in proportions were analyzed by two-tailed test. P values < 0.05 were regarded as statistically significant.

RESULTS

Participant baseline characteristics and laboratory test findings

A total of 34 patients were included from September 2019 to September 2022 (Figure 3). After randomization, 24 of the patients were included in the treatment ("hUC-MSC") group and 10 were included in the control ("placebo") group. The clinical characteristics and baseline laboratory test findings are shown in Tables 2 and 3, respectively. No significant differences were observed between the two groups.

Table 1 Quality control standards of human umbilical cord-mesenchymal stem cells

Test	Final frozen product	Final infusion product
Visual inspection	NA	Absence of visible, particle
Morphology	Fibroblastic	NA
Viability	≥ 90%	≥ 85%
Cell count	(4.5-6.0) × 10^7	According to clinical needs
Pathogen tests		
Sterility	Negative	Negative
Mycoplasma	Negative	Negative
Endotoxin	< 0.5 EU/mL	< 0.5 EU/mL
Cell surface markers		
CD73	≥ 95%	-
CD90	≥ 95%	-
CD105	≥ 95%	-
CD29	≥ 95%	-
CD34	≤ 2%	-
CD45	≤ 2%	-
CD79a	≤ 2%	-
CD14	≤ 2%	-
HLA-DR	≤ 2%	-

Not available.

Table 2 Baseline characteristics of participants

Variable	All patients	Placebo group	hUC-MSC group	*Z*	*P* value
	n = 34	*n* = 10	*n* = 24		
Sex					0.45
Male	28 (82.4)	8 (80.0)	20 (83.3)		
Female	6 (17.6)	2 (20.0)	4 (16.7)		
Age in yr	52 (45.5, 56.0)	49.0 (44.0, 58.5)	52.0 (46.0, 56.0)	-0.19	0.85
Duration of T2DM in yr	10.00 (4.00, 14.00)	7.00 (3.75, 11.75)	10.00 (4.50, 14.00)	-0.97	0.33
BMI in kg/m^2	24.21 (23.34, 26.25)	23.72 (23.00, 26.25)	24.61 (23.25, 26.50)	-0.42	0.68
FPG in mmol/L	8.95 (8.13, 10.14)	8.82 (7.81, 9.97)	9.13 (8.18, 11.91)	-0.42	0.68
HbA1c as %	7.95 (7.40, 8.50)	8.00 (7.28, 8.35)	7.95 (7.45, 8.50)	-0.49	0.70

Data are presented as median (minimum, maximum) or number of cases (percentage). BMI: Body mass index; FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin; hUC-MSC: Human umbilical cord-mesenchymal stem cell; T2DM: Type 2 diabetes mellitus.

Primary safety outcome

Safety was evaluated through occurrence of adverse events (AEs) observed within 24 h after each infusion and each visit, including *via* clinical examinations and measurement of vital signs. No serious complications associated with the hUC-MSC infusion were observed.

Four patients (16.67%) from the hUC-MSC group and none from the placebo group experienced transient fever, which primarily occurred within 24 h after the second and third infusions. There was no statistically significant difference between the two groups (P = 0.169) (Figure 4).

Table 3 Baseline laboratory tests of participants

Variable	Placebo group *n* = 10	hUC-MSC group *n* = 24	*Z*	*P* value
Routine blood				
WBC	6.48 (5.71, 8.17)	6.87 (5.52, 8.13)	-0.227	0.821
PLT	232.0 (199.5, 253.8)	214.0 (163.0, 258.3)	-0.718	0.473
RBC	5.15 (4.58, 5.46)	4.94 (4.79, 5.45)	-0.076	0.94
NEU	3.80 (2.95, 4.89)	4.20 (2.90, 5.15)	-0.189	0.850
L	2.21 (1.69, 2.44)	2.23 (1.96, 2.61)	-0.454	0.65
MONO	0.45 (0.34, 0.53)	0.36 (0.31, 0.44)	-1.192	0.233
NLR	1.89 (1.41, 2.36)	1.64 (1.43, 2.13)	-0.227	0.821
Liver function				
ALT	21.00 (18.00, 31.25)	25.50 (16.50, 48.25)	-0.606	0.545
AST	20.50 (17.00, 23.00)	21.50 (18.00, 31.75)	-0.872	0.383
γ-GT	27.00 (20.75, 31.00)	34.50 (23.75, 52.00)	-1.703	0.089
TP	73.70 (68.88, 76.93)	71.70 (68.13, 76.78)	-0.529	0.597
GLB	29.25 (24.63, 30.98)	25.75 (23.93, 28.33)	-1.966	0.049
Renal function				
Urea	6.51 (4.68, 7.23)	5.87 (5.38, 6.61)	-0.227	0.821
Cr	65.00 (58.00, 73.00)	67.00 (56.25, 81.00)	-0.227	0.82
eGFR	106.59 (97.44, 111.71)	104.52 (84.42, 111.99)	-0.454	0.65
Blood fat				
TC	4.61 (4.06, 5.38)	3.95 (3.55, 5.40)	-1.247	0.212
TG	1.67 (1.20, 3.04)	1.87 (1.13, 2.79)	-0.094	0.925
LDL-C	2.91 (2.53, 3.29)	2.48 (2.12, 3.72)	-1.077	0.281
HDL-C	1.14 (0.91, 1.33)	1.12 (0.88, 1.46)	-0.019	0.985
Coagulation function				
PT	12.25 (12.00, 12.78)	12.15 (12.00, 12.60)	-0.494	0.621
APTT	34.05 (32.68, 37.60)	34.10 (32.58, 35.45)	-0.284	0.777
TT	17.95 (17.45, 19.00)	18.00 (17.65, 18.90)	-0.265	0.791
FIB	2.95 (2.61, 3.53)	3.07 (2.29, 3.22)	-0.302	0.762
DD	0.22 (0.22, 0.26)	0.22 (0.22, 0.30)	-0.36	0.719
Infection screening				
HBsAg	0.00 (0.00, 0.00)	0.00 (0.00, 20.31)	-1.081	0.28
HCV-Ab	0.05 (0.04, 0.06)	0.09 (0.04, 0.14)	-1.679	0.093
HIV-Ag/Ab	0.07 (0.06, 0.08)	0.06 (0.05, 0.08)	-0.731	0.465
TP-Ab	0.04 (0.02, 0.06)	0.04 (0.04, 0.05)	-0.776	0.438
Tumor marker				
AFP	2.80 (2.20, 3.85)	1.95 (1.65, 3.10)	-1.494	0.135
CEA	1.85 (1.40, 3.50)	2.15 (1.58, 3.08)	-0.416	0.677
CA199	8.45 (5.20, 17.33)	5.55 (3.35, 15.90)	-0.643	0.52

