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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

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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.

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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.