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Multiple intravenous infusions of bone marrow mesenchymal stem cells reverse hyperglycemia in experimental type 2 diabetes rats

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ABSTRACT

The worldwide rapid increase in diabetes poses a significant challenge to current therapeutic approaches. Single-dose mesenchymal stem cell (MSC) infusion ameliorates hyperglycemia but fails to restore normoglycemia in diabetic animals. We therefore hypothesized that multiple intravenous MSC infusions may reverse hyperglycemia in type 2 diabetes (T2D) rats. We administered serial allogenous bone-marrow derived MSC infusions (1×10^6 cells/infusion) via the tail vein once every 2 weeks to T2D rats, induced by high-fat diet and streptozocin (STZ) administration. Hyperglycemia decreased only transiently after a single infusion in early-phase (1 week) T2D rats, but approximated normal levels after at least three-time infusions. This normal blood level was maintained for at least 9 weeks. Serum concentrations of both insulin and C-peptide were dramatically increased after serial MSC infusions. Oral glucose tolerance tests revealed that glucose metabolism was significantly ameliorated. Immunofluorescence analysis of insulin/glucagon staining revealed the restoration of islet structure and number after multiple MSC treatments. When multiple-MSC treatment was initiated in late-phase (5 week) T2D rats, the results were slightly different. The results of this study suggested that a multiple-MSC infusion strategy offers a viable clinical option for T2D patients.

1. Introduction

Diabetes mellitus is a complex metabolic disease with an estimated worldwide prevalence of 285 million cases in the adult population [1]. Type 2 diabetes (T2D) accounts for 95% of diabetes cases worldwide and is characterized clinically by uncontrolled hyperglycemia resulting from both progressive and inexorable β-cell dysfunction superimposed on insulin resistance [2]. Although oral agents and exogenous insulin ameliorated the hyperglycemia, they show limited ability to restore progressive β-cell damage. Therefore the challenge is to develop new strategies to antagonize insulin resistance and promote β-cell regeneration.

Mesenchymal stem cells (MSCs) are a population of self-renewable cells with the capacity to differentiate into various cell types [3]. They can be easily isolated and rapidly expanded *ex vivo* [4], and have also been shown to be relatively un-immunogenic, thus they allowing allogeneic transplantation [5]. These properties mean that MSCs have been studied as a potential therapeutic strategy for treating diseases [6–8]. Recent studies have indicated that MSCs could potentially exert anti-diabetic effects, which resulted in the partial recovery of pancreatic islet, increased blood insulin secretion, and correction of hyperglycemia [9–14].

In NOD mice, the administration of a single dose of adult MSCs was sufficient to prevent the onset of type 1 diabetes (T1D) and to retard its progression by suppressing the accumulation and function of effector T cells [11]. The variety of trophic cytokines produced by MSCs improved the pancreatic microenvironment and promoted the expansion of endogenous pancreatic stem cells, resulting in temporarily lowed blood glucose and increased islets in mice [12]. Another study found that MSC infusion restored the immune balance and increased the production of pancreatic islets from endogenous cells. Blood glucose levels fell in the MSC-treatment group, but did not reach normal levels [9]. In a previous study, we also found that a single MSC injection only ameliorated hyperglycemia in T2D rats for a short time, by improving insulin sensitivity in the peripheral tissue [13]. However, transplantation of one dose of MSCs only exhibited short-term effects and failed to restore normoglycemia in diabetic animal models. Because

Abbreviations: T2D, type 2 diabetes; MSCs, mesenchymal stem cells; T1D, type 1 diabetes; BM-MSCs, bone-marrow-derived MSC; STZ, streptozocin; OGTTs, oral glucose tolerance tests; IPITTs, intraperitoneal insulin tolerance tests.

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diabetes mellitus is a chronic, progressive disease, which is possible that serial MSC infusion may improve the effect by maintaining a long-term reduction in hyperglycemia. Indeed, a second MSC infusion further reduced blood glucose levels in our previously study, suggesting that multiple intravenous infusions of MSCs may reverse hyperglycemia.

In this study, we investigated the effects of multiple MSC infusions in T2D rats. We administered serial allogenous bone-marrow-derived MSC (BM-MSC) infusions to streptozocin (STZ)/high-fat diet-induced T2D rats and monitored the effect soon hyperglycemia. Results of reverses hyperglycemia may provide important evidence for future clinical use of MSC therapy for T2D.

2. Materials and methods

2.1. Induction of rat T2D model

Male Sprague–Dawley (SD) rats, 8 weeks old and weighing approximately 200 g, were selected for the experiments, and were obtained from the Chinese PLA General Hospital. Rats were housed for 5 days in a cage with a 12:12 h of light/dark cycle at an ambient temperature of 22–25 °C. A high-fat diet were fed for 5 weeks consisting of 40% fat, 41% carbohydrate, and 18% protein. Rats were fasted for 12 h with free access to water, and then injected intraperitoneally with STZ (40 mg/kg in 0.1 mol/L citrate-buffered saline, pH 4.5) to induce T2D. STZ-treated rats had free access to high-fat foods and water for 1 week and were subsequently subjected to 12 h of fasting. Rats showed fasting glucose levels of $\geqslant$16.7 mmol/L and were considered to be T2D rats as described previously [15].