γ-GT: Gamma-glutamyl transferase; AFP: Alpha-fetoprotein; ALT: Alanine transaminase; APTT: Activated partial thrombokinase time; AST: Aspartate

aminotransferase; CA199: Carbohydrate antigen 199; CEA: Carcinoembryonic antigen; Cr: Serum creatinine; DD: D-dimer; eGFR: Estimated glomerular filtration rate; FIB: Fibrinogen; GLB: Immunoglobulin; HBsAg: Hepatitis B surface antigen; HCV-Ab: Antibodies against hepatitis C virus; HDL-C: High-density lipoprotein cholesterol; HIV-Ag/Ab: Combined detection of antigen and antibody of human immunodeficiency virus; hUC-MSC: Human umbilical cord mesenchymal stem cell; L: Lymphocyte absolute value; LDL-C: Low-density lipoprotein cholesterol; MONO: Monocyte absolute value; NEU: Neutrophil absolute value; NLR: Neutrophil-to-lymphocyte ratio; PLT: Platelets; PT: Prothrombin time; RBC: Red blood cell (erythrocyte); TC: Total cholesterol; TG: Triglycerides; TP: Total protein; TP-Ab: Specific antibody against *Treponema pallidum*; TT: Thrombin coagulation time; Urea: Urea nitrogen; WBC: White blood cell.

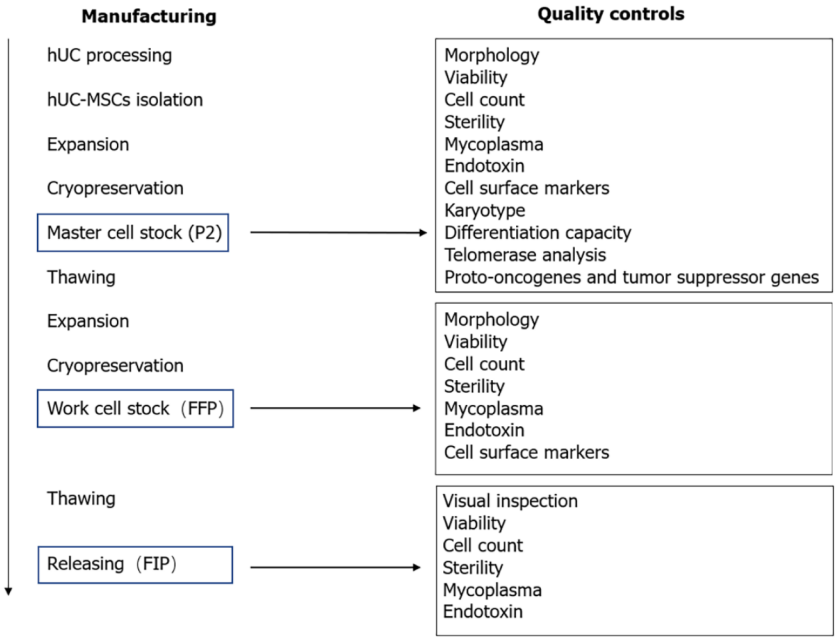

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Figure 1 Stem cell manufacturing and quality control processes. FFP: Final frozen product; FIP: Final infusion product; hUC: Human umbilical cord; MSCs: Mesenchymal stem cells; P2: Second passage.

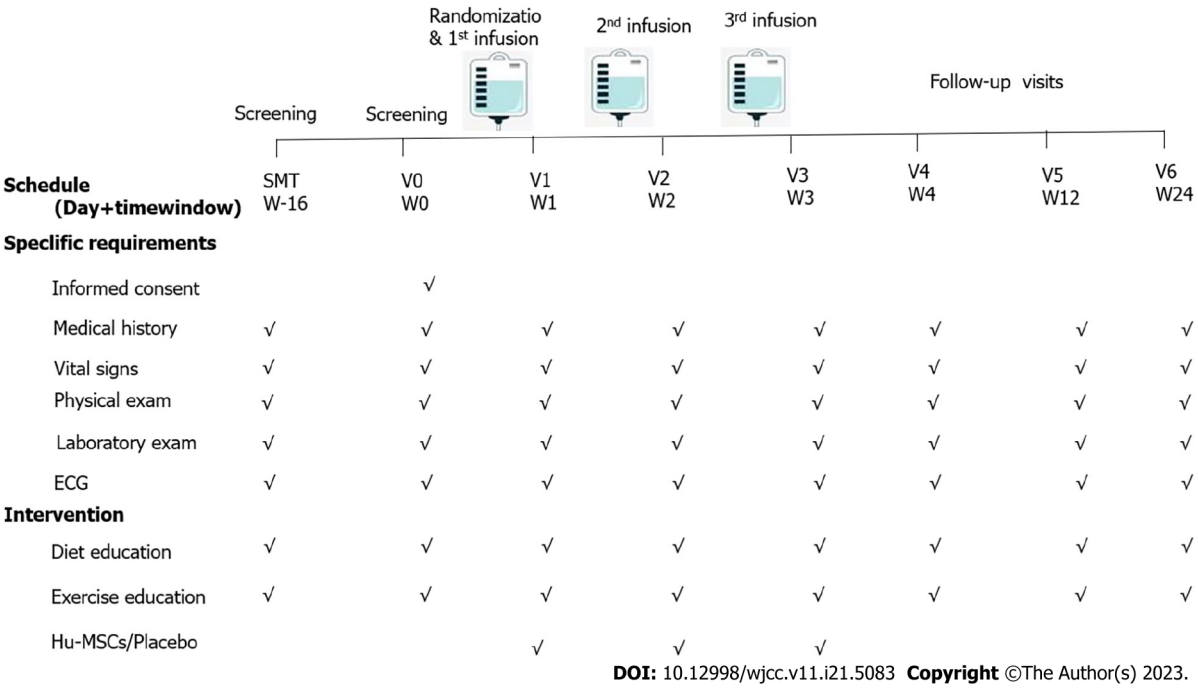

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Figure 2 Flow chart of the study procedure. The patients enrolled in the present study received three infusions on days 7, 14, and 21. They participated in four visits, occurring on days 0, 28, 84, and 180. ECG: Electrocardiogram; Hu-MSCs: Human umbilical cord-mesenchymal stem cells.

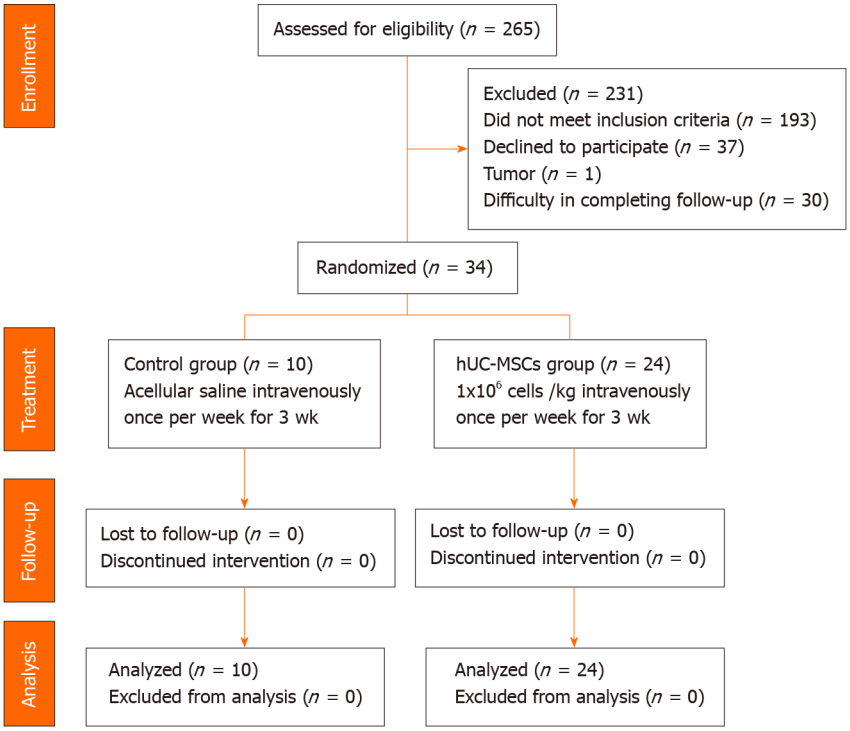

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Figure 3 Flow diagram for patient recruitment. Thirty-four patients were enrolled and completed the entire study procedure. No enrolled patient was excluded from the safety analysis. hUC-MSCs: Human umbilical cord-mesenchymal stem cells.