2.2. Isolation, culture, and identification of BM-MSCs

Fresh BM cells were harvested from the femurs of 6-weeks-old male (SD) rats (220–250 g) by flushing with DMEM-LG (Gibco BRL, Grand Island, NY, USA) containing 1% penicillin–streptomycin (Gibco BRL) as described previously [16]. The surface immunophenotype and multipotency of the MSCs were also confirmed as described previously [17]. The third or fourth passage cells were identified by flowing cytometry and used for all experiments.

2.3. Preparation of BM-MSC conditioned medium (CM)

BM-MSCs were seeded at 5000 cells/cm^2 and incubated in DMEM-LG (Gibco BRL) supplemented with 10% fetal bovine serum (Gibco BRL), 100 U/ml penicillin, and 100 μg/ml streptomycin overnight. The attached cells were washed three times with phosphate-buffered saline (PBS), and the medium was replaced with serum-free DMEM to generate CM. Incubation was then continued for 48 h, and the medium was harvested. The CM was concentrated on 40-fold using Amicon Ultra-15 centrifugal filter units with exclusion size of 3 kDa (Millipore Billerica, MA, USA). The concentrated CM was frozen and stored at −80 °C for future use.

2.4. BM-MSC intravenous administration

T2D rats were divided into three groups: T2D, T2D+PBS, and T2D+MSCs. To investigate the relationship between the infusion phase and the effectiveness of MSCs, we performed single and multiple MSC infusions. For single MSC infusions, 1×10^6 MSCs were suspended in 0.2 ml PBS and injected into rats via the tail vein at 1 week after STZ injection. For multiple MSC infusions, a series of MSC infusions was performed at 2-weeks intervals starting at early-phase (1 week) or late-phase (5 week). Control T2D rats were infused with 0.2 ml PBS at the same time points.

2.5. Blood glucose, insulin, C-peptide, oral glucose tolerance tests (OGTTs), intraperitoneal insulin tolerance tests (IPITTs)

Rats were starved for 3 h before the measurement of blood glucose levels. Tail capillary blood glucose levels were monitored throughout the experiments using one touch ultra (LifeScan Inc., Milpitas, CA, USA). Whole blood was collected from the tail vein, and the plasma was collected after centrifugation at 5000 rpm for 20 min. Serum levels of rat insulin and C-peptide were measured by enzyme-linked immunosorbent assay (ELISA) (rat insulin, C-peptide ELISA Kit, Millipore, Billerica, MA, USA) according to the manufacturer's protocols.

To assess oral glucose tolerance, rats were fasted overnight and serum glucose responses to the oral administration (by gavages) of a solution of 30% D-glucose (1 g/kg) were determined. Glucose levels were determined in tail blood samples taken at 0, 30, 60, 90 and 120 min after oral glucose administration. Animals were not anesthetized for this procedure.

To assess intraperitoneal insulin tolerance, rats were fasted overnight and injected intraperitoneally with insulin (0.5 U/kg) delivered in 1 ml/kg saline. Blood glucose was measured at 0, 30, 60, 90 and 120 min after injections using a hand-held glucose analyzer. Values were presented as a percentage of initial glucose level.

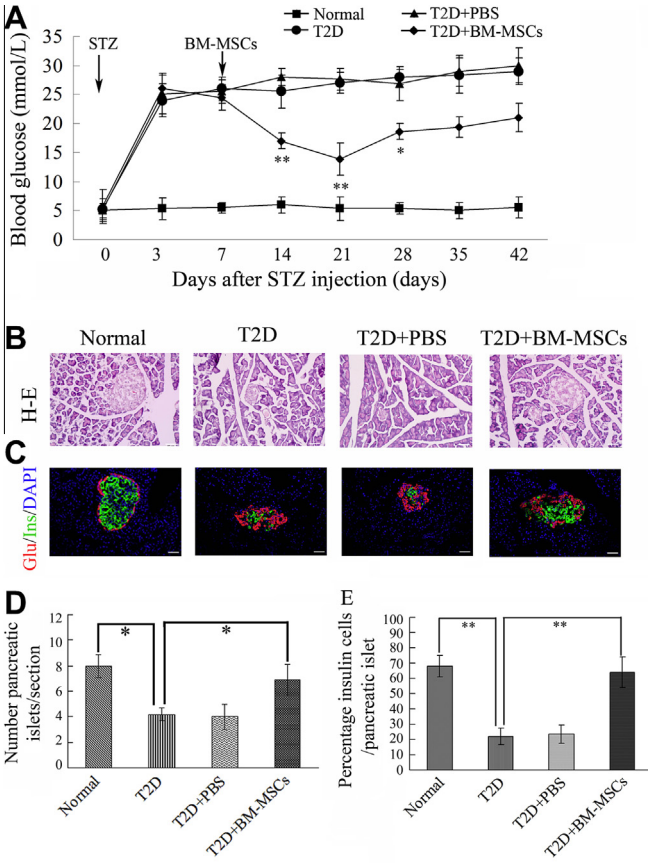

Fig. 1. Single-dose BM-MSC infusion ameliorated hyperglycemia in T2D rats. (A) Blood glucose levels were determined consecutively in fasted rats. (B) Morphology of pancreatic islets stained with H–E. (C) Pancreatic islets were characterized by immunofluorescence according to the presence and distribution of insulin- (red) and glucagon-producing (green) cells. (D) Pancreatic islets observed in H–E-stained sections were quantified in T2D rats administered MSC infusions. (E) β-Cells in pancreatic islets were quantified in T2D and MSC-treated T2D rats administered MSC infusions. Scale bar: 50 μm. Data are shown as mean ± SD (n = 5 sections per group) ($^*P < 0.05$ and $^{**}P < 0.01$).