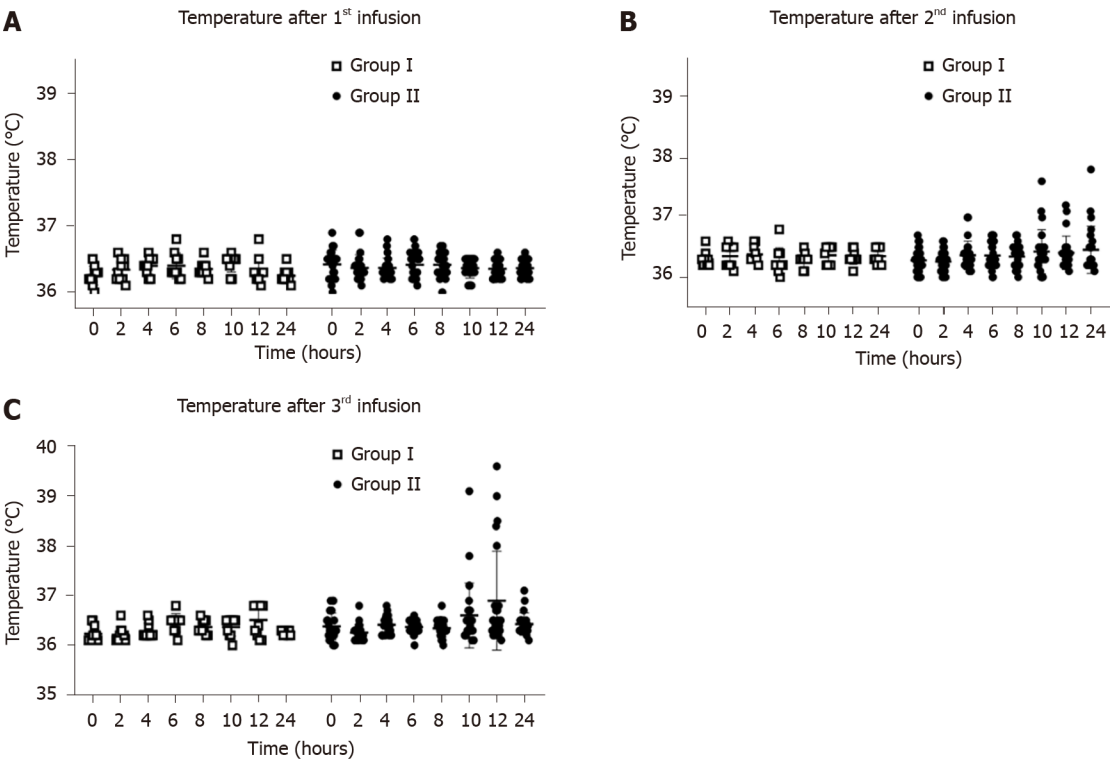

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Figure 4 Fever occurrence within 24 h after infusion. A: Temperatures of the patients within 24 h after the first infusion; B: Temperatures of the patients within 24 h after the second infusion; C: Temperatures of the patients within 24 h after the third infusion. Group I: Placebo group; Group II: Human umbilical cord-mesenchymal stem cell infusion group.

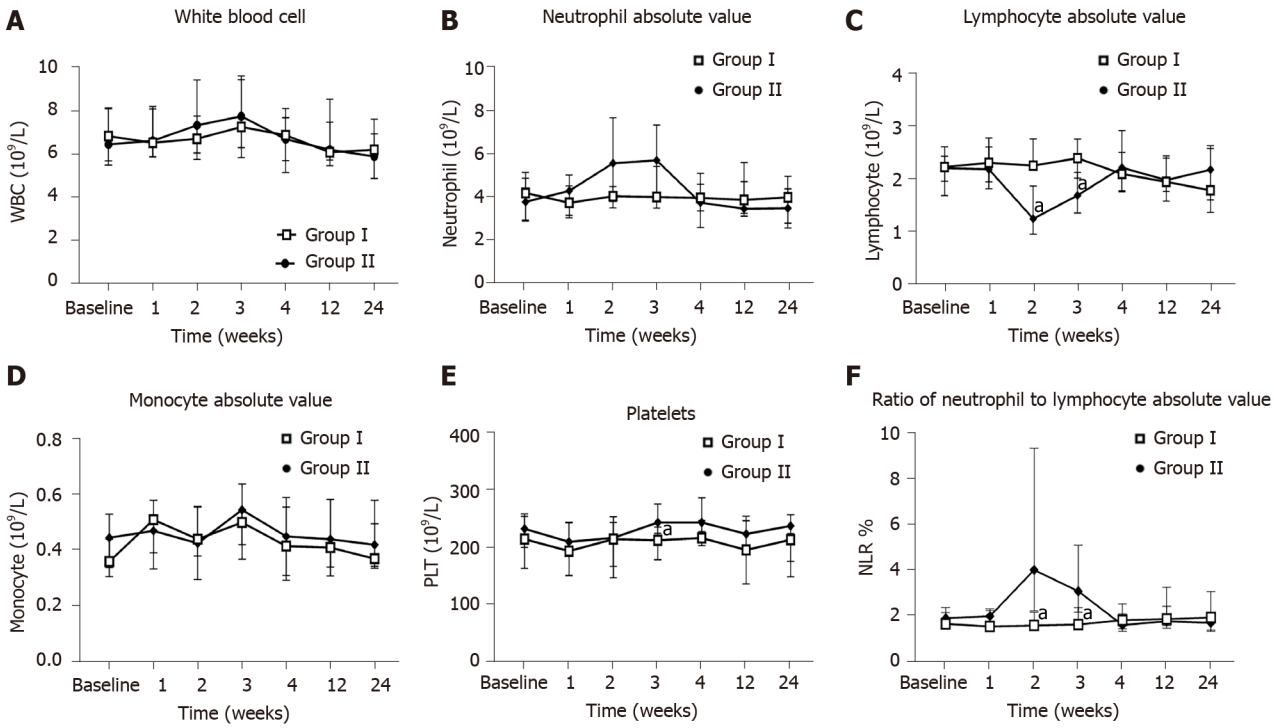

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Figure 5 Influence of the human umbilical cord-mesenchymal stem cell treatment on routine blood measurements. Measurements for all indicators were taken at baseline and weeks 1, 2, 3, 4, 12, and 24 after the first infusion. A: White blood cell count; B: Neutrophil absolute value; C: Lymphocyte absolute value; D: Monocyte absolute value, E: Platelet level; F: Neutrophil-to-lymphocyte ratio. $^{a}P < 0.05$. Group I: Placebo group; Group II: Human umbilical cord-mesenchymal stem cell infusion group; WBC: White blood cell; PLT: Platelet; NLR: Neutrophil-to-lymphocyte ratio.

One patient (4.17%) from the hUC-MSC group and none from the placebo group experienced nocturnal hypoglycemia. The lowest blood glucose recorded for that single patient was 59.4 mg/dL, and the level returned to normal without intervention or food intake. During the follow-up period, that patient reduced their insulin dose and did not experience hypoglycemia again.

Four patients (16.67%) in the hUC-MSC group and none from the placebo group experienced fatigue within 3 d after the first infusion; in each case, it did not affect daily activities or work. In all, the AE was relieved gradually without any intervention.

hUC-MSC infusion decreased lymphocyte levels and increased neutrophil-to-lymphocyte ratio

From the second week, patients in the hUC-MSC group showed a decrease in lymphocyte levels, with a return to the normal range after week 4. The lymphocyte levels in the hUC-MSC group were significantly lower than those in the placebo group at week 2 [1.26 (0.97, 1.87) *vs* 2.26 (2.20, 2.76); $P < 0.05$] and week 3 [1.70 (1.36, 2.13) *vs* 2.40 (2.02, 2.76); $P < 0.05$]. The neutrophil-to-lymphocyte ratio was significantly higher in the hUC-MSC group than in the placebo group at weeks 2 and 3 ($P < 0.05$ for both). The platelet levels were also significantly higher in the hUC-MSC group than in the placebo group at week 3 [243.00 (224.00, 275.25) *vs* 212.00 (178.25, 235.25); $P < 0.05$]. At the follow-up visits at weeks 4, 12, and 24, there were no statistically different findings between the two groups for the aforementioned indicators. There were also no significant differences between the two groups for white blood cell, neutrophil, and monocyte counts (Figure 5).

hUC-MSC infusion affected coagulation markers

D-dimer values were significantly higher in the hUC-MSC group than in the placebo group at all timepoints (weeks 1, 2, 3, 4, 12, and 24; $P < 0.05$ for all). The fibrinogen levels were also significantly higher in the hUC-MSC group than in the placebo group at week 2 [3.18 (2.75, 3.69) *vs* 2.78 (2.46, 2.99); $P < 0.05$] and week 3 [3.37 (3.20, 3.79) *vs* 2.87 (2.61, 2.99); $P < 0.05$]. The thrombin coagulation time was significantly lower in the hUC-MSC group than in the placebo group at week 2 [17.40 (17.00, 18.35) *vs* 18.60 (18.23, 19.10); $P < 0.01$], decreasing and stabilizing to baseline at week 3. The prothrombin time (PT) and activated partial thrombokinase time (APTT) were also monitored during treatment, but there were no significant differences found between the two groups ($P > 0.05$) (Figure 6).

hUC-MSC infusion increased immunoglobulin and did not affect liver function, renal function, or blood lipids

The immunoglobulin levels were significantly higher in the hUC-MSC group than in the placebo group at week 1 [29.60 (22.83, 31.60) *vs* 23.40 (21.98, 27.10); $P < 0.05$], week 2 [27.35 (24.93, 29.50) *vs* 23.75 (21.65, 25.60); $P < 0.05$], week 3 [28.00 (25.55, 29.65) *vs* 23.55 (22.03, 27.95); $P < 0.05$], and week 4 [28.70 (26.10, 32.60) *vs* 25.00 (23.70, 27.13); $P < 0.05$]. The liver function indexes (blood alanine transaminase, aspartate aminotransferase, gamma-glutamyl transferase, and total

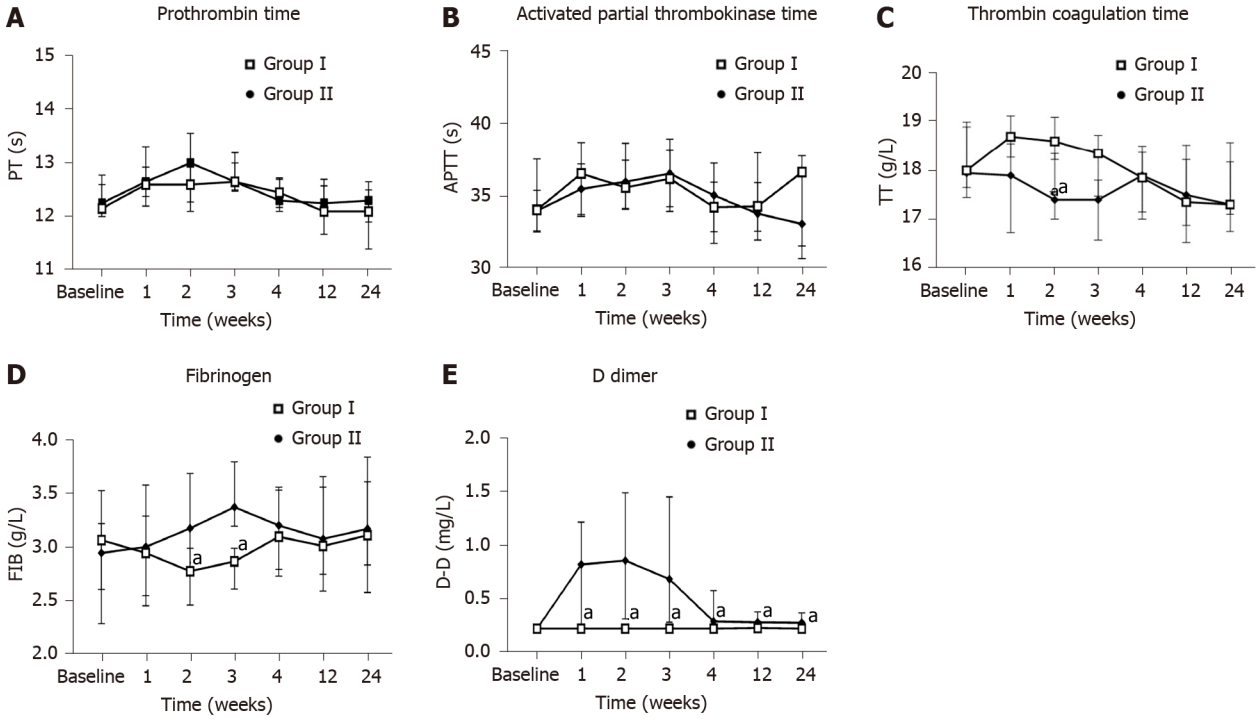

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Figure 6 Influence of human umbilical cord-mesenchymal stem cell treatment on coagulation function. Measurements for all indicators were taken at baseline and weeks 1, 2, 3, 4, 12, and 24 after the first infusion. A: Prothrombin time; B: Activated partial thrombokinase time; C: Thrombin coagulation time; D: Fibrinogen; E: D-dimer. $^{a}P < 0.05$. Group I: Placebo group; Group II: Human umbilical cord-mesenchymal stem cell infusion group; PT: Prothrombin time; APTT: Activated partial thrombokinase time; TT: Thrombin coagulation time; FIB: Fibrinogen; D-D: D-dimer.

protein), renal function indexes (serum creatinine, estimated glomerular filtration rate, and urea nitrogen), and blood lipid indexes (triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol) showed no significant differences between the two groups ($P > 0.05$) (Figure 7).

hUC-MSC infusion had no effect on tumor markers or on special infectious diseases

The AFP, CEA, and CA199 tumor markers, used to evaluate tumor trends, showed no differences between the two groups at any of the timepoints (Figure 8). At baseline we detected hepatitis B surface antigen in 2 patients in the placebo group and 1 patient in the hUC-MSC group. No patients in either group showed positivity for antibodies against hepatitis C virus, combined detection of HIV antigen and antibody, or specific antibody against *Treponema pallidum*. There were also no changes detected for these indicators during any of the follow-up visits after infusion.

Image test outcome

Chest X-ray and liver color ultrasound examinations showed no changes after the infusion at any of the timepoints. In addition, no nodules or tumors were detected in any patient.

DISCUSSION

Currently, MSC-based cellular therapies are considered effective for many diseases, including DM and its complications [11,21,22]. Our previous study showed that intravenous infusion of hUC-MSCs could improve blood glucose, glycated hemoglobin, and islet function in T2DM[18]. However, clinical research on the safety of hUC-MSCs in treating T2DM has been insufficient. The purpose of this study was to evaluate the safety of hUC-MSC intravenous infusion for T2DM patients. The results indicated good tolerability and safety of hUC-MSC infusion in T2DM patients; the potential disadvantageous responses observed were decreased lymphocyte counts, which may relate to escape from immune attack, and an effect on coagulation function, which may lead to thrombotic disease.

After three hUC-MSC infusions, no patients developed a thromboembolic event. Only mild AEs that spontaneously resolved or with minimal intervention (see fever below), were detected in a small number of patients. Generally, this indicates safety (without any acute infusion-related issues, allergic reactions, delayed hypersensitivity, or secondary infections)[23]. Furthermore, no patients experienced a fever after the first infusion, although 4 patients (16.7%) experienced transient fever after the second and third hUC-MSC infusions. The highest temperature recorded was 39.5 °C. The patients' body temperatures returned to normal naturally or gradually after administration of nonsteroidal anti-inflammatory drugs. Some patients also reported fatigue and hypoglycemia after the hUC-MSC infusions. Thus, post-

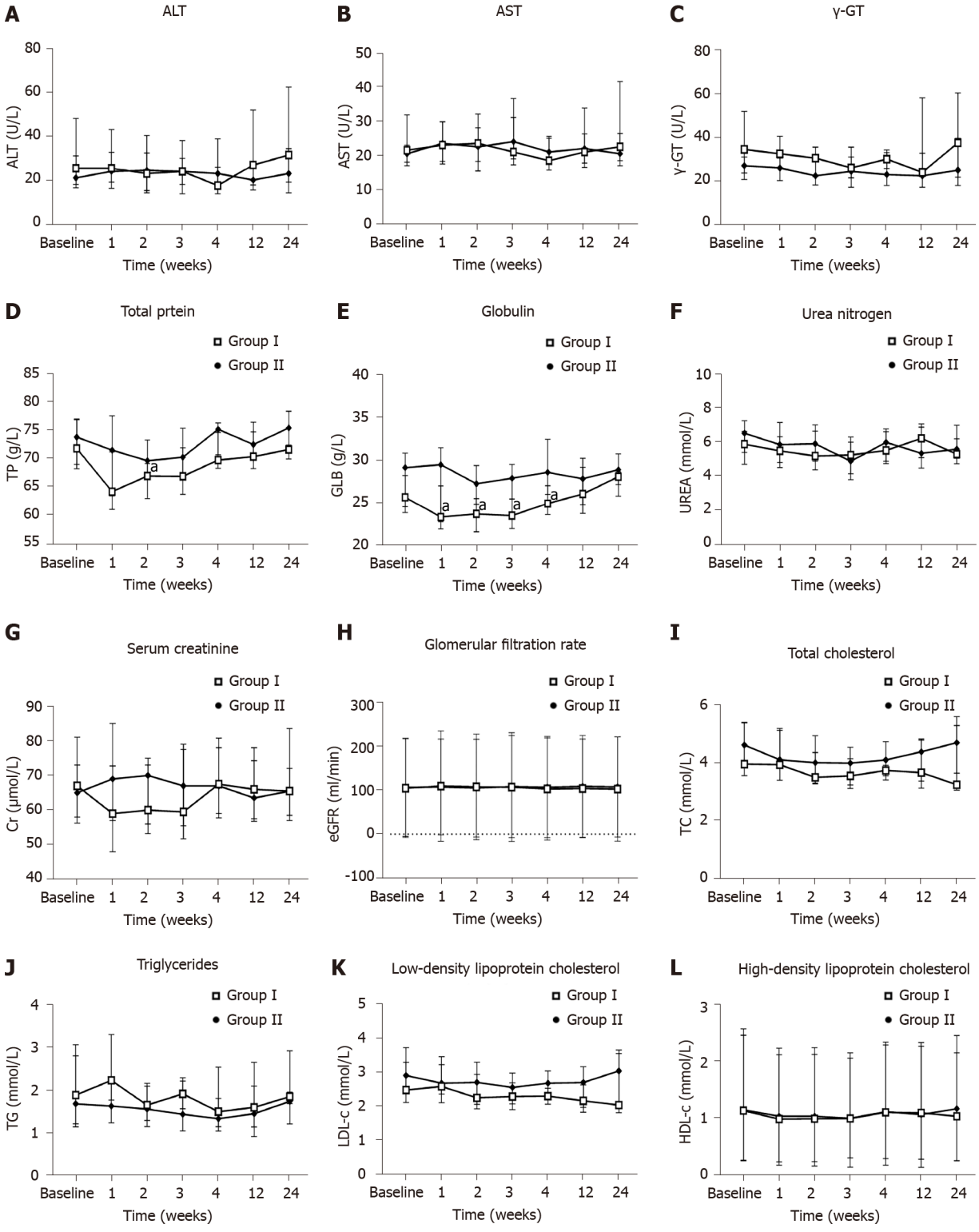

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Figure 7 Influence of the human umbilical cord-mesenchymal stem cell treatment on liver function, renal function, and blood lipids. Measurements for all indicators were taken at baseline and weeks 1, 2, 3, 4, 12, and 24 after the first infusion. A: Alanine transaminase; B: Aspartate transaminase; C: Gamma-glutamyl transferase; D: Total protein; E: Immunoglobulin; F: Urea nitrogen; G: Serum creatinine; H: Estimated glomerular filtration rate; I: Total cholesterol; J: Triglycerides; K: Low-density lipoprotein cholesterol; L: High-density lipoprotein cholesterol. $^{a}P < 0.05$. Group I: Placebo group; Group II: Human umbilical cord-mesenchymal stem cell infusion group; ALT: Alanine transaminase; AST: Aspartate transaminase; γ-GT: Gamma-glutamyl transferase; TP: Total protein; GLB: Immunoglobulin; UREA: Urea nitrogen; Cr: Serum creatinine; eGFR: Estimated glomerular filtration rate; TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol.

intravenous hUC-MSC infusion immune responses were considered as mild symptoms. No novel acute cardiovascular and cerebrovascular events or tumors/nodules occurred during the follow-up.

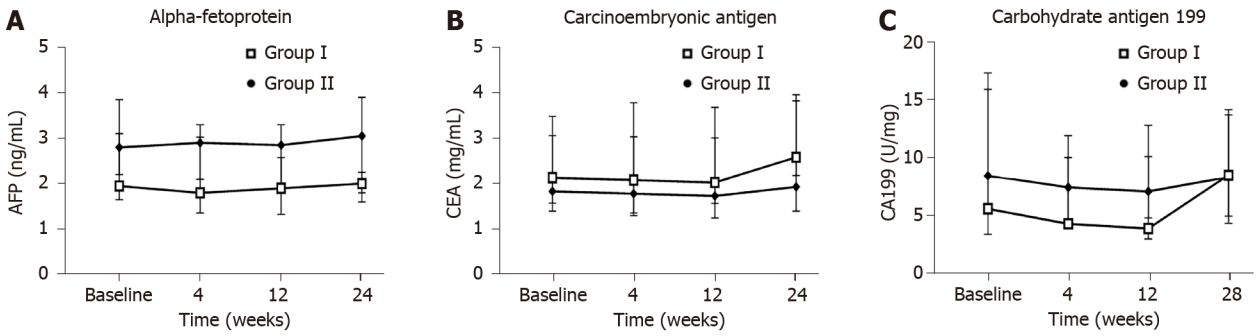

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Figure 8 Influence of the human umbilical cord-mesenchymal stem cell treatment on tumor markers. Measurements for all indicators were taken at baseline and weeks 4, 12, and 24 after the first infusion. A: Alpha-fetoprotein; B: Carcinoembryonic antigen; C: Carbohydrate antigen 199. Group I: Placebo group; Group II: Human umbilical cord-mesenchymal stem cell infusion group; AFP: Alpha-fetoprotein; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 199.

Lymphocytes are an essential component of the immune system. Peripheral blood lymphocytes primarily include T and B cells, which constitute a significant component of the immune system and can produce specific immune responses. MSCs are reported to be closely related to immune cells, like lymphocytes. Di Nicola *et al*[24] found that bone marrow MSCs can inhibit T lymphocyte proliferation and apoptosis. Thus, reactivation stimulates efficient proliferation, which may be relevant to soluble factor production after MSC infusion. Our study revealed that patients experienced a reduction in peripheral blood lymphocytes after 2 wk of continuous intravenous hUC-MSC infusion and recovered spontaneously after discontinuation. The hUC-MSC therapy did not impact the absolute values of lymphocytes during long-term follow-up, consistent with the findings by Di Nicola *et al*[24].

It has also been reported that MSCs can induce T cell apoptosis *via* the FAS/FASL pathway. Moreover, MSCs are known to regulate immune response intensity and promote regulatory T cell production through various paracrine effects and cell-cell contact. This induced immune tolerance diminishes the adverse impacts of lymphocytes on MSCs[24-27].

The application of embryonic stem cells is currently limited because of related teratoma formation[28]. Although research has shown that MSCs do not induce malignant transformation[29,30] and some studies have even explored the efficacy of MSC therapy on tumors[30], concerns about possible tumorigenic actions remain undeniable. Guan *et al*[23] conducted a 3-year follow-up observational study on the efficacy of hUC-MSC treatment of T2DM, and no MSC-related malignancies occurred. Similarly, we did not observe an elevation in tumor-associated antigens (AFP, CEA, and CA199) in patients treated with MSCs. Additionally, during the follow-up period, no nodules/tumors occurred in the lungs, liver, gallbladder, spleen, nor pancreas. However, our follow-up duration was relatively short, and we plan to extend it out to 3 years to thoroughly investigate potential transplantation-related complications.

To date, the roles of MSCs in coagulation and inflammation have been inadequately explored. It has, however, been shown that MSC production may lead to the release of procoagulant tissue factors (TFs) at different levels during the treatment[31]. TFs are significant determinants of cell product blood compatibility and are crucial regulators for the extrinsic coagulation pathway of cytokines. TFs can trigger negative systemic inflammatory responses under some conditions. MSCs themselves can trigger platelet production and promote platelet activation, posing a risk of thrombosis [32]. In particular, an MSC dose of > 1×10^6 cells/kg has been shown to extend PT and APTT and to reduce concentrations of fibrinogen and factor VIII[33]. Small-dose MSC transfusion also affects PT but not APTT, indicating that MSCs mainly activate coagulation *via* TFs and extrinsic coagulation pathways.

Our study revealed that the D-dimer level was significantly elevated following hUC-MSC (1×10^6 cells/kg) intravenous infusion for three consecutive weeks and that elevation persisted to the 24th wk. Continuous transfusion can lead to transient fibrinogen elevation and thrombin coagulation time reduction. The levels recovered after discontinuation of the hUC-MSC treatment, without affecting PT and APTT. We, thus, considered this situation to be correlated with the hUC-MSC dosage and frequency. Previous studies have also found MSC dosage increases to be associated with acute AEs, including microvascular embolization[34,35].

For future clinical application of hUC-MSC treatment for T2DM, we will explore the interactive mechanism between hUC-MSCs and lymphocytes and the effect of cell dose, culturing and passaging techniques, and route of administration on the inflammatory mechanism; these findings may help to reduce and avoid related AEs in these patients. The sample size of the current study was small. At the same time, a small number of participants had delayed visits due to the coronavirus 2019 pandemic, which could have affected our results. We used a single-blind study design, and there may have been deviation during the follow-up. Reducing the error was a priority. Multiple patients were consulted, and follow-up registrants and examiners were single-blinded.

CONCLUSION

Our study demonstrated that hUC-MSCs were well tolerated during T2DM treatment and elicited no serious AEs. Transient fever, hypoglycemia, and fatigue may occur in the short-term, but no long-term AEs were detected.

Lymphocyte levels decreased and inflammatory factors increased after the intravenous infusion of hUC-MSCs. The results of our study are expected to provide a more solid theoretical basis for the continued pursuit of clinical application of MSCs in the treatment of T2DM.

ARTICLE HIGHLIGHTS

Research background

Cellular therapies represent a new opportunity for the treatment of type 2 diabetes mellitus (T2DM) and its complications. However, the safety of human umbilical cord-mesenchymal stem cells (hUC-MSCs) in clinical application has not been fully assessed.

Research motivation

We conducted a trial to evaluate the safety and tolerance of hUC-MSC infusion in T2DM treatment.

Research objectives

We hypothesized that hUC-MSC infusion may cause fevers or nodules, affect inflammatory mediators, and induce hypercoagulability. We conducted the present trial to treat T2DM patients with an hUC-MSC infusion and evaluated the safety of the hUC-MSC therapy.

Research methods

T2DM patients were enrolled and received hUC-MSC (1×10^6 cells/kg) once per week for 3 wk. The safety was assessed by clinical symptoms and signs, laboratory tests, and imaging tests. The laboratory tests included routine blood parameters, coagulation indexes, and liver function, renal function, and tumor markers. Imaging tests included electrocardiogram, chest X-ray, and ultrasound of the liver, bile, spleen, and pancreas.

Research results

During the 24-wk follow-up period, there were no serious adverse events observed. However, a few patients experienced fever, fatigue, and hypoglycemia. The lymphocyte levels were significantly decreased in the hUC-MSC group compared to the placebo group. The D-dimer level, neutrophil-to-lymphocyte ratio, and immunoglobulin level were significantly increased in the hUC-MSC group compared to the placebo group.

Research conclusions

Our study suggests that hUC-MSCs are safe for the treatment of T2DM, with only mild adverse events occurring. hUC-MSC treatment can affect immunity and inhibit lymphocytes. The influence of hUC-MSC on coagulation requires and warrants further research.

Research perspectives

Lymphocyte subsets and the inflammatory mediators of the patients in this study will be extensively followed for further analysis to elucidate the mechanisms of the observed decrease in lymphocyte numbers and the influence on immune function.

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FOOTNOTES

Author contributions: Zhang F designed the report; Lian XF, Lu DH, Liu HL, Liu YJ, Yang Y, Lin Y, Xie F, Huang CH, Wu HM, Long AM, Hui CJ, Shi Y, and Chen Y collected the patients' clinical data; Hui CJ provided cell technical support; Lian XF analyzed the data and wrote the paper; and all authors read and approved the final version of the manuscript.

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Clinical trial registration statement: This study is registered in the Chinese Clinical Trial Registry, Registration No. ChiCTR2200057370.

Informed consent statement: The participants were recruited and enrolled from among patients admitted to Peking University Shenzhen Hospital for diabetes mellitus, and all provided written informed consent.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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